Nicola LATRONICO Frank A. RASULO Andrea CANDIANI

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Preface

BRAIN (BRescia, Anaesthesia, Intensive care & Neurosciences) *is born with the intent to create a discussion forum where clinicians pertaining to various specialties (Anesthesia, Neurosurgery, Neurology, Neuroradiology, Rehabilitation Medicine, Intensive Care), but also epidemiologists, philosophers, basic scientists, experts in ethics, journalists, and, last but absolutely not least, patients and their relatives together discuss important problems and most recent acquisitions in the fields of Neuroanaesthesia, Neurointensive Care and the related sciences.*

Working in the field of Neuroanaesthesia and Neurointensive Care means daily confrontation with the important topics of life and death; it also means finding oneself alone in his or her decision. BRAIN, therefore, wants to be the occasion to share knowledge and reflections.

Much energy has been dedicated to the birth of BRAIN and we hope that the mild climate of the Franciacorta area helps it grow well, despite the fact that the local beverage is not quite what is most suitable for a newborn. Another reason to grow more quickly!

A warm thank you to all who have helped us.

Nicola Latronico Frank Rasulo Andrea Candiani

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CONSCIOUSNESS AND COMA

Nicola LATRONICO

Key words: consciousness, coma, vegetative state, minimally conscious state.

Without consciousness the mind-body problem
would be much less interesting.
With consciousness it seems hopeless.
Thomas Nagel[1]

One, a hundred, a thousand consciousnesses

Coma is the absence of consciousness. If we define what consciousness is, then we should understand the term coma. A common explanation, as put forward by Plum and Poster[2], is that consciousness has two components: a state of wakefulness (or vigilance or alertness) and its content. The state of wakefulness is defined by the opening of the eyes and is normally easy to demonstrate (trauma to the face or bilateral paralysis of the third cranial nerve which makes it impossible to lift the eyelids are possible exceptions). A first point, therefore, is that even patients with serious alterations of consciousness are not defined as being in coma if they still open their eyes, whether that be spontaneously or not[3]. The vegetative state is one in which the content of consciousness is typically absent, like in coma, but the state of wakefulness is present. Patterns of sleep and wakefulness persist so that patients open their eyes when awake and close them when asleep.

It is more difficult to define what the contents of consciousness are or, rather, lay down a set of criteria on which to base decisions about whether those contents are completely lacking or not. Which inevitably brings us back to the question of what consciousness is. From an etymological viewpoint, consciousness (cum, with and scire, to know) seems to imply a sharing of knowledge, as if to stress that with an etymology like that there can be no real consciousness in the ab-

Prof. Nicola Latronico
Istituto di Anestesia e Rianimazione – Università di Brescia, Spedali Civili
Piazzale Spedali Civili, 1 – 25123 Brescia, Italy
Tel. +39(030)3995 570 – Fax +39(030)3995 570 – latronic@med.unibs.it

sence of a relationship, in the absence of interaction with another being or with the environment. The logical consequence is the existence of a type of behaviour tending towards interaction and communication. By the same token, absence of this type of behaviour is the absence of consciousness.
In actual fact, the definition of what consciousness is is still a long way from reaching an acceptable synthesis if cognitive scientists like Johnson-Laird[4] can still say "no one knows what consciousness is, nor whether it serves any purpose" and philosophers like Chalmers[5] can say that our ignorance about what consciousness is probably represents "the largest outstanding obstacle to a scientific understanding of the universe".
Diametrically opposed to what the etymology of the word suggests, consciousness could also be interpreted as experience, the sum of everything that enables a person to feel they are who they are and not somebody else. In this sense, consciousness would be made up of qualitative elements, the qualia, whose essential property is that of having a certain effect on whoever – animal or human – they belong to. Thus, in the often-quoted chapter by Nagel "What is it like to be a bat? [1], for bats, perceiving the world through their sonar system will have a particular effect. For others, like humans, who are not bats, knowing perfectly well how a sonar system works does not make it any easier to know what it feels like to perceive the world through a system like that.
In Jackson's[6] example, Mary is a neuroscientist who lives in a black and white world and has no idea of what colour is. Mary studied neuroscience for ages, becoming the most famous authority in the world on cerebral processes governing the function of colour, but it is not until she ventures into the outside world for the first time that she learns something new: what it is like to see red . *It is clear that consciousness as defined in qualitative and therefore subjective terms is inaccessible to external observation and therefore cannot be demonstrated.*

Consciousness can be described as self-awareness and this in turn covers various aspects like knowledge of one's own limits, awareness of one's standing within a group, recognition of one's own body and even awareness of one's own awareness. Self determination is linked to self-awareness: "you are free to eat from any tree in the garden, but you must not eat from the tree of knowledge of good and evil for

when you eat of it you will certainly die." (Gen 2,16-17)". Man heard the word "die" for the first time and along with the Holy Father John Paul II[7] we may well ask ourselves whether man, who, in his original state, knew only the experience of existing and therefore of life, would have been able to understand what was meant by the words "you will die". The choice, nonetheless, depended on him, his own conscience and free self-determination. Conscience can also be taken as the moral dimension and is often used in everyday language in phrases like "His conscience was always clean. It had never been used", "Conscience is that inner voice that warns us someone might see us", "you've got pangs of either conscience or hunger"[8]. A more learned quotation about consciousness as man's moral conscience comes from the Catholic Church's Catechism[9]: "Conscience is the most secret and sacred nucleus within man, where he finds himself alone with God, whose voice echoes within his own intimacy". Even in this sense, with consciousness meaning personal experience, consciousness is something totally internal, an inaccessible sanctum. Consciousness can also be interpreted as mind. In this case, any mental state with proactive content counts as consciousness; thus whatever we may think, desire, hope, believe, want or remember[10]. Memory is not in itself consciousness, but without memory "consciousness would be broken up into as many fragments as we have lived seconds"[11]. This maybe described the condition of primordial unicellular organisms whose chemical memories only lasted a few seconds, or even that of modern-day homo sapiens when illness has cancelled out his memory.
No less fascinating is the idea of unconscious, subliminal or implicit perception which complicates the issue of consciousness even more. Seemingly an oxymoron, unconscious perception refers to what a person or animal achieves without being aware of it, unconscious learning can be objectively demonstrated as having taken place because of new facts or actions which are performed. The reader who wants to look at this in more depth is referred to other sources[10,12-14].

The neural correlate of consciousness

As Blackmore[15] said, consciousness is the skeleton in the neuroscientist's cupboard, in the sense that it is only recently that neuroscientists have become so enthusiastic about a topic that had been almost entirely left to psychologists and philosophers. Crick's Astonishing hypothesis dates back to 1994[16]. 'You', your joys and your sorrows, your memories and your ambitions, your sense of personal

identity and free will, are in fact no more than the behavior of a vast assembly of nerve cells and their associated molecules. As Lewis Carroll's Alice might have phrased it: "You're nothing but a pack of neurons". But where is the pack? This is the question we have to ask before we ask "what is the neural correlate of consciousness?" [17], or rather, what is the minimum set of neuronal events that gives rise to a conscious experience. A method of study for defining the neural correlate of consciousness is now available in the form of the study of cerebral activation, whereby the central nervous system is studied before and after adequate stimulus, whether that be the presentation of ambiguous visual stimuli[15], the transition from general anaesthetic to waking up[18] or the transition from a vegetative state to one of minimal consciousness (see below). For example, the return to function of the high frequency oscillatory thalamocortical circuitry in a patient who was initially vegetative and then minimally conscious confirms the importance of the thalamic connections (intralaminar nuclei) and the associative cortices in maintaining consciousness in human beings[19].

Ascending reticular activating system and the state of arousal

Consciousness is such a determining function of human beings that it would be unreasonable to imagine it was contained in a limited region of the brain. Since the early Forties in the last century, thanks to work by Moruzzi and Magoun[20] as well as others (see[10,21,22] for reviews), we have known that the ascending reticular activating system (ARAS), which resides in the dorsal part of the upper brainstem, projects to the intralaminar thalamic nuclei where the widespread thalamic-cortical projections originate. This system widely activates the cerebral cortex and creates the state of arousal and alertness which is a preliminary condition essential to the contents of consciousness.

Compared to what was traditionally believed, today we are in the possession of important knowledge which I will briefly introduce. First of all, the ARAS is no longer viewed as a monolithic structure, nor a system limited to the "reticular" nuclei in the brain stem. The activating structures extend caudally as far as the spinal cord and cranially as far as the cerebral hemisphere[10]. The ARAS, on the other hand, is made up of a series of nuclei, each with distinct anatomical, physiological and chemical features[22]. Secondly, the definition of ARAS as mesencephalic is not correct as many of the brainstem nuclei which

modulate cerebral cortex function are actually found in the upper two thirds of the pontine tegmentum (there are also some in the inferior third of the pons and medulla)[21]. This explains why isolated lesions in the pons can cause coma in patients with ischemic stroke even in the absence of mesencephalic lesions[22]. Thirdly, the thalamus can be seen as the most rostral part of the ARAS and, as such, mediates the majority of its effect on the cerebral cortex. Fourthly, some of the brainstem nuclei bypass the thalamus and connect to the basal forebrain cortex where the widespread, bilateral projections to the cerebral cortex originate. Still other nuclei bypass both the thalamus and the basal forebrain cortex to connect with large areas on the cerebral cortex. Other nuclei are connected to the thalamic reticular nucleus rather than the intralaminar nuclei[10,21,22]. Fifthly, the ARAS is responsible for much more than simple desynchronisation of the cerebral cortex. It is essential for a state of alertness and attention and, in particular, the non-specific thalamocortical projections are important for generating the oscillatory high frequency thalamocortical circuitry which is believed to be critical for consciousness[19,23-26]. Finally, the ARAS, through spinal cord afference and the vestibulary system, receives information about the state of the internal environment and the viscera, vestibular and muscolo-skeletal systems as well as about changes to the organism whenever it interacts with objects[21]. Thus the ARAS not only wakes up the cerebral cortex and, through the thalamus, is important in generating the contents of consciousness, it also directs the contents to specific areas of the cortex and uses them to create a subjective sensation.

Awareness

Awareness is critically dependent on the cerebral cortex and its subcortical connections. The recent advent of cerebral activation studies (PET, functional magnetic resonance imaging, magnetoencephalography, EEG, event-related potentials) represents an important step forward in the study of fundamental mechanisms governing awareness and its content. These studies have shown that in patients in a vegetative state,the connectivity between various areas of the brain wich are normally interconnected has been lost, especially between the primary cortical areas and the multi-modal associative areas (prefrontal, premotor and parietotemporal association areas and posterior cingulated cortex and precuneus)[27] or between these and the

thalamus[28-31]. Basically, the vegetative state can be described as a *disconnection syndrome*[32], pockets of isolated neuronal activity without awareness. A similar loss of connectivity is associated with a substantial reduction in cerebral metabolism (see[33] for a review). On the other hand, in patients who are minimally conscious, who actually have awareness, functional tests have shown that the connectivity between primary cortical areas, associative cortex and thalamus are maintained (or recovered) and therefore the anatomical-functional substrate is preserved so that integrative cerebral processes necessary for conscious activity can be carried out[30,34,35].
As often happens with new discoveries, a new world has opened up thanks to the study of cerebral activation and there are more questions than answers for the moment. Thus, Kotchoubey [30] shows that in a subgroup of patients who are clinically vegetative, cortical activity as regards information analysis is still present, and so these patients are, in fact, minimally conscious. At the other extreme, Schiff [36] shows that word formation, which in itself excludes a diagnosis of vegetative state, may be nothing more than a complex automatism, *words without mind*, pockets of isolated neuronal activity without awareness. Of course it begs the question: how small does the pocket have to be for it not to count? I am not the only to pose myself the question. When Donald Hebb defined the neural correlate of consciousness as "neuronal cell-assembly"[37], Adam Zeman asked himself: "how large must an assembly be to rise to consciousness?"[10].

Coma

It is clear from the previous paragraphs that it is not only extensive bilateral lesions to the cerebral cortex but also, and more likely, small lesions to the mesencephalic pontine tegmentum or paramedian bilateral lesions to the thalamus that can cause coma.
In clinical practice the comatose patients is one who, as a result of brain damage, does not open his/her eyes, does not follow simple commands and does not utter any words that make sense even under intense stimulus. With coma, therefore, there is no trace of either wakefulness or contents. The definition, albeit rudimentary, is definitely practical and lends itself to the urgency that coma demands in medicine. It should be remembered that coma is one of the commonest pathological conditions requiring medical assistance in Accident and Emergency departments and that its causes are manifold and complex[2]. Although a patient's life may be in immediate danger, the situa-

tion can also be put right[38]. This also explains the success of very basic coma scales like the Glasgow Coma Scale[39], which, despite a few problems[40], has not only stood the test of time but outperforms more recent scales[41,42].
Whether a patient like this has total absence of consciousness is a subject for debate, especially in view of the possible definitions of consciousness offered above. There is no doubt, on the other hand, that a patient in coma according to the given definition of the term, has a worse prognosis compared to a patient who is not. This is true regardless of the cause of the cerebral damage, whether it be head trauma[43], subarachnoid hemorrage[44], spontaneous intracerebral hemorrage[45], anoxia[46], or ischaemic stroke[47,48]. The nosological independence of coma is therefore not being questioned.

Vegetative or minimally conscious state

Coma has a limited duration. As Zeman said[10], coma is a transitional state en route towards brain death, full recovery of consciousness or a vegetative state. After 6-8 weeks, patients who have survived acute brain damage open their eyes again[12]. If this return to a state of wakefulness is not accompanied by recovery of the content of consciousness the patient is said to be in a vegetative state[49-51]; if recovery of the content is partial the patient is said to be minimally conscious or minimally responsive[52]. Patients in a vegetative state open their eyes and move them but they do not follow moving objects or people, they grimace, and sometimes cry or laugh, but never as an appropriate response to a stimulus, they may shout but never utter words that make sense[49]. The vegetative state can also be a transitional stage on the way to gradual recovery of consciousness. If it lasts for more than 3[53]-6[50,51] months after an anoxic event or more than a year after head trauma[50,51] the vegetative state is defined as permanent even though the data on this is not unanimous[54]. Minimally conscious patients are unable to communicate in consistent fashion, but they have the content of consciousness. As the Neurobehavioral Conference Workgroup[52] pointed out, patients in this state carry out simple commands, give yes/no answers using either words or gestures, utter words which make sense and demonstrate intentional behaviour albeit not consistently. Differential diagnosis between vegetative state and minimally conscious state is often difficult in practice[3,54] and diagnostic errors which can prove dramatic are not uncommon[55,56]: If the clinical diagnosis is not accurate one in every 3 vegetative patients is actually con-

scious or at least minimally so[57]. With the aim of making clinical assessments more reliable, different methods have been proposed such as the Coma Recovery Scale-Revised[58], the Sensory Modality Assessment and Rehabilitation Technique (SMART)[59], the Disorders of Consciousness Scale (DOCS)[60,61] or batteries of neuropsychological tests[62]. These have recently been reviewed by Laureys[63].

Conclusions

Talking about consciousness and coma brings us face to face with one of the great unresolved mysteries of human life. Recent progress in the field of functional imaging is promising, but "every solution to a problem brings up new, unresolved problems: the more problems arising, the more profound the original problem and the more we desire the solution. The more we learn about the world, and the deeper our learning, the more conscious, specific, and articulate will be our knowledge of what we do not know, our knowledge of our ignorance. For this, indeed, is the main source of our ignorance – the fact that our knowledge can be only finite, while our ignorance must necessarily be infinite[64]".

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ANESTHESIOLOGY AND THE STUDY OF CONSCIOUSNESS

George A. MASHOUR

Key Words: Consciousness, science of consciousness, anesthesiology, mechanism of anesthesia.

Introduction

The study of human consciousness will likely be one of the most important scientific and philosophical endeavors of the 21st century. Although the rigorous investigation of consciousness is still in its early stages, the pursuit has regained scientific legitimacy and the field of anesthesiology is poised to play an important role. Anesthesiologists have the most direct experience in the modulation of consciousness, have the pharmacologic tools to explore consciousness, and stand to gain the most by the scientific understanding of consciousness. Despite these considerations, we have not yet fully embraced the question as central to our field. There is a dearth of formal discourse on the science of consciousness in the literature of anesthesiology and little teaching on the subject during training. There are several reasons why this is the case and these reasons need to be examined and overcome. First, there has been a *lack of need* to consider consciousness. Pressing issues such as safety, adequate monitoring and cardiopulmonary consequences of general anesthesia have required our attention throughout the 20th century. Second, there has been a *lack of legitimacy* associated with the question of consciousness. The dominating cognitive model of behaviorism in the 20th century gave little consideration – and in fact actively discouraged – the investigation of consciousness. Given these reasons, it is therefore clear why the field of anesthesiology has not traditionally maintained the question of consciousness as a central focus of inquiry. What is also clear, how-

George A. Mashour, MD,PhD
Chief Resident, Department of Anesthesia and Critical Care – Massachusetts General Hospital
Clinical Fellow in Anaesthesia – Harvard Medical School
55 Fruit Street – Boston, MA 02114 U.S.A.
Phone: 617-726-8808 – Fax: 617-724-8500 – gmashour@partners.org

ever, is that these reasons are no longer valid. The longer we fail to recognize this, the greater chance we have of losing an opportunity to enrich our field and enhance our scientific stature.

Reconsidering the Study of Consciousness in Anesthesiology

The Question of Need

In both the clinical and scientific facets of anesthesiology, there is now a clear need to explore the question of consciousness. The event of intra-operative awareness—a problem that is tantamount to undesired and undetected consciousness—is a problem that is achieving increased attention.[1] The objective evaluation of consciousness in the intra-operative setting is currently a topic of controversy and will likely require a more sophisticated understanding before further progress will be made. Another clinical concern intimately related to consciousness is that of post-operative cognitive dysfunction.[2] How does general anesthesia, which ideally should only modify consciousness for a short and reversible time period, lead to persistent changes in cognitive function? As the geriatric population increases, so too will the need to understand the relationship between anesthesia and consciousness in the aging brain. The question of consciousness has relevance to critical care, as well. The suppression of consciousness (sedation) and derangement of consciousness (delirium) are important clinical problems in the setting of the intensive care unit.[3] Finally, some of our most challenging clinical situations result from the need to interrupt consciousness through the induction of anesthesia, while leaving cardiovascular or pulmonary functions intact. A more specific pharmacologic targeting of conscious processes that spares systemic processes would be a clear advance for clinical care.

While the question of consciousness is merely one of many clinical concerns for the practicing anesthesiologist, its centrality in the fundamental scientific pursuits of anesthesiology cannot be overstated. First, the nature and mechanism of general anesthesia is inextricably linked to the question of consciousness.[4] Without a fundamental understanding of the neural, physical, and cognitive basis of conscious processing, no theory of general anesthesia will be capable of explanatory power or causal implications. It is important to note that there are markedly divergent opinions on the viability of a "science of consciousness" that have relevance for our field. Although some investigators are confident that neuroscientific investigation will solve the

problem,[5] other thinkers regard the question as principally insoluble.[6] Accordingly, if the identification of the neural correlates of conscious perception is sufficient to understand consciousness, then the mechanism of general anesthetic action could be understood within this context. On the other hand, if no degree of neuroscientific data will ever explain conscious subjectivity, then no neuroscientific explanation will ever suffice for an understanding of the mechanism of general anesthesia.

Divergent theories of consciousness suggest that neuroscience may not be the only approach to the question of consciousness. A number of theories have been developed in the past decade that suggest quantum physics to be a better explanatory model for the many enigmatic aspects of consciousness.[7] Again, there are implications for the mechanisms of anesthesia: if quantum physical processes underlay mechanisms of consciousness, then anesthetics must in some way also act at a quantum physical level.[8] The mechanism of consciousness and anesthesia cannot be meaningfully separated.

It is important to note that these considerations are pertinent not simply to anesthesia but to analgesia as well. The mechanism of pain, another fundamental scientific pursuit in anesthesiology, is directly linked to the mechanism of consciousness. Although there is currently an intense focus on the molecular mediators of nociception and pain, these molecular considerations must ultimately be related to the level of conscious representation. A complete understanding of pain as a *subjective* phenomenon will only be meaningful within the broader discourse of consciousness. The inter-relationship between the nature of pain and the mind-brain problem has been noted and employed by a number of philosophers.[9]

The Question of Legitimacy

It has been suggested that the 19th century German philosopher Edmund Husserl initiated the modern philosophical focus on consciousness.[10] For Husserl, consciousness was not the pinnacle, but the very *foundation* of all scientific pursuits. With the advent of behaviorism, however, consciousness and science were placed on opposite ends of a spectrum. Behaviorism ascribed no value to interior experiences, but rather regarded the relationship between cognitive input and behavioral output as the foundation for a scientific psychology.[11] As such, consciousness lost both primacy and legitimacy. Consciousness as the scourge of psychology was to persist through the latter part of the 20th century, until related concepts started to reap-

pear in psychology and neuroscience. Thus, the scientific perspective of the mind in which modern anesthesiology developed was openly hostile to consciousness. It is little wonder, therefore, that the interest and exploration of consciousness and anesthesia did not develop in parallel.

In the 1990s, a series of events reinvigorated the study of consciousness and a rigorous scientific approach to the question developed. In 1994, a landmark interdisciplinary conference of philosophers, neuroscientists, cognitive scientists, physicists and physicians converged to discuss the possibility of a rigorous study of consciousness.[12] One of the main organizers of this meeting was the anesthesiologist Stuart Hameroff, known for his quantum theories of consciousness. During this time, a number of prominent scientists publicly turned their attention to the study of consciousness, including renowned physicist Roger Penrose, as well as Nobel Laureates Gerald Edelman and the late Francis Crick. Books began proliferate on the subject and articles in preeminent journals such as *Nature* discussed the neuroscience of consciousness directly.[13] Due to the confluence of these events, the scientific pursuit of consciousness regained legitimacy. Articles in *Anesthesiology* over the past several years discussing the neurophysiology of consciousness and anesthesia speak to the arrival of the science of consciousness in our own field.[14,15] Of note, similar articles discussing mechanisms of consciousness and anesthesia have in the past been published in journals related to consciousness or psychology, rather than anesthesiology.[16,17]

Basic Problems and Positions in the Study of Consciousness

There are a number of unique enigmas surrounding the investigation of consciousness that differentiate it from other scientific subjects of inquiry and a number of philosophical positions have been elaborated in order to explain them. The most fundamental of these enigmas is the so-called *mind-brain problem*: how do the objectively observable physical constituents of the brain give rise to the seemingly non-physical subjective phenomena of the mind? (For a current discussion of the relevance of the mind-body problem to psychiatry, see Kendler.[18]) This mind-brain schism is commonly attributed to the French philosopher Renee Descartes, who divided the world into the *res extensa* (extended and physical) and the *res cogitans* (non-extended and thinking). This is the most salient example of the position

known as dualism, a position no longer taken seriously in its strongest form, but with notable proponents in the 20th century such as philosopher Karl Popper and Nobel Laureate J.C. Eccles (discussed in their collaborative book *The Self and its Brain*).[19]
The close relative of the mind-brain dichotomy in the modern study of consciousness is referred to as the "hard problem." Discussed extensively by philosopher David Chalmers,[20] the hard problem of consciousness is the following: while the neural correlates of consciousness may in principal be elucidated (although with great practical difficulty), how could even the complete knowledge of neural activity associated with consciousness explain its subjectivity? In a formulation of the question that is germane to our field, how, for example, do the various *nociceptive* processes from the peripheral to the central nervous system become translated into the subjective quality of *pain*? Even assuming a complete description of pain processing, the quality of "what its like" to feel pain seems to be something beyond this objective description—and thus there exists what has been termed an *explanatory gap*. Explaining such subjective qualities, or *qualia* in the current terminology of consciousness, is considered to be among the most daunting philosophical questions of the field.
Some philosophers, such as Colin McGinn,[6] regard this hard problem as principally insoluble: due to our limited cognitive abilities (or *cognitive closure*), we will never be able to explain our own consciousness in a satisfactory way. Investigators from the discipline of neuroscience, however, tend to see no fundamental intractable mystery.[5] Once the "easy problem" of consciousness is solved, i.e., once the underlying neural correlates of consciousness are identified (a task deemed "easy" only in terms of philosophical principal rather than scientific practice), the "hard problem" will become tractable.

The Relevance of Anesthesiology to the Study of Consciousness

Although it has been stressed above that there is an emerging need for anesthesiology to draw upon the advances in the science of consciousness, it is equally clear that anesthesiology can play a crucial role in the understanding of consciousness. Tens of millions of times each year, the state of waking consciousness is being rapidly and reversibly eliminated or modulated in operating rooms throughout the world – there is no other controlled setting that can boast a more frequent manipulation of conscious processes. This vast clinical experi-

ence and its accompanying investigation may serve to elucidate some of the basic scientific and philosophical questions of consciousness.

As we pursue the specific neural correlates of general anesthesia, we are in a position to understand further the specific neural correlates of consciousness. Neural networks or anatomic sites that are reversibly and consistently inhibited during general anesthesia present themselves as potential mediators of consciousness (for review see Prichep and John).[14] Of equal importance, as we understand the neural activity that *persists* under general anesthesia, we are also in a position to *eliminate* functional or anatomical candidates for the neural correlates of consciousness. The hypnotic component of general anesthesia essentially eliminates qualia, or perceptual qualities. Thus, if particular neural activities are present in the absence of such qualia, they are likely to be poor contenders for the neural correlates of consciousness. It has previously been demonstrated that activation of primary visual cortex V1 is not sufficient for consciousness in the non-human primate.[13] Studies of the visual system under anesthesia have further elucidated that gamma-band oscillations persist through the visual system during general anesthesia and that information may still be transferred in the "feedforward" direction to the rostrum of the brain.[21] Based on these findings under anesthesia, therefore, visual consciousness cannot be accounted for simply by the activation of the caudorostral visual pathway. This example clearly demonstrates the potential for anesthesiology to contribute to the understanding of the neural correlates of consciousness.

It should be clear, however, that the study of general anesthesia is limited in its contribution to the study of consciousness. Since presumably no qualia exist during general anesthesia, the hard problem of consciousness cannot sufficiently be addressed. Another aspect of anesthesiology that can address this question, however, is the field of pain research. The phenomenon of pain is a state of consciousness in which one perceptual quality (or *quale*) dominates. The experience of pain and its underlying neurobiology therefore presents a model for the study of consciousness that can employ the combined first-person (patient complaint) and third-person (neurologic examination, neuroimaging) approaches. The underlying neurobiology of consciousness may mediate between the two predominant contributors of pain: molecular events and psychological predispositions. It is of interest to note that the renowned 20th century logician and philoso-

pher Saul Kripke used the phenomenon of pain as a means to explore the identity of mind and brain.[9]

Conclusion

Consciousness is our organ

We share the airway with our colleagues in emergency medicine, laryngology, and general surgery. We share the lungs with our colleagues in pulmonology and thoracic surgery, and the heart with cardiologists and cardiac surgeons. Consciousness, however, is truly our "organ" – our ability to modulate this functional process from light sedation to deep anesthesia is the art that distinguishes us from all other physicians and health-care providers. This clinical art is now in the position to create a foundation for an emerging science. Furthermore, appropriating the question of consciousness provides anesthesiology with a focus that extends its role beyond the operating suite and into the heart of a fundamental intellectual pursuit. While clinical anesthesia serves as a means to an end in the operating room, resolving the mysteries of consciousness is a scientific and philosophical end unto itself.

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WHAT IS IT LIKE TO BE UNCONSCIOUS?

**Boris KOTCHOUBEY, Simone LANG,
Jerome DALTROZZO, Niels BIRBAUMER**

*Try to fill your consciousness with the representation
of no-consciousness, and you will see
the impossibility of it*
Miguel de Unamuno, "Tragic Sense of Life"

To be open learning something novel

The title of this paper is an allusion on the famous article of Thomas Nagel "What is it like to be a bat?" [1]. In that article, Nagel argued that even if we carefully study all objective characteristics of bats' sensory and perceptual processes, we cannot make a picture of *what it is like*, i.e., how it feels to be a bat and to perceive the world in terms of ultrasounds rather than visual and other natural (for us) images. At best, we can probably imagine how *we* would feel using the same sensory apparatus – but this is not the same as how *bats* feel.

But besides the bats, there are other beings, which are (or at least should be) much closer to us – but whose inner world remains exactly as closed and unimaginable for us, though they are humans and thus their brains share much more features with, e.g., my brain than the brain of a bat does. These are patients in low consciousness states – coma, vegetative state, minimally conscious state (MCS), or akinetic mutism.

But *do* they possess any inner world at all? Does not unconsciousness

Boris Kotchoubey
Institute of Medical Psychology and Behavioral Neurobiology – University of Tübingen, Germany
boris.kotchoubey@uni-tuebingen.de

Simone Lang
Central Institute of Mental Health – Mannheim, Germany

Jerome Daltrozzo
Department of Functional Diagnostics of the Nervous System – University of Strasbourg, France

Niels Birbaumer
Institute of Medical Psychology and Behavioral Neurobiology – University of Tübingen, Germany
National Institute of Mental Health – Washington, DC, USA

simply imply the complete lack of any subjective experience, any trace of pain or pleasure, any intentional state? Of course it is so, if we define it in this way. But then we step on the circular road that can only bring us back to our starting position [2 3]. Science is meaningless if each investigation can only prove the thesis already implied in the original definition.

This circularity is broadly spread in the domain of disorders of consciousness. When we submit a manuscript with data demonstrating a significant difference in some brain function between conscious and unconscious patients, we am sure that it will be accepted for publication with a rationale like "it contributes to understanding of neurophysiological difference between conscious and unconscious brain". But the things get hard if we try to report an important physiological function which – strangely – appears to work similarly in patients with different diagnoses, or even in patients and controls. The usual argument is: "Therefore, this function is not related to diagnosis and thus irrelevant. The manuscript, therefore, is of no use." This implies that we should only be interested in what does *not* work in low consciousness states, not in what does. But, then, we'll never be able to understand what it is like.

An example is the issue of pain sensation. The key dogma in the doctrine of "persistent vegetative state" (PVS) is that these patients cannot experience pain, hence, they cannot suffer. You may, therefore, leave them die of thirst, because they experience nothing. At the same time, in many Western countries it is forbidden to conduct experiments with PVS patients using painful stimuli. The ethical reasoning is the following: the results of such experiments can be useful for the next generation of PVS patients, but not for participants themselves. Therefore, the participants are exploited for the sake of other people. But the use of one human, without his or her consent, as a tool to help another human is unethical. Hence, it is unethical to examine PVS patients in order to test the assumption that they cannot experience pain. But it is fully ethical to take this assumption without testing, as a belief, and to behave toward these patients accordingly, e.g., leaving them in a condition which, if the none-experience assumption is incorrect, would result in unbelievable torment. This is the logic of scientific ethics, quite different from the naïve morals of laymen.

But even if we share the Nagel's idea that the complete objective knowledge does not yield the necessary feeling of what it is like to be in a completely different state of consciousness, we may nevertheless be-

lieve that objective neurophysiological data can at least help us to make a step toward this understanding. Regarding the issue of pain, convergent evidence has been obtained in imaging studies indicating the brain areas most consistently related to processing of pain, e.g., the somatosensory cortex, the insula and parietal operculum, the anterior cingular gyrus, the supplementary motor area [4-8]. These are not the structures most typically injured in PVS. But there is one exception: the thalamus is a formation which frequently responds to pain stimulation and, on the other hand, the thalamus is destroyed in many PVS cases. Thus it appears plausible that the presumed PVS patients' inability to experience pain is caused by their thalamic pathology. This hypothesis, however, have consequences. First, it may imply that about *two-thirds* of PVS patients, mostly of traumatic and anoxic origin, whose thalamus is *intact*, may feel pain [9]. Second, it means that direct nociceptive stimulation of PVS patients should not result in a thalamic activation. Indeed, this result was obtained by kassubek [10]who used a positron emission tomography (PET) and found, in 7 patients, pain responses in almost all typical "pain areas" (both primary and secondary) somatosensory cortex, insula, anterior cingular gyrus), but not in thalamus. Surprisingly, however, the other study recording PET in PVS with pain stimulation, came to the opposite conclusion: activation in the thalamus was found in each and every patient, but in the cortical areas, in none of them [11]. To sum up, the neurophysiological evidence cannot, of course, prove that PVS patients can feel pain; but these data hardly support the thesis that they cannot.

In search for the features of the conscious brain

Early conceptualizations about the nature of unconsciousness assumed the lost or profound suppression of all cortical functions. This suppression can result from the immediate destruction of the most part of the gray matter (e.g., in anoxia), the snap of most connections between different cortical areas (diffuse axonal injury), or the pathology of the brain stem whose activity is necessary to keep the cortex awake [12-17]. The last factor plays the main role in acute coma, while the cortex (or its large parts) can be structurally intact but switched off without the tonic activation of the brain stem reticular formation.
Starting from the 90ies, a number of studies demonstrated, however, various kinds of cortical activity in coma and PVS. As regards the former, event-related brain potentials (ERPs) were found to indicate the ability to discriminate auditory stimuli at different levels of complex-

ity, as manifested in ERP components P300 and mismatch negativity [18-24]. P300 reflects deep processing of stimulus information and is generated by a complex network including frontal and parietal cortex and the hippocampus; the mismatch negativity (MMN) is related to more shallow tone discrimination and is largely generated by the auditory cortex. Both P300 and MMN recorded in coma are reliable predictors of regaining consciousness.
As regards the later, several groups reported PVS patients whose patterns of regional blood flow varied consistently as a function of stimulation in the visual and auditory cortex [25-33]. Such data indicate that at least in some PVS patients, high-level processing operations (i.e., the processing of stimulus meaning) can take place. These findings gave rise to two non-exclusive hypotheses about the possible mechanisms underlying the loss of consciousness, exemplified in PSV. According to one of them, isolated cortical areas may work in unconscious patients; what is lacking is their coordination into a distributed but integrated network [34, 35]. According to the other hypothesis, complex stimulus processing depicted above is limited to primary sensory and motor areas; what is lacking, is their collaboration with secondary and more complex associative areas [36] (reviewed by Kotchoubey [37]).

Event-related brain potentials in low consciousness states

ERPs as a method of testing cortical functions possess several important advantages (see Kotchoubey [38]) . First, they have a high temporal resolution and allow the investigator to follow stimulus processing in real time. Second, they can be recorded in a consecutive row of hierarchically arranged stimulation paradigms, thus testing the cortical processing from the simplest to the most complex functions.
Last but not least, they can easily be registered immediately at a patient's bedside, also in PVS patients who cannot remain motionless during the examination, because movement artifacts can in most cases be removed during data analysis.We applied this method in 50 patients, thirty-eight MCS patients, and forty-four patient in acute non-traumatic coma. In addition we examined a patient with akinetic mutism, and several patients with severe paralysis including four who were completely paralyzed (including total gaze paralysis).
ERPs are event-related EEG activity, thus the analysis always began

with the description of a general EEG pattern and sensory evoked potentials. Brain stem auditory evoked potentials were intact or only slightly delayed in all patients – this was the criterion for patients to be included in the study, as otherwise the presentation of complex auditory stimuli would not have much sense. Most patients demonstrated moderate EEG slowing with the predominant theta (3.5-7.5 Hz) rhythmic activity, which did respond, or only slightly responded to stimulation. Similar slowing also characterized paralyzed patients. We called this pattern, very typical for many patients with diffuse brain disorders, "the benign EEG". A different pattern was found in 12 PSV and 15 coma patients: a diffuse, non-responsive delta-waves (1-3 Hz) or flat EEG, indicating that the most important cortico-thalamic connections were broken in these cases. We labeled it as "the malign EEG".

How frequent is cortical processing?

The lowest auditory level is the arrival of an acoustic stimulus to the auditory cortex, as manifested in an ERP complex P1-N1 with the latency of the positive component (P1) of about 70 ms, and that of the negative component (N1) of 100-140 ms. To sum up, 84% of patients diagnosed as PVS reliably showed at least one kind of stimulus-related cortical activity. After excluding those with the prevailing delta waves, cortical responses were found in 100% PVS and MCS patients. Thus whereas several previous studies using various techniques of cognitive neuroscience demonstrated the presence of cortical responses in *some* PVS patients, we showed that in an important subgroup with this diagnosis selected by the lack of prominent delta activity, cortical responses to auditory stimuli are present in *each* patient [39].

Stimulus discrimination

The MMN as an index of primary, largely automatic discrimination between tonal frequencies was recorded in 52% of PVS patients (in 65% of patients with benign EEG) and in 35% of MCS patients. P300, as an index of complex target-related stimulus discrimination, was recorded in 24% of PVS patients (32% with benign EEG) and in 36% of MCS patients.

Semantic processing

This was investigated using two kinds of stimulation: word pairs and sentences. Word pairs were either closely related ("day-night", "cat-dog"), or unrelated ("man-night", "woman-dog"). Simple 5- to 7-word sentences contained either a congruent, highly expected ending, or an incongruent, meaningless ending. In both cases, semantic discor-

dance is typically expressed in a negative ERP component called N400. This component was found in 14% of PVS patients (22% with benign EEG) and in 25% of MCS patients, demonstrating that the brain of these patients was able to analyze verbal stimuli according to their meaning.

Habituation

A simple paradigm was used in which 10 runs were presented, each run consisting of 10 tones. Finally, the 11th run was presented with tones of a different pitch (1500 versus 800 Hz). ERPs were averaged both across trials (i.e., to the 1st run, 2nd run, … 11th run) and across ten runs (i.e., to all 1st stimuli, all 2nd stimuli, …all 10th stimuli). Both analyses resulted in a significant decrement of the amplitude of the N1 component with stimulus repetition in the group of 33 PVS patients. This amplitude decrement cannot be accounted for by adaptation or refractoriness of neurons, first, because there was no similar decrement for the later ERP component P2 (having a longer latency, P2 should be more susceptible against refractoriness than N1); second, because of the highly significant correlation between the rate of the N1 decrease and its recovery to the stimuli of changing pitch (in the 11th run). Therefore, the N1 amplitude habituated with stimulus repetition, which is the first indication, in these patients, of a very simple *learning process* in the auditory cortex [40].

Predictive value

PVS and MCS are chronic conditions so that it is difficult to find significant predictors of the change in the patients' state – because in many patients there is no change in their state. Nonetheless, the presence of the MMN turned out to be significantly related to clinical improvement during the following six months after the examination – as mentioned above, similar data had consistently been obtained in acute coma. We also observed a patient who regained consciousness after 20 months in PVS, which is a very rare case, given that this condition is defined as "permanent" (that is, irreversible) after 12 months. In that patient we recorded stable P300 (to physical stimuli) and N400 (to semantic stimuli) ERP components three times, beginning from 6 months after the injury, i.e., more than one year before the clinical signs of improvement. Surely, the importance of this single-case observation should not be overvalued, but due to the rarity of such a late awakening, accumulation of a large data set will be very difficult.

Acute coma

The most representative studies of coma patients (e.g., [18, 19, 21, 41] found

the frequencies of tonal discrimination (as manifested in the MMN and P300) similar to those obtained in our investigation of PVS and MCS. Those studies, however, never tested semantic abilities in acute coma. Our study (Daltrozzo et al., submitted) was the first in which the corresponding stimulation (i.e., congruent versus incongruent word pairs, and meaningful versus meaningless sentences) was used. Unfortunately, we did not examine a representative group. In contrast to the PVS/MCS study described in the preceding paragraphs, and to the above-mentioned large coma studies, we did not examined a well-balanced group of different etiologies; rather, our sample mostly contained severe patients in coma due to brain anoxia, encephalopathy, or poisoning. Given a possible alpha-error, singular finding of a "significant" ERP component can be obtained by chance, and the observed frequency of the MMN and P300 in our sample did not substantially differ from the chance expectancy. However, the occurrence of the N400 wave to semantically incongruent word pairs significantly exceeded the figure expected by chance (7 patients, i.e., 16%), indicating that the processing of word meaning is also possible in acute coma. Like in the study of PVS, we also found a significant habituation in coma patients.

Locked-in

One may speculate on possible cognitive changes in locked-in-state, depending on its etiology. In the classical locked-in-syndrome after a stroke in the anterior part of the pons cerebri no primary cognitive disorders are expected, but patients' subjective reports reveal severe disturbances of perception and attention, probably related to the sudden loss of the movement-related proprioceptive and, in part, exteroceptive afferentation. We observed virtually normal ERP components, including semantic responses, in three patients with the typical locked-in-syndrome with preserved communication ability, and in a patient with a chronic Guillain-Barré syndrome (GBS) who had been completely paralyzed for three years before the examination[42] . Resulting from a peripheral polyradiculoneuropathy, GBS also should not yield a primary, direct disorder of consciousness. However, possible consequences of the total immobility and the complete lack of communication during three years have never been studied and cannot be imagined.

The total locked-in state can also be caused by neurodegenerative diseases like amyotrophic lateral sclerosis. The issue of possible disorders of consciousness in such patients is very speculative. Abnor-

mal states of consciousness might results from the chronic immobility and the lack of communication, or from the primary degenerative processes outside purely motor areas (e.g., in the frontal cortex), or a combination of both. Only two decades ago there was no such patients; all of them died of respiratory paralysis long before they could attain the stage of complete immobility. In the last year, however, more and more patients with amyotrophic lateral sclerosis decide to remain alive being artificially ventilated and fed. We found three such patients who (like the GBS patient above) were unable to use even minimal eye movements or blinks for communication. ERPs were close to normal in one patient, slightly abnormal but with consistent responses to all kinds of stimuli in the second one, and clearly abnormal (no P300 or N400 was elicited) in the third patient [43]. Thus it cannot be ruled out that severe motor disorders can also result in, at least temporary, disorders of consciousness whose nature should be investigated by objective neurophysiological methods.

Akinetic mutism

This very rare and virtually mysterious syndrome is typically caused by a bilateral lesion to the frontal areas related to initiation and organization of action: the premotor cortex, anterior cingular gyrus, and the supplementary motor area. Patients have a normal sleep-waking cycle but do not perform any voluntary movement although they are not paralyzed. Being unable to follow instruction, the patients nevertheless have marked following gaze movements, which strikingly distinguishes them from PVS. The traditional conceptualization implied in the term akinetc mutism assumes the essence of the syndrome in the disorder of movement intention. The term mutism describes patients who can understand language but do not possess expressive speech. From this point of view, mental processes may not be disordered in this syndrome; what is lacking is the transition from cognition to action [44, 45]. In contrast, many authors regard akinetic mutism as a severe primary disorder of consciousness close to that in PVS (e.g.)[46, 47].

The results of two examinations of a patient who suffered from akinetic mutism after a rupture of the ramus communicans anterior, hardly agree with any of these two extreme points of view [48]. On the one hand, ERPs in several paradigms were clearly pathological. On the other hand, there were significant responses to different kinds of complex stimuli including semantic stimuli. Also [24] later found a distinct P300 in a patient with akinetic mutism.

Conclusion

To sum up, what the brain of patients in low conscious states can do? It can process a lot of stimuli at the cortical level. It can distinguish between frequent and rare stimuli, respond to rare deviants as targets, analyze verbal stimuli according to their meaning. It is able to elementary cortical learning and to recognition of emotional prosody (unpublished data of recording ERPs to affective exclamations). Semantic processing can also be found in a significant part of coma patients.
However, the question formulated in the preceding paragraph is not the same as that posed at the title of this paper. Indeed, the issue of brain (or cortical) functions of patients in low consciousness states should only be a mean to answer the question, *what it is like* to be in such a state. As regards this latter question, the progress in our knowledge is less obvious. In particular, it remains unknown whether, and to what extent, the cortical activations found in PET studies are related to conscious experience of the perceived events. The activity reflected in cortical auditory components N1 and the MMN can run completely automatically, without any participation of consciousness. The same holds true for such a simple learning process as habituation. Surprisingly, more complex processes of associative learning have never been investigated in patients in low consciousness states – though even evidence for building up conditioned associations would not shed much light on the issue of subjective experience.
Likewise, most findings of the processing of semantic information in PVS and coma were obtained in the word pair paradigm (see above). At the same time, evidence has been accumulated that this kind of semantic analysis is, at a most part, attributed to the process of "automatic spreading activation", i.e., uncontrolled and largely unconscious flow of activation from excited semantic modules (e.g., "cat") to associatively related modules (e.g., "dog" and "mouse"). This spreading activation may, or may not involve conscious experience of word meaning (review see [49]. Much less frequent are positive findings in the sentence paradigm, in which, in contrast, ERP effects appear to be more closely related to conscious apprehension of word meaning. As concerns P300, this component is most usually observed in relation to active, controlled information processing. But even these findings are not definitive since very small but significant P300 has been reported in REM sleep [50, 51] and in response to subthreshold stimuli [52].
"After a brief consideration I conclude that I, in fact, am wiser than all other men. – confessed Sokrates. – "I don't know what is Truth,

Beauty, Justice, etc., and they don't know either. But at least, I know that I do not know it, whereas they, though not know, fancy that they know. Thus I believe that in this certain sense I can claim that my knowledge is larger than theirs; for even if I know nothing, I know this, and they do not" (cit. for) [53].

Following the pioneer work of Jennett on coma and PVS in the 70ies (e.g., [54-57] reviews in [58, 59], several decades past during which low consciousness states have been investigated carefully at morphological, functional, electrophysiological, social and ethical level. It appears that after all these studies, we are now approaching the wisdom of Sokrates. We still have no idea of what it is like to be in a coma, PVS or MCS. But at least, we begin to realize that we don't know it. Hopefully we shall not arrogantly state any longer, that some patients "definitely" do not possess even minimal elements of conscious awareness. What is needed is a new, *positive neuropsychology*, whose main interest is not a patient's deficits, but his or her remaining abilities and rehabilitative possibilities; not (only) lost functions, but functions preserved; not what patients can *not*, but also, and particularly, what they can. Finally, understanding of what somebody is able to, is perhaps the only way to grasp what it is like to be in this state.

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FUNCTIONAL IMAGING IN PATIENTS WITH ALTERED CONSCIOUSNESS

Adrian M. OWEN, Martin R. COLEMAN, John D. PICKARD

Keywords: vegetative, auditory, PET, fMRI, intelligibility, semantic, awareness, consciousness.

Running Head: Residual auditory cognition in persistent vegetative state.

Introduction

The vegetative state (VS) is arguably one of the least understood and most ethically troublesome neurological conditions in modern medicine. The term describes a rare disorder in which patients who emerge from coma appear to be awake, but show no signs of awareness[1]. An accurate and reliable evaluation of the level and content of cognitive processing is of paramount importance for the appropriate management of patients diagnosed with VS. Objective behavioural assessment of residual cognitive function can be extremely difficult, as motor responses may be minimal, inconsistent, and difficult to document, or may be undetectable because no cognitive output is possible. In recent years, a number of studies have demonstrated an important role for functional neuroimaging in the identification of residual cognitive function in vegetative patients. Until recently, the majority of these studies used either flurodeoxyglucose (FDG) positron emission tomography (PET) or single photon emission computed tomography (SPECT), and reported widespread reductions of up to 50% in (resting) cerebral blood flow and glucose metabolism[2-4]. In some cases, however, isolated 'islands' of metabolism have been identified in circumscribed regions of cortex, which may suggest residual cog-

Adrian M. Owen
MRC Cognition and Brain Sciences Unit – 15 Chaucer Road, Cambridge CB2 2EF, U.K.
adrian.owen@mrc-cbu.cam.ac.uk
Wolfson Brain Imaging Centre and the Cambridge Coma Study Group – Addenbrooke's Hospital
University of Cambridge – Hills Road, Cambridge CB2 2QQ, U.K.

Martin R. Coleman
John D. Pickard
Wolfson Brain Imaging Centre and the Cambridge Coma Study Group – Addenbrooke's Hospital
University of Cambridge – Hills Road, Cambridge CB2 2QQ, U.K.

nitive processing in a subset of patients[5]. While metabolic studies are useful in this regard, they can only identify functionality at the most general level; that is, mapping cortical and subcortical regions that are *potentially* recruitable, rather than relating neural activity within such regions to specific cognitive processes[6-7].
On the other hand, methods such as $H_2{}^{15}O$ PET and functional magnetic resonance imaging (fMRI) can be used to link residual neural activity to the presence of covert cognitive *function*. In short, functional neuroimaging, or so-called 'activation studies', have the potential to demonstrate distinct and specific physiological responses (changes in regional cerebral blood flow or changes in regional cerebral haemodynamics) to controlled external stimulation in the absence of any overt response on the part of the patient. In one of the first of such studies, $H_2{}^{15}O$ PET was used to study covert *visual* processing in response to familiar faces in a patient diagnosed as VS[8]. An area of the right fusiform gyrus, the so-called human 'face area', was activated when the patient was shown familiar face stimuli, but not when shown meaningless visual images[9]. In other cohort studies, both noxious somatosensory stimuli[10] and auditory stimuli[11] have also been shown to systematically activate appropriate cortical regions in patients meeting the clinical criteria for the vegetative state.
In this chapter we will demonstrate that functional neuroimaging studies in patients meeting the clinical criteria for VS should be conducted hierarchically; beginning with the simplest form of processing within a particular domain (e.g. auditory) and then progressing sequentially through more complex cognitive functions. By way of example, a series of auditory paradigms will be described that have all been successfully employed in functional neuroimaging studies of vegetative patients. These paradigms increase in complexity systematically from basic acoustic processing to more complex aspects of language comprehension and semantics. We suggest that such a hierarchy of cognitive tasks provides the most valid mechanism for defining the depth and breadth of preserved cognitive function in patients meeting the clinical criteria for persistent vegetative state and discuss how such an approach might be extended to allow clear inferences about the level of 'awareness' or consciousness to be made.

A hierarchical approach to the assessment of cognitive function in VS

Speech perception in healthy volunteers has been widely investi-

gated using functional neuroimaging. Most commonly, studies of speech perception involve volunteers being scanned while listening to spoken words (experimental condition), or signal correlated noise sounds (acoustic control condition), or no auditory stimulus at all (silence condition). When speech is compared to signal correlated noise, activation is typically observed along both superior temporal gyri, extending ventrolaterally into the superior temporal sulcus[12]
We have recently used the same approach in small numbers of patients meeting the clinical criteria for VS[9,13]. In several patients, the comparison of speech sounds with noise bursts revealed significant rCBF increases on the superior temporal plane bilaterally and posterior to auditory cortex (see Figure 1), in the region of the planum temporale. These findings correspond extremely closely with the results reported in healthy awake control volunteers while performing the same task.
While such results suggest that some level of covert linguistic functioning may be preserved in some patients with VS, the tasks used do not allow any conclusions to be drawn about *comprehension*; that is, whether speech is processed beyond the point at which it is identified as speech. Davis and Johnsrude[14] have recently developed a task to look at speech comprehension in healthy volunteers using a test of

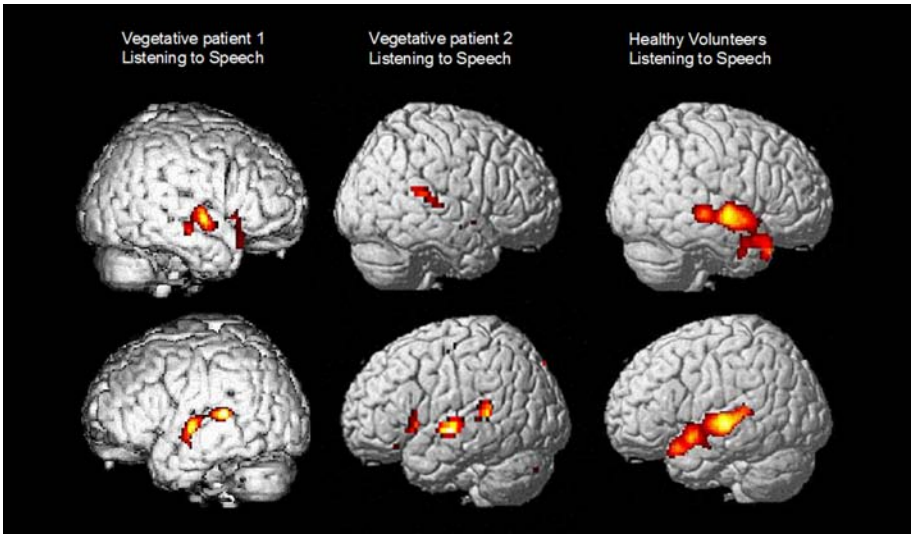

Figure 1 - *PET data illustrating the comparison of speech with signal correlated noise. On the right, control data (adapted from Mummery et al., 1999), and on the left data from two patients meeting the clinical criteria for vegetative state.*

graded intelligibility. During the task, volunteers listen to sentences that have been distorted such that they vary in the extent to which they can be understood by the listener. Speech *intelligibility* was found to correlate with activation in a region of the left anterior and superior temporal lobe; as intelligibility increased (and more sentences were understood), so did signal intensity in this region.
We have recently applied the same paradigm to small numbers of patients meeting the clinical criteria for VS[15]. Like healthy volunteers, in several patients, when low intelligibility sentences were compared to high intelligibility sentences, changes in the brain were observed in the superior and middle temporal gyri of the left hemisphere suggesting some *comprehension* of spoken language. However, whether the responses observed really reflect understanding of the contents of spoken language or a more basic response to the acoustic properties of intelligible speech can not be determined on the basis of this data alone.
Understanding natural speech is ordinarily so effortless that we often overlook the complex computations that are necessary to make sense of what someone is saying. Not only must we identify all the individual words on the basis of the acoustic input, but we must also retrieve the meanings of these words and appropriately combine them to construct a representation of the whole sentence's meaning. This process of selecting appropriate word meanings is important because most words in English are ambiguous[16]. Recently, an fMRI study in healthy volunteers has used this phenomenon to identify the brain regions that are involved in the semantic aspects of speech comprehension (i.e. understanding *meaning*)[16]. Relative to low-ambiguity sentences, high-ambiguity stimuli (involving more words with multiple meanings) produced increases in signal intensity in the left posterior inferior temporal cortex and inferior frontal gyri bilaterally.
We have recently applied this same paradigm to small numbers of VS patients[13,15]. In one study, for example, this semantic ambiguity task was used to assess a patient who had exhibited a normal pattern of PET activation in the graded complexity intelligibility task described above[13]. When the activation during the high-ambiguity sentences was compared to that during the low-ambiguity sentences, a change was observed in the left posterior inferior temporal region similar to that reported in healthy volunteers, although no consistent changes were observed in the inferior frontal gyrus. These results provide compelling evidence for partially preserved high-level linguistic processing in a patient meeting the clinical criteria for VS and suggest that

some of the processes involved in *understanding* spoken speech may be intact, despite the clinical diagnosis.

Discussion

A number of recent studies have clearly demonstrated that functional neuroimaging has the potential to elicit distinct and specific physiological responses from patients meeting the clinical criteria for VS, in the absence of any overt behavioural response. They also clearly demonstrate, however, that the technique poses a number of unique methodological, ethical and procedural problems. For example, as noted above, motor responses are often minimal, inconsistent or absent in patients considered to be in a VS and, by definition, cannot be elicited directly (i.e. wilfully) by external stimulation. In addition, even assuming that some level of residual cognitive processing does exist, there is no reliable mechanism for ensuring that the presented stimuli are actually *perceived* by the patient. Many VS patients suffer serious damage to auditory and/or visual input systems, which may impede performance of any 'higher' cognitive functions (e.g. voice discrimination), which place demands on these 'lower' sensory systems (e.g. hearing). Like patients with any form of serious brain damage, VS may also be accompanied by a significant reduction in attention span (assuming some level of cognitive processing remains), which may further complicate the assessment of higher cognitive functions. Spontaneous movements during the scan itself may also compromise the interpretation of functional neuroimaging data, particularly scans acquired using fMRI. Where PET methodology is employed, issues of radiation burden must also be considered and may preclude longitudinal or follow-up studies in many patients. Finally, data processing of functional neuroimaging data may also present challenging problems in patients clinically diagnosed as VS. For example, the presence of gross hydrocephalus or focal pathology may complicate coregistration of functional data (e.g. acquired with PET or fMRI) to anatomical data (e.g. acquired using structural MRI), and the normalisation of images to a healthy reference brain. Under these circumstances statistical assessment of activation patterns is complex and interpretation of activation foci in terms of standard stereotaxic coordinates may be impossible.

In this lecture, I have attempted to illustrate the benefits of conducting functional imaging studies in VS hierarchically; beginning with the simplest form of processing within a particular domain and then

progressing sequentially through to more complex cognitive functions. This strategy, when applied to individual patients in a longitudinal manner, has the power to define the depth and breadth of preserved cognitive function in vegetative state. One question which arises, however, is the extent to which the presence of 'normal' activation in patients diagnosed as VS can be taken to indicate a level of conscious 'awareness'. In our opinion, this issue will only be addressed directly using tasks that tap volitional (or consciously 'willed') aspects of behaviour. In all of the examples discussed above, from face processing to speech perception and even the detection of semantic ambiguous sentences, under normal circumstances cognitive processing is relatively *automatic*. That is to say, it occurs without the need for wilful intervention on the part of the patient (you can not choose to *not* recognise a face as a face, or to *not* understand speech that is presented clearly in your native language). To begin to address this issue, we have recently developed a series of mental imagery tasks in healthy volunteers which do not rely on such automatic responses, but rather, require decisions to be made by the participant about specific thoughts and mental 'actions' that are known to produce reliable and robust patterns of neural activity[17]. Importantly, because activation in these tasks depends on a response to an instruction given *prior* to each trial, it can only reflect the *intentions* of the participant, rather than an altered property of the outside world (such as the auditory presentation of a sentence). In this sense, the response is an act of *willed intention* and, therefore, clear evidence for awareness of self and surroundings in these healthy volunteers. If the same task were to yield positive results in patients meeting the clinical criteria for VS, it would have to be argued that a similar level of conscious awareness was present. Of course, *negative* findings in the same circumstances could not (and should not) be used as evidence for lack of awareness; false negative findings in functional neuroimaging studies are common even in healthy volunteers. Current studies are seeking to evaluate these tasks in patients diagnosed as VS.

In summary, there is a clear need to improve our characterisation of the clinical syndrome of VS, not only to redefine diagnosis, but also to stratify patients in terms of prognosis and possible responses to novel therapies that may emerge in the future. The use of functional neuroimaging in this context will clearly continue to present logistic and procedural problems. However, the detection and elucidation of re-

sidual cognitive function in this group of patients has such major clinical and scientific implications that such an effort is clearly justified.

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HOW IS THE SOFTWARE BUILT-UP?

Alonso PEÑA

Keywords: software, cerebral blood flow, biomechanics, brain imaging, hydrocephalus, interdisciplinarity.

1. Introduction

The *Encyclopaedia Britannica* defines a computer program as: «*A detailed plan or procedure for solving a problem with a computer; more specifically, an unambiguous, ordered sequence of computational instructions necessary to achieve such a solution. The distinction between computer programs and equipment is often made by referring to the former as software and the latter as hardware*».

In this context, it would be seem that writing a computer program should be rather straightforward. The idea of a sequence of computational instructions – an algorithm – is perfectly accurate but misleading. Even though it is true that building some software involves the precise definition of a series of tasks that the computer must perform, this is not the most important part of the process of writing software. In fact, it could be argued that it is the least important part. As any computer scientist can tell, the most important part of building software is its *design*. And, at this level, we must widen our perspective to properly answer the question that is the title of this contribution. Writing software is not simply following a recipe for converting inputs into outputs. It involves a wider attitude of combining skills, techniques, and methods from different fields. In fact, to the question "How is the software build-up?" the proper answer is "Interdisciplinarity".
Interdisciplinarity can be defined as a type of academic collaboration in which specialists drawn from two or more academic disciplines work together in pursuit of common goals.

I am myself neither a software engineer, nor an information technology

Alonso Peña PhD
Academic Neurosurgery Unit & Wolfson Brain Imaging Centre – University of Cambridge
Addenbrooke's Hospital – Cambridge CB2 2QQ, U.K.

specialist. I am by training a physicist but I have been involved in the development of computer programs for various clinical applications. In this contribution I would like to comment on the interdisciplinary nature of this work, and attempt to isolate some lessons that were useful, at least in our case, for the development of software for clinical applications.

In the following sections I will discuss this process, based on my experiences at the intersection of mathematical modelling, programming and clinical applications at the University of Cambridge, UK. I have had the privilege of collaborating with a number of senior scientists including Prof John D Pickard, Dr Marek Czosnyka and Prof Malcolm D Bolton that have made this possible. Many past and present colleagues from very many departments of the University of Cambridge have been involved including the Academic Neurosurgery Unit, the Wolfson Brain Imaging Centre, the Brain Repair Centre, the Department of Anaesthesia, the University Department of Radiology, the Department of Engineering and the Cambridge High Performance Computing Centre. I would also like to acknowledge the financial support of the Wellcome Trust through a Training Fellowship in Mathematical Biology held in the Neurosurgery unit.

In the following we present examples from interdisciplinarity activity in which we have been directly involved, covering (a) cerebral blood flow, (b) diffusion tensor imaging, (c) head injury and (d) hydrocephalus.

2. Examples

2.1 - Example 1: cerebral blood flow

In the following set of studies, conducted mainly in collaboration between Neurosurgery and the Wolfson Brain Imaging Centre, we investigated some of the factors involved in the evolution of head injury in children the relationship between cerebral blood flow (CBF) and cerebrospinal fluid (CSF) pressure.
In the first study[5], we used a combination of cerebral blood flow measurement using (15)O-water positron emission tomography with magnetic resonance co-registration and CSF infusion studies to study the global and regional changes in CBF with changes in CSF pressure. Fifteen patients with normal pressure hydrocephalus were considered. With increases in CSF pressure, there was a variable increase in arterial blood pressure between individuals and global CBF

was reduced, including in the cerebellum. Regionally, mean CBF decreased in the thalamus and basal ganglia, as well as in white matter regions. These reductions in CBF were significantly correlated with changes in the CSF pressure and with proximity to the ventricles. A three-dimensional finite-element analysis was used to analyze the effects on ventricular size and the distribution of stress during infusion. We concluded that to study regional cerebral autoregulation in patients with possible normal pressure hydrocephalus, a sensitive CBF technique is required that provides absolute, not relative normalized, values for regional CBF and an adequate change in cerebral perfusion pressure must be provoked.

In a second study[4], we investigated the distribution of the regional peri- and paraventricular white matter CBF (WM CBF) in NPH at baseline and during a controlled rise in intracranial pressure (ICP). In the past, the mean cerebral blood flow (CBF) has generally been demonstrated to be lower in normal pressure hydrocephalus (NPH) than in normal controls. Twelve patients with idiopathic NPH (mean age 69 years) underwent a CSF infusion study. CBF was measured by H2(15)O PET at baseline and then during the steady-state plateau of raised ICP. The PET images were co-registered and resliced to 3D structural T1-weighted MRIs. Ten healthy normal volunteers served as control subjects for baseline CBF determination only. Profiles of the regional distribution of the baseline WM CBF and of the percentage change in WM CBF as a function of distance from the ventricles were plotted. The global mean baseline CBF in patients (28.4 ± 5.2 ml/100 ml/min) was lower than in the control subjects (33 ± 5.4 ml/100 ml/min) ($P < 0.005$). In patients, the profile of the regional WM CBF at baseline showed an increase with distance from the ventricles ($P < 0.0001$), with a maximal reduction adjacent to the ventricles and progressive normalization with distance, whereas in controls no relationship was apparent ($P = 0.0748$). In 10 patients, the rise in ICP during the infusion produced a fall in cerebral perfusion pressure (CPP) and a significant decrease of the global mean CBF from 27.6 ± 3.1 to 24.5 ± 2.9 ml/100 ml/min ($P < 0.0001$). The profile of the percentage changes in regional WM CBF in patients showed a U-shaped relationship with distance from the ventricles ($P = 0.0007$), with a maximal decrease skewed on the side of the lateral ventricles at around a mean distance of 9 mm. The WM CBF is reduced in NPH, with an abnormal gradient from the lateral ventricles towards the subcortical WM. An excessive decrease in CBF is brought

about by reductions in CPP and appears to be maximal in the paraventricular watershed region.

In a further study[6], regional cerebral blood flow (CBF) was studied with O(15)-water positron emission tomography and anatomic region-of-interest analysis on co-registered magnetic resonance in patients with idiopathic (n = 12) and secondary (n = 5) normal pressure hydrocephalus (NPH). Mean CBF was compared with values obtained from healthy volunteers (n = 12) and with clinical parameters. Mean CBF was significantly decreased in the cerebrum and cerebellum of patients with NPH. The regional analysis demonstrated that CBF was reduced in the basal ganglia and the thalamus but not in white matter regions. The results suggest that the role of the basal ganglia and thalamus in NPH may be more prominent than currently appreciated.

COMMENTARY

Our work covering CBF involved brain imaging (both PET and MRI), multimodal infusion studies and finite element analysis. Each technique on its own is not innovative, but their combined application to the problem of periventricular blood flow is new. The Interdisciplinarity here consists in the convergence of different techniques to the solution of a single problem. It is in a sense a "classical" interdisciplinary case study in which experts from different fields converge to the solution of a common problem. Even though in this instance this formula worked very well, it is not the only or even the better interdisciplinary approach as we will see in the following sections.

2.2 - Example 2: diffusion tensor imaging

Magnetic resonance (MR) diffusion tensor imaging (DTI) is a technique that allows the *in vivo* measurement of water diffusion in biological tissues from which tissue microstructure can be inferred. It has been used successfully to investigate a number of neurological disorders that involve the disruption of white matter fibres, including schizophrenia, head injury, multiple sclerosis and stroke. In addition, DTI data can be used with a set of computational techniques called "tractography" to reconstruct in vivo white matter tracts in the human brain, which is a very promising field, for example to investigate their disruption due to an expanding tumour. In the following set of studies, conducted mainly in collaboration between Neurosurgery, the Wolfson Brain Imaging Centre and the Department of Radiology, we

investigated new ways in the interpretation of DTI data, as well as their application to clinical problems, in particular tumour growth.

Many scalar measures have been proposed to quantify magnetic resonance diffusion tensor imaging (MR DTI) data in the brain. However, only two parameters are commonly used in the literature: mean diffusion (D) and fractional anisotropy (FA). In the first study[10] we introduced a visualization technique which permits the simultaneous analysis of an additional five scalar measures. This enhanced diversity is important, as it is not known a priori which of these measures best describes pathological changes for brain tissue. The proposed technique is based on a tensor transformation, which decomposes the diffusion tensor into its isotropic (p) and anisotropic (q) components. To illustrate the use of this technique, diffusion tensor imaging was performed on a healthy volunteer, a sequential study in a patient with recent stroke, a patient with hydrocephalus and a patient with an intracranial tumour. Our results demonstrate a clear distinction between different anatomical regions in the normal volunteer and the evolution of the pathology in the patients. In the normal volunteer, the brain parenchyma values for p and q fell into a narrow band with $0.976 < p < 1.063 \times 10(-3)$ mm2 s(-1) and $0.15 < q < 1.08 \times 10(-3)$ mm2 s(-1). The noise appeared as a compact cluster with (p,q) components (0.011, 0.141) x 10(-3) mm2 s(-1), while the cerebrospinal fluid was (3.320, 0.330) x 10(-3) mm2 s(-1). In the stroke patient, the ischaemic area demonstrated a trajectory composed of acute, sub-acute and chronic phases. The components of the lesion were (0.824, 0.420), (0.884, 0.254), (2.624, 0.325) at 37 h, 1 week and 1 month, respectively. The internal capsule of the hydrocephalus patient demonstrated a larger dispersion in the p:q plane suggesting disruption. Finally, there was clear white matter tissue destruction in the tumour patient. In summary, the p:q decomposition enhances the visualization and quantification of MR DTI data in both normal and pathological conditions.

In the second study[13] the p:q decomposition technique was applied to tumour growth imaging. In this field, the inherent invasiveness of malignant cells is a major determinant of the poor prognosis of cerebral gliomas. Diffusion tensor MRI (DTI) can identify white matter abnormalities in gliomas that are not seen on conventional imaging. By breaking down DTI into its isotropic (p) and anisotropic (q) components, we can determine tissue diffusion "signatures". We character-

ised these abnormalities in peritumoural white matter tracts. Thirty-five patients with cerebral gliomas and seven normal volunteers were imaged with DTI and T2-weighted sequences at 3 T. Displaced, infiltrated and disrupted white matter tracts were identified using fractional anisotropy (FA) maps and directionally encoded colour maps and characterised using tissue signatures. The diffusion tissue signatures were normal in ROIs where the white matter was displaced. Infiltrated white matter was characterised by an increase in the isotropic component of the tensor (p) and a less marked reduction of the anisotropic component (q). In disrupted white matter tracts, there was a marked reduction in q and increase in p. The direction of water diffusion was grossly abnormal in these cases. We concluded that diffusion tissue signatures might be a useful method of assessing occult white matter infiltration.

In a third study[12] our aim was to determine whether diffusion tensor imaging (DTI) of brain tumours can demonstrate abnormalities distal to hyperintensities on T2-weighted images, and possibly relate these to tumour grade. Twenty patients with histologically confirmed supratentorial tumours, both gliomas (high and low grade) and metastases, were imaged at 3T using T2-weighted and DTI sequences. Regions of interest (ROI) were drawn within the tumour, in white matter at various distances from the tumour and in areas of abnormality on DTI that appeared normal on T2-weighted images. The relative anisotropy index (RAI)-a measure of white matter organization, was calculated for these ROI. The abnormality on DTI was larger than that seen on T2-weighted images in 10/13 patients (77%) with high-grade gliomas. New abnormalities were seen in the contralateral white matter in 4/13 (30%) of these cases. In these high-grade tumours the RAI in areas of white matter disruption with normal appearance on T2-weighted images was reduced (0.19 ± 0.04). Even excluding patients with previous radiotherapy this difference remains significant. In all non high-grade tumours (WHO grade II gliomas and metastases) the tumour extent on DTI was identical to the abnormalities shown on T2-weighted imaging and RAI measurements were not reduced (0.3 ± 0.04). Subtle white matter disruption can be identified using DTI in patients with high-grade gliomas. Such disruption is not identified in association with metastases or low-grade gliomas despite these tumours producing significant mass effect and oedema. We suggest the changes in DTI may be due to tumour infiltration and that the DTI may

provide a useful method of detecting occult white matter invasion by gliomas.

COMMENTARY

Our work in magnetic resonance diffusion tensor imaging (MR DTI) we imported a methodology which originated in geomechanics to quantify the results from the diffusion tensor in the brain. The technique involves the mathematical decomposition of the diffusion tensor into isotropic and deviatoric components. This decomposition allows the analyst the construction of multiple measures to quantify the wealth of information contained in the DTI signal. This increased flexibility is fundamental, as we have shown in these studies, because the quantification of changes in the structure of the brain using one technique rather than another, could lead to very different results. Interdisciplinarity in this case involved "go out hunting". A priori we did not know what methods could be useful to quantify this type of changes. Looking for information that might be helpful – without exactly knowing what – is the first step towards this approach. Louis Pasteur famously said, "chance favours only the prepared mind"[15].

2.3 - Example 3: head injury

The heterogeneity of the initial insult and subsequent pathophysiology has made the study of human head injury exceptionally difficult. The combination of multimodal bedside monitoring and functional brain imaging positron emission tomography (PET) and magnetic resonance (MR), incorporated within a Neurosciences Critical Care Unit, provides a resource required to study critically ill patients after brain injury from initial ictus through recovery from coma and rehabilitation to final outcome[11]. In the following set of studies, conducted mainly in collaboration between Neurosurgery, the Wolfson Brain Imaging Centre and the Department of Paediatrics, we investigated some of the biophysical factors involved in the evolution of head injury in children.

It is well known that diffusion tensor imaging (DTI) provides a unique insight into the cellular integrity of the brain. While conventional magnetic resonance imaging underestimates the extent of pathology following closed head injury,

Diffusion-weighted imaging has been shown to more accurately delineate the extent of cerebral damage. There have only been a few case studies of DTI in chronic head injury survivors. In this study[14] we used

DTI to investigate changes in anisotropy and diffusivity in survivors of head injury at least 6 months after injury. The relationship between cognition and diffusion abnormality was also investigated. The voxel-based analysis revealed significant bilateral decreases in anisotropy, in major white matter tracts and association fibers in the temporal, frontal, parietal and occipital lobes. Statistically significant increases in diffusivity were also found in widespread areas of the cortex. A significant positive correlation was found between diffusivity and impairment of learning and memory in the left posterior cingulate, left hippocampal formation and left temporal, frontal and occipital cortex. The common pattern of abnormality despite heterogeneous injury mechanism and lesion location in the group suggests that these cellular changes reflect secondary insults. The importance of diffusion abnormalities in head injury outcome is emphasized by the significant correlation between a learning and memory index and diffusivity in areas known to be associated with this cognitive function.

Vulnerability of the hippocampus to traumatic brain injury (TBI) in adults is related to severity of injury and white matter atrophy. The aims of this second study[16] was to determine features of anthropometry and cerebral morphometry late after TBI in childhood and to assess whether hippocampal volume is related to severity of initial ictus and changes in white matter at follow-up. Thirty-three patients underwent magnetic resonance imaging 4.9 y after severe TBI that necessitated intensive care; 23 had mechanical ventilation and intracranial pressure monitoring longer than 3 d. Magnetic resonance imaging analyses included volume of brain, hemisphere, ventricles, and hippocampal and perihippocampal regions; spatial distribution of voxel-based morphometry differences in white matter; and eigenvalues of diffusion tensor imaging diffusivity. Patients with longer intensive care ictus had smaller-than-expected occipitofrontal head circumference. Eight of these, identified by voxel-based morphometry, had periventricular white matter loss and smaller-than-expected brain volume for OFC, suggesting “atrophy”; the remainder had expected volume for a smaller OFC, suggesting “growth disturbance.” Ninety-three percent of the variation in right hippocampal volume was accounted for by factors related to severity of injury and white matter atrophy. It is concluded that anthropometry and cerebral morphometric measurements late after severe TBI in childhood provides useful outcome data and indicate that, despite

adequate growth in stature, effects of TBI on brain growth and hippocampal volume may extend into adulthood.

In our final study[9] we applied fractal analysis to the study of intracranial pressure (ICP). Fractals are non-regular geometric shapes that have the same degree of non-regularity on all scales. Several natural objects, such as trees, rivers and cloud formations, have been shown to have fractal characteristics[3]. There is evidence that physiological signals may also have a fractal temporal structure. In this work we applied fractal analysis to the study of intracranial pressure (ICP). Thirty-three patients with head injury were studied (age=27±12 years). All patients were sedated, paralysed and ventilated to achieve mild hypocapnia. ICP was monitored invasively for periods of 20 min. The fractal dimension (D) of the signal was calculated using the method of relative dispersion [3]. A value of D=1.0 indicates a uniform signal, D=1.5 a random signal and 1.0<D<1.5 a fractal signal. Two parameters that characterize cerebrovascular responsiveness were calculated: the linear correlations between changes in ICP pulse amplitude and mean ICP (termed RAP) and CPP (termed RAC). Results. Mean ICP=22±13, ABP=96±11, CPP=79±14 mmHg, and GCS=8. The mean D for all patients was 1.21±0.09 (range 1.05 to 1.43). D was significantly correlated with RAP ($r=-0.46$; $p<0.006$) and RAC ($r=0.53$; $p<0.0013$) indicating that derangement of normal cerebrovascular responses was associated with an increase in the chaotic nature of the ICP waveform. Our results show that the fractal dimension of the ICP waveform correlates with progressive disturbance of cerebrospinal dynamics. Further work is required to clarify the possible role of D in clinical investigation.

COMMENTARY

Our work in studying head injury was twofold: first, it involved the application of DTI to investigate the structural brain damage in children following head injury; and, second, it involved the application of novel mathematical techniques to quantify changes in the waveform of ICP. The first study is a classical example of direct knowledge transfer: different fields converging to illuminate the problem from different angles. The problem being head injury and the different techniques being psychology, brain imaging and clinical assessment. These three specialities contributed each with a "piece of the puzzle" to clarify the interpretation of the evolution of head injury in children. The second

stud was more unusual. Fractals are not the classical methodology used to assess waveforms – this is usually done using time series analysis, such as Fourier transforms. In this case the application of interdisciplinarity required using a novel technique in an unusual setting. Even though there is some literature on the application of fractal analysis to other clinical fields, such as cardiology[2] the use of fractals on head injury was completely novel. In this case interdisciplinarity involved convincing people to look at the same problem with different eyes, literally, applying different equations to the same time series raw data. Interdisciplinarity is not always to bring knowledge from one field into another. It is sometimes to bring a different way of looking at the same things with new eyes. In the words of Marcel Proust "The true voyage of discovery consists not in finding new lands but in having new eyes to see."

2.4 - Example 4: hydrocephalus

The use of physics to understand the behaviour of living organisms has a long tradition of interdisciplinary endeavour dating back to the 16th century. The names of Thomas Young, Hermann Von Helmholtz immediately come to mind. We also follow this interdisciplinary spirit by attempting to elucidate some of the physical factors involved in the biomechanical behaviour of the human brain. In the following set of studies, conducted mainly in collaboration with the departments of Neurosurgery, Engineering and the Wolfson Brain Imaging Centre, we investigated some of the biophysical factors involved in the pathogenesis of hydrocephalus.

In the first study[7], using finite element analysis and positron emission tomography, we investigated a patient who developed transient hemispatial neglect following surgical drainage of a large right frontotemporal arachnoid cyst. As symptoms evolved in parallel with brain shift over the subsequent months, we hypothesised that the disorder was associated with the appearance of mechanical stresses in the cerebral mantle. To map tissue stress at the various stages of deformation, computer simulation was conducted on the basis of computed tomography scans. Our results demonstrate substantial shear and compressive stress concentrations in the parietal lobe, a region commonly associated with neglect, and where positron emission tomography confirmed hypoperfusion. Treatment with combined ventriculo-peritoneal and cysto-peritoneal shunts was accompanied by clinical recovery and

improvement of right parietal lobe cerebral blood flow. Our conclusion was that brain deformation was a contributing factor in the reversible neglect sindrome by compromising the normal flow of blood and/or the deactivation of subcortical circuits of the parietal lobe.

In another study[8] we investigated the mechanisms involved in the chronic ventricular enlargement that accompanies communicating hydrocephalus, including its normal and low-pressure forms. We proposed that this phenomenon can be explained by the combined effect of: (a) a reversal of interstitial fluid flow in the parenchyma, and (b) a reduction in the elastic modulus of the cerebral mantle. To investigate this hypothesis, these changes have been incorporated into a finite element computer simulation of communicating hydrocephalus, in which brain tissue is idealized as a sponge-like material. The fluid pressure in the lateral ventricles and the subarachnoid space was set to 10 mmHg, while the fluid pressure inside the was set to 7.5 mmHg. The elastic moduli of white and grey matter have been set to the reduced values of 1 and 5 kPa, respectively. The simulation revealed a substantial ventricular distension (6.5 mm mean outward displacement), which was accompanied by the appearance of stress concentrations in the cerebral mantle. These results support the notion that a relative reduction in intraparenchymal fluid pressure coupled with low tissue elasticity can produce both a significant ventricular enlargement and periventricular solid stress concentrations.

COMMENTARY

Our work in this field how the technical skills from engineering, in general, and continuum mechanics, in particular, could be decisive to understand the biomechanics of hydrocephalus. In this case the application of interdisciplinarity was direct: techniques from one field were used to clarify problems in another. From an intellectual point of view this would be the end of the story. However, from a practical point of view, there is much more to it. Concepts, ideas, techniques do not happen in the vacuum. The opportunity for interdisciplinarity to happen must be carefully planned. The appropriate people must be trained in both fields and time must be giving the various involved parties to develop a common language. An effort must be made by people from all the involved fields to move into the other fields, if not to learn the details, at least to know the general principles that are important. Luigi Luca Cavalli-Sforza, Emeritus Professor of Genetics at

Stanford University, expressed clearly when he said that "Scientific terminology is useful in specialist work, but it can stifle the spread of information and comprehension between different disciplines. It is important to reduce jargon to an absolute minimum, in order not to frighten off potential helpers. Terminology's only useful purpose is to make communication between experts in the same field faster and more precise."[1].

3. Conclusion

In this contribution we have overviewed a series of fields in which we have developed software to solve clinical problems. We have concluded that the main difficulty in writing the computer programs does not lie within the series of tasks that it must conduct (its algorithm) but rather in the whole *milieu* in which the software must is developed. We propose that the answer to the question "How is the software build-up?" Is not simply a recipe but an attitude. And this attitude is embodied in the concept of interdisciplinarity.

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CEREBRAL BLOOD FLOW THRESHOLDS IN ISCHEMIC STROKE PATIENTS

Horst TRAUPE

Brain is dependent on blood flow to provide oxygen and nutrients to maintain its normal function. Basic functions are: membrane ion pumping, energy metabolism and synaptic transmission. These functions fail at distinct blood flow thresholds. Synaptic transmission stops with abolition of somatosensory evoked potentials, despite difference of species, if blood flow falls below 16-18 ml/100g/min[1,2].
At this threshold ischemic impairment is reversible.
Further decrease in blood flow immediately causes paralysis which can be reversed rapidly with elevation of flow above that value[3].
Below thresholds of 8 – 10 ml/100g/min in primates and man ([2,4,6] synthesis of adenosine triphosphate (ATP), the energy source of the brain, is outstripped by demand and cell membrane pumps fail leading to massive efflux of K^+ from and influx of Ca^{2+}, Na^+ and water into the neuron, causing membrane depolarization. This threshold of membrane homeostasis is dependant of the species. In cat it is 10 to 14 ml/100g/min in the rat approximately 15 ml/100g/min and in the gerbil about 22 ml/100g/min.
As failure of the integrity of the cell membrane precedes structural damage of the cell, CBF threshold of irreversible damage of the cell can be associated with the threshold for ion pump failure. As however disturbed energy metabolism and ion pump function can fully recover, CBF threshold is close to that of infarction, thus the development of neuronal damage requires additional time. Based on experimental results from cortical neurons in cats , we estimated a curve relating the level of residual blood flow to the minimum duration of ischemia required to induce permanent loss of neuronal activity. We found that the duration of ischemia required for infarction to develop becomes progressively shorter as blood flow decreases. Below 5 ml/100g /min critical time was 20 min, below 8 ml/100g/min it was 30 min, 12ml/100g/min for 50 min and 15 ml/100g/min for 80 min. The blood flow for permanent structural damage was 18 ml/100g/min when maintained permanently.
Various parts of the brain show different sensitivity to ischemic injury,

which means that CBF must be measured regionally throughout the brain to assess the specific thresholds for infarction or viability.

Autoregulation

Within limits the brain vasculature has an intrinsic system of control to maintain cerebral blood flow normal. When cerebral perfusion pressure decreases, normal flow can be sustained by dilation of the resistance blood vessels. Autoregulation leads to an increase of CBV and is a sign of viable and still active cerebral vasculature.

In summary we find a gradation of thresholds for functional and structural damage which reaches from about 8 ml/100g/min to 20 ml/100g/min. We have to realize, that these values differ from species to species and for each species from brain region to brain region. Thresholds for structural damage during ischemia depend on the duration of ischemia: stable ischemic conditions led to infarction even with CBF > 15 ml/100g/min whereas short lasting severe ischemia can be tolerated for 10 to 20 min.

Methods, Flow Parameters

Cerebral Perfusion is typically measured as the quantity of blood (ml) perfusing a volume of brain (100 g) per unit of time (min), known as cerebral blood flow (CBF).

Classical methods for clinical measurements are Xe-Clearance with isotopes or stable Xenon CT. Stable xenon CT is based on the equilibrating indicator model. The balance of concentrations of a free diffusible tracer between blood and cerebral tissue is rapidly realized in the whole brain. Knowledge of the blood or alveolar concentration and the parenchymal concentration curves leads to the CBF values for each pixel. The method yields global values for CBF in ml/100g/min.

CT Perfusion is realized through the central volume principle. Since the first discussion by and Axel a number of methods have been developed for tissue perfusion measurement in CT. Basically the various methods can be grouped under two classes, non-deconvolution based and deconvolution based where the first makes use of the Fick Principle. The advantage of the deconvolution-based methods for the determination of CBF, CBV and MTT is its independence of assumptions concerning the underlying hemodynamics. Deconvolution methods are extremely sensitive towards noise in the TDCs. Motion correction is obligatory to correct for patients motion. Third, to measure the arterial input function the arterial TDC usually is obtained from relatively small intracranial arteries and partial volume averag-

ing will cause the arterial TDC to be underestimated. Both, CBF and CBV will also be overestimated.
Compared to Xe-Clearance-techniques CT-Perfusion gives a rather detailed insight into hemodynamic parameters in that MTT, CBV, CBF and PS (permeability surface factor) can be measured.
MTT is the mean distribution of transit times of vessels of different pathlength traversing a block of tissue. It has the unit of seconds. There is a close relation in between MTT and local perfusion pressure which is (mean arterial pressure) MAP – intracranial pressure (ICP). Prolongation of MTT means reduced perfusion pressure.
CBV is the amount of blood in the vasculature including large vessels, arterioles, capillaries, venules and veins and sinuses, rCBV the amount of intravascular blood in a distinct volume of the brain. It has the unit of ml/100g. and ranges between 2 – 6 ml/100g.
Both, CBV and MTT are linked by the central volume principle (Meier 1954): CBF = CBV/MTT.
Permeability of contrast medium in lesions with disrupted blood brain barrier can regularly be observed in ischemic lesions when structural damage of the endothelium occurred. Permeability surface area product (PS) has the unit ml/100g/min . After severe ischemia up to 2 hours duration with residual CBF values < 10 ml/100g/min increased permeability is seen experimentally and clinically . Regarding the different survival times for neurons and cerebral vasculature it is important to emphasize two points: Permeability in stroke always indicates ischemia which exceeds the survival time of neurons and secondly a risk of hemorrhagic transformation if thrombolysis is considered.

Why Perfusion Imaging?

Besides ensuring the diagnosis and excluding differential diagnoses after acute stroke, the central problem is to differentiate those neurological deficits due to ischemia that are likely to improve or reverse spontaneously from those that are likely to persist or worsen . Clinically and by neurological examination it is not possible to determine the level of persisting perfusion. A normal level of CBF suggests that spontaneous reperfusion or sufficient collateralization has occurred and that no acute thrombolysis is necessary . The same holds true for a normal MTT map indicating normal cerebral perfusion pressures together with a normal CBV map in CTP or PWI.
A level below 18 - 20 ml/100g/min indicates cell death within one hour or more . In these cases, tissue with flow values in the intermediate

zone might be salvaged if flow restauration occurs quickly either by vascular thrombolysis, by clot fragmentation or collateralization . Unfortunately the individual "therapeutic window" concerning the relation between degree of ischemia and duration of the ischemic condition is not well known. It depends above all on the amount of collateral circulation beyond the primary occluded vessel. Collateral circulation or beginning reperfusion is shown by CT–Perfusion during the first passage of the contrast bolus. The "perfused blood volume" becomes clearly visible and distal branches which are currently filled by collateralization or partial reperfusion are seen. Finally the resulting map of perfusion pressures (MTT map) in our experience provides a clear picture of hemodynamic gradients.
Quantification of rCBV provides an additional parameter. If mean transit time (MTT) is prolonged indicating low regional perfusion pressure but CBV is increased, autoregulation is intact and CBF will be low to normal. According to the Central Volume Principle if CBF is reduced, MTT prolonged but rCBV reduced a severe ischemic condition exists. It is important to realize that it is the mismatch between CBF and CBV that discriminates salvageable from infracted tissue.
Benefit of potential thrombolysis or risk of hemorrhage with mass effect depends on the condition of cerebral vasculature eg the intactness of the blood brain barrier . CTP with calculation of surface permeability in our experience provides a good estimate of the state of blood brain barrier.
CBF of less than 10 ml/100g/min cannot be tolerated for more than a few minute before infarction occurs . In Perfusion weighted CT Imaging a clear relation between reduction in CBV 2 to 4 hours from onset of vessel occlusion and minimal final infarct size exists . As cerebral perfusion pressure falls, resistance vessels dilate (increased CBV) to maintain CBF. When maximum dilatation has been reached, autoregulation fails and CBF begins to fall. Progressive increases in cerebral oxygen extraction can temporarily maintain cerebral oxygen metabolism. As perfusion pressure continues to fall irreversible ischemia develops.

Colors without sense? Can we have faith in particular quantitative techniques?

The past decade has witnessed significant advances in the management of acute ischemic stroke. By better separation of large vessel thrombembolic event from small and watershed infarcts and throm-

bolytic therapy during the first 3 – 6 hours patients prognosis and management was enhanced. In the same time several methods to qualify or quantify cerebral hemodynamics evolved.
Whereas Xenon-enhanced computed tomography (XeCT) and single photon emission tomography (SPECT) only measure CBF globally, CTP and to a less quantitative degree PWI resolve specific hemodynamic parameters: CBF for regional cerebral blood flow, MTT which for the local distribution of mean transit times and hereby of local perfusion pressures; CBV which may be increased showing vasodilation and hereby autoregulation or decreased, showing collaps of vessels and finally PS, a new parameter which stands for the condition of the vessel wall and the endothelium.
One of the biggest advantage of MR-Perfusion and above all CT-perfusion is their widespread availability. The biggest drawback of these

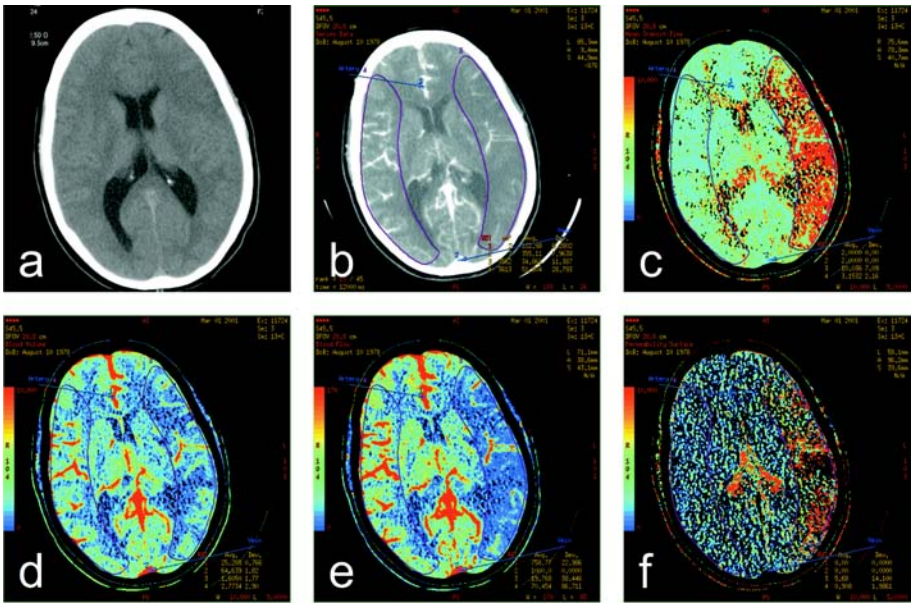

Figura 1 – *CT and CTP in a 21-year old female after cardiac embolism and complete hemiparesis of the right side. CTP was performed 2:50 hours after attack. (a) CT after 2:50 hours shows no early infarct signs. (b) during maximum of contrast bolus passage less mca-branches on the left side are visible, reduced perfused CBV in left mca territory. (c) MTT-map demonstrates prolongation MTT in the territory of left mca up to 10 sec, right side 3 sec. (d) CBV-map, affected side 1,6 ml/100g, contralateral 2,7 ml/100g. (e) flow reduction in the left mca territory to 20 ml/100g/min right side 70 ml/100g/min. (f) Permeability-map showing severe extravasation 3 hours after mca-occlusion of 5,6 ml/100g/min (opposite side 0,9 ml/100g/min indicating disrupture of the blood-brain-barrier.*

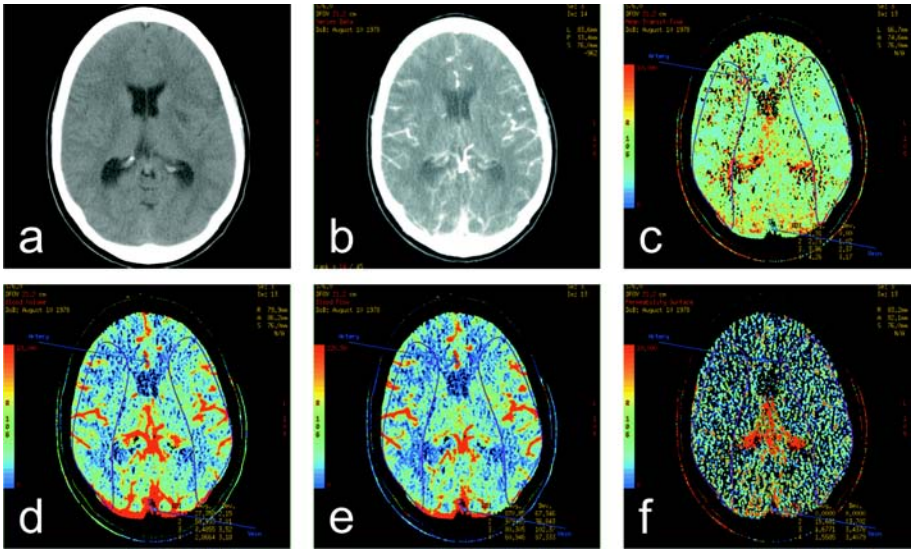

Figure 2 – *Same patient as in Fig. 1, one day later. The follow up study shows (a) a slight density decrease in the left putamen (b) compared to the previous examination all mca-branches on the left side are visible. (c) MTT has completely normalized (left mca 3,5 sec, right 4.2 sec). (d) CBV on the left side now 3,4 ml/100g, right side 2,9 ml/100g. (e) CBF now with slight postischemic hyperperfusion on the left side (80 ml/100g/min) on the right side 61 ml/100g/min. (f) Permeability has completely normalized, no more extravasation on the left side.*

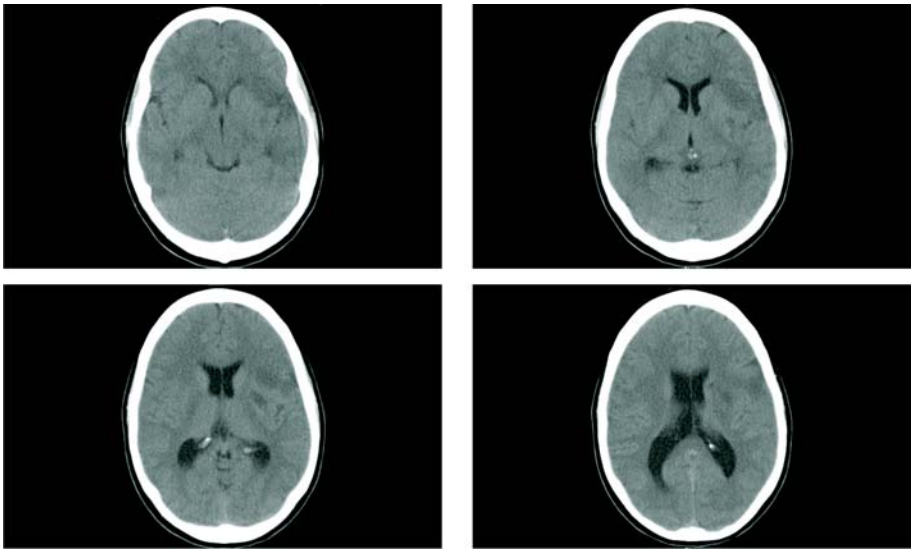

Figure 3 - *Same patient 7 days after stroke; clinically slight disturbance of coordination right hand no motor deficit, no aphasia. CT shows disseminated necrosis in all regions where earlier permeability was observed (left striatum, frontal cortex and medulla).*

methods is the lack of reproducible standards in data analysis and the promise of absolute CBF-measurements which does not exist. Does the colourful spectrum of imaging still make sense?
CTP identifies ischemic stroke immediately and separates large vessel and cardioembolic stroke from small and watershed ischemias. The location of ischemia can be detected and the volume of ischemia be quantified and irreversible ischemic injury from ischemic tissue not yet irreversibly injured be distinguished.
This is information that will help acute stroke care to evolve beyond rigid time windows and to individualized pathophysiologically based treatment .
There are still concerns about the robustness and lack of calibration of CTP especially in low flow conditions which can not be overcome but finally it is not the extreme low flow range that determines clinical outcome but the range of flow values between 10 to 20 ml/100g/min which during the first hours after an ischemic event is responsible for structural damage or not.

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CEREBRAL BLOOD FLOW THRESHOLDS FOR ISCHAEMIA AND IRREVERSIBLE DAMAGE FOLLOWING HEAD INJURY

Jonathan P. COLES

Key words: cerebral blood flow; cerebral metabolism; ischaemia; physiological thresholds.

Definition of cerebral ischaemia and the ischaemic penumbra

The sequence of events following complete arrest of the cerebral circulation is clear. Within seconds neuronal electrical activity ceases, and within minutes there is a deterioration of the energy state and ion homeostasis. If the resultant depletion of high energy phosphates, membrane ion pump failure, efflux of cellular potassium, influx of sodium, chloride and water, and membrane depolarisation persist for longer than 5-10 minutes, irreversible cell damage is likely. The events that occur following incomplete ischaemia are less clear. In this situation, it is likely that residual perfusion persists in the ischaemic area due to incomplete occlusion of the feeding vessel and local collaterals. This results in variable tissue outcomes. Experiments in models of ischaemia suggest that brain function is critically dependent on this residual blood flow, with failure of electrical activity occurring at 15 – 20ml/100g/min, and failure of energy metabolism at 10ml/100g/min. Brain tissue at risk of irreversible damage is classi-

Jonathan P. Coles PhD
Clinician Scientist and Honorary Consultant
Division of Anaesthesia, University of Cambridge
Box 93, Addenbrooke's Hospital, Cambridge, CB2 2QQ, U.K.
The Wolfson Brain Imaging centre, University of Cambridge
Box 65, Addenbrooke's Hospital, Cambridge, CB2 2QQ, U.K.
Tel 00441223217889 – Fax 00441223217887 – jpc44@wbic.cam.ac.uk

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cally described as the 'ischaemic penumbra', and exists between these perfusion thresholds. Such penumbral tissue borders the more densely ischaemic centre, in which energy failure and ion pump failure have already developed. The description of this region of brain tissue as 'penumbra' is analogous to the half shaded zone around the centre of a complete solar eclipse (Figure 1).
Unfortunately the concept of an ischaemic penumbra is not static, but dynamic and constantly changing. In fact, the development of ir-

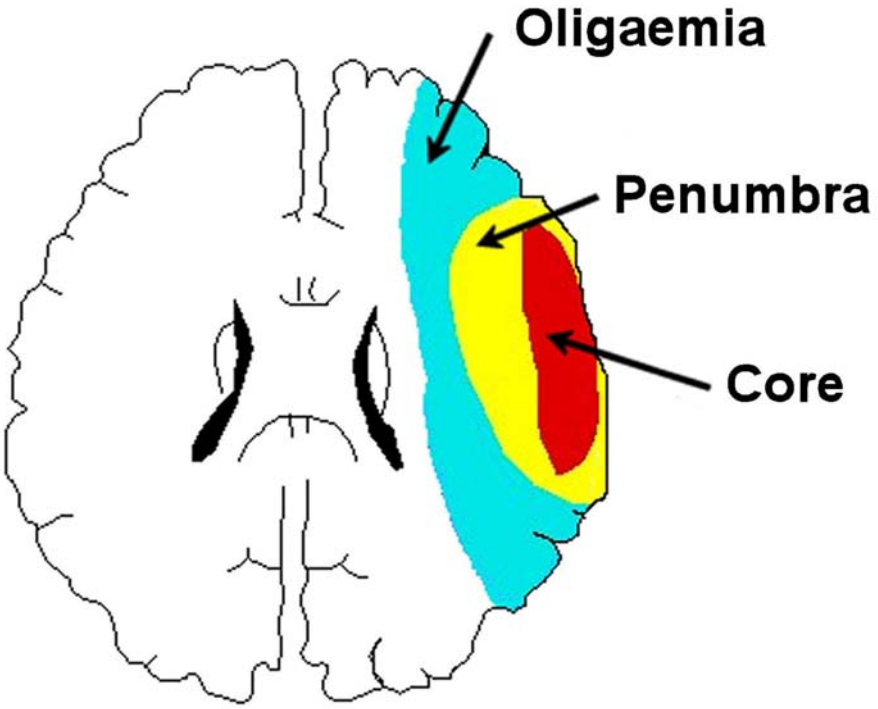

Figure 1 - Schematic to demonstrate the ischaemic penumbra. *Ischaemic core (red), penumbra (yellow) and surrounding region of oligaemia (blue) following acute vascular occlusion.*

reversible damage and cell death is critically dependent on three factors; the level of residual blood flow, the duration of ischaemia and the individual susceptibility of neurons. At flow levels close to the threshold of membrane failure the tolerated ischaemic period is short. As residual blood flow increases towards the upper defining threshold for penumbra (~20 ml/100g/min) the length of time that neural tissue can tolerate ischaemia but still recover increases progressively.
Clinical studies of acute ischaemic stroke have utilised positron emis-

sion tomography and more recently magnetic resonance imaging to identify and differentiate irreversibly damaged and ischaemic but viable tissue. These studies emphasise the dynamic nature of the ischaemic penumbra. This concept is clearly described by Baron using a model of middle cerebral artery occlusion. Immediately following occlusion the volume of ischaemic penumbral tissue is large, but as long as perfusion is promptly restored to an adequate level all brain tissue will survive. As time passes the most susceptible tissue within the core of the lesion becomes infarcted, and unless perfusion is restored, this necrotic core relentlessly increases in size until there is no salvageable brain tissue within the lesion. A dynamic definition used by Baron states that penumbra is 'a severely ischaemic, functionally impaired tissue at risk of infarction, that will be saved if reperfused before it is irreversibly damaged, but that otherwise will be progressively recruited into the core until maximum infarct extension is reached'. This definition has clinical relevance it terms of treatment. If perfusion can be restored to adequate levels, time is of the essence. The longer flow is inadequate the more neural tissue will die.

Difficulties in quantifying cerebral ischaemia following head injury

Conventional imaging approaches have used cerebral blood flow (CBF) thresholds for ischaemia, and data from clinical and experimental stroke provide useful predictive values for tissue survival and death. Unfortunately, it is difficult to translate these results to patients with head injury for several reasons. Following head injury, although the primary injury is important in terms of eventual outcome, secondary ischaemic insults are responsible for worsening of outcome in many patients. In this case it is difficult to predict thresholds based on early measurements of CBF and cerebral oxygen metabolism ($CMRO_2$) and late structural imaging with any certainty. In addition, although measurement of CBF can identify hypoperfusion, in practice, true ischaemia can only be defined in the context of a blood flow that is inappropriately low for tissue metabolism. In health, cerebral blood flow and metabolism are closely coupled and the level of oxygen extracted from the blood is relatively fixed. Both depressed level of consciousness and concurrent sedation can reduce metabolic rate (and hence coupled perfusion) in the injured brain and reduce critical CBF thresholds for ischaemia. In addition, mitochondrial dysfunction can occur following human head injury. This may

lead to coupled reductions in CBF, and present difficulties in using ischaemic thresholds based on cerebral perfusion. Conversely, epileptiform activity, or hypermetabolism associated with excitotoxicity may increase $CMRO_2$ and make "normal" CBF levels inadequate. A clear definition of ischaemia and hyperaemia in situations with primary alterations in $CMRO_2$ depends on the demonstration of compensatory increases or decreases in oxygen extraction respectively Based on the above principles, the risk of cerebral ischaemia and hyperaemia following head injury have previously been defined using measurements of CBF and global indices of the adequacy of CBF, including jugular bulb oximetry (SjO_2) and arteriojugular differences in oxygen content ($AJDO_2$). Cerebral blood flow values less than 20 ml/100g/min, SjO_2 values less than 50% and $AJDO_2$ greater than 9 ml/100ml are thought to suggest significant ischaemia. In comparison, CBF in excess of the normal range (> 55 ml/100g/min), $AJDO_2$ values less than 4 ml/100ml and SjO_2 greater than 75% have been used to suggest hyperaemia.

Finally, it is important to consider difficulties in quantifying ischaemic burden in the injured brain that arise from a lack of *a priori* knowledge regarding the location of ischaemia. Ischaemia in stroke usually conforms to topographical patterns, with identification of an ischaemic core and penumbra. While ischaemia may be prominent in perilesional areas in head injury, significant ischaemia may also be observed in structurally normal brain (Figure 2), and this may be significantly modulated by systemic physiology owing to impaired pressure autoregulation and the effect of $PaCO_2$ on the cerebral circulation.
Despite the great potential shown by focal monitors of brain oxygenation and metabolism, it is difficult to define, with confidence, what constitutes a critical reduction in brain tissue oxygen tension or evidence of ischaemia based on microdialysis measurements. In addition, although these tools allow continuous monitoring of brain oxygenation and metabolism, results are limited to the small volume of tissue within the sampling region of the probes. Whether the information provided is clinically useful within the heterogeneously traumatised brain, is dependent on accurate placement of these monitoring devices within previously identified critically ischaemic regions of brain tissue. In recent publications we have used oxygen[15] positron emission tomography (^{15}O PET) imaging of CBF, cerebral blood volume (CBV), $CMRO_2$ and oxygen extraction fraction (OEF) to define evi-

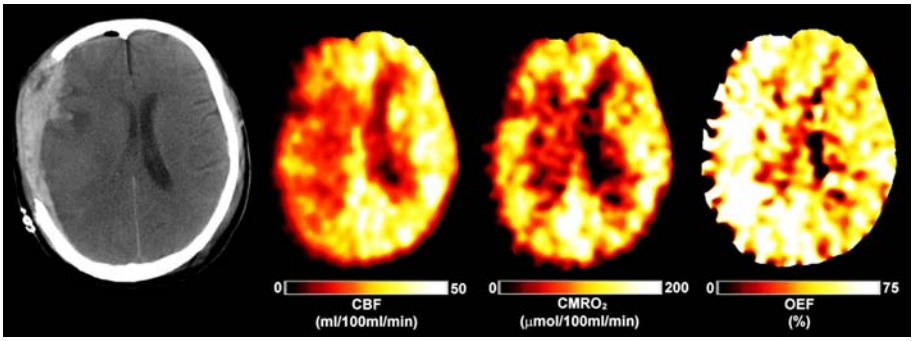

Figure 2 - Positron emission tomography images following early head injury. *X-ray computed tomography, cerebral blood flow (CBF), cerebral metabolism (CMRO2) and oxygen extraction fraction (OEF) images obtained from a 42 year old female 16 hours post injury following evacuation of a subdural haematoma. Note the marked reductions in CBF, increases in OEF and relatively maintained CMRO2, which are suggestive of ischaemia. These physiological derangements extend beyond the cortical tissue immediately underlying the evacuated subdural haematoma.*

dence of cerebral ischaemia following head injury We sought to identify brain regions with inadequate cerebral perfusion and critically increased OEF values. The imbalance in flow-metabolism coupling denoted by such high OEF values clearly characterises tissue at high risk of ischaemic injury. Although it is extremely difficult to define an OEF threshold value predictive of ischaemic injury we have used a threshold value based on an individually calculated cerebral venous oxygen content of 3.5 ml/dL that equates to an OEF of $\geq$ 75%. This is based on the best available data, and is clinically relevant in terms of the management of head injury, where we wish to identify regions *at risk* of neuronal injury. The data provide evidence that regional ischaemia (rather than metabolically coupled hypoperfusion) is present in early head injury, even in patients who achieve suggested targets for cerebral perfusion pressure and intracranial pressure control. While such macrovascular ischaemia is clinically important and potentially related to outcome there are clearly other pathophysiological mechanisms responsible for tissue hypoxia such as mitochondrial dysfunction and microvascular ischaemia. Evidence of microvascular ischaemia comes from studies using ^{15}O PET, brain tissue oxygen monitoring and electron microscopy of contused brain. These data demonstrate evidence of microvascular collapse and perivascular oedema resulting in tissue hypoxia despite normal OEF values following head injury. Further studies are still needed to determine the relative contributions of macrovascular ischaemia, microvascular ischaemia and mitochondrial dysfunction in this setting.

Definition of cerebral blood flow thresholds for irreversible neuronal injury following head injury

Although it may be difficult to quantify cerebral ischaemia following head injury it would still be useful to define the limits of irreversible injury and tissue necrosis. Indeed, classical experimental thresholds based on CBF in states of acute ischaemia, have been useful in predicting tissue outcome. These suggest that if CBF is reduced below 12 ml/100g/min and not restored within a 2-3 hour period, tissue is not able to sustain metabolism and is likely to infarct. A number of studies in acute ischaemic stroke have correlated the morphological outcome of tissue with the acute physiology and determined physiological thresholds, of CBF and $CMRO_2$, for the development of irreversible tissue damage. Using such thresholds, investigators have attempted to acutely identify irreversibly damaged, penumbral, and normal tissue and to monitor the efficacy of therapeutic strategies such as reperfusion. Following head injury, the early identification of irreversibly damaged, and penumbral tissue could have considerable therapeutic and prognostic implications. Early identification of such tissue could offer the opportunity to better study the effects of neuroprotective interventions, surgery and drugs, and formulate strategies that best preserve vulnerable tissue from secondary injury in individual patients. In particular, it may be possible to predict neurological outcome and determine whether such strategies lead to significant clinical benefits with improved neurological outcome.

In recent work we have sought to determine physiological thresholds for the development of irreversible tissue damage in contusional and pericontusional tissue following head injury. To derive these thresholds we have used similar methodology to that used within the stroke literature, and is based on identifying lesions on magnetic resonance imaging obtained at 6 – 12 months post injury. We compared the acute physiology from ^{15}O PET imaging obtained within 72 hours of injury within these regions of structural injury to matched regions of normal appearing brain. Importantly, we had hoped to differentiate between ultimately damaged and undamaged tissue based on physiological thresholds for blood flow and metabolism. The lower limits of the 95% confidence interval for CBF, $CMRO_2$ and OEF in non-lesion tissue were 15.0 ml/100ml/min, 36.7 μmol/100ml/min, and 25.9% respectively. We found that the CBF threshold below which tissue is unlikely to survive

differs from that typically following ischaemic stroke, while the $CMRO_2$ threshold was comparable. This underlines the problems of translating thresholds defined within the ischaemic stroke literature to head injury. In addition, we found that there was considerable overlap in the physiological measurements of CBF and $CMRO_2$ observed within the ultimately injured and normal appearing brain. This is consistent with previous reports using stable Xenon computerised tomography measurements of CBF within and around cerebral contusions. Furthermore, we investigated the ability of single physiological variables to predict tissue outcome, and have demonstrated that, in common with ischaemic stroke, there is no absolute threshold of any single physiological variable that provides accurate differentiation of damaged and undamaged tissue. It is important to emphasise that while we based our analysis upon regions with pan-necrosis there is evidence of neuronal injury occurring within regions of structurally normal appearing brain. In order to fully investigate differences in physiology within lesion and non-lesion areas, and possibly determine methods that separate them, it may be necessary to use follow-up techniques that are sensitive to such selective neuronal loss, such as magnetic resonance spectroscopy or ^{11}C-flumazenil positron emission tomography. Future attempts to produce predictive maps for tissue outcome may require using combinations of several physiological variables, and may need to account for time and patient variables.

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CHOOSING THE BEST METHOD TO IDENTIFY AND REPORT DIAGNOSTIC THRESHOLDS

Cosetta MINELLI, Marco BOTTERI, Nicola LATRONICO

Introduction

Diagnostic studies may address different questions, which often correspond to successive phases in the evaluation of test performance, in analogy with what happens for treatment evaluations in clinical trials. Sackett and Haynes have classified the most common questions in four phases, characterised by different study designs and methodologies. Phase 1 assesses whether the test results for a new test differ in subjects with and without the disease. Phase II and III aim at assessing how well the new test can predict the presence of the disease under ideal conditions (phase II) and in the real world setting of clinical practice (phase III). In phases II and III, the accuracy of the test is evaluated by comparing, for each subject, the result of the test with the true disease status, which is assessed through the use of a diagnostic "gold standard". Finally, phase IV addresses the issue of whether subjects undergoing the new test fare better, in terms of ultimate health outcome, than subjects who do not.

An aspect of diagnostic research which is often overlooked is that the results of a diagnostic study, in terms of test performance and impact on clinical outcome (phase II to IV), strongly depend on the choice of the threshold value of the test used to separate normal (negative) from abnormal (positive) results. Diagnostic studies aimed at evaluating the "best" threshold represent a step of crucial importance in assessing the ultimate performance of the test in guiding clinical decision-making, that is its impact on decisions about future diagnostic and

Cosetta Minelli, MD, MSc
Department of Health Sciences, University of Leicester, U.K.
22-28 Princess Road West – Leicester, LE1 6TP, U.K.
Tel.: +44(0)116 252 3287 – Fax: +44(0)116 252 3272 – cm109@le.ac.uk
Dott. Marco Botteri, MD
Prof. Nicola Latronico, MD
Istituto di Anestesia e Rianimazione – Università di Brescia, Italia

treatment options. It is on this type of studies that this chapter will focus. Methods used for the identification of the best threshold will be discussed, and the difference in interpretation, and clinical implications, of defining a threshold using inappropriate approaches will be illustrated using the example of Cerebral Blood Flow (CBF) thresholds in the diagnosis of ischemic stroke.

Although not addressed in this chapter, equally important in the methodology of studies evaluating diagnostic thresholds are more general issues of diagnostic studies, which include aspects related to the comparison of the new test with the reference test (e.g. independent, blind comparison with a reference standard; reference standard represented by an adequate gold standard), and the choice of the study population (e.g. patient sample including an appropriate spectrum of patients). Guidance on these methodological issues can be found in a review by Jaeschke and colleagues on the validity of the results from diagnostic studies.

Measures of test performance

In the evaluation of a diagnostic test, where a gold standard is used to assess the true disease status of each subject, the distributions of diseased and non-diseased subjects according to the result of the diagnostic test is represented in Figure 1, for a test where lower values are associated with higher probability of the disease (e.g., CBF and brain ischemia). In most situations, the distributions of values of the diagnostic test typically overlap, which means that the test cannot distinguish between diseased and non-diseased subjects with 100% accuracy. This implies, in practice, a certain amount of misclassification, which can take two different forms: a diseased subject could result negative to the test (false negative), or a non-diseased subject could result positive to the test (false positive). The probability that the test is positive in subjects with disease is referred to as sensitivity, and a test has high sensitivity when the proportion of false negatives is low; on the other hand, the probability of a negative test in subjects without the disease is defined as specificity, and a test has high specificity when the proportion of false positives is low. Table 1 shows a two-by-two contingency table, which is the standard method for the presentation and analysis of diagnostic studies, while the definition and calculation of sensitivity, specificity and a number of other measures of performance of a diagnostic test are presented in Table 2. An overview

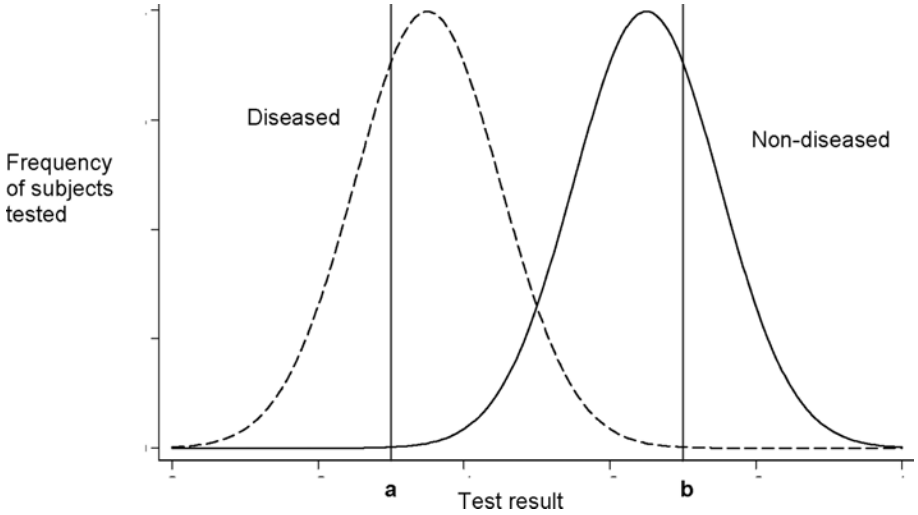

Figure 1 – *Distribution of test results for diseased and non-diseased subjects. If the threshold is* ***a****, the specificity of the test is 100% (no false positives) but sensitivity is low. If the threshold is* ***b****, the sensitivity of the test is 100% (no false negatives) but specificity is low. Thresholds used in clinical practice will vary between these two extremes.*

Test results	**Disease**		
	Present	Absent	
Positive	True positives a	False positives b	Total positive a+b
Negative	False negatives c	True negatives d	Total negative c+d
	Total diseased a+c	Total non-diseased b+d	a+b+c+d

Table 1 – *Two-by-two contingency table*.

of the utility and limitations of these different measures is provided by Farr and Shapiro. The positive and negative predictive values depend not only on sensitivity and specificity of the test but also on the prevalence of the disease in the population tested, and are thus clinically more useful when assessing the probability of the disease given the test result in individual subjects. On the other hand, all the other measures (sensitivity, specificity, positive and negative likelihood ratio, diagnostic odds ratio) are more appropriate when defining the intrinsic characteristics of the test. This is important, for instance, when com-

Diagnostic accuracy statistics	Definition	Calculation
ACCURACY	Proportion of subjects correctly classified as diseased or non-diseased	(a+d) / (a+b+c+d)
SENSITIVITY	Probability of a positive test result in diseased	a / (a+c)
SPECIFICITY	Probability of a negative test result in non-diseased	d / (b+d)
POSITIVE PREDICTIVE VALUE (PPV)	Probability of disease in subjects with a positive test result	a / (a+b)
NEGATIVE PREDICTIVE VALUE (NPV)	Probability of absence of disease in subjects with a negative test result	d / (c+d)
POSITIVE LIKELIHOOD RATIO (LR +ve)	Ratio of the probability of a positive test result in diseased and the probability of the same result in non-diseased	a/(a+c) / b/(b+d) *OR* sensitivity / (1-specificity)
NEGATIVE LIKELIHOOD RATIO (LR -ve)	Ratio of the probability of a negative test result in diseased and the probability of the same result in non-diseased	c/(a+c) / d/(b+d) *OR* (1-sensitivity) / specificity
DIAGNOSTIC ODDS RATIO (DOR)	Odds of positivity of test result in diseased divided by the odds of positivity in non-diseased	(axd) / (cxd) *OR* LR +ve / LR -ve

Table 2 – *Definitions of different measures of performance of diagnostic tests (a,b,c,d defined in Table 1)*.

paring diagnostic studies performed across a range of different study populations. Among these measures of performance that are not dependent on the prevalence of the disease, some authors have suggested that the relatively newly introduced diagnostic odds ratio might be the single best indicator of test performance.
Sensitivity and specificity of a test depend on the threshold value used, as shown in Figure 1. The position of the threshold, indicated by a vertical line, determines the definition of positivity of the test and the corresponding number of false negatives and false positives. As a rule, an increase in the sensitivity will decrease the specificity, and vice versa. In different clinical situations we may require different values of sensitivity and specificity for clinical decision-making, and therefore use different thresholds for the same test, if we wish to minimise one specific misclassification problem. The choice of the "best" threshold for any specific setting may be straightforward if the evidence on the performance of the test is presented using ROC curves, where sensitivity and specificity are plotted for different threshold values considered.

Identification of the "best" threshold using the ROC curve

The "Receiver Operating Characteristic" (ROC) analysis was developed during World War II to analyse radar images. The Receiver Oper-

ating Characteristics represented the ability of radar receiver operators to decide whether a blip on the screen constituted an enemy target, a friendly ship, or just noise. It was not until the 1970's that this approach was adopted in medicine to represent the ability of a diagnostic test to diagnose a disease. The curve provides the "receiver" of the information (i.e. the reader) with the "characteristics" of the test (i.e. sensitivity and specificity) for any threshold value considered.

The ROC curve is a plot of the true positive rate (sensitivity) against the false positive rate (1-specificity) for the different possible thresholds of a diagnostic test, and the Area Under the Curve (AUC) represents the *average* value of sensitivity for all possible values of specificity, and vice versa. A curve close to the diagonal, i.e. an AUC close to 0.5, indicates that the test has no accuracy; the accuracy increases with the curve getting closer to the left upper corner, i.e. AUC close to 1. An example of ROC curve is shown in Figure 2, which describes the results of a study evaluating cerebral blood flow thresholds in the diagnosis of ischemic penumbra. By labelling the different threshold values on the curve, the plot shows the trade-off between sensitivity and specificity for each threshold. This allows the identification of the threshold value of the

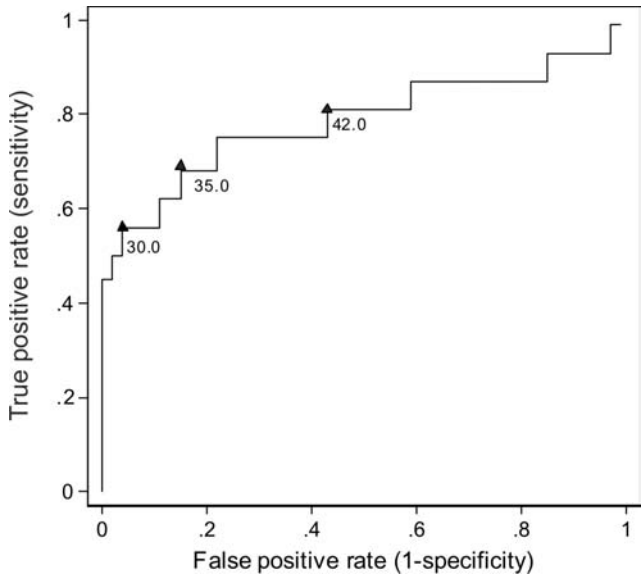

Figure 2 – *Example of ROC curve for the evaluation of cerebral blood flow thresholds in the diagnosis of ischemic penumbra. The values labelled on the curve represent three different thresholds (from Grandin et al., 2001[6]).*

test which maximises its performance under specific situations, where a certain level of sensitivity or specificity is required. For a diagnostic test where the likelihood of disease increases with the decrease in test values, Figure 1 shows how a higher threshold will imply fewer false negative (equivalent to say that a broad diagnostic criterion is adopted in order to identify all possible cases of disease), while a lower threshold will imply fewer false positives (a strict diagnostic criterion is adopted in order to avoid to diagnose as diseased subjects who are indeed healthy). Further information on the ROC curve and how to calculate the AUC can be found in a number of papers dedicated to this topic.

The point on the curve corresponding to the left upper corner is the best compromise between sensitivity and specificity when sensitivity and specificity are assumed to have the same importance. Although this point is often taken as the "best" threshold, the choice of the optimal threshold should rather be made based on the expected benefits and risks corresponding to the use of each threshold considered. We could answer the question "How many healthy subjects are we willing to erroneously diagnose as diseased in order to correctly diagnose one diseased subject?", and thus define a benefit-to-harm ratio, by evaluating the clinical (and/or financial) "cost" associated with the use of each threshold value. This can be addressed using a decision-analysis framework similar to that used to choose between different treatment options.

Illustrative example: CBF thresholds in the diagnosis of ischemic stroke

In acute ischemic stroke, a CBF reduction below certain values is the critical event leading to functional, biochemical and structural changes, which culminates into neuronal death. The ischemic area is constituted by an infarct core surrounded by ischemic tissue that is still viable although functionally impaired, and which can be divided into areas that recovers spontaneously (benign oligemia) and areas that progress to irreversible changes, unless effective treatment is used (penumbra).
Although intravenous thrombolysis with recombinant tissue plasminogen activator (rtPA) is the recommended treatment in patients with acute ischemic stroke, in clinical practice the use rtPA is limited to a minority of such patients. This is not only due to the narrow time window for which rtPA treatment is currently approved (less than 3 hours after the onset of symptoms) and a certain number of contraindica-

tions, but also to uncertainties as to which patients might actually benefit from thrombolysis. It is for this reason that active research has been recently focusing on imaging methods to discriminate between infarct core and surrounding potentially salvageable tissue, with the aim of better identifying patients suitable for treatment. This might allow restricting rtPA use to those patients with large penumbra and small infarct core even beyond the 3-hour time window.
Magnetic Resonance Imaging (MRI), including perfusion and diffusion-weighted imaging (PWI, DWI), has been used as diagnostic tool for hyperacute stroke before therapy decision; however its capability to discriminate between infarct core and penumbra is still controversial. CBF, which can be measured using several imaging methods (Xenon-Enhanced CT, Single Photon Emission CT, Positron Emission Tomography, CT Perfusion, and PWI), can help characterising at risk tissue in terms of salvageable potential. CBF thresholds of 17 and 10 mL/100g/min are routinely used to discriminate between benign oligemia and penumbra, and between penumbra and infarct core, respectively. However, these thresholds are mainly based on experimental studies in animals, and their diagnostic accuracy in humans has never been established.
In a recent review, Bandera and colleagues systematically reviewed the medical literature to evaluate the evidence available on CBF thresholds and its methodological adequacy in adults with acute ischemic stroke. They included studies on adult stroke patients if they compared CBF measurements with a diagnostic gold standard (follow-up brain CT/MRI) and reported CBF thresholds, and 7 studies met these criteria. The optimal reported CBF thresholds varied widely, from 14.1 to 35.0 and from 4.8 to 8.4 mL/100g/min for penumbra and infarct core, respectively. Among the possible explanations for such heterogeneity in study results, which included differences in measurement techniques, patients' case-mix and quality of the studies, the review identified the methodology used to derive the threshold values as an important source of variability.
For illustration, we will consider here only the threshold for penumbra (distinguishing between benign oligemia and penumbra), for which more evidence was available. Only the four most recent studies included in the review provided sensitivity and specificity for each threshold value reported. In two studies the threshold was derived from a ROC curve, in one study from cumulative probability curves, and in another study from a discriminant analysis. Although cumula-

tive probability curves and discriminant analysis represent valid approaches to the identification of the optimal threshold for a diagnostic test, they do not provide the reader with as much information as the ROC curve. Since what represents an optimal threshold in a specific clinical setting might not be the ideal one in a different setting, the graphical representation of the relationship between sensitivity and specificity of the test for different thresholds provided by the ROC curve is crucially important for the generalisability of study results. Indeed, even in situations where discriminant analysis is preferred, such as the evaluation of a test constituted by a combination of diagnostic variables (e.g. a score), still ROC curves might be best employed to display the results, in terms of sensitivity and specificity, for different thresholds. In the other three studies included, the approach used to identify the threshold did not allow a direct interpretation of the results in terms of sensitivity and specificity. In particular, in two studies, the threshold was defined as the lowest CBF value measured in the area of benign oligemia or the highest in the area of penumbra, which implicitly aims for a specificity of 100% (no false positive) at the expense of sensitivity, or a sensitivity of 100% (no false negatives) at the expense of specificity, respectively (see Figure 1). In another study the threshold was defined as the mean CBF value in the area of penumbra; this approach, although useful for descriptive purposes, has no rationale when used for threshold definition, and cannot be interpreted in terms of sensitivity and specificity.

The authors of the review could not perform a quantitative synthesis of the results, that is a meta-analysis based on a summary ROC curve, due to insufficient data. They summarised graphically the results of the four studies for which sensitivity and specificity data were available, by plotting the ROC curve (two studies) or the values of sensitivity and specificity associated with the single threshold reported (two studies), according to the information available (Figure 3). Inconsistencies in the optimal thresholds reported by the four studies could partially be explained in terms of differences in sensitivity and specificity. The optimal thresholds reported by Rohl et al. and Liu et al. were similar (29.5 and 24 mL/100g/min, respectively) and similar were the corresponding sensitivity and specificity (sensitivity of 91% and 88%, and specificity of 73% and 66% respectively), while the threshold reported by Heiss et al. was substantially lower (14.1 mL/100g/min), but indeed corresponded to a lower sensitivity (72%) and higher specificity (90%). On the other hand, the high value of the optimal threshold

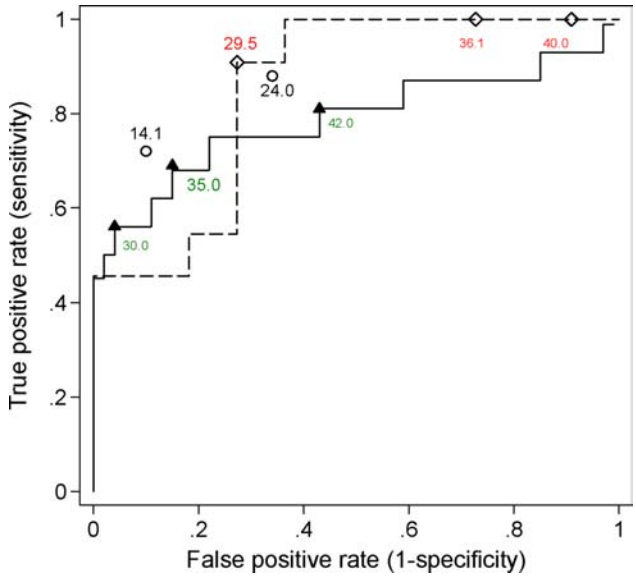

Figure 3 – *Graphical display of the results of four studies in the review by Bandera and colleagues. The threshold values written in bigger font are those chosen by the authors of each study as the optimal threshold (published with permission from Stroke).*

reported by Grandin et al. (35 mL/100g/min) could not be explained in terms of differences in sensitivity and specificity (69% and 85%, respectively).
The conclusion of this review was that currently suggested diagnostic criteria to discriminate between infarct core, penumbra and benign oligemia are based on weak evidence, and further evaluation is needed before CBF thresholds can be reliably used in commercial software for imaging methods to detect acute brain ischemia.

Discussion

Although the quality of any health care intervention highly depends on the quality of the diagnostic process, the methodological aspects of the evaluation of diagnostic procedures have received disappointingly very limited attention compared to the evaluation of treatments. Indeed, the usual formal requirements for the adoption of new treatments are often lacking for diagnostic tests. The result of this situation is reflected in the methodological flaws which have been repeatedly described in diagnostic studies . This chapter addresses a crucial issue in the evaluation of a diagnostic test, the choice of the threshold value which maximises the performance of the test.

The example of the recent review by Bandera and colleagues on CBF thresholds in the diagnosis of ischemic stroke illustrates how the methodological issues surrounding the evaluation of diagnostic thresholds are often not fully understood, which can impact the quality of a study and make it difficult to interpret its results. In this review, the high heterogeneity in study results could be partly explained by the difference in the methods used to derive the threshold values, and some differences in CBF thresholds could be interpreted in terms of differences in the corresponding sensitivity and specificity. The methods used varied from defining the threshold as the mean CBF value in affected areas, to obtaining the threshold based on ROC curve, cumulative probability curves, or discriminant analysis.
In clinical decision making, selection of optimal diagnostic strategies is based on a number of factors; accuracy of the test, assessed through sensitivity and specificity; prevalence of the disease in the relevant population; relative benefits and harms resulting from application of the test and consequent treatment choices. The threshold value used for a diagnostic test is crucially important in determining the balance between sensitivity and specificity, and the decision of where this balance should lie (e.g. are sensitivity and specificity equally important or should we favor one over the other?) strictly depends on the particular diagnostic setting. In practice, the optimal compromise between sensitivity and specificity depends on the prevalence of the disease and on the clinical consequences of the two kinds of incorrect test results, false-negative and false-positive. In the treatment of hyperacute stroke with rtPA, for example, where CBF measurement can be used in commercial software to detect ischemic areas that are salvageable with therapy, thresholds with high sensitivity but poor specificity would expose a number of patients to the risk of receiving drugs with associated side effects without any potential benefit, while thresholds with low sensitivity but high specificity would exclude from treatment patients with salvageable ischemic tissue. In specific situations, clinicians might aim at a higher sensitivity at the expense of specificity or vice versa, as might happen, for example, in patients with baseline increased hemorrhagic risk, for which a high specificity threshold might be desirable in order to avoid severe side effects of rtPA administration. For these reasons, a study aimed at identifying the optimal threshold for a specific diagnostic test which reports only a single threshold value will not maximize the informative potential of the study, since its results might not be generalisable. The use of a

ROC curve does address this issue, by providing the reader with all relevant information gathered by the study.
It is important to note that although the "best" threshold based on a ROC analysis is often chosen as the point on the curve closer to the left upper corner, by assuming equal importance of a false-negative and a false-positive diagnostic result on the patient's outcome, in practice this assumption will rarely hold. The optimal threshold should indeed be chosen as the point on ROC curve for which the benefits (clinical and/or financial impact of a correct diagnosis) exceed the harms (impact of an incorrect diagnosis) in any specific situation.
The choice of the approach to be used for identifying and reporting diagnostic thresholds, although crucial, is not the only methodological issue involved in studies evaluating diagnostic thresholds. It is interesting to note that in the review on CBF in ischemic stroke, among the 73 studies evaluating the performance of CBF measurements in the diagnosis of brain ischemia in patients with ischemic stroke, only 11 investigated CBF threshold values for penumbra and/or infarct core. Among the 11 studies, only 7 fulfilled the basic methodological requirement of comparing the test under evaluation with a gold standard. Moreover, the sample size of these 7 studies was small, with only one having more than 25 patients, and none of them satisfied all the suggested criteria for the optimal design of diagnostic test evaluation: a prospective, blind comparison of the index diagnostic test with an accepted gold standard in a consecutive series of patients from a relevant clinical population.
The conclusions of the review by Bandera and colleagues, in line with what highlighted by other authors , were that there is a need for methodological improvement in diagnostic studies, not only in terms of study design (prospective studies on larger patient samples with blind comparison between index and reference test), but also in terms of analysis and reporting of study results. The ideal approach to analyse and report the results of a study evaluating diagnostic thresholds is the use of the ROC curve. The ROC curve provides information not only on the performance of the test for different threshold values, but also on the *overall* accuracy of the test, which can be measured by the area under the curve. Moreover, the ROC curve allows direct comparisons between two or more different diagnostic tests for a disease. Finally, a widespread use of the ROC curve in primary studies would allow results from different studies to be pooled in meta-analyses using summary ROC curves (SROC curves). In fact, although evidence on

diagnostic tests can be combined using classical accuracy measures such as sensitivity and specificity, this approach suffers from the same problem described for primary studies, that is the dependence of the pooled result on the threshold used. In SROC curves, unlike ROC curves, each data point on the curve represents a separate study, and the curve shows the trade-off between sensitivity and specificity for different thresholds corresponding to different studies. The SROC curve allows to assess whether the variability in the threshold used by different studies could explain the variability in study results in terms of diagnostic performance. SROC curves represent a powerful tool to synthesise the available evidence on diagnostic tests and provide the reader with the information needed for an effective evidence-based diagnostic decision-making.

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REDUCING MORTALITY RATES IN BRAIN TRAUMA: NEUROSURGERY HOLDS THE KEY

Franco SERVADEI

Every year in Europe an average of 235 patients every 100.000 inhabitants ends up in a Hospital as a result of brain trauma[1] . Around 80% of these are minor cases (GCS14-15) , 10% moderate trauma (GCS 13-9) and the other 10% are serious (GCS below 9)[1] . The percentage of patients with cranial haematoma rises from 3% in those patients with minor trauma to 10-15% in those with moderate trauma and up to 30-40% when the trauma is serious. Mortality and disability rates also rise accordingly.

These patients end up in many different departments (Emergency, Neurology, General surgery, Orthopaedics, Neurology, Neurosurgery, Intensive Care). Only one person sees the patients with potential complications and that person is the neurosurgeon. The neurosurgeon's first job therefore is to check out "patients at risk" within the referral area of the Specialist Hospital.

Although there is a slight risk of complications, all cases of post-trauma haematomas identified in asymptomatic patients are curable if the haematoma is evacuated before the clinical situation worsens. The published guidelines[2,3] give indications as to when to do a CT in an asymptomatic patient. It is up to the neurosurgeon, along with the doctors in the Emergency Unit and the neurologists, to make sure these guidelines are widely available and observed. Increasing the use of CTs with this type of pathology has led to a drastic reduction in the number of "talk and die"[4] patients and reduced to practically zero mortality rates in patients with extradural haematomas[5].

Another important element in reducing mortality rates is in the selection of patients to be sent to Neurosurgery . In many areas of Italy and Europe, Neurosurgery is very centralised, and patient selection is done either by phone or through systems of image transmission. Unfortunately, there are no evidence-based international guidelines to inform

Franco Servadei
Neurochirurgia Traumatologica, Ospedale M. Bufalini, Cesena

selection. Aspects taken into consideration are patient's age, the GCS referred from the outlying hospital and the CT results as seen from the transmitted image. All of these can lead to the wrong decisions: recent studies show how the value of the GCS has been lost over time as tubing and sedation techniques make it impossible to assess the patient on entry [6]. This is proved by telemedicine data in the Piemonte Region (North East Italy) A careful study of follow-up in patients referred from an outlying hospital to Neurosurgery and presenting with a GCS3 on arrival shows that 35% of these patients recovered perfectly without any therapeutic procedure [7] making the initial GCS 3 assessment very questionable. Patient age is the major criterion when deciding which patients to transfer[8]. Once they are admitted to Neurosurgery, however, the treatment is the same as for younger patients[9]. So are we sure that this is such a valid criterion?

An initial normal CT generally manages to help prevent unexpected developments. Patients with lesions compressing the cisterns, or which create shift or a mass effect are transferred in accordance with the guidelines. But what should be done with patients with minor contusions/widespread damage? The current approach is to leave the patient where he/she is. One patient of 7[10] presenting with what are initially non-surgical lesions can evolve, with obvious consequences for the prognosis if this happens when the patient finds him/herself a long way from anyone who might be able to treat them. The solution is not, however, to delegate the decision as to whether a patient should be transferred or not to someone else. The most significant mistakes in failing to transfer patients occur with those patients who initially are in the low risk category (minor traumas) which have no bearing on intensive care.

Finally, the role of the neurosurgeon in severe cranial trauma cases. In most parts of Europe these patients are cared for by colleagues in Intensive Care and the role of the neurosurgeon appears to be a supporting one. However, whether to monitor intracranial pressure including the insertion of catheters, whether to intervene surgically when a lesion is evolving and whether to opt for a decompressive craniectomy are all part of the typical remit of the Neurosurgeon. This does not mean that integration is not necessary. When a decompressive craniectomy is indicated, for example, a series of steps needs to be taken[11] which combine both medical and surgical treatment. No surgical decision can be taken without knowing the medical treatment level reached by Intensive Care colleagues. Even a forthcoming,

randomised study (www.rescueicp.com) says that certain medical and surgical steps have to be followed before any randomisation can be introduced.
The conclusion is that the neurosurgeon is a key figure in the treatment of trauma. Compared to other professionals in the field, the neurosurgeon has a few advantages:

1) They see all traumas with intracranial lesions, including those with a high GCS (the Emergency doctor only assesses and treats patients with high GCS but without intracranial lesions, the intensivist only sees coma patients or those who are not seriously ill on entry but later become so). It is obvious that collegues in the Intensive Care Unit (ICU) have a tendency to consider as serious any patient with significant lesions.
2) They are the only person who can decide to refer the patient to a special unit. We recognise that this decision is often made on the basis of too rigid criteria with devastating results[12] . It is the "modern" neurosurgeon's job, in collaboration with colleagues in ICU, to estabilish protocolls for patient transfer which mean that any patient requiring specialist monitoring of any type, not only "surgical" monitoring, are sent to Neurosurgery Units.
3) Evacuating a haematoma or decompressing a patient are technically simple procedures, much simpler than organising sophisticated intensive care. In this sense, the neurosurgeon can be of help to patients suffering from brain trauma wherever they are, even if resources are very limited.

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DECREASING MORTALITY IN HEAD TRAUMA PATIENTS: THE KEY IS THE NEUROINTENSIVIST

Arturo CHIEREGATO, Doriano ZAPPI, Luigi TARGA

Key words: head injury, intensive care, mortality, outcome.

Introduction

Several sources in medical literature suggest that in civilized countries mortality rates for severe head injury are decreasing where patients are treated in specialized centres [1-4]. Systematic tratment of patients with head injury (TBI) began in period 1970-1980 when artificial ventilation become available [5]. Overall mortality remained unacceptably high, however [6]. From 1980-1990, neurological examination (GCS), the progressive introduction of CT, and the beginning of ICP monitoring helped to reduce mortality [7,8]. During the period 1990-2000 the management of ICU was enriched by the monitoring and treatmento of cerebral perfusion pressure [9] and the monitoring of metabolic or perfusional parameters. Since the year 2000, the main aim has been to introduce evidence-based medicine into clinical management and guidelines. Data regarding the decrease in mortality, however, refers only to academic or specialist centres, while data regarding the management of this type of patients in non-specialist centers, or centers or centers without neurosurgery, is less readily available. Consequently the overall prognosis for severe TBI patients still has to be defined. Most patients are not admitted to trauma centres [10] and many are treated in hospitals without neurosurgical or neurocritical care facilities [11].

However, studies based on large number of patients show that admission to trauma centers does reduce mortality rates in trauma [10] and that mortality in severely TBI patients is reduced when these patients are admitted to neurosurgical centers [11]. Admission to a neurosurgical center is usually

Arturo Chieregato, MD
Doriano Zappi, MD Luigi Targa, MD
UO Anestesia e Rianimazione, Ospedale M.Bufalini - AUSL-Cesena, Italy
achiere@ausl-cesena.emr.it

determined by the presence of a mass requiring emergency or urgent evacuation, especially when identified on the initial CT, or by the presence of lesions which could get worse. It is current practice not to transfer patients with injuries deemed life treatening to a neurosurgical center even though isolated reports suggests that even TBI patients who are initially considered very severe may show encouraging improvement when aggressively treated [12]. Admission are also influenced by the availability of the necessary facilities for neurosurgical intensive care. It seems probable that patients with surgical lesions are more likely to be referred to specialist centers compared to TBI patients with diffuse lesions because of shortage of beds in neurosurgical centers. However, roughly half of all patients with TBI have widespread lesions, and Patel and colleagues [11] showed that even in this subtype of patients, outcome may be improved by medical and surgical treatment in a specialist neuroICU. All this converging evidence suggests that centers which specialise in neurosurgery and neurointensive care could improve outcome for this type of patient. The relevance of the neurosurgeon is obvious, while the significance of a specialized team of neurointensivists may be more difficult to apreciate. The aim of this study was to report and evaluate a preliminary description of outcome improvement in our ICU following the introduction of a specialist neurointensivist, and to review the current literature which analyzed the effect of specialized neurocritical care on the outcome of TBI.

Material and Methods

Effect of changes neurocritical and general management

The ICU at the Bufalini Hospital in Cesena admits patients from a large referral area (from 1 to 2 million people, figures show some seasonal variation due to tourism). It was set up in 1989 and has admitted 7,295 patients since then. The Hospital is the only neurosurgical hospital in the region and from the year 2000 onwards, patients with TBI were admitted on the basis of image transmission. Since June 2002, the hospital has been classified as a level I trauma center as part of a re-organization of the regional trauma system (SIAT) thus confirming the center's role and allowing for the introduction of a new protocol for the admission of patients. In May 1997, a new head of the Anestesiology Department was appointed and, in September 1997, the management of the ICU was entrusted to a new specialist in neurocritical medicine. He implemented a new management scheme based on the idea that acute neurological disease needs to be evaluated and interpreted and its natural history known and

predicted , so that suitable treatment plans can be worked out for minimizing damaging side-effects. A protocol to control intracranial hypertension was implemented and adapted to the human and technical know-how within the ICU. Monitoring techniques such as invasive arterial pressure monitoring, $ETCO_2$, SpO_2 and internal T° became routine for all patients and intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were also misured and jugular bulb monitoring carried out. Recorded physiological multivariate data for three patients at a time can be sent to the computer through an AD converter (MacLab, World Precision Instrument), sampled at a rate of 20 Hz. Ventricular drainage is the preferred option for monitoring ICP as it is more reliable and allows for CSF drainage. Serum sodium was strictly controlled three times a day in any patient at risk of ICP elevation.

In the initial years, from 1998 to approximately 2002, a stair case protocol was worked out in agreement with the American and European Brain Injury Consortium [13,14] and all patients were managed with a stair protocol to maintain ICP below 20 mmHg and CPP above 70 mmHg. The first level of therapy (standard) consisted in sedation and analgesia to reach poor reactivity of ICP to noxious stimulation, intermittent CSF drainage, tight control of serum sodium to maintain values toward the upper normal limits, normocapnia, and CPP around 70 mmHg with crystalloids input and norepinephrine and dobutamine. Long-acting benzodiazepines (diazepam) were preferred, giving the patients more stability. The protocol for the second staircase (reinforced) level of therapy when ICP was deteriorating included a bolus of mannitol, serum sodiu elevation, continuous propofol infusion in asociation with benzodiazepines, cooling of the skull surface with ice, and mild to moderate hyperventilation. Whenever non-evacuated parenchymal lesions were potentially responsible for ICP elevation, these were evacuated in collaboration with the neurosurgeon. If the lesions were in the language areas or situated very deep, it was sometimes considered preferable to prolong medical therapy or use external decompression rather than evacuation. The final level of therapy (extreme) was applied in cases of refractory ICP and involved the use of barbiturates.

External decompression was an option for cases of bilateral frontobasal contusion or bilateral contusions in temporal lobes. External decompression was rarely used for diffuse swelling.

Since the year 2000, the regional and global cerebral blood flow (CBF) can be assesed in our center using Xenon-CT.

The direct measurement of global and regional CBF, as well as accompanying changes in CPP guide-lines, have led to the lowering of CPP levels and to the personalisation oftreatment regimes for each patient. At the same time, in isolated cases, the detection of global hyperemia with accompaying refractory intracranial pressure meant that selective use of indomethacin was indicated.

General management

The general management of the patients aimed to allow for the best possible control of infections, to ensure adequate ventilation and to allow a good nutritional status.

A conservative use of antibiotics was planned by using therapy predominantly after the bacteriological diagnosis. Antibiotic prophylaxis was not used, except during initial intracranial surgery. First level antibiotics were used predominantly. A large use of tracheostomy was used to allow for safer ventilation and potentially better prevention of infection. The patient's position was changed regularly to prevent pulmonary complications and more recently prone position has been allowed even in patients with active treatment of intracranial hypertension. A high isotonic fluid intake has been used to allow the maintenance of CPP targets and to prevent any deleterious effect of vasoconstrictor agents on renal perfusion. Enteral nutrition was started from the first day post injury and was the only source of calories. Invasive hemodinamic monitoring with Swan Ganz has been introduced in ICU and applied in patients with ARDS or with continuous barbiturate infusion.

General ICU organization

The ICU now has two major meetings every day where cases are assessed and planning is discussed. The ICU director is on call 24 hrs a day so that an opinion can be sought in more complex cases. Daily meeting with the neurosurgeons has been scheduled to allow for updates on the level of medical treatment and to re-evaluate any further surgical options after the emergency one, usually carried out in the first 24 hours. The nurses, whose duties were formerly very very clearly spelled-out, have gradually acquired much more autonomy in their work. Where patients with TBI and ICP monitoring are

concerned, the nurses are in charge of the modality of intermittent CSF drainage to ensure an ICP of 20 or 25 mmHg, and they also adjust norepinephrine infusion to ensure CPP levels of 70 mmHg and also give extra boluses of fentanyl when necessary to prevent ICP elevation during risky procedures.
Human resources have improved over the years in line with the more intense management. More nurses were available moving from a nurce/3 beds to a nurse/2 bed ratio. The nursing staff has been enriched by a head nurse and by a nurse responsable for materials, technological maintenance and information technology development.
Improvements in medical resources were less but, nonetheless, changed, during the daily shift, from 1 attending physician/4-5 patients to 1 attending physician/3-4 patients. There has been one resident specialist every year since 2000.

Data collection

Electronic data are available for patients admitted since 1990. Before September 1997, data was obtained from the hospital administrative data base of the hospital andlacked most of the clinical information we need to allow a suitable severity-adjusted analysis.
Historical data has been compared for ICU mortality and for mortality within 48 hours. Since data regarding initial severity was lacking, it was assumed that the patients who died within 48 hours were hopeless cases where changes in clinical management could do little to change clinical outcome.
From 1989 onwards the neuro ICU had seven beds until June 2002 when the number was increased to eleven beds.

Intervention measurements

Very little data is available before 1997 except for tracheostomy procedures. Data which describes general approaches to patient management rather than individual cases is available from the pharmacy services in the form of drug comsumption.
From September 1997 data was prospectively collected regarding injury severity (GCS, pupil reactivity, the presence of multiple injuries, hipoxia and hypotension in the early phases, the CT classification according to Marshall [15], type of intervention levels). From June 2002, data on outcome measured using the Glasgow Outcome Scale (GOS)[16] has been available by means of either telephonic interview, postal questionnaire or surgery examination. This allowed for analysis of data collected from June 2002 to December 2004 regarding all patients admitted to our hospital with the exception of patients with bilateral dilated un-

reactive pupils on arrival.
Data from GOSE was put into three categories: poor, which included death and persistent vegetative state and severe disability; moderate disability; and good recovery.

Results

Comparison between two historical series

From 1990 to the present day, March 2006, 1601 patients with moderate or severe TBI have been admitted to our unit. Of these, 1112 were classified as being severe TBI. In Table 1 we show the best available data to allow for comparison between the two historical phases.
In the "basic" neuroICU readmission was more prominent and the lenght of stay (LOS) was shorter. A decline in mortality has been observed in the advance neuroICU epoch. Admission to the basic neuroICU was associated with a higher probability of death in ICU (OR 1.45, CI 95% 1.15-1.819). When the probability of delayd death was considered, the OR was more elevated (OR 2.92, CI 95% 2.1-4.21). This trend may be better appreciate when the data is specific to each year (fig 1). In each of the two periods in the initial years the mortality was higher than in the following years but in the following years mortality declines from 14.9% to 5.5%. The probability of delayed death in the plateau phase was OR 2.9 (CI 95% 1.79-4.986) consistent with an absolute relative risk reduction (ARR) of 9.36% and relative relative risk reduction (RRR) of 169.3% (the number needed to treat, NNT, was consistent with 11 patients). A lower pobability of early death has been associated with the basic neuro ICU era (OR 0.79, CI 95% 0.59-1.048).

Results from advanced ICU management

Results of the management of severely head injured patients in June 2002-December 2004 are reported in table 2, in which 162 consecutively admitted are analyzed out of which 34 were excluded because they had bilaterally dilated unreacive pupils on arrival. The GOS was missing in 17.3% of patients. Considering the bias, a good outcome was observed in 47.7%.
Case mix has been associated with an elevated proportion of patients with masses (54.9%) and swelling (16%) as well as pupil abnormalities 59.9%. Also Hypoxia (42.6%) and hypotension (24.1%) were relevant. In 54.3% of patients, multiple injuries were present, which were sufficient alone to justify their admission to ICU.
A higher proportion of patients (62.1%) were treated with the highest

		basic neuroICU	advanced neuroICU	
		1990-aug1997	Sept 1997-2005	
patient admitted with moderate or severe TBI		704	897	
age	years, n (%)	42.4 (23)	41.9 (22.2)	p=0.4853
male	n (%)	523 (74.3%)	680 (75.8%)	p=0.4853
readmission	n (%)	27 (3.84%)	5 (0.56%)	p≤0.0001
LOS	median (IQR)	5 (10)	8 (14)	0.0156
severe TBI	n (%)	496 (70.5%)	616 (68.7)	p=0.4425
dead in ICU	n (%)	197 (28%)	190 (21.2%)	p=0.0016
dead within 48 hrs post injury	n (%)	88 (12.5%)	138 (15.4)	p≤0.0001
dead after 48 hrs post injury	n (%)	109 (15.5%)	52 (5.8%)	
tracheostomy	n (%)	86 (12.2%)	445 (49.6%)	p≤0.0001
tracheostomy when LOS ≥3 days	n (%)	84 (18.8%)	438 (76.8%)	p≤0.0001

Table 1 – *Comparison between the two ICU phases.*

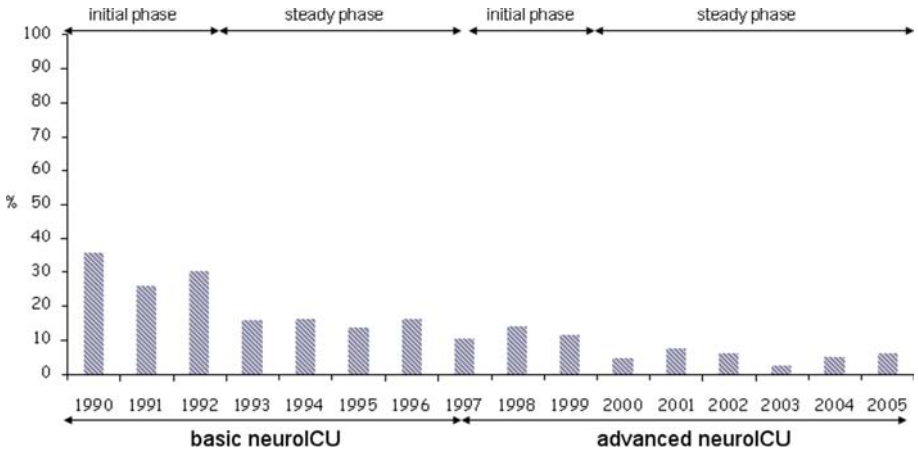

Figure 1 – *The time corse of ICU mortality evaluated after 48 post injury per year for patients with severe TBI. A progressive decline of mortality may be appreciated. The first reduction may be observed after the three initial years (2000-2003) of the basic neuroICU, the second one after the two initial years (1998-1999) of the advanced neuroICU. It may be possible to hypotize that, in both the neuroICUs, initially the mortality was higher because specialistic knowledges and organization has not fully developed. At this phase the "steady state" mortality has been stabilized and in advanced neuroICU (years 2000-2005, 5.5%) the mortality was reduced to one third in respect to basic neuro ICU (years 1994-1997, 14.9%).*

levels of the stair case protocol (extreme and reinforced) allowing meanvalues of ICP below 20 mmHg (18.8 mmHg, SD 11.1) and a mean CPPof 66.9 mmHg.

Discussion

The current data suggest two findings: 1st) an association between a marked reduction in ICU mortality after two-day survival in TBI patients after the introduction of a specilist team of neurointensivist and 2nd) fairly good results in outcome in a case mix of patients usually characterized by poorer prognosis.

variables				missing
Patients			162	
Age	years	Mean (SD)	43.1 (22.4)	
male		n (%)	130 (80.3%)	
LOS		Median (IQR)	12 (12)	
Dead in ICU		n (%)	22 (13.6%)	
Dead after 48 hrs post injury		n (%)	9 (5.5%)	
GOS	poor	n (%)	49 (34.5%)	20 (12.3%)
	severe disability	n (%)	27 (19.0%)	
	good	n (%)	66 (46.5%)	
Type of admission	direct	n (%)	79 (49.1%)	
	secondary	n (%)	71 (44.1%)	
	deferred	n (%)	11 (6.8%)	
ISS		Mean (SD)	28.0 (10.7)	16 (9.9%)
hypotension		n (%)	39 (24.5%)	3 (1.9%)
hypoxia		n (%)	69 (43.4%)	3 (1.9%)
worst CT	D I	n (%)	9 (5.8%)	8 (4.9%)
	D II	n (%)	30 (19.5%)	
	D III	n (%)	19 (12.4%)	
	D IV	n (%)	7 (4.5%)	
	EML	n (%)	71 (46.1%)	
	NEML	n (%)	18 (11.7%)	
worst pupils	normal	n (%)	64 (39.75%)	1 (0.62 %)
	unilaterally dilated unreactive	n (%)	93 (57.75%)	
	dilated unreactive	n (%)	4 (2.5%)	
Best GCS		Median (IQR)	7 (5)	1 (0.62 %)
Worst GCS		Median (IQR)	4 (3)	1 (0.62 %)
Best mGCS		Median (IQR)	4 (2)	1 (0.62 %)
Worst mGCS		Median (IQR)	2 (3)	1 (0.62 %)
ICP monitoring		n (%)	121 (74.7%)	1 (0.62 %)
Mean ICP, the mean	mmHg	Mean (SD)	18.8 (11.1)	65 (53.7%)
Max ICP, the mean	mmHg	Mean (SD)	26.7 (14.2)	65 (53.7%)
Mean CPP, the mean	mmHg	Mean (SD)	66.9 (11.3)	65 (53.7%)
Min CPP, the mean	mmHg	Mean (SD)	54.7 (11.7)	65 (53.7%)
TIL	none	n (%)	12 (8.2%)	14 (8.6%)
	basic	n (%)	44 (29.7%)	
	advanced	n (%)	52 (35.1%)	
	extreme	n (%)	40 (27.0%)	

Table 2 – Patient consecutively admitted to our ICU with severe head injury from june 2002 to december 2004, during the phase of "specialized" neuro ICU phase. Patients presenting with bilateral dilated unreactive pupils has been excluded.

Decline of TBI mortality

The comparison between the historical phases of the ICU has to be considered with caution because the case mix, in terms of severity of patients' condition is not fully defined for either of the two observation periods. Furthermore, multiple evidence suggests that not only neurocritical care but also overall medical management of the ICU has improved over the years.

The long period of observation (15 years) also means that the results are potentially affected by improvements in pre-hospital care as well as in the organisation of the trauma system.

The lower probability of early death observed in the basic neuro ICU phase is a result that needs to be carefully evaluated. One possible explanation, though this cannot be proved, could be the admission of less severe patients and/or a higher frequency of pre-hospital death in the basic neuro ICU phase.

The lower probability of early death observed in the basic neuroICU phase is a result that needs to be carefully evaluated. One possible explanation, though this cannot be proved, could be the admission of less severe patients and/or a higher frequency of pre-hospital death in the basic neuroICU phase. Over time, advanced life support has improved and become more widely available and, from 2002 onwards, helicopter transport of patients to specialist centres has been more common. Even more relevant may be the fact that, in the early phase of the basic neuroICU, in peripheral hospitals the most severe patients might have been considered unsaveable and consequently not centralized. Albeit not part of the written protocol of the basic neuroICU, it was common practice to refuse admission to the neuroICU for the secondary admission or deferred referral patients from outlying hospitals if they presented with bilaterally fixed midriasis on arrival. They were sent back to their original hospital instead. In fact until 1995 donor management was not developed in basic neuroICU. These multiple reasons suggest that early mortality is not a reliable outcome measure in this data setting.

To evaluate mortality after the initial two days, only LOS and frequency of tracheostomy may be compared. The short LOS in the basic neuroICU seems to reveal a tendency to plan for a short observation period as well as limited management objectives, especially in operated patients. Less widespread use of ICP monitoring, even though not documented, is a further factor explaining early discharge of patients to peripheral hospitals. This practice may potentially introduce a further bias in data collection. In fact, the data regarding mortality collected here refers to that of the neuroICU and not that of the hospital or the latest ICU. Consequently, since LOS in the basic neuroICU phase is shorter, more deaths may occur in later ICUs and therefore go undetected.

The spread of tracheostomy is the only change in treatment which enables us to compare the two ICU phases. The lower ratio of tracheostomy over the years on the part of the neuroICU may probably be due to shorter LOS as well as to less profound sedation, and also becouse regular wards are unable to accept and correctly care patients with tracheostomy. The less specialized treatment plan for neurological patients may also be of relevance. The limited use of percutaneous tracheostomy in the early 1990s might be a further reason. It is now clear that tracheostomy is a safe strategical procedure for neurocritical patients allowing a safe patency of airways throughout the prolonged phase of their recovery and may help early rehabilitation. More recently, early tracheostomy has been also supported as a preventive treatment useful for reducing the rate and frequency of pulmunary infections.

Review of literature

Several fields od research aim to assess whether professional, ndicated, specilist treatment can improve mortality rates and outcomes for severely TBI patients. Paper available in the literature were selected for review on the basis of the following criteria: a) comparative analysis of outcome in neurosurgical centres versus patients not admitted to neurosurgical centers, (paper also dealing with non-TBI patients were also included) and studies analyzing systematic data bases which observe the effects of trauma centers on trauma mortalityor evaluate the effect of neurosurgical hospital admission on the outcome of TBI patients. b) comparative analysis of outcome in centers with aggressive versus non-aggressive monitoring and centers in ICU, c) single center study showing an improvement in outcome, d) comparative analysis of changes in outcome in an ICU following the introduction of specilized neurointensivist consultant or team (paper dealing with non-TBI patients were also included).

Regarding type a) studies, data from Nascot[10] suggests that admission to trauma center level I is associated with improvement in mortality rate especially in more severe patients (those with elevated AIS). Unfortunately, the study does not address the question of whether patients with severe head injury benefit from specialized trauma care. Conversely, the study from TARN data base[11] suggests that patients with TBI who are admitted to a neurosurgical hospital have a lower risk of mortality. The results suggest that limitation in centralization is one of the possible explanation for the lack of further improvements in prognosis for patients in UK. Studies of type b), analyzing intervention and monitoring from multiple center forming part of a trauma center network[17], suggest that centres in which aggressive treatment is applied have lower mortality rates. Recently, however, a study from the Netherlands[18] compared two ICUs admitting a similar case mix of patients, but applying different monitoring and treatment policies. In one ICU there was monitoring of ICP and CPP and a strict protocol in place to control the values within well-defined thresholds, in the order , "non-aggressive" management was applied. The study showed no difference in mortality and outcome. Type c) studies[4] involve the association in a single center between aggressive management of ICP and highly desiderable percentage of good outcomes. This kind of study suggest that

specialist management contribute to a continuous improvement in outcome in comparison to historical series[6,7].
The type d) studies[1,2,3] support the idea that the introduction of new, more updated or aggressive protocol for ICP and CPP treatment in the same ICU or the introduction of a single neurointensivist or a team of specialist in neurocritical care could be associated with an improvement in lengh of stay or even in mortality, or with a reduction in disabilities. Many limitations affect this kind of study. Firstly the studies are frequently retrospective, tough sometimes data is collected prospectively in general ICU data base. Secondly, it seems difficult to detect and measures even relevant changes in organization and general care which help to improve the quality of treatment. For example, changes in pre-hospital management may be underestimated as well as improvements in rehabilitation. Sometimes the case mix of the patients may be not perfectly comparable, and for example the admission of less severe cases might potentially be a cofactor in the improved results[2]. Finally, it seems reasonable to assume that only changes in ICU management policy or new protocols which had a positive effect on outcome were submitted for pubblication thus introducing a substantial bias. Notwithstanding these major limitation, the results suggest that the role of the neuro-specialist is relevant in term of patients improvement. Perhaps the strength of this kind of study is that they assess the impact of the protocol on everyday intensive care practice as opposed to an artificial and rogorously controlled research setting. Similar findings have also been found when similar changes are applied to a broad range of categories of neurosurgical patients[19,20,21].

Outcome data from advanced ICU management

Data from a single center is difficult to compare due to differences ein case mix. The case mix showed in the present setting of the advanced neuroICU management suggest that within the TBI patients the case mix admitted is particulary severe. In fact the patients are affected predominantely by focal lesion presenting with signs of impending herniation. Most patients are treated with high levels of specialist medical and surgical treatment and, in the perception of the autor, this is the reason why ICP and CPP levels generally seem to be controlled.
The outcome may be considered fairly good, at least compared to older series[15] in which focal lesion were predominant. Stocchetti and

collegues[4] showed slightly better results potentially associated with some differences in patients mix and patients not being admitted consecutively but selected on the basis of availability of continuous digital data recording.
Contrary to results suggesting that specialist neuroICU may improve outcomes, a recent study[18] questioned the relevance of such specialist management by suggesting that ICP and CPP monitoring does not improve outcome. The study showed a similar ratio of good recovery (46.4%) in the specialist center in which ICP was measured compared to a center in which ICP was not measured (50%). Except for the potentially simplicistic explanation that ICP monitoring is not strictly necessary, a more reasonable interpretation of the result may be that in some cases ICP management, and more probably high CPP directed protocols, which work too hard to maintain ICP and CPP within predefinied limits, may be associated with undue medical complication that can reduce the potential benefits of the monitoring.

Conclusion

Converging evidence suggest that neuro-intensive care units and neurocritical care specialists seem to improve the quality of treatment of TBI patients. Given that TBI is a heterogeneus disease, further work need to be planned so that indication and treatment are specifically monitored.

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IS MORTALITY FOR SEVERE HEAD INJURY REALLY DECREASING?

Hiren C. PATEL, Omar BOUAMRA, Fiona E. LECKY
on behalf of the Trauma Audit and Research Network

Keywords: Severe Head Injury, mortality trends, TARN.

Introduction

Over 1.000.000 people per year are assessed following a head injury in the United Kingdom. Of these, approximately 250,000 patients are hospitalised, 7500 receive neurosurgical care, and 4000 die[1,2].Headnjury deaths account for 1% of all deaths, and 20% of all deaths in those under the age of 40 years making it the foremost cause of death in this age group[1,4] . Morbidity is also high following head injury, with an estimated prevalence rate of 100 per 100,000 reported[1,2]. Whilst there has been a significant decline in injury related deaths in England and Wales over the last 3 decades, with increasing reliance and global expansion of motor vehicles, trauma deaths and therefore deaths from head injury are predicted to become the second commonest cause of disability adjusted years of life lost by 2020[5].

The reduction in mortality observed from epidemiological surveys has been attributed primarily due to preventative measures (improvements in road engineering, increased car safety, use of seatbelts and helmets, alcohol control measures etc)[6,7]. Improvements in post trauma care have also led to improved morbidity and mortality. Unfortunately no single pharmacological agent has been shown to significantly reduce this disease burden despite over 250 randomised trials and improvements in head injury outcome appear to be limited to specialist centers[6,8-10]. In 1995, following recognition that head injury care was variable, guidelines for the management of severe head injury

Hiren C. Patel, PhD
Department of Neurosurgery, Hope hospital, Salford
Omar Bouamra PhD
Trauma Audit and Research Network, University of Manchester, Hope Hospital, Salford M6 8HD, UK
Fiona E. Lecky, PhD
Trauma Audit and Research Network, University of Manchester, Hope Hospital, Salford

(SHI) based on evidence or 'best' practice were published and disseminated by both European and American brain trauma consortia/foundations[11,12]. These clinical pathways, aimed at standardising care, have improved outcomes in those institutions that have embraced and instituted them[9,13,14]. However, whether there has been a wide-ranging impact on mortality is not known.
Our collaboration, which represents the largest European trauma database, has previously demonstrated that the improvements in post trauma care have led to a 40% reduction in mortality[15,16]. This has been attributed to increasing involvement of senior medical staff and better integration of acute services. We have also observed that head injury accounts for only 13% of all trauma patients but has a 10-fold increased mortality when compared to trauma patients without head injury[17].Using the approaches used previously, the aim of this study was

a) to determine the impact of SHI on trauma deaths in England and Wales;
b) to observe the temporal trend of mortality following SHI since 1989.

Method

The TARN (Trauma Audit and Research Network) database records the data of patients injured by blunt trauma within England and Wales. The records of all patients presenting between 1989 and 2004 and treated by participating hospitals were studied. TARN includes patients of any age who sustain injury resulting in immediate admission to hospital for three days or longer. Glasgow coma scale, blood pressure and respiratory rate are recorded when the patient enters the Emergency Department in order to calculate the revised trauma score (RTS-measure of physiological derangement)[18].Every injury is recorded and defined according to the abbreviated injury scale dictionary (AIS)[19]. This is used by trained coders to enable calculation of the injury severity score (ISS)[19,20] . Each hospital tranfer leads to the generation of a separate record, which is attached to the records from the initial presentation. The patient's age (but no patient identifier) is also recorded and outcome in terms of survival or death is based on assessment at discharge or 93 days, whichever is first. Patients over 65 years with isolated fracture of the femoral neck or pubic ramus and those with single uncomplicated limb injuries are prospectively excluded. Patients submitted to TARN but transferred to non participating hospitals were subsequently excluded from this analysis.

The temporal course (from 1989-2004) of the crude mortality and adjusted odds of death were studied for patients with and without SHI. For this purpose patients presenting with a GCS < 9 were defined as having a SHI. All other patients were considered not to have sustained a SHI.

Statistical Analyses

A logistic regression model based on the new TARN outcome prediction model was used to calculate the 95% confidence intervals for the odds of death in each year (1990-2004) compared with the 1989 baseline for a) all patients, b) all SHI patients and c) all patient without a SHI. The odds of death were adjusted for variations in ISS, GCS, age and gender. These factors were entered as independent variables in the model. ISS was entered as continuous variables, age (7 bands; <16 years, 16-44, 45-54 years, 55-64 years, 65-74 years, 75-84 years and >84 years) and GCS (5 bands; 3, 4-5, 6-8, 9-12, 13-15) as categorical variables. Linear regression was used to seek a yearly trend in the log odds of death for each group.

Results

The database had one hundred and eighty three thousand three hundred and fifteen patient records by the end of 2004. These represented the contribution of 110 hospitals, and represented 50% of all trauma receiving hospitals in England and Wales. From the database, 173,383 (87.5%) had no head injury, 12,549 (6.3%) had a mild or moderate head injury (AIS head >2 + GCS 9-15) and 12,273 (6.2%) had a SHI.

Patients with a SHI were younger, had a higher ISS and were more likely to have an acute physiological disturbance (hypoxia, hypotension) at presentation. Forty percent of patients with a SHI were transferred to a neurosurgical centre for further care.

Figure 1 illustrates the trend in case mix adjusted odds of death for all trauma deaths. The crude mortality for all trauma patients over the whole study period was 5.7%. There was a significant reduction in the adjusted odds of death (4% reduction per year p=0.0004) from 1989 –2004. The overall crude mortality for patients with SHI was 44.7%. Whilst SHI patients contributed to only 6.2% of all patients, SHI patients accounted for 47% of all trauma deaths with patients with SHI having a 2-fold increased adjusted odds of death compared to those presenting with a GCS>9. Temporally, a significant reduction in adjusted odds of death was observed for SHI patients although the gains observed were less than for those without a SHI (3.3% reduction

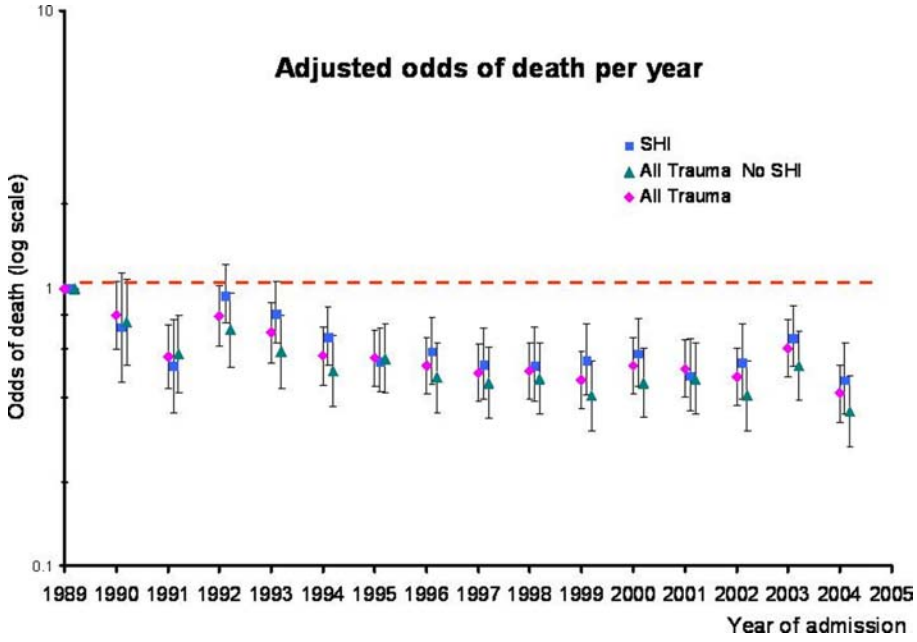

Figure 1.

per year p=0.005; see below) from 1989-2004 (Figure 1). No significant gains in the odds of death were observed over the last decade.
Figure 1 also illustrates the same analysis applied to all patients that did not have a SHI. In this group, overall mortality was 3.4%, and by 2004 the case mix adjusted odds of death was 64% lower than that of 1989 (OR=0.36, 95%CI 0.27–0.48). The trend analysis from 1989-2004 suggested a significant reduction of 4 % per year in the odds of deaths (p<0.001). An 8 % reduction by year occurred between 1989-1994 (p=0.02), whilst the trend since (1994-2004) was not been significant p=0.08.

Discussion

The presence of a SHI was associated with a thirteen-fold increased mortality when compared to patients without a SHI, demonstrating the dominant position of head injury in trauma deaths. Improvements in hospital care introduced since 1989 have significantly reduced the adjusted odds of death following SHI. However, the gains observed between 1989-1994 have plateaued over the last decade.
This study is not a population-based study, but it has the advantage of being based on a large sample of patients. Further, the trends ob-

served are from data supplied by approximately 50% of trauma receiving hospitals in England and Wales, and therefore we feel that this is a good representation of the national trend. Despite this or indeed because of the wide coverage and volume of patient data submitted to TARN, data quality and completeness may influence the trends we have observed. Each hospital that submits data to TARN is responsible for data acquisition and submission. TARN regularly runs consistency checks, and runs training courses to ensure coder reliability. It remains impossible however to ensure that all eligible cases are submitted, particularly those that die. Because each hospital pays for the analysis provided by TARN we assume that accurate collection of data is in each centre's self interest. Twenty hospitals have provided complete data since the inception of TARN, and trends from these hospitals mirrors those observed overall suggesting that data collection and submission is consistent. Analysis of the number of annual cases expected per hospital according to A&E admissions and those submitted to TARN per hospital has also suggested that the majority of patients are appropriately submitted and coded.

The difficulties of comparing differing epidemiological studies due to the differing case definitions, variability in study periods, differing end points and changing patterns of care, have led to calls for collaborative studies and increased standardisation of traumatic brain injury epidemiological studies[21] .Because of the problems stated above, it is difficult to identify comparable data studying mortality following SHI over time. National surveys in particular are limited because they cannot determine whether changes in mortality are due to changes in medical care or are reflective of successful preventative public health initiatives.

Epidemiological studies from national surveys from Europe and America have however demonstrated a reduction in traumatic brain injury (TBI) related mortality[6,22,23]. The TBI related mortality from the USA has declined by 11.4% from 1989 to 1998, and continues a trend of reducing traumatic brain injury related mortality observed from the 1970's. A recent report from Denmark has also demonstrated a reduction (27% between 1986 to 1996) for TBI death. These reductions in overall mortality from population based studies have mirrored our observations of in-hospital case fatality. The most striking improvement in mortality from both these studies was observed in younger patients. The data also suggested that this reduction was mainly due to a reduced motor vehicle accident related TBI, although a significant

firearm related reduction was observed from the USA. These improvements have been attributed to general injury preventative initiatives rather than due to improved hospital care because in the Danish study, there was no temporal change in the hospital case fatality rate, and in the USA, the decline in TBI related deaths paralleled the decline in age adjusted injury related mortality of all types that occurred during the same period. Importantly these studies also reported that fall-related TBI deaths observed primarily in the elderly were increasing, and that the elderly increasingly contributed to the proportion of TBI deaths. SHI in the elderly is associated with a poor outcome and their increased representation in the TBI population may explain why hospital case fatality rates for TBI have not been observed[6,22,23] (Table 1).

AUTHOR	STUDY	YEAR	N. OF PATIENTS	MORTALITÀ
Clifton et al	Hypothermia	2001	178	27%
Marshall et al	Trilizad	1998	479	26%
Morris et al	Selfotel	1998	331	21%
Marshall et al	TCDB	1990	746	36%
Murray et al	4 centres	1999	976	39%
Jennett et al	Scottish	1979	700	51%
Jennett et al	ICDB	1977	2959	49%

Table 1

Another approach to determining whether mortality following SHI has reduced is to look at whether the mortality published in either the placebo arms of randomised controlled SHI trials, or reports on outcome from prospectively collected data has changed over the years. A review of these studies (table 1) suggests that overall mortality has reduced since the report of Jennett et al who reported a mortality of 51% and supports our observation that in-hospital care has resulted in a reduction of mortality over time. The list of studies tabulated is by no means exhaustive, and these have been chosen because they best represent the outcomes observed from the 1980's and the 1990's. Further, these studies are limited by the fact that they report on outcome in a selected group of patients and should be viewed as a group to observe a trend rather as individual comparable reports[24,30].
The crude mortality in our series is significantly higher than those reported from either the placebo arm of phase 3 trials or those reported

from most of the other contemporary data from the 1990's[24,28,29]. The mortality reported in this study is from an unselected population, and included 60% of patients that have not been transferred for treatment in specialist neurosurgical centres. Further, the database does not record pupillary responses, and therefore a certain proportion of patients will be those who sustained SHI deemed incompatible with life.
The initial improvements in mortality following SHI observed between 1989-1994 have been reported for trauma overall and are the result of the implementation of recommendations from the Royal Colleges, increased involvement of senior medical staff and better integration of trauma services[7]. The reason for the plateau over the last decade however is unclear and is likely to be multifactorial. One argument may be that the plateau represents the limit of medical care in relation to the severity of injury sustained. This may be true for blunt trauma patients without a head injury in whom the mortality is low at 3.4%. However, given the difference in crude mortality observed, our series compares poorly to the mortality reported from the best centres in the world, suggesting that there is room for further improvement.
Guidelines aimed at reducing the variability of care and use of therapies shown not to be beneficial in treating patients with SHI have reduced mortality in centres that have instituted them[9,13,14]. As illustrated by the EBIC observational study, there is significant variability in care across Europe, with a poor overall uptake of the guidelines. In England and Wales only 48% of patients presenting to a non-neurosurgical centre with a SHI receive neurosurgical/neurointensive care. Those patients that are not treated in a specialist setting have a >2 fold increased probability of death[17]. These patients are treated on intensive care units, but do not have intracranial/cerebral perfusion targeted therapy, and therefore are less likely to be treated along protocols developed from guidelines issued on the management of SHI. We have estimated that, assuming the mortality was reduced from 61% to 35% (as observed in patients treated in neurosurgical units), 2,000 lives would have been saved over the 8 years of the study period (250 per year, average age 28-34).
Finally, the rising incidence of head injury in the elderly is increasing and represents yet another challenge with regard to reducing mortality following SHI. Age is well accepted as being a negative prognostic predictor of outcome, and reports have suggested a conservative treatment strategy for SHI in the elderly given the low incidence of functional survival in this cohort[32-35]. The elderly may therefore be where

medical intervention may be limited. With increasing contribution to head injury series, the plateau in mortality observed may be due to increased numbers of elderly patients with a SHI. Approximately 16% of SHI patients on the database were over the age of 65 with an average crude mortality of 78%.

The data presented here suggests that mortality following SHI in England and Wales has improved with time since 1989. The improvements have mirrored national population based results, and those presented in table 1. The improvement in our series has plateaued since 1994, and we would argue based on previous observations that neurosurgical/ neurointensive care intervention appears pivotal to improving care for SHI patients, and represents the best strategy for improving case fatality in hospitalised blunt trauma patients

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TRAUMA CARE RESEARCH AND THE WAR ON CHANCE

Ian ROBERTS

Trauma is a major global health issue

For people at ages 5 to 45 years, trauma is second only to HIV/AIDS as a cause of death.[1,2] Every day world-wide some 300,000 people are severely injured, about 10,000 of whom die. In those who survive to reach hospital, exsanguination accounts for nearly half of all deaths with central nervous system injury and multi-organ failure accounting for most of the remainder.[3] Road crashes are the leading cause of major trauma, followed by suicide, interpersonal violence and war. The global number of road deaths is forecast to rise by 65% between 2000 and 2020 and the number of conflict related deaths has increased steadily over the past five centuries, with the twentieth century being the most violent on record.[4] Despite the best preventive efforts, providing effective trauma care and rehabilitation will remain a major challenge for healthcare professionals.

What works in trauma care?

There is considerable potential to reduce death and disability after trauma by using clinical audit to improve the organisation of trauma services.[5] For some trauma care interventions the benefits so obviously exceed the risks that randomised controlled trials are unnecessary. No trial is needed to know that an obstructed airway must be cleared, although the best method of maintaining the airway is open to question. There are other trauma care interventions where the balance of risks and benefits is less obvious and have never been assessed reliably. One example is acute blood loss following trauma. This leads to a reduction in tissue perfusion and oxygen delivery that if prolonged causes lactic acidosis and organ failure. Treatment of

Ian Roberts
CRASH Trials Coordinating Centre
London School of Hygiene and Tropical Medicine
Keppel Street – London WC1E 7HT
Phone: 020 7958 8128 – Fax: 020 7299 4663 – ian.roberts@lshtm.ac.uk

shock involves maintaining blood pressure and tissue perfusion until bleeding is controlled. However, maintaining blood pressure may worsen bleeding. Raising the blood pressure may increase tissue perfusion and oxygenation but the increased pressure may impair the formation of new blood clots or dislodge existing clots.[6] Most patients with significant haemorrhage receive fluid resuscitation but there is little reliable evidence on the most appropriate resuscitation targets or on which fluids should be used. A systematic review of randomised trials of the timing and volume of fluid resuscitation in bleeding trauma patients concluded that due to the small number of available trials there is continuing uncertainty about the best fluid administration strategy.[7]

The number of clinical trials in trauma is small compared with the disease burden. A 2002 survey sought to evaluate whether the amount of clinical research on various medical conditions was related to the burden of disease and health needs of the local populations, in this case for sub-Saharan Africa.[8] A total of 1179 randomised controlled trials conducted in the past 50 years were identified. For a range of different disease categories, the authors calculated the burden of disease (1000 DALYS) per trial participant (Table 1). The correlation between

Disease category	Burden of disease in 2000 (1000 DALYs)	No of trials	No of participants	Ratio of burden of disease (1000 DALYs)	
				Per trial	Per participant
Malignant neoplasms	8 114	46	128 786	176	0.06
Nutritional deficiencies	8 389	105	111 922	80	0.07
Infectious diseases	131 327	540	813 305	243	0.16
Conditions arising during perinatal period	18 700	30	28 381	623	0.66
Cardiovascular diseases	13 390	99	5 648	135	2.37
Respiratory diseases	9 037	33	3 320	274	2.72
Congenital anomalies	5 224	2	1 321	2612	3.95
Neuropsychiatric conditions	15 788	41	3 580	385	4.41
Injuries	58 352	31	2 887	1882	20.21

Table 1 – *Disease burden and evidence from controlled trials for main categories of human disease in sub-Saharan Africa.*

randomised trial evidence and burden of disease was good for most disease categories but this did not hold for others. The worst correlation was for injuries. Researchers have also estimated the correlation between the number of systematic reviews and the global burden of disease.[9] Injury is again one of the most neglected.
The small size of clinical trials in trauma also contributes to the uncertainty about treatment effectiveness. For example, few if any of the pharmacological agents that are currently used in the treatment of brain and spinal cord injury have been shown to be effective in clinical trials. The available evidence from clinical trials is compatible with both modest benefits and modest harms.[10] A 2000 survey of the size and quality of randomised controlled trials in head injury found that the average number of participants per trial was 82 with no evidence of increasing size over time.[11] None of the existing trials were large enough to detect reliably a 5% absolute reduction in the risk of death or disability and only 4% were large enough to detect an absolute reduction of 10%. Randomised trials in head injury are in general too small to detect or refute reliably, realistically moderate but nevertheless important treatment effects.

The need for some large trials in trauma care

Systematic reviews and meta-analyses of available trials increase the precision of estimates of treatment effects. However, publication bias and the inclusion in meta-analyses of poor quality trials can lead to misleading conclusions with serious consequences. Corticosteroids have been used to treat head injury for over 30 years. In 1997, a systematic review suggested that corticosteroids reduce the absolute risk of death by 1% to 2% but the 95% confidence interval was from 6% lower mortality to 2% higher mortality. The CRASH trial was designed to reliably confirm or refute such effects by recruiting 20,000 patients.[12] After 10,000 patients had been recruited, the data monitoring committee disclosed the un-blinded results to the Steering Committee which then stopped recruitment. The effect of corticosteroid allocation was a highly significant 18% (95% confidence interval 9–27) increase in the risk of death from all causes within two weeks. It has been estimated that some 10,000 patients may have died because of the inappropriate administration of corticosteroids to treat head injury.[13]
To avoid modest random errors clinical trials in trauma care must recruit sufficient numbers of patients and this implies the need for large international collaborative trials. The CRASH trial was conducted in

250 hospitals in 48 countries. The overall result from this 48 country trial can be thought of as the pooled estimate from a meta-analysis of the effects of corticosteroids on death after clinically significant head injury in 48 identical trials conducted in each of the participating countries (Figure 1). In this meta-analysis most of the effect estimates are non-significant with only four of the 48 reaching $p<0.05$. Moreover, there is no significant heterogeneity in the treatment effects by country. This suggests that the results are compatible with the view that each country provides an estimate of the same treatment effect albeit strongly influenced by the play of chance. A criticism of multicentre trials is that there may be important differences in the standards of trauma care in the participating hospitals so that an overall estimate of treatment effect is worthless.[14,15] The data presented in Figure 1 do not support this interpretation. The effect of the trial treatment can be estimated in each country and this effect is independent of the national survival rate which might be influenced by trauma aetiology, clinical facilities and management strategies.

Do trial results change clinical practice?

Providing reliable estimates of the effects of treatments is necessary but not sufficient to improve patient care. The results of research will only improve patient care if they are disseminated and taken into account by practitioners. Pharmaceutical companies that stand to gain from trial results spend large amounts of money ensuring that results are disseminated but if there is no financial incentive the situation is different. However, international collaborative trials involving hundreds of doctors around the world have major advantages when it comes to dissemination of results. Whereas small trials might remain unpublished and contribute to publication bias, particularly if the results are 'negative', this is less likely with large trials.[16] Moreover, in international trials, collaborators around the world have a personal investment in the results and are empowered to disseminate them in their local and national contexts. Media releases filed by the national co-ordinators helped to ensure that the results of the CRASH trial were reported in national newspapers in Albania, Argentina, Australia, Canada, China, Cuba, Egypt, France, Germany, Ireland, India, Spain, Serbia, Uganda and the United Kingdom (see www.crash.lshtm.ac.uk for details). There is also evidence that hospitals participating in multi-centre trials are more likely to implement the trial results.[17] For these reasons, international multi-centre trials

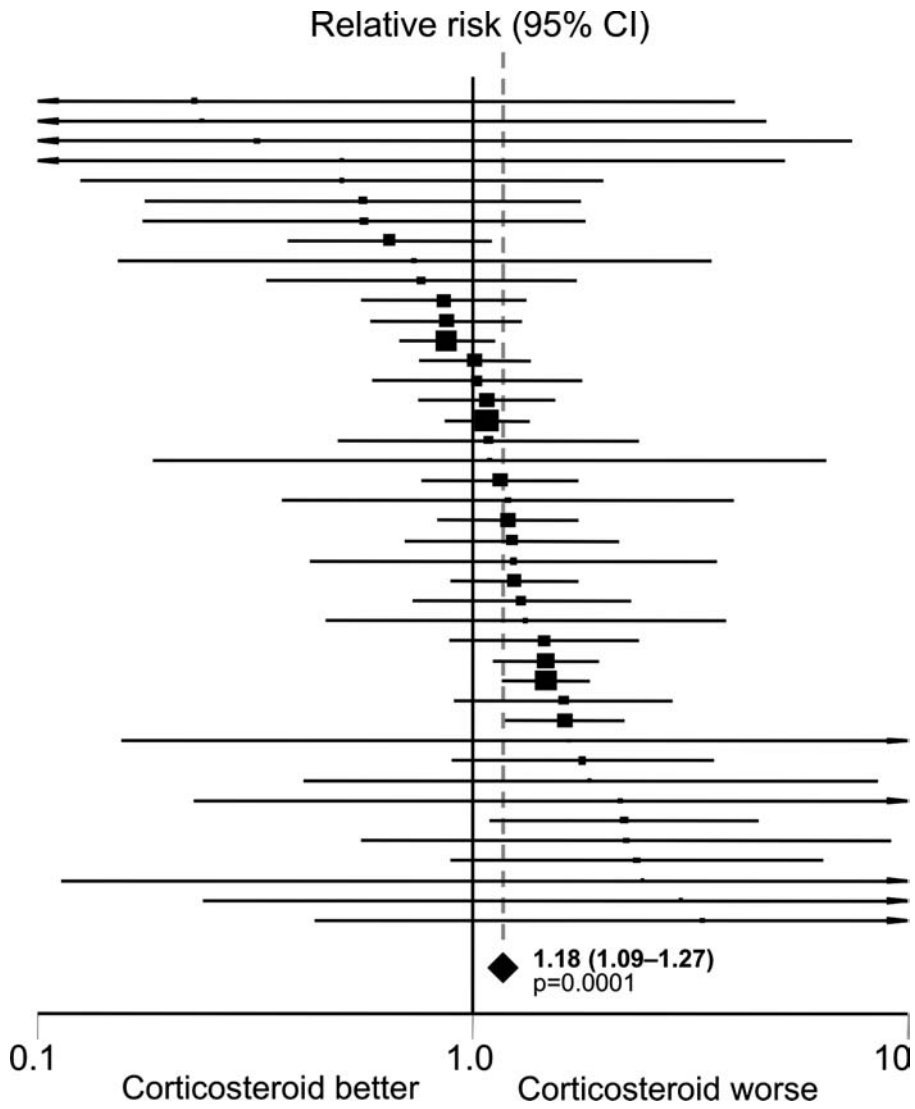

Figure 1 – *Country specific estimates of effect of corticosteroid allocation on deaths from all causes within 2 weeks (MRC CRASH trial).* [Heterogeneity chi-squared = 47.9 (41 d.f.) p=0.21]

Notes: *(a) For each country, relative risks are plotted (black squares with area proportional to the amount of statistical information contained) comparing outcome among participants allocated corticosteroids to that among those allocated placebo, with 95% confidence intervals (horizontal lines ending with an arrow head when the confidence interval extends beyond the scale); (b) The overall CRASH trial result is represented by a black diamond with relative risk (95% CI) given alongside; (c) In six countries the relative risks of death within 2 weeks were not estimable (there were no deaths observed); (d) the names of countries and country-specific data have been omitted in order to maintain anonymity.*

can be expected to have a substantial impact on clinical practice, with the evidence disseminated widely within and out with the trial network.

Barriers to large scale trials in trauma care

Money

Compared to the disease burden, funding for clinical trials in trauma is less than for any other cause of human suffering. Unlike most other major health problems, few large research charities specifically target trauma care. In 1996 a WHO expert committee estimated research funding for nine specific health topics using the capture-recapture method and compared the level of funding with both current and projected (2020) disease burden (Table 2).[18]Although the expert committee did not suggest that there was excessive funding for any of the health problems examined, their results clearly indicated that there was relative under-funding of research on road traffic crashes.

Marketing

Doctors in many countries would be pleased to collaborate in large international trials but as yet there are no well established ways for bringing such trials to their attention. Trial investigators will try to get editorials about their trials published and will present them at conferences, but some medical journals are reluctant to publish editorials 'advertising' the conduct of trials or else are reluctant to publish editorials when the authors have a stake in the trial they are writing about, which they necessarily do. Formal advertising of clinical trials has been shown to increase trial participation. This suggests that many more doctors would take part in large collaborative trials if only they knew about them (Figure 2).

Regulation

Conducting a clinical trial involves the successful negotiation of a number of regulatory, ethical and logistic hurdles. Most countries have trial regulations geared towards the development of new drugs and most use the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996) as the basis of their regulations. However, few have legislation in place for the special situation of clinical trials in trauma patients. These trials take place in the emergency situation and often involve patients who are unable to give informed consent. Many of these patients are unaccompanied by a person who can make decisions on their behalf. As few countries have exceptions to the requirement for obtaining in-

Topic	1990 spend/DALY (US$)	2020 spend/DALY (US$)
HIV/AIDS	85.2	26.2
Asthma	13.2	10.8
Blindness	10.1	5.4
Malaria	1.9	3.9
Acute respiratory infections	0.5	1.4
Tobacco use	4.3	1.3
Diarrhoeal diseases	0.3	0.9
Tuberculosis	0.7	0.6
Road traffic injuries	0.8	0.4

Table 2 – *Global R&D funding for selected topics.*

formed consent from each participant, or his or her legally authorised representative, prior to initiation of a trial intervention, these laws inappropriately preclude trauma patients from clinical trials.
International trials typically require that national ethical committee approval is obtained as well as the approval of the local ethics committees of participating hospitals. Servicing these committees has substantial resource implications and can involve long delays. The CRASH trial provided materials and personnel support for the completion of over 500 ethics application forms in 50 countries worldwide.[12] Although appropriate ethical oversight is of considerable importance the opportunity cost of servicing ethics committees is considerable. The drug regulatory process of each country can also be a barrier to conducting clinical trials. Even trials examining the efficacy of widely available treatments which already have a product licence are required to provide pre-clinical data and clinical data which might only be available to the manufacturer as part of the regulatory assessment. In countries where this is a legal requirement, trials that do not have the support of the relevant commercial company face considerable difficulty.

Rewards

The reward systems in universities and the authorship formats used by journals may be disincentives to trial collaboration. In some universities promotion is related to the number of first author publications, a model of publication which encourages competition rather than collaboration. Collaborative trials often cite the entire collaborative group rather than named individuals. Although many journals accept this model, problems still arise in indexing.[19] The International Com-

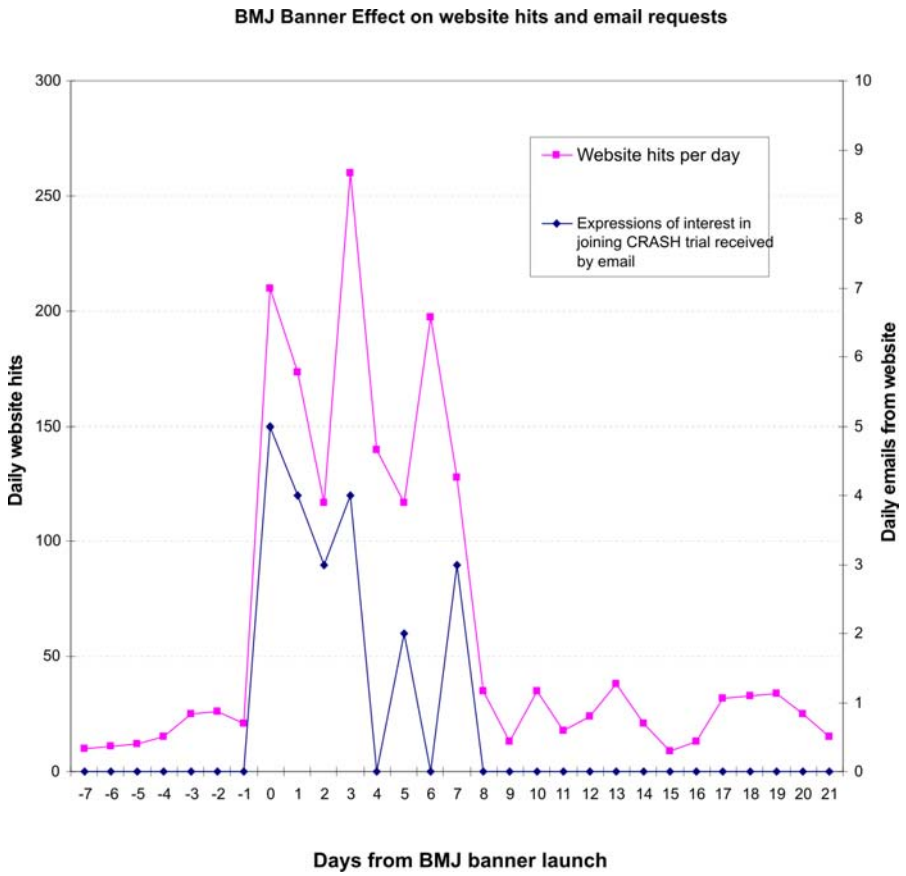

Figure 2 – *To increase participation in the CRASH trial, we purchased a 30% share of the banner advertisement on BMJ.com. For one week, 30% of visitors to BMJ.com were exposed to one of three advertisements: (1) Road traffic crashes = 6,000,000 head injuries worldwide. Join the international head injury trial now! (2) Steroids in head injury? Join the international head injury trial now! (3) Emergency physicians ... Neurosurgeons ... Anaesthetists ... Intensivists ... Join the international head injury trial now! The advert linked to the trial web-site which gave trial information in 10 languages and where doctors could join the trial by sending an e-mail to the trial office. The advert cost £4,550. The figure shows the number of trial web-site hits and requests to join the trial, before, during and after the advert. In the week before the advert there was an average of 17 hits per day but no requests to join the trial. During the week of the advertisement there was an average of 168 hits per day and 21 requests to join. In the week following the advertisement, there was an average of 25 web-site hits per day and no requests to join.*

mittee of Medical Journal Editors (ICMJE) "Uniform Requirements" read in part: *"When a large, multi-centre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript ... These individuals should fully meet the criteria for authorship defined above and editors will ask these individuals to*

complete journal-specific author and conflict of interest disclosure forms. Journals will generally list other members of the group in the acknowledgements." The National Library of Medicine indexes as authors those individuals that the group has identified as being responsible for writing the manuscript. However, as a result of improved methods of trial reporting, in particular the CONSORT statement, trial reporting has become increasingly algorithmic and there is little scope for creativity. The main responsibility therefore, is not for the writing, but for the integrity of the trial data which depends on the entire collaborative group (most trials will use a variety of methods to ensure the validity of the data). Citing as authors only a writing group is not a suitable method of apportioning either responsibility or credit. The existing reward systems favours the conduct of small trials with named authors even though this increases the risk of making inappropriate inferences due to the play of chance and publication bias.[16]

Summary

Trauma is a major global health issue and there is an urgent need to improve the evidence base for the medical care of trauma victims. Many if not most treatments for trauma are of unproven effectiveness. Large scale clinical trials can provide important information in this respect but in order to wage war on the play of chance we need large collaborations of equals rather than self-interested individuals. Doctors from all around the world can join such 'collaborations of the willing' but in order to conduct large international trials there is a need to find better ways to bring trials to the attention of doctors world-wide, to reduce the regulatory burden and to more appropriately reward collaboration. Most importantly, the mismatch between the level of funding for trauma research and the public health burden from injuries must be addressed.

Conflicts of interest

The authors have no conflicts of interest in relation to this paper.

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ATTENTI ALLE BUFALE (BEWARE OF RED HERRINGS) OR HOW TO MAKE EVIDENCE-BASED MEDICINE WORK FOR YOU

Tom JEFFERSON

Introduction

During the year busy doctors and healthcare workers have little time for reading or reflecting. This fact conflicts with the need to keep up to date with what is going on in medicine and to track the avalanche of paper, sound and electronic inputs we receive. The obvious starting point is a topic of interest, but we also need to select something which has some kind of credibility, either scientific or ethical or both (although some tell me there is no difference between the two)[1,2]. So we now have two linked problems: quantity and quality of the scientific works that we read.

The quality issue seems to be an easy one to solve: if something is in print it has undergone "routine quality control measures" such as peer review and should be good enough, especially if published on the more prestigious journals. And if something has passed peer review then the quantity issue can be solved quickly by reliance on reviews, meta-analyses, editorials, evidence-based journals and the good old network of friends and colleagues who "give you the wink" on a good piece of research.

Alas, not everything is so easy in life, research or medical practice[1,2,3,4,5,6]. Often what seems is not what is. I shall explain what I mean by taking the issues one at a time. Let me first start by quality control mechanisms as they apply to the publication of research papers and then move on to the issue of quantity. I need to start off with quality (speaking about editorial peer review, its main control mechanism) as it is the key to understanding the issue of quantity.

Tom Jefferson
Cochrane Vaccines Field and Cochrane Acute Respiratory Infections Group
00061 Anguillara Sabazia (Rome) Italy
www.attentiallebufale.it

Editorial peer review

The custom of sharing scientific production with one's peers or friends has a long and honourable tradition. In modern science it probably goes back to the age of reason[7,8,9,10]. I have in mind Boyle's physics experiments in Oxford, just after the end of War of the three kingdoms, the English civil war. The design and results of the experiments were shared with Boyle's peers and friends. In medical practice the use of one's "peers" is firmly rooted in the time-honoured custom of the bedside consultation in which the treating doctor, usually a generalist, called one or more eminent specialist colleagues to elicit opinions on a difficult case. The masterful Collodi gives us a precious insight into the dynamics of the consultation in his masterpiece "the Adventures of Pinocchio":

> *The Lovely Maiden with Azure Hair sends for the poor Marionette, puts him to bed, and calls three Doctors to tell her if Pinocchio is dead or alive*
> *One after another the doctors came, a Crow, and Owl, and a Talking Cricket.*
> *"I should like to know, gentlemen," said the Fairy, turning to the three doctors gathered about Pinocchio's bed, "I should like to know if this poor Marionette is dead or alive."*
> *At this invitation, the Crow stepped out and felt Pinocchio's pulse, his nose, his little toe. Then he solemnly pronounced the following words:*
> *"To my mind this Marionette is dead and gone; but if, by any evil chance, he were not, then that would be a sure sign that he is still alive!"*
> *"I am sorry," said the Owl, "to have to contradict the Crow, my famous friend and colleague. To my mind this Marionette is alive; but if, by any evil chance, he were not, then that would be a sure sign that he is wholly dead!"*
> *"And do you hold any opinion?" the Fairy asked the Talking Cricket.*
> *"I say that a wise doctor, when he does not know what he is talking about, should know enough to keep his mouth shut. However, that Marionette is not a stranger to me. I have known him a long time!"*

Now you may ask: what does all this have to do with peer review? If you imagine that the naughty Marionette Pinocchio is a manuscript

sent to the editor of the Journal of Very Important Results (the Fair Maiden) and the Crow, Owl and Talking Cricket are three external reviewers perhaps with Cricket playing the part of the statistician you have a fair representation of the essential workings of peer review[11]. Three experts and probably three different opinions which have to be mediated and summarised by the editor-fair maiden who has to make a decision on whether to publish Pinocchio on the Journal of Very Important Results or send it back to the author.

Now, this very gentle representation of what goes on is still valid today, as peer review has evolved little in the last 200 years. Electronic submissions and evidence-based medicine have not changed the essence of peer review and the Fair Maiden, the Crow, Owl and Talking Cricket are still working their way through thousands of Pinocchios[7]. But the world around them has changed and in the fiercely competitive research environment of today they are struggling and failing to do an effective job. As each one of us has a nefarious tale to tell about peer review and the number of retracted fabricated papers mounts, we all suspect that something is not quite right. When Frank Davidoff, Liz Wager, Phil Alderson and I published the results of our Cochrane review on the evidence of the effects of peer review on JAMA in 2002, there was a deafening silence[8,9]. The review showed that there was flimsy proof of the effectiveness of peer review and in a linked piece of work we also reported that its objectives were also unclear[8,9]. Updates of the reviews have failed to find evidence to change the conclusions.

In the BMJ Christmas issue of 2003 we published a board game called Get Peered! that parodied the situation showing the pitfalls of modern publishing from plagiarism to salami slicing (or redundant publication as it is more correctly known, one of the many ways of padding one's impact factor). The aim of the game is to become a peer or Lord as soon as possible by any means. Although a parody, the game had a serious message: publishing is a lottery[12].

Despite such offerings I still hold the view that the main problem is the mediocrity of what is published today and the extreme cases of plagiarism or data fabrication are still, thankfully, relatively few.

So we are left with a more complex problem of summarising a lot of research of variable quality. From a practical point of view of a busy clinician the problem becomes: who do I trust?

Quantity vs Quality

So how can we reconcile the contrasting requirements of selecting

Figure 1 – *The board of the game Get Peered*[12].

uptodate good, reliable knowledge from a huge offer and very limited reading time?

Six months ago I published a book called "Attenti alle Bufale" ("Beware of red herrings", in Italian)[11]. In italian slang bufale are red herrings but in real life bufale are female water buffaloes. Their milk is the basic ingredient of the famous mozzarella cheese (mozzarella di bufala). Apart from the obvious reasons for writing a book (very poor returns on my time and getting my name banned from every library in the Italian republic given the risqué humour of some of the jokes in the text) my motive was to publish a series of basic quick instruments based on my experience as an Evidende Based Medicine (EBM) warrior. These instruments in turn had two functions. First I wanted to help folk who realise that a very high percentage of what we read or hear is a bufala, to spot the bufale and chuck them where they belong, thereby limiting the intellectual damage. Second I wanted to try to make EBM relevant to busy healthcare workers who have little time. (www.attentiallebufale.it).

I called the chapter presenting the instruments "Bufala spotting" and structured it in the following manner. For each type of communication (lecture, editorial, research paper etc) I described a quick version of the instrument, designed to be applied in two minutes. Next I described a longer and more detailed version for folk who have more time (a lot more time in some cases). I then explained the rationale for my approach. Next to each section I placed a number of bufala heads to signify the danger of being taken for a bufala ride: four bufala heads mean extreme danger, one bufala head means low danger. You can see an example at the bottom of the paper.
Finally I issued some basic health warnings so that readers did not take the content as absolute gospel and approached the issue with what I think is the right mentality:

- everything and anything we read or hear in biomedical sciences has to be approached critically. This is the fastest and most reliable universal instrument. Trust but verify, as Ronald Reagan used to say. A critical mentality is developed and nurtured, it does not grow overnight.
- all quick instruments work, but you only have my word for it. True, I have read thousands of papers, reviews, editorials etc critically but it is still only one man's experience. I have no references to offer for the quick instruments, but some of the elaborate ones have been validated and are beginning to make an impact on the quality of published science.
- if you do not want to bother typing in all the URLs of the full instruments, visit www.attentiallebufale.it, where you have quick drill-through links to the various links.
- finally if you do not like what I am about to propose, develop your own instruments. If you do, try it. Either way, let us know what you think.

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Bufala spotting - from "Attenti alle Bufale"[11]

Assessing a guideline (danger of red herrings: three bufale)

Quick instrument

Beware of guidelines prepared by single scientific societies or groups. Bin guidelines with no methods chapter, conflict of interest statement and are not drafted by a multidisciplinary group.

Full instrument

AGREE (appraisal of guidelines research and evaluation) is a complex but bufala-unfriendly instrument (www.agreecollaboration.org/instrument/). It is made up of six specific domains reflecting key aspects of a guideline (scope and purpose, stakeholder involvement, rigour of development, clarity and presentation, applicability to real-world situations and independence of the editorial group that developed the guideline).

Rationale

Guidelines are a flourishing industry. Everyone writes them, but few will withstand the rigours of the quick instrument and even fewer of AGREE[13]. Clinical guidelines are supposed to apply to real world situations, so the idea that they can be written by members of a single discipline is ridiculous. Bear in mind that even the best guideline has a heavy qualitative component and manipulations are still possible.

INFORMATION AND AWARENESS-RAISING CAMPAIGNS, PROMOTION AND MARKETING OF ILLNESS

Roberto SATOLLI

Keywords: disease mongering, medicalisation, awareness-raising campaigns

Premise

A campaign to make people aware of the risks involved in neck artery obstructions and to encourage them to get themselves checked out to make sure they have no problems might be seen as a worthwhile preventative measure. To my knowledge a "carotid week" hasn't yet been organised but I wouldn't be surprised if it happened in the near future. In reality, it is questionable whether looking for obstructions in the carotids, using an ecograph for example, in people who have no symptoms, is of any benefit. In fact, there are grounds for wondering whether it might not be dangerous for the majority of people tested. The US Preventive Services Task Force is currently updating its recommendations, which date back to 1996 and no longer reflect the latest literature. It is also possible that they modify their view that "there is insufficient proof to determine whether screening in asymptomatic individuals is advisable or not" in favour of a more interventionist stance.

Regardless of the positive or negative effects that this type of campaign might have on people's health (which, as we said, in this specific case is still dubious and the subject of research), making a big fuss about one particular illness can spark off a flurry of health spending: check-ups, laboratory tests, ecographs, drugs, treatments, therapies, other medical care and/or operations as a result of complications, the use of medical premises and medical products etcetera. In other words, raising public awareness of a health problem could be seen as a powerful marketing tool for anyone involved in the produc-

Roberto Satolli
presidente di Zadig, agenzia di giornalismo scientifico
via Calzecchi 10 – 20133 Milano
027526131 – 0276113040 – satolli@zadig.it

tion or supply of goods or services in some way connected to that particular medical sector. This phenomenon, which in English is termed "disease mongering", is becoming the major way in which information reaches the public in the field of medicine.
Obviously not all health education campaigns are also (or solely) aimed at commercial gain. In the past there were genuine, spontaneous projects (and there still are, albeit very few) which reflected a desire on the part of doctors and patients to try and do something about this or that illness, so they informed people what the illness was like and what they could do to fight it. Unfortunately, marketing experts quickly realised the enormous advertising potential of initiatives like these, and they started to propose campaigns which were financed or even promoted by the health industry itself.

Health Industry

Health, as well as having intrinsic individual and collective value, also represents the raison d'etre for one of the most flourishing and profitable economic sectors in developed countries. Pharmaceutical companies constitute the real life-source of this system, companies which have developed and joined forces over the last few decades to become bigger than was even imaginable until a few years ago. They currently provide work for more than 520,000 people[1].
The number of producers of instruments and consumer goods has also grown: diagnostic machines, surgical equipment, chemical agents, disposable instruments etc etc. In terms of service providers, there are clinic and hospital chains, both public and private as well as diagnostic centres, laboratories, health centres and centres specialising in specific illnesses, rehabilitation centres, nursing care both at home or in centres, pharmacies etc etc.
Overall we are talking about a growing business, which here is simply termed "the health industry", which in a city like Milan alone, for example, in the year 2003, included at least 3,200 companies employing 54,000 people with an annual turnover of about 10,000 million Euros.
It appears as a heterogeneous mix, but it isn't. Within the system, single elements compete with each other on a horizontal level (product suppliers and similar services) but they also work in synergy on a vertical level (businesses which mutually complement and boost the other).
From this point of view the health industry behaves more like a closely-knit chain. Once one link starts working, it sets the surrounding links in motion too, often creating a kind of circular movement

(whether that be a vicious or virtuous circle depends on your point of view) which tends to be self-perpetuating and proliferating. All the players in the chain have interests in common; specialists who can increase patient numbers and thereby their income, reputation or power; administrators of diagnostic or treatment centres who get more patients through the doors therefore increase their turnover; producers of diagnostic machines and tests; suppliers of surgical materials or prostheses; and not least the pharmaceutical companies who are frequently responsible for powering the whole chain. In other words, the explicit and carefully-directed work which the single element carries out to promote its own particular products and services, is only the last stage of a whole marketing process which turns out to be very sophisticated and to have its origins much further back. This can be seen on a daily basis with campaigns where certain science communities of doctors or certain patient groups seem to join forces in a more or less spontaneous way. If we dig deep, hidden behind this front of a public-relations based exercise, we find the financial backing of large companies, especially pharmaceutical companies. And the aim is nearly always the same; to exaggerate the importance of this or that illness (how serious or widespread it is; the social and economic implications etc.) to increase patient numbers, multiply the number of treatments, fill up health centres and surgeries and boost business.
If this is the overall picture, it is worth dissecting and analysing the mechanism in detail so people are in a better position to defend themselves from the harmful effects it can have on their lives and health.

Language and messages

Athens, Greece, 19 September 2005 – Annual Assembly of European Federation of Neurological Societies "Restless leg syndrome (RLS) is an incredibly common but under-diagnosed pathology which has a negative effect on the lives of millions of people all over the world," explained Professor Dr. Wolfgang, MD, Oertel, Head of Neurology Department, Centre for Diseases of the Nervous System, Philipps University in Marburg, Germany.
RLS is one of most common neurological pathologies in the world but it is curable. It is estimated that the condition affects one in ten people aged between 30 and 79.
RLS sufferers often say that their illness has serious repercussions on their daily activity and life in general.

"The data presented today is very important because it offers further proof as to the beneficial effects of pramipexolo, not only for symptom relief but also for improving the quality of patients' lives."

The text in the box above is a press release which was circulated during a recent scientific conference and then taken up and used in various ways by the different media. It is a typical example and therefore serves well for our analysis to try and understand the language used in illness awareness-raising campaigns and how the advertising message is normally delivered.

Source and context

The source appears to have great authority: an important scientific community's conference. In reality, the information probably emerged from a symposium within the conference. This symposium was probably sponsored by a pharmaceutical company wanting to present the results of trials of its new drug, trials which they also financed. We will see from the paragraph below (entitled Players, sponsors and intermediaries) how the various protagonists typically share roles.

Exaggerating the problem and engendering fear

The starting point is a growing number of people who suffer from a particular illness or condition. Numbers usually hit the "many millions" mark (in Italy, in Europe, in the world) and is quoted at the beginning to get people's attention. The figures are increasing every year, as more and more specialists in the field start to focus their attention on even newer, more silent and more benign conditions. There is a growing tendency in all branches of medicine to eliminate the dividing line between normal and pathological, to make earlier and earlier diagnoses, and to consider certain conditions anomalous which were previously thought of as normal.

Apart from this, data given in press reports and other promotional messages is often not checked, nor can it be, in the sense that the source is not given so the reliability of the information cannot be checked up on. Often the data is simply invented on the basis of very rough estimates, as is obvious from the fact that figures regarding the same problem (the most famous example is the number of people suffering from depression in Italy) can vary enormously from one source to another.

If we tried to calculate all the millions of people suffering, according to the promoters behind the various campaigns, from one or other of

even only the most significant and chronic conditions, the total figure would be enormous, and imply the kind of paradox that every Italian must be suffering from several serious diseases simultaneously.
The second point is that the majority of these sufferers is actually unaware of the fact that they are ill; they are "under-diagnosed". This is a classic argument of medicalisation dating back to when Jules Romains, the French playwright put the words "a healthy person is a sick person who doesn't know they are ill" into his Doctor Knock's mouth in 1929. More recently, the British Medical Journal, in an article on "disease mongering" said: "a lot of money can be made by telling healthy people they are sick"[2].
A frequent corollary of this point is that family doctors often don't recognise the problem in time, hence the obvious conclusion that it is better to consult a specialist first.
After exaggerating how widespread and frequent the problem is, it is normal to stress its seriousness, in terms of negative effects on health, well-being, the economy, work, social relationships etc.

Encouraging people to go for check-ups and tests

The slogan of an awareness-raising campaign a few years ago (1996) was: "the normal symptoms of hepatitis are very common; healthy aspect, normal appetite, absence of pain. If you feel well, go and get yourself checked out". The obvious implication was that millions of Italians were suffering from a serious disease, were clearly at risk without knowing it, and should quickly go and find a doctor (or better still, a specialist, because, as we know, family doctors tend to ignore the problem) and have a check-up and get themselves some tests prescribed.
Tests are the real life-source of the medicalisation machine. Tests reveal some kind of abnormality in a large percentage of people who considered themselves healthy before being labelled as sick within the ever-growing parameters of that definition as explained above.
These people represent the "hidden part of the iceberg" but in reality they have only a certain probability of developing any problem or infirmity in the future; and the risk is even less the earlier, more silent and more benign the condition ascribed them. In the meantime, however, the "clinical cascade" is already under way with its series of check-ups, medical treatments or surgery, complications, side-effects, further tests, new treatments and so on. "A hundred" people are treated just so that one person can avoid a medical problem in the future. The other ninety-nine will only experience the disadvantages. To what extent this is beneficial for the individual who gets caught up in this chain of

events would be worth working out, if anyone took the trouble to inform and seek consent from the person before getting them involved.

Simplifying the answers

The conclusion is usually easy, often simplistic in fact. After exaggerating the risks involved in burying your head in the sand, the whole finishes with a reassuring message: don't worry, there's something (a drug, a treatment etc) which will sort everything out without problems. At this point, the report nearly always assumes a rhetorical tone reminding us of the "deus ex machina" in theatre, whereby the final intervention almost miraculously manages to solve everything. We might note that this structure is typical of promotional messages where the most important information (what the speaker cares most about) rather than being mentioned at the very beginning as in journalistic reports, often only appears at the end of a long speech which paved the way for this apotheosis. This aspect is so typical that it might even be "pathognomic". If you suspect that the writing is simply advertising in disguise, we can skip to the end. If you see the name of a drug which is going to solve the problem in the last few lines then your suspicions were probably right. Which does not necessarily mean that the last part always focuses on a specific cure. As we will see later, promoting a single product is only the final milestone along a journey which, up to a certain point, is made by lots of different people working together: people who have similar interests in focusing public attention on a certain disease, because the setting in motion of the "clinical cascade" will prove useful for all of them.

Players, sponsors and intermediaries

Doctors and scientific societies

The main players in awareness-raising campaigns are doctors, especially specialists in the illness they are raising awareness of, organised within their scientific associations.

They started running these campaigns with increasing frequency over the last few decades with the best of intentions. They wanted, each within their own field, to play an active role in disease prevention and health promotion rather than limiting themselves to the passive role of helping patients once they are ill. The attraction of the word "prevention" is so strong (even when it is not really a question of combating causes of illness – like smoking, unhealthy diet or sedentary lifestyle – but rather one of identifying the risk factors or making an earlier diagnosis) that it has so far blinded us to two important facts:

that this initiative on the part of doctors breaks with a thousand-year-old tradition and that this change has its negative sides which we should all be aware of. "Medicus non accedat nisi vocatur" (the doctor should keep away unless called): since the time of Hippocrates, doctors have felt able to offer their services only when a patient is ill and turns to them for help. This veto has formed a defence against the intrusion of medicine for centuries now, and acted as an almost automatic guarantee of patient consent.
This deontological principle is no longer in force today but there has never been a collective debate to clarify what the change implies.
First: when doctors and medicine take the decision to intervene (even if only with information) in healthy subjects, or people who believe they are healthy, they must be in possession of absolute proof of the benefits that can be expected and the absence of possible risks[3]. Unfortunately, this is true of very few preventative measures today, and yet they continue to run loads of campaigns, in defiance of this principle.
Second: specialists in any particular illness have a vested interest in identifying sufferers before they show symptoms or when there is only a chance that they might develop them. In this way they can increase patient numbers, improve their business, and benefit in terms of professional, career and economic success. In other words "preventative" care constitutes an intrinsic conflict of interests for doctors between looking after their patients' health and their own gain. This is currently aggravated by the closer relationships which are being established between the various components within the health industry and in particular with the pharmaceutical companies which, as we will see shortly, are often present behind the scenes providing the inspiration and funding for the campaign.
Whatever the circumstances, however, the message is always conveyed to the public by the reassuring and authoritative face of science, in the shape of the individual opinion leader or with the guarantee of the specialist group.

People, patients and their associations

Along with the white coats, ordinary mortals are also called on to perform various tasks and roles in awareness-raising campaigns.
People from the world of television or media, or other kinds of celebrities, can take on the more or less dramatic, and more or less direct, role of sufferer from the illness in question. In addition, citizens' groups or specific organisations for patients and their families often function as pressure groups in opposition to the government, admin-

istration or society in general to get more attention focused on the illness they are concerned with.
Obviously their activities act as fodder for the wheel of commercial promotion which is set in motion as a result. So why do they allow themselves to become party to this?
Even so-called lay-persons, whether they be individuals or organisations, mobilise themselves in good faith, with the best intentions of doing good but, like the doctors, they are unaware of how delicate an issue it is to intervene (even if only with information) in the case of healthy people. A chronic need for money to support their activities leads the organisations to look for and accept funding from the most willing parties, that is, the pharmaceutical companies, without having the necessary clarity about the conflict which this sort of funding implies or seeing the necessity for maximum transparency.
On the other hand, those who represent the sufferers, have an interest, either as individuals or as organisations, in getting more support from civilised society (including economic support) and in encouraging more interest in the particular illness they defend. Added to this is a kind of cultural awe for medical specialists and a lack of independent information with the result that these people more or less unwittingly also become instrumental in the medicalisation process[4]. This is something the Mario Negri institute along with the Italian Cochrane Centre and the scientific journalism agency Zadig are trying to address with their "Partecipasalute"[5] project.
The launch of new drugs for high emotional impact illnesses like Alzheimers and Multiple Sclerosis often sees producers and representatives of patients or their families forming an alliance against the health authorities who would like more consistent proof of the clinical relevance of the new treatment in question before agreeing to use public money to fund it.
Examples of cases of promotional campaigns which were apparently led by independent institutions, ordinary people and patients, yet in reality financed by industry are: the campaign to encourage screening for prostate cancer which was organised in nine countries by "Us Too! International"; the pressure campaign for new anti-inflammatory drugs to be more widely-prescribed (COX 2) led by Arthritis Care; pressure to make drugs to combat erectile difficulties more widely-available led by Impotence Association; etcetera.
Fortunately, many organisations recognised the risk implicit in this type of alliance and today are trying hard to free themselves of cul-

tural subjugation, if not financial, and work out their own independent point of view, thanks to careful and critical use of EBM information which is widely available nowadays, and to participation in research. A decisive step in this direction consists in recognising the potential conflict of interest if funding is accepted from industry, and the need to establish rules regarding the transparency of the alliance/conflict. Some federations of organisations are working on guidelines for managing the relationship with the pharmaceutical companies. One example is the guidelines published by the British Long Term Medical Conditions Alliance, which can be seen on the organisation's website [www.lmca.org.uk/docs/pharmgds.htm].

Producers of drugs or other health supplies

One question which we should always ask ourselves when we see any kind of public project within the health sector is: who's paying? If we exclude small-scale projects which are limited in both time and space, and conducted on a voluntary basis with very little money, health campaigns these days are generally complex programmes which cost a lot to organise. This is why funding nearly always comes from industry, especially the pharmaceutical industry.

This is not always obvious, because the sponsors often prefer to be seen as little as possible, if at all, afraid that the source of the funding might throw suspicion on the campaign's message. That's why at press conferences, in printed material, on internet sites, at various events etc. only the names of doctors or maybe patient representatives appear, whereas you have to look very carefully or even ask specifically if you want to find the names of the sponsors.

It is obvious that profit-making, limited companies do not finance scientific projects or patient associations out of charity. They do it in part (and this is still a valid motive) to keep on good terms with various groups whose opinion matters to them for various reasons. But most of all, marketing strategists have realised that encouraging and supporting the enthusiasm of specialists and patient representatives is very remunerative in terms of improving business and finding new clients.

Often the same sponsor part-finances different projects set up by various scientific organisations or lay-person groups which actually form part of a bigger overall promotional programme whose complexity the individual groups are unaware of. The campaign to convince women to take hormones during the menopause has been going on for decades and it was, and is, orchestrated by various producers who, from the 1940s onwards, paid a famous clinic to write a book which be-

came a bestseller about the eternal youth pill (and this detail only emerged recently).
Sometimes, especially when it's an individual company who is doing the financing, their investment is coupled with a demand for the addition of what we termed "the last promotional milestone", in other words, a more or less explicit direct incentive to use a particular product.
More often, however, the campaigns aim to create a favourable cultural environment rather than promoting this or that product. It is what the experts term "cause-related marketing" or "passion branding" and this can happen when various sponsors come together to work on a single project, and each of their contributions forms part of their overall strategy.
In these cases, all the parties involved always declare that they are convinced that the contents of the campaign were not influenced in any way by the sponsors. This is plausible: once the general aim of encouraging people to go for diagnostic tests, even if they have no symptoms, has been established, the resulting cascade of spending and services which can be expected provides reason enough to ensure cooperation on the part of all the various links in the health industry chain.
In general, commercial companies are so very much in favour of patient representatives (along with medical specialists) asking for "more money to be spent" on a certain illness or condition, that they get involved ever earlier along the line, often actually setting up new organisations or allying existing ones to larger groups. For example, the efforts that went into constituting a group of international coordinators responsible for patients' organisations which in 1999 culminated in the establishment of the International Alliance of Patient's Organisations [www.patientsorganisations.org], were financed by the pharmaceutical industry right from the first meeting.

Public relations agencies

Public relations agencies act as the intermediary between the sponsors, who provide the funding, and the patient or medical organisations who offer their authority and credibility. Their role in health advertising campaigns is also to set up some kind of screen between the contents of the message and the commercial interests which are at its heart.
PR experts organise meetings, enlist opinion leaders for conferences and editorials, provide informational material in both paper and electronic form, organise events etc. For this they use the so-called "third-party" technique, which, in the words of the chairman of one of the leading international companies in the field, means "presenting the

facts without making it obvious they are being manipulated by the pharmaceutical industry."
Any communication with journalists, from the press conferences to launch a new campaign, to information about a particular event or action, is always handled in person by the PR company and they discuss the campaign topic and the people heading it, both scientific and lay, but often they do not even mention the financers. The journalists, who in reality know very little about how these agencies operate, risk writing an uncritical report on messages created by marketing experts as if relaying information from an independent source which is completely unbiased and therefore deserves maximum credibility. It is because of this that many promotional campaigns to do with health find their way onto television screens or into newspapers, having exactly the effect the sponsors desired.

Mass media

The mass media act as the speakers for medical advertising campaigns, broadcasting information about them, practically unaware of the commercial motivation underpinning them. They are encouraged to do this by their natural awe of anybody who wields some kind of power be that economic, academic or political; by a triumphalist and hyperbolic concept of medicine and its progress; and by their blind faith in the power of technology and authority of science.
These "cultural" stigmata are sufficient to explain the major faults in medical journalism, which often fails to view the information it is handling with the critical eye it deserves. Current problems, according to the analysis of the medical journalist Ragnar Levi[6], are:

- Limiting themselves to the opinion of experts
- Treating specialists as omniscient
- Confusing facts and fantasy
- Being misled by the numbers
- Taing anecdotes as proof
- Uncritical reading of study results
- Applying research to practice
- Emphasising the clinical relevance
- Confusing risk factors with illness
- Presenting risks in a misleading way

Tools and channels

Since there are more and more, and ever more frequent, health awareness-raising campaigns, organisers are having to raise their voices as if to make themselves heard above the background noise. The fundamental message needs to be broadcast at different times using different means and tends to cover the whole multimedia spectrum of communication tools available and use every type of event imaginable.

Days, weeks, months, years

According to available resources, promotional efforts can be concentrated in the one day (Parkinson's disease day, epilepsy day, myelin diseases day, memory disorders day) or spread out over a longer period: a week (Multiple Sclerosis week, Brain week, Alzheimer's week), a month (eye-testing, water retention, dental health) or even a year (brain year).

The calendar of events reminding people to focus attention on one or another illness is already so full, especially in the spring and autumn, that sometimes National and International days overlap, sometimes with unintentionally comic results. Obesity day falls the day before world hunger day. There is currently complete anarchy as regards the calendar. Anyone can choose a certain day or period to promote an illness and the success of the venture depends solely how much money is spent on supporting it. Tongue in cheek, but not completely, an authority (preferably international) could be appointed to assign calendar days based on objective criteria which take into account not only the size of the sponsor's wallet but also how relevant the problem is and how serious the campaign.

Joking apart, this artificial linking of a health problem to a specific date is based on a feature of the media which communication theory experts are very familiar with: namely that it's easier to make news out of events happening in the short-term than tendencies which develop over a long period. This explains why the number of "dates" is proliferating. Another reason is the desire to transform a single campaign into a recurring event, imprinted on people's minds, as well as the need to condense the organisational effort into a clearly-defined period, including events and actual encounters.

Town-centre check-ups

These days, the organisers of health awareness-raising campaigns, when designing their events package, nearly always make sure they include the following: the mobilisation of white coats in the major cit-

ies who offer passers-by information, consultations, check-ups and examinations. This kind of action has strong local impact, reducing the distance between campaign organisers and ordinary people, with an almost "door-to-door" intensity. The effectiveness of this type of campaign has long been known by the organisers of election campaigns and now it is being applied to medicine.
However, even here, as in many other cases, the organisers seem unaware of the negative consequences of these "close encounters".
The first concerns the possible success of these "town centre" events. Whatever the means used (based mainly on information or intervention) the principle objective is to encourage people who thought they were healthy to go for tests or check-ups that they wouldn't otherwise have gone for. In this sense it is like screening, albeit an illegitimate form because it lacks what it takes to be a proper programme i.e. a coordinated and controlled series of actions. It is well-known that non-organised screening is less efficient and effective because without the necessary quality control at each phase (from the invitations to the validity of the tests to the surgical or other operations/treatments) possible benefits are reduced and possible harmful effects, which are always a risk, are massively increased. We know, for example, that the people targeted belong mainly to low-risk groups (young, healthy, careful about their health) who stand to gain the least. If this can sway the balance in the wrong direction even when the screening programme is of proven effectiveness (organised and controlled conditions), it is obvious how dangerous this might be when the screening is of dubious value. Another fear which emerges is that of the close contact that is established between specialist and public, and in an inappropriate place, one which by-passes the family doctor / mediator and thus potentially ruins the triangular relationship (GP, patient, specialist) whose delicate equilibrium, in terms of duties and competencies, is indispensable to the survival and correct functioning of the National Health Service.
We should also be concerned about image. Town-centre events, like many other aspects of awareness-raising campaigns regarding illnesses, introduce advertising language into the rapport between doctor and patient and institutions and the public. This is yet another of the increasing mass of factors which make the public consider health as a product and the health service as a market and the consumer spending that derives from this.

Adverts, brochures, posters and other advertising materials

The availability of copious funds allows campaign organisers to use high-level professionals to produce their publicity material and information. The effectiveness of their language and messages has already been mentioned. Here we would like to stress that the ambiguity surrounding the real commercial nature of the campaign, disguised as public good, often opens the doors to communication channels which ought to be limited to genuinely independent programmes of public interest.

It often happens that advertising space on television or in newspapers is given free when it concerns a health problem, maybe through the Progressive Publicity channel. It is equally easy to find institutions like the Post Office or the Railways willing to act as distribution channels for the material. Even schools open their doors to marketing initiatives. For the last two years, the Italian association of gynaecology and obstetrics (SIGO) has organised workshops on personal hygiene in Middle and High schools as part of National Gynaecological Prevention Day, publicising one particular soap product produced by the company which sponsors the programme.

As in the case of patient associations and the mass media, the willingness of these institutions to help out is explained in many cases by their very real conviction that they are propagating the verb health against illness. The marketing objectives, which are not particularly well-hidden, are not perceived. The most enterprising campaigns even get patronage, though not necessarily conviction, from high-level health authorities like the Ministry of Health or the World Health Organisation.

Professional culture and critical function

Signs of intolerance towards disease-mongering are starting to be seen, as a result of a growing unease between health workers and their patients.

As far as doctors are concerned, professional and ethical considerations seem to become more of an issue the simpler and less serious the cause of the conflict – a conflict between the need to act in the patient's best interests and that of furthering their own interests. It is no coincidence that family doctors are often the first to express criticism of medicine's excesses and invasiveness through their professional associations or their most enlightened leaders. There are many reasons for this counter-current attitude but there are two main ones.

The first is ideological, and derives from the fact that they are more patient-centred and look at patients' general health needs rather than focusing on a specific illness like specialists do. The second is material: the family doctor's salary or career is not particularly affected by the number of patients he sees (for treatments, check-ups, prescriptions, hospitalisations) which is what happens, in a variety of ways to the groups of specialists. In fact, the family doctor is usually applauded if his patients are healthy or perceive themselves as such. And so they are naturally unwelcoming, or even hostile, when it comes to more aggressive and invasive approaches.

An illustration that this critical attitude towards the intrusiveness of medicine forms part of the cultural heritage of a family doctor can be seen in the European definition of General Practice/Family Medicine published in 2002 by the "European Society of General Practice/Family Medicine. In this document, when referring to the doctor's role of "defending patients' interests"it says that family doctors "need to protect patients from the harm that over-medicalisation of their problems can do, and, if necessary, stop them from doing screening, exams and treatments which are unnecessary."

Other groups of medics are also beginning to express their dissatisfaction and there is ample evidence of this in the literature. The idea that emerges is that in the industrial era of medicine, the doctor is little more than a technician and they are slaves to the market and forced against their will to live in a permanent state of conflict.

"Why are doctors so unhappy?" was the title of an editorial in the British Medical Journal[7]. The pages of comment which it provoked in the major journals shows that discontent amongst doctors is rife in every country from America to Europe independent of the type of Health System. And it was not simply a question of low pay or long working hours, though these are obviously both important. One of the major problems is the continuous battle with the authorities and politicians who always want to cut costs, and clearly the negative image which is constantly given by the media is also irritating. But the main reason for their discontent is the discrepancy between what a doctor was trained to do and what the health industry demands of them.

Maybe this could be an incentive for formulating a new and different "social contract" between the healers and their patients which takes account of today's changing world. Here are some of the points that the new contract should focus on according to the BMJ editor:

– Illness, pain and death are part of life;

- Medicine has limited power, especially when it comes to solving socially-related problems, and it can be dangerous;
- Doctors do not know everything and they should make their decisions (questionable) with the help and support of the patient;
- Patients cannot hand over their problems to their doctor;
- Doctors need to be honest and admit their own limits.

It is possible that in the near future the very excesses that the process of medicalisation is heading towards will lead to a new awareness and cultural and ethical "renaissance" on the part of the medical profession, thus bringing a halt to the dynamics we have illustrated.

Civilised society: existential resistance and information

As far as individual citizens are concerned, it is unlikely that they will be able to develop an effective resistance to the incursions of medicine on the basis of knowledge and information except for small minority groups. Especially considering the dominant culture and the lack of independent and critical information.

A different kind of resistance is growing, however, one which could be termed existential and which is linked to a feeling of saturation or fatigue on the part of the public who are being told that they need treatment for a growing number of diseases or risks even when they feel perfectly well.

Every time the threshold level for diagnosis is lowered or brought forward, it causes a reduction in the sector of the population willing to be recruited into the ensuing series of treatments and interventions. For example, the recent reduction in arterial pressure levels that should be reached after treatment means that the patients who are being "well-controlled" are now only a third of those treated rather than the 50% they represented before[8].

In other words, overall compliance on the part of the population has intrinsic limits where medicalisation is concerned and it is probably difficult to improve on these figures. This does not stop market strategists from pursuing their aims until they achieve a net increase in the number of patients seeking treatment. From a commercial point of view it matters little that these new recruits belong to sectors of the population for whom the interventions are likely to be useful, yet for whom the risk of harmful effects remains the same. This ought to worry the Public Health Authorities.

A rather different critical role could be played by the media and citizens' associations or patient groups if they were not so culturally and

financially subjugated to the interests of the industry. We only have to think of groups like the American Public Citizen and the information it produces about drugs[9]. Unfortunately, although in Italy there is a chance that certain independent associations might become more incisive even within the Health sector, we have to assume that information like this, at least in the near future, will continue to be much less than that produced or influenced by the health industry. The same can be said of the media and specialist press, though a detailed analysis of the nonetheless interesting national and international sources of critisicm is beyond the scope of this paper.

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   But surely the fundamental promise we make when we actively solicit individuals and exhort them to accept preventive interventions must be that, on average, they will be the better for it. Accordingly, the presumption that justifies the aggressive assertiveness with which we go after the unsuspecting healthy must be based on the highest level of randomized evidence that our preventive manoeuvre will, in fact, do more good than harm. Without evidence from positive randomized trials (and, better still, systematic reviews of randomized trials) we cannot justify soliciting the well to accept any personal health intervention." D. Sackett. The arrogance of preventive medicine. CMAJ 2002; 167: 363.
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MULTIMODALITY MONITORING AND TELEMONITORING IN NEUROCRITICAL CARE

Paul M. VESPA

Introduction

There has been a great deal of progress in Neurocritical Care in the last decade. Much of this progress has focused on advances in monitoring of the brain and directing our treatment specifically to the brain. Monitoring takes many forms, including continuous electroencephalography (cEEG), brain oxygenation (jugular venous and brain tissue oxygen pressure-PbrO2), cerebral microdialysis, intracranial pressure, and the clinical examination. One may use this monitoring in any neurologic critical illnesses including brain trauma, stroke, subarachnoid hemorrhage, status epilepticus, coma and brain hemorrhage. Multimodality monitoring refers to simultaneous monitoring of several parameters of brain physiology and function and the application of this information to direct critical care. The intention of my discussion is to focus on the integration of these methods together with novel approaches to telemedicine in neurocritical care.

Intensive Care Unit cEEG

Over the past 10 years, it has become increasingly apparent that seizure activity commonly occurs in critically neurologic patients. Based on an extension of intraoperative monitoring, continuous electroencephalography (cEEG) was started at UCLA around 1994. Vespa et al[1] initially demonstrated that over 20% of patients with traumatic brain injury demonstrated seizures while in the ICU, half of which were nonconvulsive seizures that were detected only by the use of continuous EEG. These seizures can be regional in the area of the primary insult, such as adjacent to a temporal lobe contusion, or can be more widespread involving the entire brain. Other centers have started to do this

Paul M. Vespa, MD
Associate Professor of Neurosurgery and Neurology – Director of Neurocritical Care
UCLA School of Medicine – Division of Neurosurgery
10833 Le Conte Ave – CHS 18-218 Los Angeles, CA, U.S.A.
Phone: 310-206-6969 – Fax: 310-794-2147 – Pvespa@mednet.ucla.edu

too[2]. In a similar study, Vespa et al[3] demonstrated that over 26% of patients with non-traumatic intracerebral hemorrhage demonstrated seizures on cEEG, and that these seizures were independently associated with progressive brain edema and midline shift. Thus the seizures may be eliciting progressive secondary injury and promoting clinical deterioration.
Secondary deterioration and cell death has been seen in laboratory studies of induced post-traumatic seizures[4]. Vespa et al[5] demonstrated that post-traumatic seizures are not benign and may lead to additional neurologic injury and cell death, especially in vulnerable regions of the brain such as the hippocampus. Claassen[6] used similar cEEG methods and detected an 18% incidence rate of seizures in a mixed neurocritical care population. The incidence rate of between 20-30% has been reported in several studies[3,7].
Intensive care cEEG requires several fundamental elements: 1) Dedicated high quality EEG machines with capacity of 8 or more electrodes. 2) An expert physician who can formally interpret the EEG, 3) A trained nursing and critical care physician group that can understand the basic features of EEG, such as seizures. 4) An automated seizure detection system to enhance the diagnosis of seizures. 5) A internet-based system to remotely access to EEG for real-time reporting. Several new digital EEG instruments are currently available that provide for remote access as well as automated seizure detection. In our hands, we have launched an internet-available remote expert review system that permits remote access and assessment of the EEG. This telemedicine approach has resulted in decreased interval between event detection and intervention. Direct feedback to nurses in the form of confirmation of seizure detection and record review as well as the ability to teleconference increases accuracy of seizure detection. In addition, the telemedicine approach creates the potential for expanding the limited list of experts to centers that presently lack sufficient human experts. Figure 1 demonstrates the telemedicine cEEG system.

Cerebral Oxygenation

Preserving cerebral oxygenation in neurocritical care patients is the primary goal for most patients. Several lines of evidence from clinical brain injury research suggest that ischemia occurs frequently in traumatic brain injury. However, this is controversial since several elegant recent studies suggest that the occurrence of ischemia occurs only

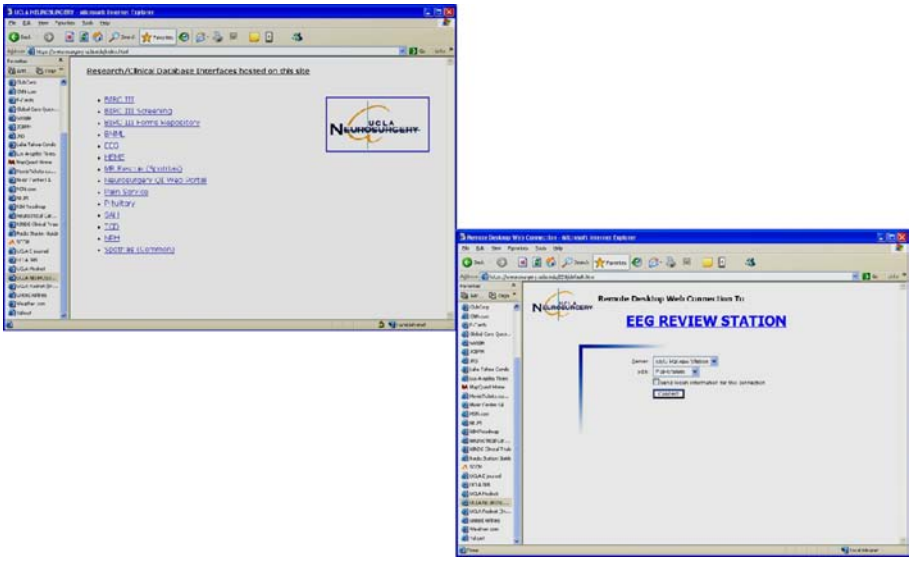

Figure 1 – *UCLA cEEG Network.*

during marked physiological distress such as with excessive hyperventilation or hypotension. Diringer and colleagues[8] performed PET studies at a mean of 12 hours post injury and demonstrated areas of potential ischemia. Similarly, Coles and colleagues[9] reported that a significant portion of the brain may be ischemic, and that this volume of ischemic brain tissue increases with provocative hyperventilation. Until recently, cerebral perfusion pressure (CPP) has been used to estimate the degree of cerebral blood flow and to avoid ischemia. However, several studies have questioned the validity of CPP[3,5,10]. Most recently, perfusion computerized tomographic imaging has been done early after brain injury and demonstrated that the expected relationships between CPP and cerebral blood flow do not hold with some patients having ischemic range cerebral blood flow despite CPP >70 mm Hg [10] and that the degree of pressure autoregulation is impaired and cannot be predicted based upon baseline values of cerebral blood flow or CPP. Thus, direct monitoring of tissue oxygenation and oxygen utilization appear to be better than monitoring CPP alone to determine the adequacy of cerebral oxygenation.

Presently, there are two modes of monitoring brain oxygenation: jugular venous saturation (SjO2) monitoring (a global method) and brain tissue oxygen (PbrO2) pressure monitoring (a regional method). SjO2 monitoring has been well established to have prognostic significance

and clinical utility in detecting ischemic as well excessively hyperemic states of cerebral blood flow and oxygen supply. Indeed, hyperemia was the most common disturbance of cerebral blood flow as associated with alteration of brain metabolism[11], in which cerebral blood flow is increased disproportionately to the need for oxygen utilization. Oxidative metabolism (CMRO2) is uniformly reduced after brain injury and the extent of reduction in CMRO2 is a significant prognostic indicator. Hyperemia is thought to occur because of impaired cerebral autoregulation and has been associated with poor outcome, especially in pediatric brain injury populations[12]. The ability to monitor cerebral blood flow in real time and to adjust blood pressure and ventilation parameters to avoid either extreme of hyperemia or brain ischemia has become a powerful tool in the treatment of global brain edema resulting from brain injury.

In contrast to global cerebral oxygenation monitoring, PbrO2 monitoring provides regional assessment of oxygenation, in the format of a brain sensor that resembles an intracranial pressure monitor. The PbrO2 value is thought to represent the partial pressure of oxygen in the brain and is considered a marker of the balance between oxygen supply and utilization. Normal ranges of PbrO2 are 20-45 mm Hg in normal brain tissue. The PbrO2 is not a measure of oxidative metabolism per se, but in general can reflect changes in oxygen utilization as a function of the oxygen supply. In the last year, several interesting papers on PbrO2 have been published and have resulted in several new concepts. 1) PbrO2 can decrease even in the setting of adequate cerebral perfusion pressure[13] and different CPP thresholds are required between different patients to maintain the PbrO2 in the normal range[14]. 2) Decreases in PbrO2 indicate tissue metabolic distress or brain ischemia[15] and the threshold of PbrO2 below 10 mm Hg, was considered critical. 3) Critical PbrO2 threshold, of 10 mm Hg, have been validated in studies of prognosis after brain injury and in physiologic studies. 4) PbrO2 can be increased by increasing the amount of supplemental oxygen during mechanical ventilation[16] or by delivering hyperbaric oxygen[17]. This finding suggests that the brain may not have adequate oxygen delivery despite normal arterial oxygen content. PbrO2 during prone positioning or other maneuvers to increase arterial oxygenation provides direct guidance to treatment endpoints in patients with hypoxemia resulting from acute lung injury[18]. PbrO2 is now being used routinely in neurocritical care[19,20,21,22].

Brain Microdialysis

Cerebral microdialysis is the method of placing a small semipermeable membrane catheter into the brain tissue and sampling metabolites and amino transmitters from the extracellular space to determine changes in brain metabolism. Cerebral microdialysis has been used clinically since 1990 and currently in clinical use[23,24]. Cerebral microdialysis is often in conjunction with PbrO2, to determine if brain ischemia or other alterations in metabolism exist. In the past year several findings have begun to crystallize the importance of this monitor: 1) Extracellular glucose is reduced after traumatic brain injury and may be related to poor outcome[25] and may be related to insulin treatment, seizures or spreading depression in the brain[26,27]. 2) The lactate/pyruvate ratio is a good descriminator of altered brain metabolism or ischemia, and can be used in the setting of subarachnoid hemorrhage to diagnose and track cerebral vasospasm[28,29,30,31,32]. Increases in the lactate/pyruvate ratio or the lactate/glucose ratio indicate brain ischemia associated with vasospasm after subarachnoid hemorrhage. The precise thresholds for these parameters have not been agreed to and some have used relative percent changes as the indicator for ischemia, whereas others have used absolute thresholds. Validation of these specific parameters is yet to be done and but is needed in order to make best use of this technology in the future. 3) Cerebral microdialysis is variable depending on the tissue in which it is performed. Pericontusional tissue will have much different levels of metabolites compared with normal appearing brain tissue[5,32,33]. Thus selection of the site in which monitoring is to be performed, as well as the specific methodology of microdialysis, such as the perfusion and sampling rates, are critical factors that must be considered to perform this technique with validity.

Telemedicine

Remote access to the patient and to all of the neuromonitoring technology is important to neurocritical care. The principal goal is to make the information from the bedside accessible and available remotely. At UCLA, we have extensive experience in developing this remote telemedicine system. We have an internet web-portal for remote access to on-line vital signs including imaging and laboratory studies. This system is called Global Care Quest® (http://gcq.ucla.edu/index_pc.html) which enables wireless access to all data via handheld personal digital assistant and/or tablet personal computers. This per-

mits the physician to access on-line data from any of the monitors mentioned above and to integrate the information and display it as an array of important data, similar to a pilot's cockpit array (See figure 2). The cockpit array permits the physician to integrate and compare data that are time-locked and to begin to interrogate the data in order to best plan the next step of therapy. In Figure 2, the physician is able to simultanesouly and remotely see the vital sign monitoring, the brain CT scan, the chest CT scan and any number of trended lab values, such as glucose.

The second approach that we have undertaken is to have remote access to the ICU. Some centers have made use of the electronic intensive care unit that permits a clinician to watch a series of monitors for a variety of patients. The electronic ICU has been shown to be associated with decreased mortality, lower length of stay in the ICU and decreased cost[35]. In contrast to this we have made use of the concept of telepresence. Telepresence is concept that the clinician can be present at the bedside in spirit if not in person, and is able to observe and respond in a life-like fashion as if the clinician were in the room itself. Telepresence is facilitated though the use of robot® (In Touch Health,

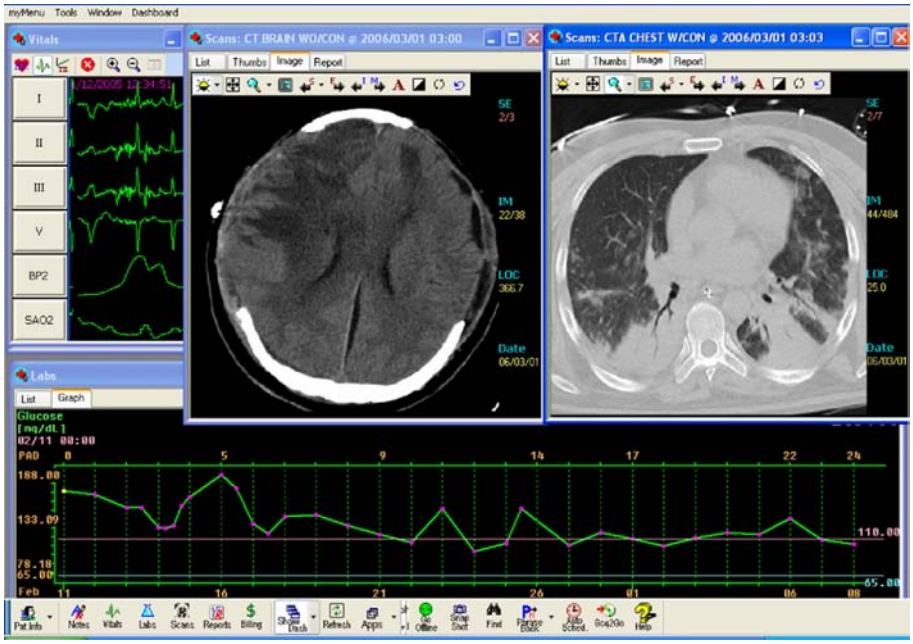

Figure 2.

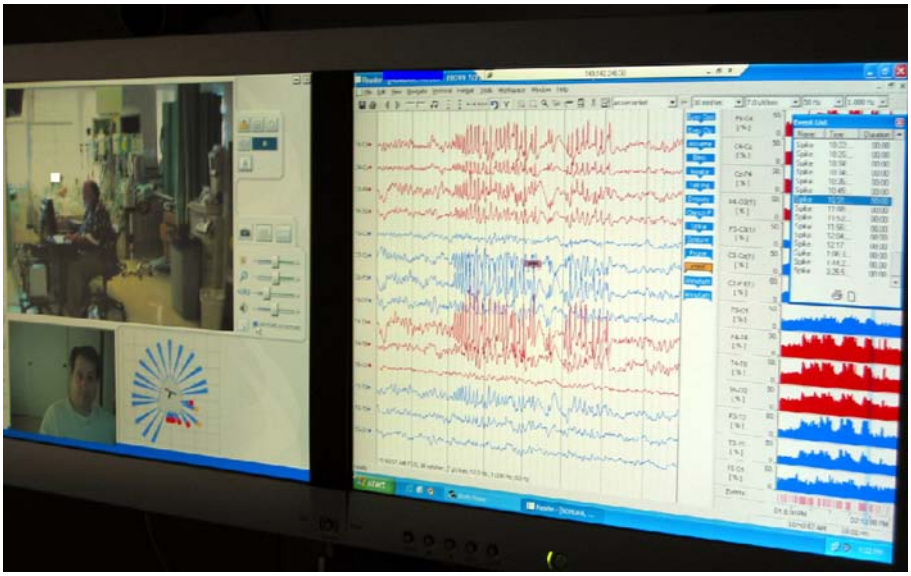

Figure 3 – *UCLA Robotic Telemedicine System.*

Inc. Santa Barbara, California) which is remotely controlled via an internet connection. The telepresence robot permits the clinician to walk from bedside to bedside, look at all available charting information, perform face-to-face discussion with patients, nurses, and families, all from a remote site (See Figure 3). The remote site can be anywhere that is connected to internet technology. The telepresence robot can be used to augment communication and obtain visual and behavioral information that is otherwise lost by telephonic communication. The robotic telepresence is interestingly quite easy to use and is quickly accepted by nursing staff, patients and families. The secret strength of telepresence seems to be the ability to capture visual and verbal behavioral clues that occur in face-to-face interaction that is otherwise missing in telephonic or computer-based chat-room interactions. The ability of the robot to move permits freedom to wander the intensive care unit and be animated, which further creates the ambience of actually being present.

Summary

In summary, multimodality monitoring enables the intensivist to directly monitor and treat the brain much in the way we treat the cardiopulmonary system (ie the heart and systemic blood pressure). We can treat the patient, experiment with different treatment goals

and thresholds, and attempt to improve brain metabolism and function during the most critical phase of the critical illness. As intensivists, we now have new opportunities to affect outcome and given the widespread availability of information technology, we may be able to direct this care from remote sites at all times of day or night.

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NEUROCHEMICAL MONITORING

Nino STOCCHETTI, Sandra MAGNONI

Key words: microdialisis, cerebral metabolism, head trauma, subarachnoid haemorrage.

Monitoring objectives

Over the last few decades, investigation into cerebral damage has been on a different scale. While early investigative techniques capable of analysing changes in cerebral morphology (pneumoencephalography, angiography) were indirect and macroscopic in nature, the advent of CT scan and then MRI has allowed for a level of discrimination and detail which was previously unthinkable. Morphological investigation has thus closed the gap on functional exploration, which had a very detailed semiology in the field of neurology.
Developments in neurointensive care meant that specific investigations were necessary, either because of the severity of the damage or the difficulty/impossibility of testing the patients themselves.
The objective of the various investigative techniques used in intensive care was to establish indicators which were particularly significant or dangerous, for example, intracranial pressure. However, identifying the causes of intracranial hypertension required new parameters which were not readily available. An increased intracranial volume could be caused by intracranial masses or the dilation of the cerebral vessels, but also by an altered blood-brain barrier with vasogenic edema or damage to ionic pumps with cytotoxic edema. The ensuing intracranial hypertension could be tolerated or could in turn cause a reduction of the perfusion pressure or the perfusion itself.
The main objective of the monitoring is to measure cellular integrity

Prof. Nino Stocchetti, MD
Milan University, Neuroscience ICU
Dipartimento di Anestesia e Rianimazione, Terapia Intensiva Neuroscienze
Ospedale Maggiore Policlinico, Mangiagalli, e Regina Elena, Fondazione IRCCS
Via F. Sforza 35 – 20122 Milano, Italy
Tel: 0039 0255035517 – fax. 0039 0255035560 – stocchet@policlinico.mi.it
Sandra Magnoni, MD
Neuroscience ICU
Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Fondazione IRCCS – Milano, Italy

or damage. Neurochemistry plays a crucial role in this because it enables us to measure single metabolites or components of the cell structure.

Neurochemistry

Neurochemistry, as applied to intensive care, first uses "global" analysis of cerebral tissue, investigated through the cerebro-spinal fluid (CSF) or arteriojugular lactate difference[1].
In the case of ischaemic damage, for example, raised lactate levels were noted in the CSF and this finding was confirmed as a prognostic indicator for trauma[2,3].
It was quickly established, however, that CSF functioned as a kind of "washbasin" collecting metabolites from various regions of the brain and mixing and diluting them. A further limitation was caused by the presence of subarachnoid haemorrhage, frequent in trauma, which "contaminates" the CSF by changing its metabolite concentrations.
By 1987, extracellular pH was already being measured in patients during neurosurgical operations and it was noted how different values were compared to CSF[4].

Microdialysis

Significant progress was made when microdialysis techniques, which had been used in laboratories for years, were brought into intensive care[5].
Microdialysis consists of bringing small amounts of fluid into contact with the fluid in the extra-cellular space across a membrane whose permeability is known. A fraction of the solutes present outside the membrane cross the membrane and can then be measured. The amount of solutes recovered in this way (dialysed) depends on the nature of the solutes, especially their molecular weight, the permeability of the membrane, and the speed at which the liquid flows within the dialysis catheter[6].
A device which is capable of determining glucose, glutamate, lactate, pyruvate and glycerol concentrations is much smaller nowadays and can be used at the bedside. Once connected to the computer the results can quickly be collected, compared and visualised.

Concentrations of metabolites and clinical condition

The study of cellular energy metabolism is possible through measurement of the concentrations of glucose, lactate and pyruvate. In physiological conditions, concentrations of glucose are dialysed which are

indicative of a good supply of substrate, and concentrations of lactate and pyruvate linked to adequate functioning of the Krebs cycle. When there is ischaemia, on the other hand, concentrations of extra-cellular glucose are reduced or disappear, and the lactate/pyruvate ratio increases. This has been found both in head trauma and subarachnoid hemorrhage[7,8,9,10,11], as well as in other pathological conditions[12].

Results and prospects

Microdialysis has meant that a detailed study of cerebral metabolism can be carried out at the patient's bedside. In this way, cellular "health" and function have become explorable and the effects of both illness and treatment can be seen in neurochemical terms. One of the main limitations of microdialysis is that only a small amount of cerebral tissue can be explored. The concentration of solutes can only be determined in the areas immediately adjacent to the catheter, while information regarding areas more than a few centrimetes away cannot be obtained[13].

Although information collected at a specific point may reflect the global state of cerebral metabolism when the cerebral damage is widespread, in the case of focal lesions only catheters adjacent to the lesion can indicate the metabolic state. The complexity of the sampling technique is a further limitation, which requires meticulousness and time. In a series at the Baylor College of Medicine (Houston), 13% of samples were not adequate for analysis because of technical details[14]. When venturing into the neurochemistry, the amount of intellectual work needed to properly evaluate the data is substantial, particularly in complex cases. Finally, microdialysis has a temporal resolution of hours at the commonly used perfusion flow of 0.3 microlitres/minute. With this perfusion flow, at least 30 minutes are needed for the interstitial fluid to diffuse over the dialysis membrane, and twice that time to have a sufficient amount of dialysate to analyse. This temporal resolution, which is adequate for pathophysiological studies, is not appropriate when the objective is the identification of secondary cerebral insults. In this latter scenario, even minutes are important.

In vivo monitoring to correct neurochemistry derangements is an important step forward. The microdialysis is a potent research tool, however there are limits for its routine use.

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NON-INVASIVE ASSESSMENT OF CEREBRAL PERFUSION PRESSURE AND INTRACRANIAL PRESSURE

Marek CZOSNYKA, Bernhard SCHMIDT, Eric SCHMIDT

Key words: Transcranial Doppler Ultrasonography, Cerebral Perfusion Pressure, Intracranial Pressure, head injury.

Although many factors may affect outcome in the head injured patient, an increase in intracranial pressure (ICP) and a reduction in arterial blood pressure (ABP) are both independently predictive of poor outcome. Both systemic hypotension and intracranial hypertension lead to a reduction in cerebral perfusion pressure (CPP), provoking potentially a decrease in cerebral blood flow, a cerebral energy failure, increasing the rate and severity of secondary ischemic brain insults[1,2]. The intensive care of the head injured patient aims to optimise CPP and reduce the risk of intracranial hypertension, and CPP-targeted therapy has been shown to be an effective method of improving outcome after head trauma.

In neurocritical care invasive measurement of CPP, as the difference between ABP and ICP, requires introducing invasive pressure transducer into the brain[3]. The measurement can be biased for various reasons. Errors in the measurement of ICP may result from long-term or temperature drifts of the transducer, or – in the case of using contemporary microtransducers – from uneven distribution of intraparenchymal pressure within the brain. The less invasive epidural ICP probes have been shown to produce case – and time – dependent errors of up to 20 mmHg. There are also potential errors in the measure-

Marek Czosnyka, PhD
Academic Neurosurgical Unit, Addenbrooke's Hospital
Hills Road, Cambridge CB2 2QQ, U.K.

Bernhard Schmidt, PhD
Department of Neurology, Klinikum Chemnitz, Germany

Eric Schmidt, MD
Department of Neurosurgery, Hospital Purpan, Toulouse, France

ments of the ABP. Arterial pressure at the level of the brain is often underestimated as almost all invasive pressure-monitoring devices obtain readings from peripheral vessels. Although considered as a 'golden standard' invasive CPP may be inaccurate in clinical practice. Therefore, the 'real CPP' should be considered not as a number (i.e. measured mean ABP minus ICP) but rather condition for cerebral blood to flow.

Beyond obvious clinical emergency i.e. following lower grade stroke or subarachnoid haemorrhage, in hydrocephalus, idiopathic intracranial hypertension, it would be very helpful to measure intracranial pressure or cerebral perfusion pressure without invasive transducers in accident and emergency observation units, in many pediatric cases, liver failure, pre-eclampsia, etc.,

Historically ICP has been estimated non-invasively using various methods: tympanic membrane displacement[4], by fontanometry[5] or by analysis of pulse waveform of TCD[6]. None of these methods was considered to be precise enough to be routinely used in clinical management of intracranial hypertension. They rather provide supporting data which may be helpful in making decisions regarding direct measurement.

Various methods of non-invasive measurement of ICP have been proposed:

- Time of flight of ultrasound through the skull (reported 95% confidence interval for prediction 12 mm Hg;[7]),
- Change in skull diameter[8],
- Change in blood flow velocity in straight sinus[9]
- Methods based on electroencephalography (reported 95% limits for prediction 8 mm Hg,[10]).
- Methods based on MRI accounting of volume of CSF, arterial and venous blood[11]
- Prediction of intracranial pressure from non-invasive transocular venous and arterial hemodynamic measurements[12]

Our own experience is mainly based on methods using transcranial Doppler ultrasonograhy. This methodology will be discussed more in detail.

Aaslid's design and description of transcranial Doppler sonography in 1982[13] permitted bedside monitoring of cerebral blood flow velocity, which may be used, under assumption of absent vasospasm, an index of the cerebral blood flow. The measurement may be conducted

non-invasively, repeatedly, and even continuously. The problem is that this is a 'big tube technique', which measures flow velocity in branches of the circle of Willis, most commonly the Middle Cerebral Artery (MCA). Compliant branches of the MCA can be compared to two physiological pressure transducers. The pattern of blood flow within these tubes is certainly modulated by transmural pressure (i.e. cerebral perfusion pressure) and the distal vascular resistance (also modulated by CPP). But what is the calibration factor and how should we compensate for unknown non-linear distortion?

There is reasonable correlation between the pulsatility index of the Middle Cerebral Artery blood flow velocity and cerebral perfusion pressure after head injury but absolute measurements of cerebral perfusion pressure cannot be extrapolated[14]. Also correlation between pulsatility index and mean ICP has been reported[6] but the error is probably huge when ICP is only moderately increased (below 30 mm Hg).

Other sources have suggested, that 'critical closing pressure' (CCP) derived from flow velocity and arterial pressure waveform approximated the ICP[15]. The accuracy of this method has, however, never been satisfactory[16].

Aaslid proposed[17] that an index of cerebral perfusion pressure could be derived from the ratio of the amplitudes of the first harmonics of the arterial blood pressure and the middle cerebral artery velocity (detected by transcranial Doppler sonography) multiplied by mean flow velocity. However the 95% confidence limit for predictors is as wide as ±20 mm Hg.

CPP affects the shape of blood flow velocity waveform. However, arterial pulse waveform, heart rate, tension of arterial CO2, distal vascular resistance, even age affect FV waveform as well. Some simple formulas to assess CPP non-invasively from ABP and FV waveforms have been proposed in the past.

Out of these, a particular one has reached a possibly satisfactory accuracy (error less than 10 mm Hg in more than 80% measurements;[18]):

$$nCPP = MAP * FVd/FVm + 14$$

(FVd- diastolic FV; FVm- mean FV)

nCPP is useful both to estimate absolute CPP and to monitor changes in CPP in time. 95% confidence limit for estimation of CPP is 12 mm Hg[18]. Although this seems to be satisfactory for CPP, such a precision would be not good enough to estimate ICP.

Pulsatility index increases with rising ICP[6]. Prediction of absolute ICP

using PI is not accurate enough as many other factors may influence PI (arterial pulse, heart rate, PaCO2, vascular tone, proximal stenosis, spasm, etc)[19].
However, a recent publication[20] suggests a very narrow 95% confidence limit for predictor in head injured patients: ±4.2 mm Hg. The same prediction limit derived from our own (Cambridge) data is ±20 mm Hg. Such a huge discrepancy ought to be explained.
Estimation of ICP using formula[17]:

*MAP-A1/F1*FVm*

where F1 and A1 are first harmonic components of flow velocity and arterial pressure pulse waveforms

gives 95% confidence limit of around ±20 mm Hg.
Moving-average model of transmission between ABP and ICP, modified by the relationship between ABP and FV gives mean absolute error around 6 mm Hg and 95% confidence limit for predictor around ±12 mm Hg[21]. The method is based on analysis of a large data-base of patients with homogenous pathology undergoing full ICP, ABP and FV direct monitoring and is most probably pathology-dependent. Changes in PaCO2, spasm and proximal stenosis are confounding factors.

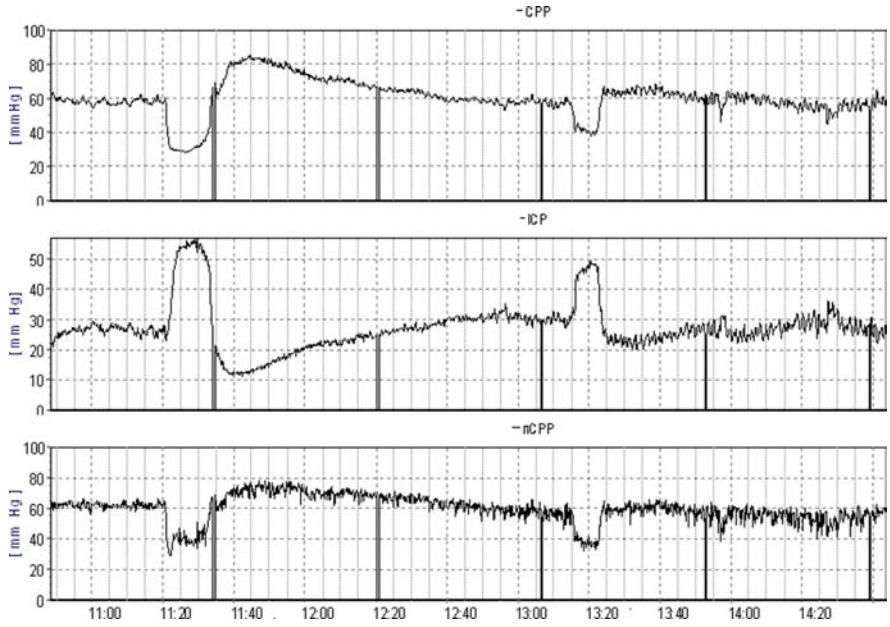

Figure 1.

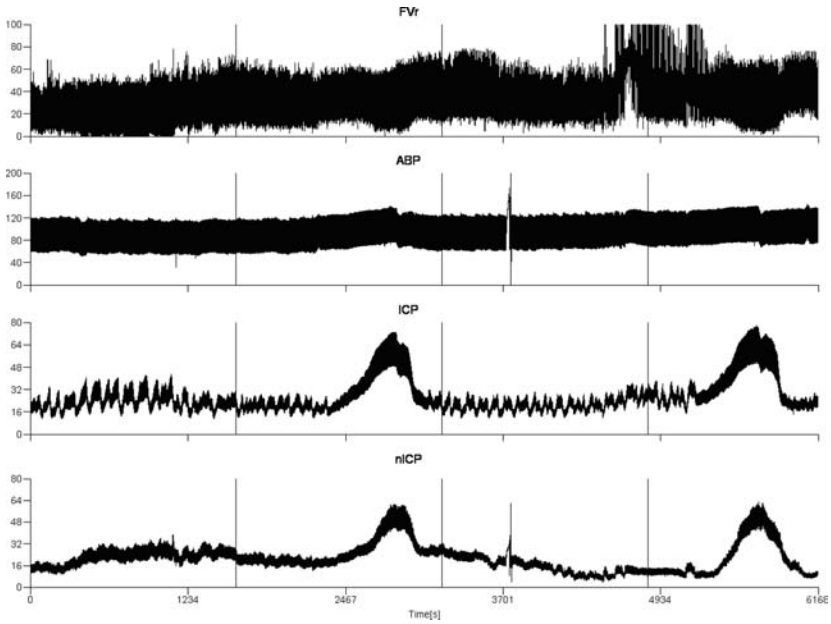

Figure 2.

TCD methods (with easy access to bi-lateral Doppler machines) allow us theoretically to assess interhemispherical gradients of ICP or CPP. As long as CSF communicates freely between different fluid cavities within the brain there should not be any substantial differences in regionally measured ICP. Direct measurements of pressures in two CSF compartments are performed rarely. In head injury intrahemispherical pressure gradients were reported[22].

There are intrahemispherical pressure gradients in critical closing pressure and non-invasive CPP associated with midline shift, side of contusion (assessed using CT) or side of craniectomy[18,23].

Both nCPP and CCP measurements indicate that cerebral perfusion (nCPP) or level of cerebrovascular dilatation (CCP) is greater on the side of contusion or expanding brain in case of midline shift or on the side of craniectomy.

This may support the hypothesis that not a brain tissue volume increase but a vascular expansion is associated with side-to-side differences seen on CT scan[18]. This hypothesis may be further supported by the fact that cerebral autoregulation is worse on the side of contusion or brain expansion[24].

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Interests:

MC was acting (1997-2002) as a consultant to Deltex Ltd (UK) which was trying (in vain) to commercialize 'Neuro-Q' – apparatus for non-invasive measurement of CPP. Now this methodology is incorporated into ICM+ software (www.neurosurg.cam.ac.uk/icmplus) licensed by University of Cambridge Enterprise Ltd. MC has an interest in a fraction of the license fee.

CONTINUOUS MONITORING OF CEREBROVASCULAR AUTOREGULATION

Frank A. RASULO

Keywords: Cerebrovascular autoregulation, Continuous multimodality monitoring, Correlation coefficients.

Introduction

One of the greatest challenges for the neurointensivist is that to avoid secondary brain damage resulting from cerebral metabolic and hemodynamic reactions following initial brain insult. An important tool at hand for the clinician during the neurocritical care management of these patients is neuromonitoring, which must be initiated as early as possible. During the last decade, significant progress has been made in the area of neuro-management. Better understanding of important pathological mechanisms leading to secondary brain lesions and consequences of therapeutics agents on brain physiology have led to changes in standard practice. The development of multimodality monitoring, has contributed positively to these changes, however, while the body of knowledge in regard to central nervous system function and/or the mode of action of centrally active agents on neuronal function has increased, new techniques on how to measure such changes have not developed in parallel through these years. Furthermore, since the aging population of patients admitted to the ICU is rising, so does the risk of cerebral badperfusion or minute signs of degradation of the aging central nervous system. Besides the pathological events to which these patients may be more vulnerable, the exact effect that anesthetic agents and many drugs used in the intensive care unit, like catecholamines, have on brain physiopathology are still being explored. Therefore, it is fundamental

Frank A. Rasulo MD, Medical Researcher
Istituto di Anestesia e Terapia Intensiva – Spedali Civili, Ospedale Universitario di Brescia
Piazzale Spedali Civili, 1 – 25125 Brescia, Italy
Home: +390302522635 – Mobile: +393393366290/+393930057049
Hospital: +39-030-3995 570/764/563
http://www.med.unibs.it/anest/

to continuously monitor the brain which represents the organ with the highest vulnerability and likely to deteriorate faster.

Continuous monitoring

The monitoring of important "neuro" parameters continuously is becoming more and more popular in neurointensive care units (NICU). Besides parameters such as intracranial pressure (ICP) and cerebral perfusion pressure (CPP) , new devices capable of monitoring parameters such as brain tissue oxygenation (PtiO2), brain temperature, cerebral blood flow velocity (CBFv) and brain metabolites, and the gaining popularity of continuous brain functional monitoring have significantly expanded the number of continuously monitored parameters. Furthermore, the adjunct of continuous assessment of the cerebrovascular autoregulation (CVA) status helps to implement a more targeted therapeutic strategy for each individual patient aimed at avoiding or treating physiopathological mechanisms which may lead to secondary brain damage.

Continuous monitoring of ICP

Through the years continuous ICP monitoring has slowly gained popularity in centres throughout the world due to recommendation by experts in the field[1,2]. Despite the association of high ICP and case fatality, there is no concrete evidence demonstrating that monitoring of ICP improves outcome[3] . A recent retrospective cohort study with prospective assessment of outcome sought out to determine in 333 severe head trauma patients whether ICP/CPP-targeted intensive care had any effect on functional outcome and therapy after severe head injury. They conclude that ICP/CPP-targeted intensive care results in prolonged mechanical ventilation and increased levels of therapy intensity, without evidence for improved outcome in patients who survive beyond 24 hrs following severe head injury[4].

Continuous monitoring of CPP and CBF

Great effort has been applied in the monitoring of CPP.
Decreases in CPP can occur either through decrease in BP or increases in ICP. The evidence from recent studies showing that a CPP of 60 mm Hg is adequate for most patients and the fact that cerebral ischemia can occur despite CPP being above this threshold, challenges the present knowledge of CPP oriented therapy in head injured patients by demonstrating no relationship with outcome at CPP levels above 60 mmHg[5,6]. Therefore more specific methods which provide information regarding adequate cerebral blood flow (CBF) are desirable. The ideal monitor for cerebral ischemia is yet to be in-

vented and it should provide information regarding regional CBF, since there can be marked regional differences in CBF (after brain trauma for example). It is important to have this information at hand continuously since CBF evolves over time after injury. Measurement of CBF through imaging techniques such as the stable-xenon-enhanced computed tomography (XeCT), CT perfusion, magnetic resonance perfusion imaging, single photon emission computed tomography (SPECT) and positron emission tomography (PET) is of great help for physicians by providing important insights into the evolution of injury, regional information regarding CBF and the effects of treatments which may alter CBF such as hyperventilation[7]. However, they represent a mere single snap shot in time of CBF. Two commercially available bedside methods for monitoring CBF continuously seem to have produced promising results in literature; the thermal diffusion and laser Doppler flowmetry methods. Unfortunately, in both these methods CBF is measured in a small volume of the brain, which may not reflect the whole brain or the region of importance.

Continuous monitoring
of brain tissue oxygen tension and brain metabolites

Continuous measurement of brain tissue oxygenation has been validated extensively in literature. Gupta A. et al. studied severe head trauma patients who had brain tissue oxygenation monitored and who underwent PET scanning and found that no correlation existed between end capillary oxygen tension and brain tissue oxygen pressure, but found a positive correlation between change in end capillary oxygen tension and change in brain tissue oxygen pressure[8]. A recently published paper concludes that the use of multimodality monitoring with both ICP and brain tissue oxygen tension monitors can be associated with a reduced patient's mortalityrate after severe traumatic brain injury[9] .These findings, however, are based on preliminary results. The exact lower limit of brain oxygen tension is not well defined. Johnston et al. used positron emission tomography, brain tissue oxygen monitoring, and cerebral microdialysis to assess the effects of cerebral perfusion pressure augmentation on regional physiology and metabolism in the setting of traumatic brain injury. Cerebral perfusion pressure augmentation significantly increased levels of brain tissue oxygen and significantly reduced regional oxygen extraction fraction. However, these changes did not translate into predictable changes in regional chemistry. Their results suggest that the ischemic level of brain tissue oxygen may lie at a level below 14 mm

Hg (1.8 kPa); unfortunately, the data did not permit the authors to be more specific[10].

With the event of cerebral microdialysis it is now feasable to measure the concentration of metabolites of brain tissue damage in the extracellular space directly and continuously[11]. There is evidence in literature suggesting that the biochemical changes typical for ischemia may be detected prior to elevation in ICP[12,13]. The future success of microdialysis as a diagnostic tool depends on the choice of biomarkers, their sensitivity, specificity, and predictive value for secondary neurochemical events, and the availability of practical bedside methods for chemical analysis of the individual markers[14].

Continuous monitoring of brain function monitoring

Another form of continuous neuromonitoring is represented by continuous EEG which has become an interesting and useful component of patient monitoring in the neurointensive care unit. In conjunction with other components of multimodality neurologic monitoring, including ICP, CBF, brain tissue oxygen tension monitoring, Transcranial doppler, and microdialysis,it provides unique data regarding the electrical activity of the brain. Fourier-transformed continuous EEG data are being used in ICUs to continuously monitor global cerebral activity and cortical function permitting the intensivist to diagnose nonconvulsive seizures as they appear [15,16].

Continuous somatosensory evoked potentialmonitoring is useful in the intensive care unit (ICU-SEP); one of its confirmed applications is for the prognosis of coma. Brainstem auditory evoked potentials are helpful in distinguishing structural from nonstructural causes of coma and can supplement ICU-SEP in predicting outcome[17,18].

Continuous assessment of cerebrovascular autoregulation

A key in understanding the pathophysiological mechanisms which eventually may lead to secondary brain damage is knowledge of CVA and how it plays an important role in these mechanisms. Many methods exist to assess both static and dynamic CVA, most of which use TCD to assess changes in either resistance in blood flow velocity following changes in blood pressure or direct carotid artery manipulation[19].

More recently, several authors[20-25] have developed new methods of continuously assessing cerebral autoregulation based on spontaneous and small oscillations in arterial blood pressure and CBF velocity. The later, which generate the so-called “B waves”, are determined by

cerebral vasomotor activity in response to fluctuations in intracranial volume. The high-pass filter model described by Diehl and Linden analysed the phase shift angle between oscillations in CBF velocity and MAP obtained by a controlled breathing pattern at a rate of 6/min for 60 seconds[20]. They were able to define the normal pressure reactivity as a positive phase shift angle between MAP and FV of 30° to 70° by testing this method in healthy volunteers and in patients known to have impaired autoregulation. Czosnyka et al define a new index of cerebral autoregulation (Mx)[22] : a moving correlation coefficient between 36 consecutive samples of CPP and mean FV collected at 5-seconds intervals over a period of 3 minutes. The accuracy of this method relies on using CPP and not MAP in order to reduce the chances of detecting "false autoregulation". When autoregulation is impaired, increases in MAP result in a concomitant increase in ICP, leaving CPP unchanged. Therefore, a change in MAP may not result in an increase in FV giving the impression that autoregulation is intact when in fact the lack of change in FV may be the result of an unchanged CPP.

Steinmeier et al used cross-correlation function analysis to study the correlation and the time-delay between periodic oscillations of arterial blood pressure and ICP[23].They concluded that autoregulation is impaired when a positive correlation with zero time is present between periodic oscillations of arterial blood pressure and ICP. When autoregulation was intact, a negative correlation between the two signals with a time delay of 7 seconds was detected. A similar relationship between arterial blood pressure and FV was also detected.

Based on the above principle the Cambridge group introduced the pressure reactivity index (PRx) which is calculated as the moving correlation coefficient between 40 consecutive ICP and MABP measurements occurring every 5 seconds and can be used as an indirect measure of cerebral autoregulation by reflecting changes in cerebral blood volume and ICP in response to spontaneous waves of arterial blood pressure[28,25].This index has been validated as a reliable parameter for continuous estimation of CVA[24]. The Prx varies within a standardized limit ranging from -1 to +1; a negative value indicates an inverse correlation between MABP and ICP, and therefore representing intact CVA. Variations of ABP and CPP induce changes in the arteriolar vascular bed causing active vasoconstriction or vasodilation affecting the ICP which moves in the opposite direction. On the contrary, a positive value of Prx, suggesting a direct correlation between

these two parameters, represents a condition where the cerebral resistance vessels become filled and emptied with blood passively following variations in CPP. In clinical practice it is prefered to consider as impaired and intact CVA a value respectively above or below 0.2. A value in between reflects a transitional area, closer to an intact CVA state. In fact, as suggested by Czosnyka the Prx value should not be considered as an absolute index, but rather as a useful indicator of worsening (negative Prx) or improved (positive Prx) CVA. Prx has the potential of estimating global vasomotor reactivity and represents a more reliable predictor of outcome factor in the severe head trauma patient when confronted with the admission GCS score[22].
A worsening of the Prx has also been correlated to important increases in intracranial pressure[24] although this finding has not been confirmed in a more recent paper[30]. Prx has been confronted with another correlation coefficient, the Mx, also derived from the correlation between CPP and CBFv capable of providing continuous important information regarding the CVA[27].In this study the Prx and Mx correlated significantly. Again, CVA was considered preserved and had a value of <0.2 when the CBFv moved inversely to variations of ABP (or CPP), due to the vasoconstriction/dilation of the cerebral resistance vesselsin an attempt to guarantee constant blood delivery to the brain parenchyma. Vice-versa, when CVA was impaired, ABP (or CPP) and CBFv moved in the same direction, causing the Mx to move to a more positive value. As for the Prx the sensitivity and the specificity were respectively 100% and 90% when confronted with a static index of CVA (sRoR). Lang E. W. also confirmed a strong correlation between Mx and Prx showing that a preserved vasoreactivity was almost always associated with an intact CVA status. However, a previously published paper by Paulson et al. stated that pressure reactivity and autoregulation are not the same phenomenon, as the former may remain intact when the later mechanism is already exhausted[29]. In a study performed by Mascia L. et al. the authors underline the importance of continuous monitoring of CVA as a guide for the treatment of patients with severe head injury.

Continuous assessment of CVA as a guide for optimal CPP

Although it has been suggested that CPP-oriented therapy improves the outcome of head injured patients, the optimal CPP value remains controversial[26]. The CPP >70 mmHg value suggested by Rosner et al

in 1995 and than used by the Brain Trauma Foundation for their guidelines for managing patients with traumatic brain injury, has been questioned by Jull and others[5,6]. Jull et al demonstrated that outcome after traumatic brain injury only correlated with cerebral perfusion pressure when CPP was lower than 60 mmHg and not the 70 mmHg previously described. Furthermore, it is suggested that maintaining excessive CPP increases the risk of vasogenic oedema in patients with impaired autoregulation[21].It is likely that the optimal CPP value varies between patients. This has prompted Steiner and Lang to propose that the optimal CPP should be defined for each patient based on his or her individual cerebral autoregulation status[28,30]. Steiner investigated the use of continuous cerebral autoregulation assessment as a possible guide in optimising CPP in severe head trauma patients. He used Prx as a continuous CVA assessment parameter in an attempt to find the ideal CPP in patients with severe head injury[28]. Optimal CPP (CPPopt) was defined as the patient's CPP where CVA was most preserved, and corresponded to the most negative value of Prx. He was able to define the CPP opt in 60% of their patients. While he was able to link impaired outoregulation to poor outcome , he was unable to correlate CPP value and outcome, thus, confirming the findings of Jull's et al. Furthermore, he clearly demonstrated that the relationship between CPP and pressure reactivity index is similar to that described by Lassen's autoregulation curve, although the upper limit of autoregulation was lower then previously described.

Our experience

At the Institute of Anesthesiology and Intensive Care of the University Hospital of Brescia, Italy, assessment of CVA is done through use of various techniques which assess both static and dynamic CVA. Until recently, the Trans Hyperemic Response Test (THRT) by means of transcranial Doppler was the method most oftenly used. After acquiring the Intensive Care Monitoring system plus (ICM+®,University of Cambridge UK), software system capable of elaborating a large amount of data derived from multimodal parameters, we have been continuously monitoring the CVA status in our patients through use of both the Prx and Mx correlation coefficients. To investigate whether it is possible to find the most suitable CPP for each individual patient, we have adopted into practice Steiner's proposed optimal CPP protocol[28],and evaluated if such a protocol based on the patient's CVA status is capable of improving patient treatment and therefore outcome.

During the year 2005 a total of 30 patients who fulfilled the inclusion criteria (critically ill, comatose patients who underwent invasive intracranial pressure monitoring)was enrolled. Besides severe head trauma (n=14), we extended our selection of patients to include also subarachnoid hemorrhage (n=10), intracerebral hematoma (n=4) and ischemic stroke (n=2). Mean age was 43.2 [S.D.17.5] years, ranging from 17 to 78 years. Outcome at ICU discharge was 7 dead and 23 alive. Mean monitoring time was 100.1 [S.D. 62.6] hours per patient, ranging from 18 to 192 hours, for a total monitoring time of 3102 hours. The highest measured Prx was 0.8, the lowest -0.6. The average CPP was 78,5 mmHg (S. D. 10,5 range 33-109).

We compared Prx to CVA as evaluated by the Trans Hyperemic Response Test (THRT), first described by Giller C.A. and later validated in clinical practice by Czosnyka M, which permits assessment of CVA by means of TCD[31,32]. This is done by insonating the mean cerebral artery and observing changes in CBFv before and after brief carotid compression, providing information regarding changes in cerebral vassels radius induced by CVA. Our results confirm the literature which previously describes a good correlation between Prx and THRT testing[32].

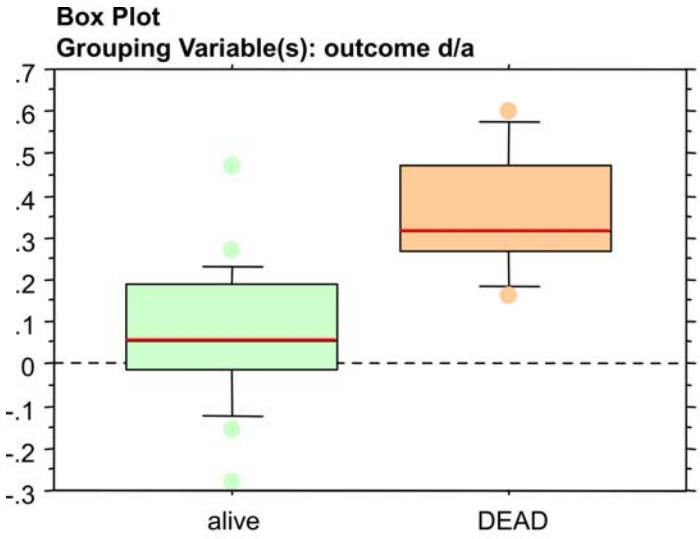
A CPPopt range was identified in 60 days, 42% monitoring time, while a trend was recognized in 49 days (34%). It was not possible to identify a CPPopt range nor a trend in 35 days (24%).

CPP was in the CPPopt range only in 26.3% of periods, below range in 37.1% and above range in 36.6% of periods, signifying that a CPPopt oriented protocol may have changed CPP management in 73.7%.

Patients who died had higher ICP ($p = 0.006$), worse mean PRx ($p = 0.0002$) and longer defective autoregulation crisis ($p = 0.005$) (figure 1). Worse outcome was associated to longer periods where CPP was lower than the CPPopt ($p = 0.0019$), was non significant to shorter periods where CPP remained within the CPPopt range ($p = 0.11$) and, quite unexpectedly, was associated to shorter periods of CPP above the CPPopt range ($p= 0.015$) (figure 2).

We also sought out to verify any correlation between Prx and the Glasgow Coma Score (GCS) on admission. As expected, the average first-day Prx correlated scarcely with the admission GCS. This finding is coherent with the literature regarding this topic[33].

In a similar way, the average Prx calculated during the entire monitoring period was correlated with the GCS on transferral from the



Unpaired Means Comparison for mean
Grouping Variable: outcome d/a
Hypothesized Difference = 0

	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
alive, DEAD	-.297	28	-4.349	.0002	-.436	-.157

Group Info for mean PRx:
Grouping Variable: outcome d/a

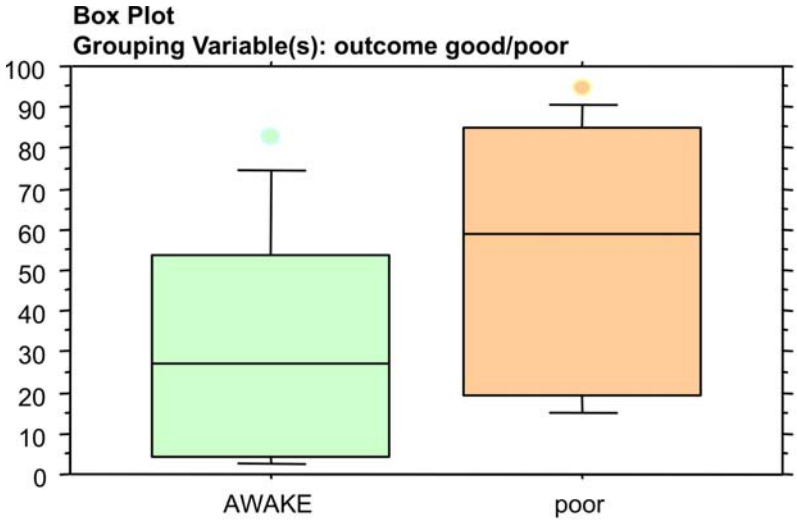
	Count	Mean	Variance	Std. Dev.	Std. Err
alive	23	.068	.026	.160	.033
DEAD	7	.365	.022	.149	.056

Figure 1 – *Box plot showing the correlation between mean Prx and outcome (grouping variables: alive or dead).*

ward for each patient and it was found to be more negative in the patients with a good outcome, therefore, showing correlation.

Discussion

Modern monitoring systems for continuous assessment of CVA are highly sophisticated and capable of elaborating an enormous quantity of data while maintaining a certain flexibility in application.
In particular, the ICM+® recently acquired by our institute is made up of a software able to register, in real time, data which permits continu-



Unpaired Means Comparison for below range
Grouping Variable: outcome good/poor
Hypothesized Difference = 0

	Mean Diff.	DF	t-Value	P-Value	95% Lower	95% Upper
AWAKE, poor	-24.270	26	-2.103	.0453	-47.997	-.543

Group Info for below range
Grouping Variable: outcome good/poor

	Count	Mean	Variance	Std. Dev.	Std. Err
AWAKE	14	30.202	773.279	27.808	7.432
poor	14	54.472	1092.156	33.048	8.832

Figure 2 – *Box plot showing the correlation between outcome and % of time in which the CPP was below the range of CPP opt. As shown, the patients who died or were in coma at the end of the ICU stay (poor outcome) had had a CPP below the CPPopt range for a longer period of time.*

ous monitoring of correlation coefficients, including Prx and Mx. Within the limitations of a small and heterogeneous population, ICM+ proved to be a very strong prognostic tool. Elevated PRx values and defective autoregulation crisis were strongly related to CPP lower than the CPPopt range and to fatal outcome. However, in our series a CPP exceeding the CPPopt range was not related to a worse outcome. We are not certain on how to interpret this finding but it may signify that a CPP slightly above the CPPopt range is at a lower risk of

secondary brain damage due to repeated episodes of reduced CPP when compared to a CPP maintained near the lower limit of CPPopt. It is noteworthy to mention that we observed a considerable variation of CPPopt not only among different patients but from period to period within the individual patient. Knowledge of these variations may permit the clinician to retarget the CPP, and therapy, towards the ideal CPP which may vary greatly from admission and throughout the ICU stay. As mentioned previously, the average Prx during the first few days after admission did not correlate with the admission GCS score, yet there was correlation between the average Prx of the whole monitoring period and the GCS score on transferral from the NCCU. We interpreted these data as being the result of the fact that in our patients their initial clinical condition may have depended from factors not related to the preserved vascular reactivity but rather to the primary brain damage. The presence of an altered CVA on admission would then contribute to the developing of secondary brain damage during the hospital stay, confirming the Prx as being an important prognostic index. A drawback of the ICM+® may be the fact that it requires an initial registration period of at least 2 hours before good quality information can be derived from this system, making it inapplicable during the first few hours after admission. Our data show that the most frequent initial range at which the ideal CPP was distributed in our group of patients was from 70-80 mmHg, suggesting this range of pressure as being the most suitable for initial CPP management.

Conclusion

Advances in technology are taking place at a rapid pace resulting in the availability of newer devices which have made neuromonitoring truly multimodal. An important role is played by cerebrovascular autoregulation monitoring and its continuous assessment may be the key which links physiopathological mechanisms of secondary brain damage to a more targeted treatment. In our study, continuous CVA assessment with the Prx confirmed itself as being an important adjunct to CVA monitoring. Although necessitating invasive ICP monitoring, It consents continuous global evaluation of CVA without carotid artery manipulation.
Larger multicenter clinical studies are needed so as to find out the best monitoring modalities capable of changing the management strategies of patients with encephalic pathologies. The main challenge for clinicians and researchers will be to understand how these

different aspects of multimodality monitoring relate to each other, and how physiologic variables such as CVA, CPP, osmolality etc. can be manipulated to optimize cerebral function and tissue survival in the setting of acute injury.

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REGENERATION FOLLOWING CNS INJURY, FROM EXPERIMENTAL MODELS TO HUMANS: WHERE ARE WE?

Simone DI GIOVANNI

Abstract

Regeneration in the adult Central Nervous System following injury is extremely limited. Traumatic spinal cord injury as a paradigm of CNS injury is characterized by a permanent neurological deficit followed by a very limited recovery due to failed regeneration attempts. In fact, it is now clear that the spinal cord as much as the brain have intrinsically the potential to regenerate, but cellular loss and the presence of an inhibitory environment strongly limit tissue regeneration and functional recovery. The molecular mechanisms responsible for failed regeneration are starting to be unveiled. This gain in knowledge led to the design of therapeutic strategies aimed to limit the tissue scar, to enhance the pro-regeneration versus the inhibitory environment, and to replace tissue loss, including the use of stem cells. They have been very successful in several animal models, while results are still controversial in humans. Nonetheless, novel experimental approaches hold great promise for use in humans.

Introduction

Spinal Cord Injury (SCI) is a major cause of disability among the young population worldwide. This also makes its social costs particularly challenging due to the high cost of the long term care and rehabilitation of these patients. In the USA there are an estimated 10.000 new cases a year and about 200.000 people living with SCI related disability. The annual aggregate direct costs including emergency care, hospitalization, supplies, medications, cost for physicians, outpa-

Simone Di Giovanni, MD, PhD
Department of Cellular Neurology – Hertie-Institute for Clinical Brain Research
University of Tubingen – D-72076 Tubingen, Germany
Department of Neuroscience – Georgetown University – Washington DC, U.S.A.

tient services, and long term rehabilitation amount to an estimated 14.7 billions dollars a year[1].
The neurological sequelae of SCI in humans lead to permanent locomotion impairment, pain, bladder, sexual, and several autonomic dysfunctions, for which a therapy is not available as yet.
Traumatic injury to the spinal cord in humans and in mammalian animal models causes a variety of reactive processes. The main common gross pathological alterations include an immediate ischemic and hemorrhagic response at the center of the injury, followed by cell loss and cavitation within days, and finally by connective tissue invasion at and around the injury epicenter in weeks and months[2-5]. The extent of these processes varies depending upon the intensity and the modality of the trauma and presents some variation between species. For example, typically a contusion injury to the spinal cord in rodents results in a less pronounced cavitation than in humans[2,6].
Unfortunately, the capacity of the Central Nervous System (CNS) to regenerate axons and re-establish a proper functional neuronal network after injury is extremely limited, and it correlates to the very poor clinical recovery[5]. Importantly though, studies in animal models suggest that a very low percentage of spared descending axonal tracts, between 5 and 10% are needed to maintain a reasonable locomotion[7]. This leads to great hope for therapeutic interventions aimed to spare or re-establish these connections.
SCI can be used as the prototype disease model for neurodegeneration, and the spinal cord the prototype location to study the molecular mechanisms and the opportunities for regeneration and re-establishment of a functional neural circuit.
In fact, the known cause of the damage: trauma, and the straightforward evaluation of the functional behavioral and neurophysiologic outcomes as compared to idiopathic disorders of the brain, make the injured spinal cord an ideal experimental model to investigate the mechanisms for the limited recovery of function following injury to the CNS. It may also constitute a good model to design and develop therapeutic strategies. Obviously, not all the lessons learnt from this model can be merely translated to disorders such as Alzheimer's, Parkinson's, or multiple sclerosis, but the basic mechanisms of response to injury seem to be quite reproducible across these different degenerative conditions.
Here, I will discuss the most relevant molecular and cellular changes following SCI and the main reasons for the failed spontaneous regen-

eration. Also, I will consider the available solutions to favor regeneration and functional recovery, and the most promising clinical applications for the treatment of human spinal cord injury.

Experimental approaches to promote regeneration and recovery of function following SCI

Traumatic injury to the spinal cord results in delayed biochemical alterations that may result in cell death but also survival/restoration, including successful target re-innervation[8-11]. In part, functional recovery reflects the number of surviving cells and fiber tracts, the extent of neural plasticity, and/or the presence of a permissive environment for regeneration. Such processes are substantially regulated by gene expression changes; temporally, these alterations include an earlier phase associated with inflammation, extension of axonal damage, cell death and loss, and a later one characterized by tentative axonal regeneration and the formation of an inhibitory environment and of the tissue scar.
Therefore, the success of the reparative processes depends upon factors involved in:

1. protecting and replacing the original cellular environment;
2. overcoming the inhibitors of axonal regeneration and limiting the scar formation;
3. facilitating the spontaneous mechanisms of neurite outgrowth and axonal regeneration;

Collectively, these factors likely determine the degree of anatomical and functional recovery after injury.

Cell and tissue transplantation

Strategies to promote axon regeneration and functional recovery include the use of cell or tissue transplantation. They aim to replace the loss of both glia and neurons with new functional cells or to provide a better environment for axon regeneration. Transplants consist of the use of peripheral nerve grafts, Schwann and olfactory ensheathing glia cells (OEG), inflammatory cells, embryonic or fetal spinal cord transplants, and stem cells.
Peripheral nerve grafts have been transplanted across the two ends of a transected spinal cord in rats and mice for quite a long time. Results have been modest as supra spinal descending fibers cannot cross the grafted tissue and form synapses[12-14]. Better luck had the combination of intercostals peripheral nerve grafts with fibrin glue soaked with

fibroblast growth factor across a complete thoracic cord transection. The creation of this nerve bridge with fibrin and fibroblast growth factor enhanced axon regeneration of the corticospinal tracts, promoted partial locomotor recovery, and resulted in amelioration in evoked motor potentials in the hindlimbs[15,16]. A similar approach has been used in human spinal cord injury in several patients, but no results were made available.

Transplantation of *Schwann cells* has the goal to provide myelinated axons in the injured cord and potentially bridge the injury site. They have been administered in suspension following contusion injury or over fibrin or matrigel substrates in the case of transection models[17-19]. When used alone Schwann cells form either rodents or primates do not provide better results than peripheral nerve grafts, but in combination with the neurotrophins BDNF and BDNF plus NT-3 were able to promote axon regeneration across the transected spinal cord[20,21].

Interestingly, human Schwann cells transplantation in combination with methyl prednisone in nude rats to limit the immune rejection, resulted in extension of supraspinal axons across the transplant and in modest regeneration of propriospinal axons and mild functional recovery[22].

OEG accompany olfactory growing axons in their entry to the central nervous system. They have specific features responsible for their regenerative properties such as the molecular composition of the membrane which express several key adhesion molecules combined with their ability to produce neurotrophic factors, reduce glial scarring and to accompany new growing axons into the host CNS[23]. Several studies reported that the implantation of OEG in a complete or partially transected rat spinal cord resulted in improved axon regeneration of supraspinal long descending fibers across the injury site and promoted functional recovery even a few months following SCI[24-29]. OEG transplantation has been performed on hundreds of patients with SCI in China, and partial recovery of function has been reported[30]. Nevertheless, interpretation of these results requires extreme caution as these studies do not follow standard clinical trials criteria.

The rationale for the delivery of *activated macrophages* in the injured cord resides in the properties of macrophages to secrete cytokines and growth factors that potentially promote axon regeneration and clear cellular debris. Pro-inflammatory cytokines and proteoglycans are also abundantly secreted by macrophages following SCI and contribute to cell loss and scar formation[31-37]. A study published in 1998

showed that the implantation of stimulated homologous macrophages following transection of the thoracic spinal cord resulted in improved axon regeneration and partial recovery of paraplegic rats[38]. In contrast several reports showed in both transection and contusion models of rat SCI that the activation of macrophages worsens the pathology and the recovery of function[34-36,39]. Also, pharmacological depletion or reduction of macrophages and microglia is actually protective following both brain and spinal cord contusion injury[37,40-42]. Therefore the rational for therapeutic use of macrophages remains highly controversial. Nonetheless, clinical trials using activated monocytes are currently taking place in human spinal cord injury and preliminary safety data has been recently released[43].

Embryonic and fetal spinal cords have a better capacity for axon regeneration as they probably replicate the plasticity of the nervous system which is proper of the developmental stages. Embryonic and fetal spinal cords transplants have been used successfully in partial transection models (hemisection) in rodents and cats in particular in association with neurotrophins injections and resulted in long term improved axon regeneration from supraspinal sites across the transplant and from the transplanted tissue itself[44-46]. Functional recovery was also present in a few cases[44,45,47].

The use of *stem cells* for cell replacement purposes following spinal cord injury is potentially an ideal approach because the capacity to differentiate into multiple cell types can accommodate the need of the spinal cord to replace the loss of both neurons and glia[48-51]. The specific differentiated phenotype of transplanted stem cells depends on several factors, including the type of stem cells (adult vs embryonic, committed or not toward a specific phenotype, in this case we can more properly define them as progenitors cells), the developmental stage of the host, and the level and features of the damage of the recipient spinal cord. Stem cells from rat or mouse embryos mainly differentiate into glia lineage after transplantation in both the injured and intact spinal cord[51-53]. To generate a significant amount of neurons, neuronal stem cells collected from specific areas of the adult brain or cord are better suited[54].

Typically, problems with stem cells transplantation are the occurrence of inappropriate differentiation of cell types in the wrong place at the wrong time in the wrong amount. The clinical implications observed in animal models are mainly the occurrence of pain and allodynia, probably due to aberrant axonal sprouting, and failed func-

tional improvements[55,56]. Nonetheless, several reports to date have documented the efficacy of stem cells transplantation in SCI[55]. Wild type or *in vitro* genetically modified to enhance their neuronal differentiation potential, neural stem cells were grafted in adult rats following contusion SCI and resulted into enhanced neurogenesis and improved functional recovery[57-59]. Transplantation of embryonic stem cells into injured rat spinal cord also resulted into improved functional recovery, and differentiation mainly towards the glial lineage . In other cases, embryonic stem cell-derived oligodendrocyte progenitors were successfully used to restore myelination and locomotion in SCI in rats . Also, delivery of neurotrophins through genetically modified neural stem cells proved to mediate a limited but significant recovery following SCI in rats. The exact anatomical reasons of these improvements are not clear yet. None of these studies actually showed an effective integration of transplanted progenitor cells into the neural circuitry until recently.

Recently, a study showed that transplantation of human CNS stem cells derived from fetal brains in immunodeficient mice induced locomotor improvements following SCI, and that stem cells survived, engrafted and differentiated into neurons forming synapses, and into oligodendrocytes forming myelin. This study represents a major leap forward in the potential use of human stem cells for spinal cord injury repair . Along the same lines, another study demonstrated that human neural stem cells transplanted into primates (marmosets) differentiated into neurons and glia and were associated with functional recovery following contusion SCI .

Lastly, an important alternative to the classical stem cell approach is the use of the stem cell like human teratocarcinoma cells that are pluripotent, replicate indefinitely and can actually differentiate into glia or neurons after transplantation *in vivo* in both brain and spinal cord . The risk for the use of these cells is their neoplastic potential, but several studies have already suggested that such risk is very low . These cells have a potential similar to embryonic stem cells but present the major advantage to be easily available, and do not have any ethical issues. Remarkably, these cells *in vitro* form synapses very similar to primary neurons , but it is not clear as yet whether they form a perfectly functional neuronal circuitry *in vivo*. These cells differentiate into glia and neurons following Retinoid Acid (RA) administration. RA signaling plays an essential role during CNS development and has recently been implicated in axon regeneration following SCI . A

recent review covers their implications for therapy in SCI regeneration .

Inhibitors of axonal regeneration and scar formation

The reason for the failed axon regeneration in the CNS as opposed to the successful regeneration in the peripheral nerves is to be found in a complex network of molecules and pathways that are specific of the CNS and limit axon regeneration post-injury . Importantly, they are also likely to regulate proper axon growth and target recognition. That may imply that the inhibition of these pathways can be quite a risky approach, as it may lead to not only to increased axon growth but also to side effects, such as pain and failed locomotor benefit, due to improper axon growth and re-connectivity into the complexity of the neural network.

Critical inhibitors of axon outgrowth and regeneration are myelin derived molecules, expressed mainly in oligodendrocytes and they include the transmembrane proteins NogoA , myelin associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) . These three proteins signal through a common receptor, the Nogo receptor (NgR). NgR forms a complex with the low affinity neurotrophin receptor p75 and with LINGO-1 , and this molecular complex activate the GTPase RhoA, that leads to the recruitment of Rho kinase which in turn mediates cytoskeleton remodeling and inhibits neurite and axon outgrowth .

Experimental evidence showed that NogoA is specifically involved in growth cone collapse and inhibition of neurite outgrowth *in vitro* . *In vivo* studies showed that axon regeneration is inhibited following peripheral nerve transection in transgenic mice expressing NogoA under the control of a P0 promoter, specific for peripheral myelin forming Schwann cells . The importance of Nogo family members, including the alternative spiced isoforms Nogo A, B and C in axon regeneration and functional recovery in the CNS is sill under debate. In fact, in 2003, three studies from three different groups reported discordant effects on axon regeneration in mice lacking two of three of Nogo isoforms in a model of spinal cord dorsal hemisection . Significant improvement in axon regeneration was found only by one group in mice lacking NogoA/B . Experiments by the other two groups showed that, although Nogo-A/B-deficient myelin had reduced inhibitory activity in a neurite outgrowth assay in vitro, tracing of corticospinal tract fibers after dorsal hemisection of the spinal cord did not result in increased regeneration or sprouting of these fibers . This data sug-

gests that other molecules and may be pathways are required to induce extensive axon regeneration in the spinal cord. In support of this hypothesis, more recently, neither NgR-nor p75(NTR)-deficient mice showed enhanced regeneration of corticospinal tract axons in comparison with wild-type controls after spinal dorsal hemisection .
The second protein binding to NogoR is MAG, which inhibits neurite outgrowth in adult or post-natal neurons, while enhances neurite outgrowth in pre-natal DRGs or embryonic retinal ganglion or spinal neurons . Importantly, following injury in the adult MAG is mainly an inhibitory molecule. MAG knock out mice show enhanced axon regeneration following transection in the peripheral nerve, but not in the spinal cord . Also, transgenic mice expressing MAG under the control of the p75 promoter show retarded peripheral nerve regeneration after transection . Again, this data is similar to what is observed in the case of Nogo: axon regeneration is affected in the peripheral nerve but not in the CNS.
OMgp, the third NogoR ligand, has been only characterized *in vitro* so far as far as its inhibition on neurite outgrowth and induction of growth cone collapse .
Nonetheless, the use of antagonist antibodies against Nogo has been able to promote axon regeneration and functional recovery in several models of SCI in rodent . Also, a small peptide that can compete with Nogo binding to the receptor can enhance regeneration of corticospinal tracts following spinal cord hemisection in rats . Importantly, a recent study with anti-Nogo antibodies in spinal cord hemisection models in primates showed improved axon regeneration of the corticospinal tracts . Also, a pilot unpublished study from the same group showed behavioral recovery following Nogo antibodies administration in monkeys in a hemisection SCI model .
Downstream from the receptor complex formed by NgR, p75 and LINGO-1 is the GTPase mediated activation of RhoA , which, through a still unclear downstream signaling, mediates growth cone collapse. Importantly, RhoA and p75 are induced following SCI in both glial and neuronal cells , and they are likely involved in both neurite outgrowth and apoptosis. Recently another co-receptor, which belongs to the TNF family members named TAJ was described. It is broadly expressed in postnatal and adult neurons, binds to NgR1 and can replace p75 in the p75/NgR1/LINGO-1 complex to activate RhoA in the presence of myelin inhibitors . *In vitro* exogenous delivery of TAJ reversed neurite outgrowth caused by myelin inhibitors molecules.

Also, neurons from Taj-deficient mice were more resistant to the suppressive action of the myelin inhibitors .
Inactivation of Rho signaling was first reported to rescue axon regeneration in a rat model of optic nerve crush . More recently, in a rat model of spinal cord dorsal hemisection, RhoA and Rho kinase inhibition using C3 transferase or Y27362 were able to promote corticospinal axon regeneration, collateral sprouting and increase plasticity in the motor cortex, in association with improved recovery in locomotion . Modulation of Rho pathway can therefore potentially represent an efficient strategy to promote axon regeneration.
Another class of inhibitors of axonal regeneration is represented by the proteoglycans, the chondroitin (CPSG), heparin (HSPG) and keratin sulfate (HSPG) proteoglycans. Their expression is linked to the proliferation of reactive astrocytes and microglia, but can be produced also by oligodendrocytes, and they represent the major players in the glial scar formation that inhibits axon regeneration from days to weeks and months following SCI . They are variably upregulated following SCI at both the mRNA and protein levels . The best characterized and potential targets for therapy are the CPSG, including brevican, neurocan and versican, which are directly produced by reactive astrocytes, and are responsible for blocking and re-directing axon growth .
Importantly, intrathecal injection of chondroitinase ABC has been used to inactivate CSPG by removing the glycosaminoglycans chains in order to neutralize the inhibitory effects of CPSG on axon growth and the formation of the glial scar. This approach increased regeneration of descending CST and ascending sensory fibers in a model of dorsal hemisection in rats along with enhanced locomotion . Improved locomotion was also observed with a similar treatment regimen in a contusion model of SCI in rats .
Recently, a possible convergence between proteoglycans and myelin inhibitors of axon regeneration was discovered through the activation of the epidermal growth factor receptor (EGFR). In fact, suppression of the kinase function of EGFR blocked the inhibition of CSPG and myelin proteins on neurite outgrowth, and blocking EGFR signaling enhanced axon regeneration in the injured optic nerve *in vivo* . Therefore, local administration of EGFR inhibitors can be now considered as an alternative therapeutic strategy to promote axon regeneration.

Pro-neurite outgrowth and potentially pro-axonal regeneration molecules

"Regeneration-associated proteins" (RAGs) appear to play a role in neurite and axon outgrowth and axon regeneration following SCI. These include transcription factors such as c-jun and p53 (personal communication), cytoskeleton associated proteins such as Tubulin, Coronin 1b and Rab 13, microtubule associated proteins, growth associated proteins (Gap-43, Cap-23) , cell adhesion molecules (N-CAM, L1, TAG1) , cytokines and extracellular matrix components (Snap25, Munc13, and cpg15/neuritin, attractin) . In some cases, these factors share common molecular pathways. For example Gap-43 and Cap-23 bind downstream to the cofactor PI(4,5)P(2), at plasmalemmal rafts, contributing to the regulation of actin and modulating neurite outgrowth in neuronal-like cell lines . The only case of successful CNS axon regeneration *in vivo* due to overexpression of pro-neurite outgrowth molecules was the combined overexpression of the growth cone proteins GAP-43 and CAP-23, which was able to increase axon extensions in transplanted DRG neurons following SCI in rats . The role of neurotrophins in neurite and axon outgrowth and regeneration has been studied for many years and has been extensively covered in several reviews . Its importance is well established as far as it concerns the development of the nervous system and peripheral nerve regeneration, but their efficacy for the therapy of the damaged CNS is still controversial. The low or absent expression levels of the three major neurotrophins receptors Trk A, B, and C following SCI make it unlikely that neurotrophins can signal through established pathways as they do during development of peripheral axonal regeneration . Nevertheless, a few studies in experimental models of SCI showed promising results. For example nerve growth factor (NGF) can promote axon growth when administered on fetal spinal cord transplants or peripheral nerve grafts . The reason of this positive effect may be due to the higher expression of TrkA receptors in these tissues than in the adult spinal cord. Delivery of brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) were also able to increase growth of descending axons in fetal spinal cord transplants . Both BDNF and NT-3 secreting fibroblast were also transplanted in the adult spinal cord following dorsal hemisection and results in improved axon growth and partial functional recovery .

The major issue for the therapeutic use of neurotrophins in humans is the induction of pain, likely mediated by the growth of the small diam-

eters peptidergic fiber afferents into the spinal cord that cause hyperalgesia. Indeed, hallucinations, depression, fever, muscle pain, and various dysautonomic responses were observed in several clinical trials using systemic administration of neurotrophins, which limited the clinical evaluation of the efficacy of the treatment . Certainly downstream effectors of neurotrophins dependent signaling pathways such as cyclic AMP (cAMP) may also be a valid alternative for facilitating axon regeneration. cAMP is a very ancient signaling molecule, produced from ATP by adenyl cyclases and is degraded to AMP by phosphodiesterases . Importantly, intracellular levels of cAMP at the growth cone regulate cone attraction (high levels= or repulsion (low levels) to guidance clues . Also, its levels are higher in developing neurons rather than in older ones, and are associated with neurite outgrowth. In fact, neurons cultured on myelin inhibitory substrates have low levels of cAMP, and induction of cAMP restores the neurite outgrowth properties of these neurons. In an elegant set of experiments it was shown that pre-conditioning lesion of the peripheral branch of DRG neurons followed by cut of the dorsal columns of the spinal cord results in increased regeneration of ascending axons, and these effects are largely mediated by the increased levels of cAMP and are inhibited by blocking PKA signaling . Also, induction of cAMP levels alone promotes axon regeneration of lesioned spinal neurons . Finally, partial functional recovery and axon regeneration through transplanted embryonic spinal cord tissue were promoted in a model of spinal cord hemisection in rats by elevation of cAMP levels following subcutaneous delivery of the phosphodiesterase inhibitor Rolipram . Also, the combination of a delivery of intraspinal cAMP analog and subcutaneous Rolipram in a contusion SCI rat model resulted in increased axon regeneration and locomotion .

The molecular mechanisms that mediate the effects of cAMP on axonal outgrowth have not been elucidated, yet modulation of cAMP seems one of the most promising way to regeneration.

A very interesting recent study showed that another molecular pathway dependent upon retinoic acid (RA) signaling, which also induces cAMP levels, plays a role in axon regeneration. In this study, signaling through retinoic acid receptor beta2 (RARbeta2), which is critical in development for neuronal growth, enabled adult neurons to grow in an inhibitory environment . In fact, overexpression of RARbeta2 in adult rat dorsal root ganglion cultures and *in vivo* increased intracellular levels of cAMP stimulated neurite outgrowth and en-

abled axons to regenerate across the inhibitory dorsal root entry zone and project into the gray matter of the spinal cord. The regenerated neurons enhanced second-order neuronal activity in the spinal cord, and RARbeta2-treated rats showed highly significant improvement in sensorimotor tasks . These findings show that RARbeta2 induces axonal regeneration programs within injured neurons and may thus offer new therapeutic opportunities for CNS regeneration.

Clinical applications for regeneration following SCI

Classical therapeutic trials in human spinal cord injury have traditionally focused on the limitation of the secondary damage responsible for cell death and tissue loss. The most comprehensive ones were three subsequent multicenter randomized clinical studies that evaluated the effects of methylprednisolone on functional recovery in SCI patients . The effects were very modest and considered by some not significant, also due to design and statistical analysis issues. To date the efficacy of the use of methylprednisolone in SCI patients is still under debate . Another trial using GM-1 gangliosides with the goal to promote protection, axon regeneration and myelination did not impact functional recovery any better than steroids, and design and statistical issues confounded data interpretation as well .

Following the example from experiments in animal models, a few preliminary clinical trials are undertaken at the moment with the goal to enhance axon regeneration and locomotor recovery. Most are still evaluating the safety and very few data are available at the moment. Following trauma, fetal tissue has been transplanted in spinal cord in several patients, safety was established but no functional gains were reported . Human fetal stem cells have been injected at the lesion site, but no data from this phase 2 trial is available yet . Reports form South East Asia of transplantation of peripheral nerve grafts and neurotrophins and of OEG cells are anecdotal, and would deserve a better and clearer experimental design.

Proneuron Biotechnology has a phase 2 trial using delivery of activated autologous monocytes below the injury site, but the use of monocytes is very controversial due to the likely inflammatory and pro-cell death effect on the spinal cord.

Preliminary endeavours or declaration of intents form biotechnology companies to transplant autologous progenitor cells into the injured cord or the modulation of the myelin inhibitory pathways appear quite promising . Inhibition of the Rho signaling by developing the avail-

able Rho and Rho kinase inhibitors, and the human chondroitinase ABC are currently under development and pledge to improve axon regeneration limiting growth cone collapse and scar formation.
Probably, the most promising approach is the use of NogoR antagonist peptide or antibodies. Studies in primates have already shown its safety and efficacy in improving axon regeneration and functional recovery , and now clinical trials in humans have been planned.
Last but not least, a list of clinical trials is available on the NIH website (http://www.clinicaltrials.gov/search/term=Spinal+Cord+Injury) and is aimed to improve the quality of life of patients with SCI that do not necessary target axon regeneration and recovery of locomotion. Levatiracetam and venlafaxina have been used to reduce neuropathic pain, oxybutyrin to improve bladder function by fighting the hyperreflxia of the detrusor, and anabolic drugs to limit muscle atrophy due to both denervation and immobilization.

Conclusions

Amazing progress has been achieved in the last decade to enhance the understanding of the biological mechanisms sustaining and limiting axon regeneration. This allowed delineating several major players in the control of axon regeneration. Also, the advent of stem cells technology combined with genetic engineering have contributed to the possibilities related to cell and tissue transplantation to favor bridging of the lesion and repopulation of the damaged environment.
Nevertheless, I believe that the main lesson we have learnt is that there is not a single factor or a single pathway that we can target to dramatically improve axon regeneration and functional recovery. Probably, the winning approach to impact recovery in patients with SCI will be the use of a combined strategy that favors axon regeneration, limits growth cone collapse and scar formation, and delivers new multipotent cells. A potential strategy to achieve such ambitious goals and promote axon regeneration could be represented by delivering embryonic stem cell genetically modified to be driven towards a neuronal phenotype, to express pro-neurite outgrowth transcription factors, to express molecules that maintain high levels of cAMP and block the axon growth inhibiting Rho pathway. This is where the future could take us and with it the hope for a real improvement for patients with SCI.

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STEM CELLS IN THE ADULT HUMAN BRAIN

Morten C. MOE, Mercy VARGHESE, Iver A. LANGMOEN

Keywords: neural stem cells, human brain, differentiation, action potentials, synaptic connections

One of the most important breakthroughs in the history of neuroscience was the discovery of the *reazone nera* by the Italian histologist Camillo Golgi. This technique made it possible for Nansen, Hiis, and Forrell to prove the neuron doctrine and for Ramón y Cajal to develop an extensive map of the neuronal connections in the central nervous system. In essence, these discoveries and studies lay the foundation for many of the developments within the field of neuroscience in the 20th century. The early neuroanatomists did, however, not observe mitotic cell divisions and other evidence for the formation of new neurons in the central system in adult mammals. This forced them to conclude that new nerve cells are not generated in adult vertebrates. Infact, Cajal was very distinct in this regard when he expressed that "*nothing can regenerate in the brain or spinal cord, everything must die"*[1].

For almost a century this remained a central axiom in neuroscience, although Altman in the 1960s suggested that new neurons might be added in certain areas even long time after birth[2]. Adequate methods for demonstrating this were, however, not available at that time and formal scientific documentation for neuronal regeneration had to await further developments in the techniques utilized by neuroscientists[3].

In the mean time Nottebohm and others discovered that vocal centers in the brain of birds increased in size prior to the breeding season, when their singing is most active[4]. Despite this, they did not see any signs of neuronal recruitment from the local cell population. By examining the whole brain, however, they did discover cell divisions in the ventricular wall and cell migration from this area to the vocal centers.

Morten C. Moe, Mercy Varghese, Iver A. Langmoen
Vilhelm Magnus Center for Neurosurgical Research, University of Oslo, Oslo, Norway

Prof. Iver A. Langmoen, MD PhD
Vilhelm Magnus Center/Department of Neurosurgery, Ullevål University Hospital – 0407 Oslo, Norway
Tel. +47 911 24 513 – laiv@uus.no

They concluded that prior to the breeding season, new neurons are produced in the vocal centers by cells emigrating from the ventricular walls.
More recently, new technical developments made it possible to reexamine the possibility of neuronal regeneration in the mammals. Firstly, the BrdU technique (BrdU is integrated in the genome of dividing cells) made it possible to identify proliferating cells[5,6], and secondly the introduction of type-specific immunohistochemica[7] stainings made it feasible to identify neurons histologically by a relatively high degree of certainty. Animals could then be exposed to BrdU for a defined period of time, sacrificed, and the brains stained for neuronal antigens and BrdU respectively. Newly generated neurons could thus be identified by double labeling and examination by confocal microscopy and orthogonal sections.
Reynolds and Weiss isolated cells from the striatum of adult mice and induced proliferation by epidermal growth factor[8]. Subsequently, subsets of the cells developed the morphology and antigenic properties of neurons and astrocytes. Some of the newly generated cells were also immunoreactive for the neurotransmitters GABA and substance P. This was followed by a number of studies indicating that neurogenesis could take place *in vivo* in adult animals, and that populations of stem or progenitor cells giving rise to new neurons on a more regular basis were found at least in the ventricular wall and in the dentate area[5]. There are also several studies suggesting that progenitor cells with a multi-linage capacity may exist in other regions of the central nervous system, but that the local environment is not permissive for neurogenesis[5,9].
It took another 13 years, however, before it was proved that the new cells were neurons. In this context it is important to realize that a functional neuron only can be identified by proving that the cell in question has the following properties: Firstly, it must have the ability to generate short lasting, low threshold action potentials, and secondly it must have the ability to communicate with other neurons by synapses[10]. Identification of neurons by histochemical techniques alone is uncertain - both we and other groups have seen multiple examples of cells staining with so-called mature neuronal markers without having the ability to produce action potentials. In 2002, however, Fred Gage and collaborators published two studies where they had identified new-born cells in the adult rodent brain which had the capability of producing short lasting, low threshold action potentials and had established synaptic connections[11,12].

The situation in the human brain was quite unclear until the late 1990s, as it for obvious reasons was unthinkable to administer research substances to living humans for the purpose of identifying newborn cells. BrdU is, however, sometimes given to cancer patients for diagnostic purposes and Ericsson and co-workers used this unique opportunity[13]. They were granted ethical permission to examine these brains post mortem and co-stained for the frequently used neuronal marker NeuN and BrdU. Doing this they could identify cells that both (1) were born during the period the patients had been given BrdU and (2) expressed neuronal antigens. Other studies, utilizing brain tissue removed for instance during temporal lobe resections due to epilepsy, showed that it was possible to develop monoclonal cultures of cells from the adult human brain and differentiate these cells into mature cells with immunohistochemical characteristics of oligodendrocytes, astrocytes, and neurons, the three principal building blocks of the brain[14-17] (Fig. 1). Still, the question remained whether the new cells were functional neurons.

In a preliminary study we utilized the stem cell culturing techniques developed by Johan Frisèn and collaborators and the immunohistochemical techniques fine-tuned by Michael Svensson and Britt Meijer, together with the patch-clamping techniques in our laboratory at the University of Oslo[18]. In this study we observed that over a period of three weeks, neuron-like cells went through a development with up-regulation of inward Na^+ currents, hyperpolarization of the cell membrane and development of quite major-looking action potentials. The average resting membrane potential after three weeks of differ-

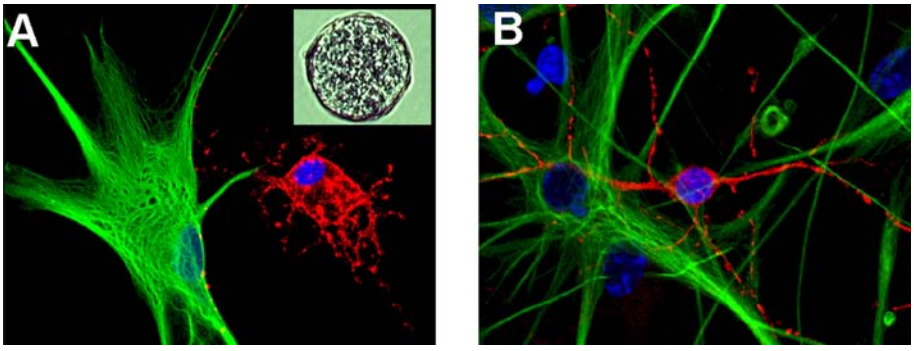

Figure 1 – *Cultivation of ventricular wall tissue from the adult human brain gave rise to neurospheres (**A** inset) containing multipotent stem/progenitor cells with the ability to differentiate into cells expressing glial (GFAP, green), oligodendroglial (**A**, RIP, red) and neuronal (**B**, βIII-tubulin, red) markers.*

entiation was -58mV and the input resistance 396 MΩ. On the other hand, glia-like cells had a more negative resting potential (-68mV), a lower input resistance (84MΩ), and a much faster time constant (8ms). Thus, we were not only able to reproduce earlier findings that cells differentiating from a proliferating cell population harvested from the ventricular wall of the adult human brain may develop into cells expressing antigens associated with oligodendrocytes, astrocytes, and neurons, but more importantly that the glia-like and neuron-like cells had functional properties of the specific cell types.
We further found cells where the nucleus stained by NeuN and the terminal of a long process by synaptophysin[19]. These results suggested quite strongly that a number of the cells developed into neurons. It was, however, still necessary to demonstrate that the cells developed functional synaptic connections, more mature action potentials, and to correlate neuronal generation *in vitro* with previous studies on functional neuron development *in utero*.
We therefore decided to prolong the period of differentiation and were able to, on a regular basis, to keep the cells for at least four weeks. The extensive epilepsy program at Rikshospitalet in Oslo provided sufficient tissue for systematic patch-clamp studies at different timepoints of development. Working on monoclonal cell populations from a number of surgical specimens we found that over a period of four weeks in culture, the cells went through characteristic steps of morphological and electrophysiological development[19,20] (Fig. 2). Early in development neurons developed a polarized appearance which eventually resulted in multiple dendrites. Patch-clamp studies of individual cells revealed that the first active membrane properties (i.e. voltage-gated channels) appeared after one week and consisted of a voltage-regulated K^+-current. Later in the second week voltage-clamp studies showed the presence of voltage-gated Ca^{2+}-channels. In current clamp, these cells fired small Ca^{2+}-driven action potentials. The first signs of a voltage-gated Na^+-channels developed in the third week, and by the end of the fourth week of differentiation the density Na^+-channels were high enough to allow repetitive action potentials with a completely mature waveform (Fig. 2A, D25). These action potentials were generated by a combined action of a TTX-sensitive Na^+-channel (I_{Na}) and two K^+-channels (I_A I_K). During four weeks the cells thus went through the same developmental steps that occur when new neurons are formed in vertebrates at the embryonic stage.

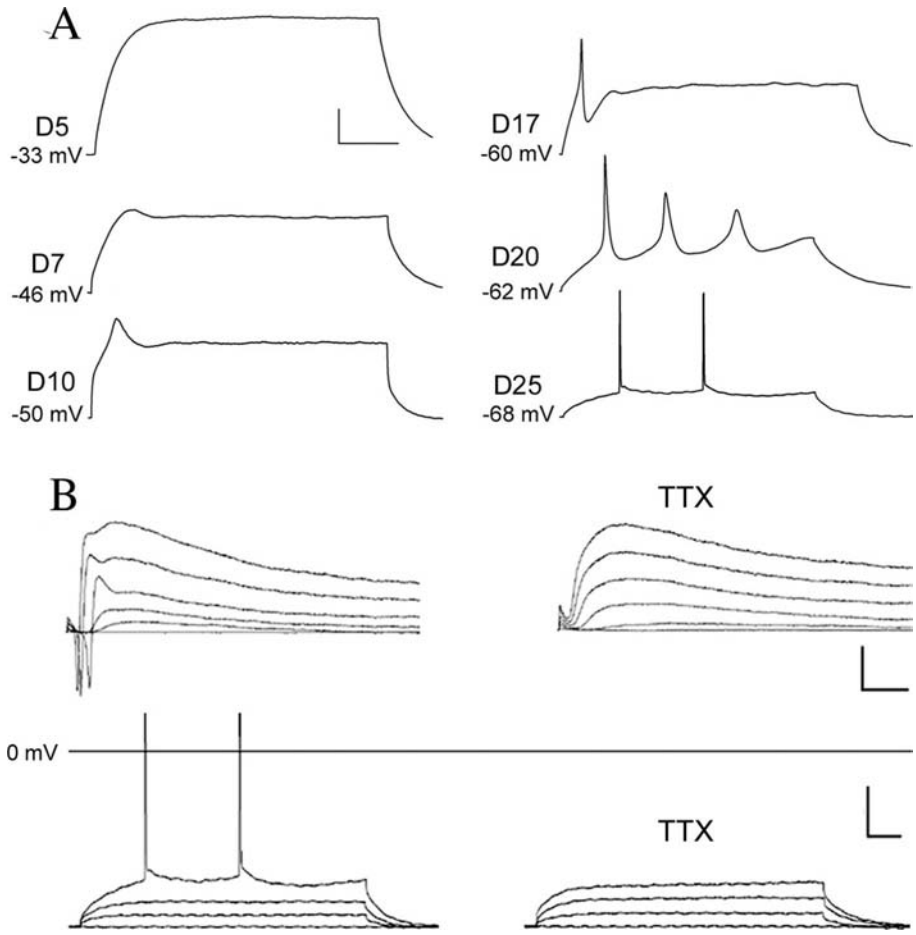

Figure 2 – *Functional neuronal differentiation.* ***(A)*** *Responses to a 0.1 nA intracellular current pulses. Note that only immature action potentials were observed during the third week of differentiation.* ***(B)*** *Neuron-like cell at D28 with mature firing pattern. Voltage-clamp (upper panel), shows a transient inward current (left) blocked by TTX (right), as well as outward currents. Current-clamp (lower panel) shows multiple short-lasting, over-shooting, low-threshold action potentials that were blocked by TTX. Scale-bars: 20 mV and 100 ms (a); 1 nA and 10 ms (b, upper panel); 20 mV and 50 ms (b, lower panel). Modified from NEUROSURGERY, 2005 Jun;56(6): 1182-8.*

Preliminary immunohistochemical studies, where we stained against glutamate-receptors, the vesicular glutamate transporter and GABA, indicated that the cells also had the capability to communicate by chemical transmission. We therefore decided to look for functional evidence of synaptic connections by patch-clamping individual cells and look for spontaneous synaptic events while neurotransmitters were directly applied though a pressure-controlled micropipette

(Fig. 3A, left). Using the latter technique, we could show that application of glutamate to the surface of the neuron-like cells resulted in a transient increase in intracellular Ca^{2+}-concentration as well as a depolarization of the neuronal membrane (Fig. 3A, right). Further, the amplitude of the depolarization depended upon the membrane potential and reversed at approximately - 6mV. The effect of glutamate application to the cell surface was thus identical to the action occurring in glutamatergic synapse *in vivo*[21].
Patch-clamping of individual neuron-like cells also revealed spontaneous potentials that depended on the membrane potential in a similar way as has previously been shown for spontaneous GABAergic and glutamatergic potentials, and was blocked by bicuculline and CNQX/MK-801, respectively . This gave further, and quite strong evi-

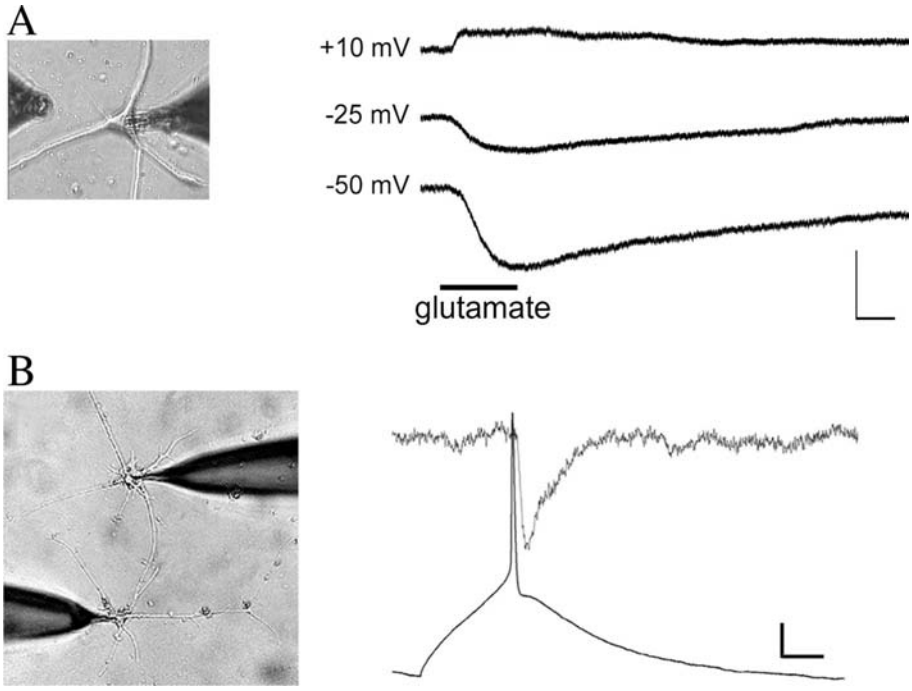

Figure 3 – *Development of functional synaptic connections.* ***(A)*** *Whole-cell voltage-clamp recordings during pressure application (inset) of glutamate at different holding potentials.* ***(B)*** *Dual whole-cell patch-clamp recording of two seemingly connected D27 neuron-like cells. The presynaptic cell (lower recording) was recorded in current-clamp, the postsynaptic cell (upper recording) in voltage-clamp. Current injection (50 msec, 70 pA) in the presynaptic cell evoked single action potentials. After a short delay, an inward postsynaptic current (PSC) was recorded from the upper neuron. Scalebars: 20 pA and 1 s (a); 20 pA/10mV and 20 ms (b). Modified from NEUROSURGERY, 2005 Jun;56(6): 1182-8.*

dence for that the cells had developed synaptic contact as both presynaptic terminal releasing the neurotransmitter and a post-synaptic membrane with the adequate receptor is required for the production of such spontaneous synaptic potentials.
Finally, we were able to perform parallel patch-clamping of several pairs of neuron-like cells simultaneously (Fig. 3B, left)[19]. In these experiments we first patch-clamped one neuron-like cell and then injected depolarizing current pulses in order to verify that the cell could produce major action potentials. Then we patched a second neuron-like cell, which seemed to be connected to the first one, and repeated the procedure with depolarizing current pulses. After identifying both cells as neurons, we generated action potentials in one cell while recording from the other cell in voltage-clamp mode. Doing this, we could identify a number of cell-pairs were action potentials in one cell generated a synaptic current in the other cell (Fig. 3B, right), thus directly demonstrating that the cells had developed functional synaptic contacts.
Hopefully, the fact that the adult human brain has a capability of producing neurons one day will lead to new treatment strategies for patients with neuro-degenerative diseases. This may be done either by transplanting stem cells, preconditioned cells or cells developing into a specific neurochemical phenotype or by recruiting endogenous progenitor cells[22-24] . So far, however, it has turned ot to be more difficult develop new neurons in the brain *in vivo* as compared to *in vitro*. This is probably at least in part due to the restrictive environment of the adult human brain, and much research will be required before such treatment alternatives may be brought into reality.

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SPINAL DESCENDING GLUTAMATERGIC AXONS CAN REGENERATE THROUGH A PERIPHERAL NERVE GRAFT AND FORM FUNCTIONAL GLUTAMATERGIC NMJ

Marina PIZZI, Giorgio BRUNELLI, Pierfranco SPANO

Key words: peripheral nerve graft; regeneration; neuromuscular junction; glutamate; AMPA receptor.

Failure of axonal regeneration after acute or chronic injury to the adult mammalian spinal cord is though to result from a nonpermessive extracellular milieu surrounding the lesion site[1-3]. However, more than twenty years ago Aguajo et al. showed that the axotomized central axons can regrow through a peripheral nerve (PN) graft, suggesting that they can regenerate if suitable substrates and growth factors are provided[4-6]. Thus, in the attempt to bypass a spinal cord lesion, diverse authors tried to directly connect the spinal cord with a skeletal muscle[7-11]. Distal stumps of severed motor nerves were connected to the spinal cord by mean of authologous PN grafts bridging the gap to the spinal cord. The protocol adapted to various types of skeletal muscles was applied to rats and primates and showed to produce motor and sensory recovery[9-11].
We started from the evidence that when a PN terminally connected to

Marina Pizzi
Professore Associato di Farmacologia
Dipartimento di Scienze Biomediche e Biotecnologie – Scuola di Medicina
Università di Brescia – V.le Europa, 11 – 25123 Brescia - Italia
Tel +39 (030) 3717501 – fax +39 (030) 3717529 – pizzi@med.unibs.it

Giorgio Brunelli
Foundation for Experimental Spinal Cord Research

Pierfranco Spano
Division of Pharmacology and Experimental Therapeutics
Department of Biomedical Sciences and Biotechnologies – School of Medicine
University of Brescia – Brescia, I 25123
IRCCS - San Camillo Hospital – Venice, I 30100

a skeletal muscle is grafted into the lateral bundle of the spinal cord, effective muscle reinnervation occurs[11]. It has been ascertained that the lateral white matter of rodent spinal cord includes afferents from red nucleus and brainstem reticular formation, while the main descending pyramidal tract runs at the base of the dorsal column of the spinal cord[12]. Since neurochemical and histochemical data suggest that all three descending tracts are glutamatergic, diverse important questions rose. First of all, are central fibers really reinnervating the skeletal muscles? What kind of fibers are responsible for that innervation? If the muscles physiologically innervated by spinal motor neurons are responsive to a cholinergic signal, what can occur in muscles that show to be functionally reinnervated by a non cholinergic transmission? To answer these questions we reproduced the nerve graft procedure in the rat. An autologous sural nerve graft was implanted into the acutely severed lateral white matter of the spinal cord and connected to the distal stump of the transected nerve of the internal obliquus abdominis muscle. Two months later, rats were examined for reinnervation[13]. We chose to pursue a functional and pharmacological approach, and our first aim was to verify the efficiency of cholinergic transmission. As normal motor endplates respond to cholinergic signal through the activation of muscular nicotinic receptors that are classically blocked by curare, we checked the responsiveness of reinnervated muscles to curare agents. For this purpose rats were anestethized and mechanically ventilated. Obliquus muscles at both reinnervated and controlateral control sides were exposed, and the compound muscle action potentials (CMAPs) in response to direct nerve stimulation were measured (Fig.1). Reinnervated muscles efficiently responded to nerve electric stimulation, though they showed CMAPs of lower amplitude and longer latency than the control sides. After the first stimulation, a group of rats was injected with the competitive neuromuscular blocking agent vecuronium (800 μg/Kg, i.v) (Fig. 1A). As expected, the control side was completely blocked by the application of the nicotinic receptor antagonist. Conversely, the reinnervated muscle appeared to be totally insensitive to the curare application. We then tried the blockade of glutamatergic transmission. Among the glutamate receptor involved in the excitatory transmission in central nervous system are the ionotropic glutamate receptors belonging to the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptor subtypes. We chose to investigate

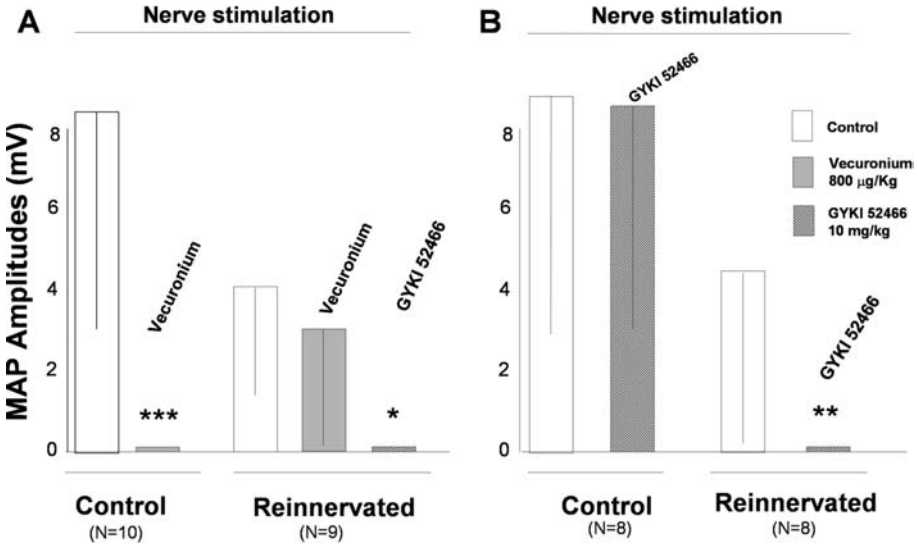

Figure 1 – *Electrophysiological recording of muscle activity in response to direct stimulation of control and grafted nerve.*

*(A) Columns represent means and standard deviation of CMAPs amplitude in control and reinnervated muscle. The intravenous injection of vecuronium completely blocked muscle response to control nerve stimulation, but not muscle response to stimulation of grafted nerve (Reinnervated). Next administration of the AMPA receptor antagonist GYKI 52466 totally abolished the muscle response resistant to vecuronium. (B) In these experiments, GYKI 52466 was administrated after the first CMAP recording. GYKI 52466 did not modify the control muscle response, but totally prevented the response of reinnervated muscle. Traces are from a representative experiment. The stimulus strength was always adjusted to obtain maximal CMAP amplitude. The difference of amplitude and area were examined with a paired Student's t test. *p<0.05, **p<0.02, ***p<0.01, vs no drug (control).*

first the involvement of AMPA receptors, as they are responsible for the spinal motoneuron activation by upper neurons, and also because AMPA-like receptors have been found to mediate the activation of glutamatergic neuromuscular junctions (NMJ) in drosophila[14]. After the admistration of vecuronium, rats receive the AMPA receptor antagonist GYKI 52466[15-16] at the dose of 10 mg/Kg i.v. We observed that the reinnervated muscles previously insensitive to curare, were totally paralysed by the AMPA receptor antagonist. Even if administered in the absence of vecuronium (Fig.1B), GYKI 52466 totally abolished the contractility of the reinnervated obliquus muscle, indicating that after reinnervation the muscle became responsive to a glutamatergic transmission.

To explore the hallmarks of the muscle-innervating fibers grown within the nerve graft, we investigated the expression of specific

markers for cholinergic neurons (choline acetyl transferase enzyme – ChAT, and vesicular ACh transporter -VAChT) and for glutamatergic neurons (vesicular glutamate transporters 1/2-VGluT1/2). Sections from control and grafted nerves were analysed by immunofluorescence technique. Compared to the control, the grafted nerve showed a lack of ChAT immunoreactivity and the appearance of both VGluT1- and VGluT2-reactivity in βIII tubulin-positive fibers. It suggested that the original cholinergic fibers had been replaced by new glutamatergic axons. Similar results were obtained by Western blot analysis of protein extracts prepared from selected portions of obliquus muscle enriched of nerve terminals. The reinnervated muscle revealed a lack of both ChAT and VAChT proteins and the expression of VGluT1/2. These data indicated that after the nerve grafting procedure, the muscle reinnervation occurred and was mainly operated by glutamatergic fibers.

Next, we investigated the expression of glutamate receptors responsible for the activation of reinnervated muscle. By a semiquantitative analysis of PCR products[17] we analysed the expression of mRNAs for the four homologous subunits forming the heteromeric AMPA receptor complexes, GluR1-4. All GluR1-4 mRNAs were present in both control and reinnervated muscle, though only GluR1 and GluR2 mRNAs displayed an upward expression trend in reinnervated muscle. This phenomenon was even more evident at the protein level. Hence, GluR1-2 proteins appeared undetectable in control muscle, but were highly expressed in the reinnervated one. The immunofluorescence analysis of GluR1-2 revealed a specific clustering of these receptors in reinnervated muscle fibers. AMPA receptors appeared as large aggregates of postsynaptic junctional receptors innervated by βIII tubulin-positive axons.

Finally, to track the origins of the axons grown into the PN graft, we injected the retrograde tracer CTβ into the reinnervated obliquus muscle. The neuroanatomical tracing confirmed that axons elongated within the PN graft belonged to supraspinal neurons originating the rubro-spinal and reticulo-spinal tracts involved in the control of motor activity. Most retrograde labelling was found in mesencephalic neurons of the red nucleus and in the following brainstem nuclei, the medullary reticular formation, the vestibular complex, the nucleus ambiguous, the dorsal and ventral medullary reticular nuclei and the nucleus gigantocellularis. No labelled neurons were found in the spinal cord either caudal or rostral to the graft insertion, and in the cortex.

In conclusion, acetylcholine is considered to be the main neurotransmitter at the mammalian NMJ where nicotinic acetylcholine receptors mediate the signalling between nerve terminals and muscle fibers[18,19]. Glutamate has a primary role in neuromuscular transmission of organisms phylogenically distant from mammals, such as invertebrates including insects and mollusks[14,20,21]. We here show that under glutamatergic transmission the rat NMJ can switch from cholinergic type synapse to glutamatergic synapse. Connecting the distal stump of a transected motor nerve to the lateral white matter of the spinal cord by mean of a PN graft produces a functional muscle reinnervation. The restored neuromuscular activity becomes resistant to common curare blockers, but sensitive to glutamate AMPA receptor antagonist. Analysis of the regenerated nerve shows the growth of new glutamatergic axons and the disappearance of cholinergic fibers. Many axons reinnervating the muscle fibers belong to supraspinal neurons located in red nucleus and brainstem nuclei. Finally, reinnervated muscle display high expression and clustering of AMPA receptor subunits GluR1 and GluR2. Our data suggest that supraspinal glutamatergic neurons can directly target the mammalian skeletal muscle. On the other hand, the skeletal muscle retains the memory of ancestral glutamatergic transmission and the plasticity to remodel and adapt the NMJ to the glutamatergic input[13].

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WHEN HOPE OUTWEIGHS FEAR

Maria Cristina VALSECCHI

Keywords: clinical trials, informed consent, amyotrophic lateral sclerosis, trauma.

I am a freelance journalist and I work for various journals specialising in scientific information including the monthly Newton magazine, part of RCS periodicals. A few months ago I was asked by Newton to collect information and write an article[1] on a controversial and rather sensitive issue: trials of a new therapy being carried out at the Xi Shan hospital in Peking, involving the injection of foetal stem cells into the nerve tissue of patients who are paralysed either as a result of trauma or degenerative disease, especially lateral amyotrophic sclerosis.
The editor who asked me to write the article said that very little has been published about the technique developed by a Chinese neurologist, doctor Hongyun Huang. However, patients talk about it as a kind of miracle cure and thousands of people from all over the world are on the waiting list to undergo the operation.
In general, when a new therapy is developed, news of it reaches the press through bulletins from specialist journals or universities, and it is only later that people get to hear about it through patient associations. The fact that things happened differently in this particular case made me suspicious from the outset. Remembering the Di Bella case, the way false hopes were inflated by the press, and the subsequent disappointment for patients and their families, made me promise to tread very carefully indeed.

Lack of published data

I started looking for information on Doctor Huang's therapy on Medline and Eurekalert. Eurekalert is an American gateway serving the press which publishes preprints from specialist magazines and bulletins

Maria Cristina Valsecchi
Giornalista scientifica free lance
Via Marcantonio Bragadin, 20 – 00136 Roma
Tel. Fax +390639726974 – c_valsecchi@yahoo.it

from research organisations from all over the world. I found information on doctor Hongyun Huang in three magazines: Lancet [2] , Nature [3], and the Chinese Medical Journal [4]. The pieces in Nature and Lancet were not scientific articles but rather reports from editorial staff who had visited the hospital in Peking and interviewed the neurologist and some of his patients. The articles are accompanied by comments from Western specialists who reveal a certain scepticism as regards the therapy. What is more, both pieces make reference to the article in the Chinese Medical Journal as the only existing work in the English language that doctor Huang has written on his experiments.
The article in the Chinese Medical Journal describes the therapy developed by the neurologist. Doctor Huang uses immature nerve cells taken from the olfactory bulb of foetuses aborted during the sixteenth week of gestation. The cells are then cultivated in vitro for ten days. During this period the cells multiply between ten and twenty times. The neurologist then injects them into the patient's nervous system: into the spinal cord for patients who have suffered trauma, and into the frontal lobe in the case of patients suffering from lateral amyotrophic sclerosis. The injected stem cells, explain Doctor Huang, do not substitute the damaged nerve tissue but release growth factors which stimulate the regeneration of the patient's neurones.
The neurologist claims that 70% of the people treated by him in this way experienced immediate improvement, even if the improvement was minimal. The results sound amazing. There is a problem, however. The study contains no detailed information concerning the patients' condition either before or after treatment. When interviewed by the journalists from the Lancet and Nature, Doctor Huang explained that the only proof he had of the success of his treatment were the videos of patients a few days after the operation where they claim they have regained sensation or mobility in parts of the body which were previously paralysed or had no feeling.
To try and get a clearer idea as to the validity of the experiment, I decided to contact an Italian specialist, professor Eugenio Parati, Director of the department of Neurobiology and Neuroreparative therapies at the Besta Institute in Milan. Dr. Parati, who knew about the Peking project, pointed out weaknesses in Huang's work. He explained that experiments had already been carried out to test the use of stem cells to stimulate regeneration of damaged nerve tissue in vitro and they had been successful. However, for the moment, the international scientific community had no available data regarding use of the same

technique on live subjects. It is the dream of every stem cell researcher, maintains Professor Parati, to get the kind of results Doctor Huang boasts about. He is sure that whoever managed to achieve these results would rush to publish the results of the experiment or, at the very least, sent out a preliminary report to colleagues in the field. Doctor Huang, on the other hand, did not publish anything in any international journal, did not present his results at any international conference and has not sent round any kind of preprint to his colleagues. Professor Parati finds this behaviour suspicious.
The famous videos with the patients' announcements only provide testimony to subjective sensations on the part of people who are directly involved. Objective tests exist which would provide quantitative measures as regards the recovery of post-surgical nerve activity in the patient. But results of these tests, the only ones to have scientific value, are not available, nor have they ever been published. The improvements that the patients talk about could simply be the result of the placebo effect of the operation. In cases like this, explains Professor Parati, only time enables us to distinguish between real and apparent improvement. All the statements on the videos were made only a few days after the operations and no data has been published regarding subsequent tests.
Professor Parati's attitude, which seems to coincide with that of most of the International community, is one of caution until further information is available.

Patient hopes

My next step was to look to patients who had already undergone the operation for information. I contacted the Italian Association for Amyotrophic Lateral Sclerosis and the Luca Coscioni Association. Both confirmed that several Italians are on the waiting list for the operation in Peking but were unable to put me in touch with anybody for reasons of confidentiality. According to them, no Italian patient had yet undergone treatment.
I then went to Internet and consulted the forums where lateral amyotrophic sufferers and people paralysed as a result of trauma write. I found a few stories. I quote two in the article. The first is that of Chris Olson, a young man from Madison, in South Dakota, paralysed from the thorax down as a result of a car accident. He had the operation and reported improvement in his respiratory capacity, mobility of the left wrist and sensation in the legs. He said he was satisfied because "having some chance is better than having none". The second

case I reported in the article was that of Ronald Abdinoor, from Windham, in Massachusetts, a lateral amyotrophic sclerosis sufferer who died on 26th October 2004 in Peking from respiratory complications as a result of the operation.
In general, what emerges from the forum about Doctor Huang's experiments is that all these patients desperately need hope. The ones who talk say that they have nothing left to lose. They are willing to face the risks of an experimental operation, to foot the bill (20,000 dollars is what the hospital charges for the treatment) as well as make the long journey despite uncertain outcomes, and frequently despite their own doctor's misgivings, in exchange for a glimmer of hope. Those people who are getting ready to undergo the operation do not think of themselves as guinea-pigs, or at least, this idea is much less important than their expectations.
I'll give you an example. The explorer, Antonio Fogar, who died 24th August 2005, had been paralysed for years after an accident and was on the waiting list for the operation in Peking. On his death, the Luca Coscioni Association published an article about Fogar in which they applauded his courage for being prepared to act as a guinea-pig for the treatment. In response, lots of sufferers sent indignant messages, saying that he was not so much a brave guinea-pig as one of the lucky few who could afford to pay for the treatment and maybe use his fame to jump the waiting-list queue.

The bioethical viewpoint

To help make sense of such a complex issue, with all its risks, uncertainties and hope, I decided to contact an expert in bioethics: Doctor Cinzia Caporale, vice-president of the National Bioethics Committee and president of the Intergovernmental Bioethics Committee for UNESCO. Doctor Caporale was not familiar with the Peking story so she preferred to discuss general issues connected to clinical trials. Here are the main points I used in the article: "the main ethical criterion which makes a clinical experiment morally acceptable is informed consent on the part of the patient. This principle implies that the patient has to receive all the information necessary to make his/her consent a free and conscious choice – and that this information is given in an appropriate form, using comprehensible language – and this includes information about the risks inherent when taking part in an experiment".
"The trial also has to have a solid scientific basis, even if the mechanisms by which a specific drug works or the exact ways in which a given proce-

dure might be successfully used do not need to be explained in minute detail. Whenever a trial is planned and carried out, it is always important to maximise the benefits and minimise the risks for the patient".
The fact that clinical trials carry a certain risk for the volunteers is inevitable, she says, and necessary for the progress of medical research.
Lots of questions remain unanswered. What are the limitations of informed consent when the people involved are desperate and ready to try anything: people whose emotional state obviously tends to make them exaggerate the chances of improvement and play down the risks? And to what extent is the consent really informed in this case, since doctor Huang is so stingy with information regarding the results of his research? Who is responsible if a treatment which is risky and whose outcomes are uncertain is perceived by patients as a miracle cure? The Doctor conducting the trial? Nobody, because information and hope spread freely, by word of mouth? The press, who highlighted news of the trial without paying much heed to the consequences?
The last person I quote in the article is Doctor Marcello Villanova, neurologist at the Nigrisoli hospital in Bologna, who met Hongyun Huang in person during his visit to Italy and who many patients consult as to the efficacy of Dr Huang's treatment.
"It is a very sensitive issue", he said, *"because it involves the emotions of people who are suffering. Hongyun Huang has not provided scientific data regarding the success of his trials so for the moment there is no way of formulating any kind of judgement regarding their effectiveness. I do not want to deprive people of hope, especially when life has already taken everything away from them, but on the other hand I do not want to give hope where it might prove to be false. My advice is to be cautious, very cautious indeed."*

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COILING IS BETTER FOR MOST RUPTURED INTRACRANIAL

Andrew J. MOLYNEUX, Richard KERR
per conto di tutti gli investigatori dello studio ISAT

Coiling is better than clipping for most ruptured intracranial;
International Subarachnoid Aneurysm Trial (ISAT)
Understanding what it says and what it does not say.

Introduction

This prospective randomised clinical trial has now been running nearly 12 years since the first patient was enrolled. It has now confirmed both short and medium term benefit for a policy of Endovascular coiling compared with a policy of Neurosurgical clipping in patients with ruptured cerebral aneurysms in whom the aneurysm is suitable for both treatments.

Methods

The primary objective of this trial was to determine the relative **clinical** benefits of the two treatment policies, in the short and long-term.
The primary outcome measure is independent survival at one year, (based on the modified Rankin scale, using a dichotomous outcome: dead and dependent versus independed. The main secondary outcome is the survival and long-term re-bleeding risk.
Enrolment/eligibility depended on the patients being suitable for both treatments based on the aneurysm anatomy and clinical condition of the patient, the ability to get consent and, crucially, the willingness of Neurosurgeons and Interventionists to recruit and consent patients.
Based on power calculations, a target of 2500 patients was judged to be required for a P value of <P= 0.01.

Andrew J. Molyneux
Consultant Neuroradiologist – The Radcliffe Infirmary, Oxford, U.K.
Richard Kerr
Consultant Neurosurgeon, The Radcliffe Infirmary, Oxford, U.K.

Results & Most Recent Findings

When recruitment to the trial was stopped on the advice of Data Monitoring and Ethics 2143 patients had been enrolled from 42 centres.
The initial paper published[1] represented only partial data because not all patients had reached a One year outcome point. The findings in the latest paper, published in the Lancet, are summarised below[2]. After all the patients had been analysed, the one year primary outcome (less than 1% loss to follow-up at one year) and medium term follow-up had been obtained.
We were able to report the one year outcomes for 1063 of 1073 and 1055 of 1070 patients allocated to endovascular and neurosurgical treatment, respectively. 250 of 1063 (23·5%) patients allocated to endovascular treatment were dead or dependent at one year, compared with 326 of 1055 (30·9%) patients allocated to neurosurgery: a relative risk reduction for dependency or death of 24% (95% confidence interval (CI) 12% to 34%) and an absolute risk reduction of 7·4% (95% CI 3·6 % to 11·2%, P=0.0001). The early survival advantage was maintained and was statistically significant (log rank P=0.03). The risk of epilepsy was substantially lower after allocation to endovascular treatment: relative risk 0·52% (95% CI 0·37 to 0·74).
The most reassuring and important aspect of the latest data are the survival curves out to 7 years, which show statistically significant and continuing benefit for coiling at this time. Whilst the risk of late re-bleeding is higher (0.2% per annum after one year), at the present rate the one year benefit is very unlikely to be lost over the lifetime of the patient.
Further data in respect of the Neuropsychological outcomes in a sub group of 500 patients will be presented[3], together with Health economic outcomes, in particular examining overall cost of care and resource use, taking into account overall lengths of stay in hospital, rehabilitation, readmissions, re-treatments, length of time off work and the relative rates of return to employment, all of which impact on the cost to society.
The frequency of seizures is also being analysed in detail to determine effects of the SAH, craniotomy, ventricular drainage, and shunting. Present data suggest a relative risk of 0.52 by treatment allocation, but even lower than this when patients avoid craniotomy and ventricular drainage altogether (RR 0.33).
Recent data from a national outcomes study in the UK has shown a major shift in practice in UK, from a rate of clipping in 2001, during the ISAT study, of about 35% coil UK wide, to a rate of about 70% in 2003-

2004. In some UK centres coiling rates are as high as 90%, depending on the willingness and experience of the operators in the use of alternative techniques.

Conclusion

ISAT has had a major effect on the management of patients with ruptured aneurysms in both Europe and North America. It has proven for the first time to Grade 1 evidence level the effectiveness of neurointerventional techniques and will continue to influence clinical practice worldwide. It could not have achieved this without the support of many Neuroradiologists and Neurosurgeons in many countries, or without the support of the Research funding bodies in UK, France and Canada.

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CAROTID-ARTERY STENTING VERSUS SURGICAL ENDOARTERECTOMY

Peter A. RINGLEB

Carotid artery stenoses are responsible for around 20% of all ischaemic strokes[1]. In asymptomatic patients event rates are between 2 and 5%/year[2]. In symptomatic patients recurrence risk is high despite medical treatment, it is stricIty dependent on the stenosis severity and the time since the neurological symptoms. In the first month recurrence risk is up to 10%, for the first year it is around 15%, and in the second year around 5%[3].

Carotid stenosis is traditionally treated by carotid endarterectomy. Multicentre randomised controlled trials have shown that surgery significantly reduces the risk of ipsilateral stroke in patients with severe symptomatic carotid stenosis. The North American Symptomatic Carotid Endarterectomy Trial (NASCET) showed a reduction from 27.6% to 12.6% at two year follow up ($p < 0.001$)[4]. The European Carotid Surgery Trial (ECST) showed a reduction in ipsilateral stroke in the surgically treated patients at three year follow up from 21.9% to 9.6% ($p < 0.01$)[5].In the Asymptomatic Carotid Surgery Trial surgery was shown to significantly reduce the overall 5 year risk of ipsilateral stroke or any perioperative stroke or death from 11.8% to 6.4% ($p<0.0001$)[2].

The results of these trials were incorporated in several guidelines and recommendations. The EUSI recommends that CEA is indicated for patients with stenosis of 70-99% without a severe neurological deficit with recent (≤180 days) ischaemic events and CEA may be indicated for certain patients with stenosis of 50-69% without a severe neurological deficit[6]. This is valid only for centres with a perioperative complication rate (all strokes and death) of less than 6%. In asymptomatic patients carotid surgery may be indicated for some patients with a

Peter A. Ringleb
Department of Neurology – University of Heidelberg
Im Neuenheimer Feld 400 – 69120 Heidelberg, Germany
Tel. +49 6221 56 7504; Fax: +49 6221 56 5348 – peter_ringleb@med.uni-heidelberg.de

60–99% stenosis of the carotid artery. The CEA-related risk of stroke or death must be less than 3% in such a situation.
Endovascular techniques for treating carotid stenosis have been developed over recent years. Initially, percutaneous transluminal balloon angioplasty was used. Later stents were invented and have been used in the carotid artery and have been used either with or without initial balloon angioplasty. These treatments have the potential of being a useful alternative to carotid endarterectomy. The advantages include avoidance of general anaesthesia, an incision in the neck and the risk of cranial and cutaneous nerve damage from the surgical incision. Surgically inaccessible lesions can be treated and both the procedure and admission time are usually shorter than for surgery, therefore reducing costs. Endovascular treatment may offer an alternative to surgery in high risk patients in whom surgery is not advisable. However, there has been reluctance to recommend balloon angioplasty or stenting of the carotid artery because of anxiety about the risk of cerebral embolism both during and immediately after the procedure[7].
Some evidence on the risks and benefits of endovascular treatments comes from non-randomised case series. A large scaled worldwide registry reported 12,392 endovascular carotid stent procedures up to 2003 included 12392 procedures involving 11243 patients[8]. Technical success rate was reported as of 98.9%. There was transient ischemic attack rate of 3.07%, minor strokes of 2.14%, major strokes of 1.20%, and procedure-related deaths of 0.64%. The combined minor and major strokes and procedure-related death rate was 3.98% based on procedure number. With nonprocedure-related death rate of 0.77%, the total stroke and death rate was 4.75%.
But to answer the question wether CEA or CAS are equivalent, randomised controlled trials are mandatory. To date only three completed randomised controlled trials comparing endovascular treatment of carotid stenosis with surgery or with medical care, involving 608 patients are published[9-11]. In addition there were two further randomised trials comparing carotid angioplasty and stenting with surgery which were both stopped early[12,13]. A meta-analysis of these five randomised trials comparing CAS and CEA found a trend for equivalence of both techniques, however the periprocedural morbidity and mortality (m+m) was unacceptably high[14]. Moreover, a recent Cochrane systematic review analyse the data from 1.269 patients[15]. Analysis of 30-day safety data found no significant difference in the odds of treatment-related death or any stroke (odds ratio [OR],

endovascular surgery, 1.33; 95% confidence interval [CI], 0.86 to 2.04), death or disabling stroke (OR, 1.22; CI, 0.61 to 2.41), or death, any stroke, or myocardial infarction (OR, 1.04; CI, 0.69 to 1.57). At 1 year after randomization, there was no significant difference between the 2 treatments in the rate of any stroke or death (OR, 1.01; CI, 0.71 to 1.44). Endovascular treatment significantly reduced the risk of cranial nerve injury (OR, 0.13; CI, 0.06 to 0.25). The review concluded that the current evidence does not support a shift away from recommending carotid endarterectomy as the standard treatment for carotid stenosis. One major problem of such analysis is the dissimilarity of the included trials. They used different techniques, e.g. CAVATAS used mainly PTA without stenting [Cavatas Group, 2001 #1037], in SAPPHIRE all patients were treated using protection systems. Furthermore different patient-cohorts were treated, e.g. in SAPPHIRE more than 40% of the entire study population were asymptomatic patients. Therefore, there is a clear need for further, large scale randomised trials of carotid stenting compared to carotid endarterectomy to convincingly establish the clincal value of carotid stenting, especially in patients with low and moderate surgical risk.

In North America the *Carotid Revascularization Endarterectomy versus Stent Trial* (CREST) is done to answer this question with the aim to include 2.500 patients. A lead-in phase was set up for an interventionalis to perform 20 stent procedures before performing procedures on randomised patients. The early results from the 749 patients treated in this lead-in phase reported a combined rate of death, stroke, or myocardial infarction of 4% within the first 30 days after intevention[16].

In Europe three trials were designed and performed to compare the efficacy of carotid surgery and stenting (see table 1).

The *Endartérectomie Versus Angioplastie chez les patients ayant une Sténose carotide Symptomatique Serrée* (EVA-3S) is a French multicenter, non-inferiority randomized trial with national research organisation funding[17]. Patients are eligible if they have experienced a carotid TIA or nondisabling stroke within 4 months before randomisation associated with an atherosclerotic stenosis within the ipsilateral carotid bifurcation of 60% or more, as determined by the NASCET method, that investigators believe is suitable for both carotid surgery and angioplasty. The primary endpoints were: (a) any stroke or death within 30 days of the procedure and (b) any stroke or death within 30 days of the procedure plus ipsilateral stroke. To join EVA3S, a centre must form a multidisciplinary team, including a vas-

Trial	EVA3S	ICSS	SPACE
Countries	France	Australia, Canada, Finland, Germany, Holland, Ireland, Israel, Norway, New Zealand, Slovenia, Spain, Sweden, Switzerland, UK	Austria, Germany, Switzerland
Year started	2000	2001	2001
No. of centers	30	33	37
Planned sample size	900	2.000	1.900
Enrolled patients	527 (Sep 05)	723 (Nov 05)	1.205 (Jan 06)
Status	Stopped	Ongoing	Ongoing
Protection device	Mandatory (after protocol-amendment)	Recommended	Optional
Web Links	eva3s.hegp.bhdc.jussieu.fr	www.ion.ucl.ac.uk/cavatas_icss/index2.htm	www.space.stroke-trials.com
Principal Investigator	J.L. MAS	M.M. Brown	W. Hacke

Table 1 – *European trials comparing carotid endarterectomy and stentprotected angioplasty for treatment of symptomatic carotid artery stenoses in patients with low and moderate surgical risk.*

cular neurologist, a vascular surgeon and an interventionalist. Operator experience must be substantiated through documentation of a sufficient number of cases performed. The vascular surgeon must document at least 25 endarterectomies in the year preceding the entry into the study. The interventionalist must document at least 12 cases of CAS or at least 5 cases of CAS and 30 cases of endovascular treatment of other supra-aortic trunks. Carotid angioplasty initially consisted of primary stenting with or without the use of cerebral protection. In January 2003, the Safety Committee recommended stopping unprotected CAS after treatment of 80 patients in the CAS arm because the 30-day rate of stroke was 3.9 (0.9 to 16.7) times higher than that of CAS with cerebral protection although numbers without vs. with protection were small (4/15 versus 5/58)[18]. Therefore the protocol was changed and from January 2003 the use of a protection device was mandatory. In the meanwhile EVA-3S has been by the DSMB stopped after 527 included patients for safety concerns in the stenting arm[19]. The precise reasons for this decision are not known to the public up to now.

The *International Carotid Stenting Study* (ICSS), also known as CAVATAS-2, was set up as an multinational, multicentre, randomised, controlled, open, prospective clinical trial[20]. Symptomatic patients are included over the age of 40 years with atherosclerotic carotid stenosis, suitable for both stenting and surgery, and are randomised in equal proportions between carotid endarterectomy and stenting. Stents and other devices must be approved by the devices committee. The protocol recommends the use of a cerebral protection system. All patients receive best medical care. Patients are followed up by neurologists at 30 days after treatment, 6 months after randomisation and then annually up to 5 years after randomisation. The primary outcome measure is the difference in the long-term rate of fatal or disabling stroke in any territory between patients randomised to stenting or surgery. Secondary outcome measures include any stroke, myocardial infarction or death within 30 days of treatment, treatment-related cranial nerve palsy or haematoma. Restenosis (>70%) on ultrasound follow-up, economic measures and quality of life will also be analysed. The sample size is estimated at 2.400 patients. Currently, more than 700 patients are included in the trial. The ICSS has been funded by grants from the Stroke Association, the MRC, Sanofi Synthelabo and the European Commission.

The *Stent-Supported Percutaneous Angioplasty of the Carotid Artery versus Endarterectomy* (SPACE) trial is investigating if CEA and CAS are equivalent in the treatment of severe symptomatic carotid stenoses since March 2001[21]. Patients over the age of 50 years with symptomatic (transient ischaemic attack or minor stroke) stenosis (above 70% local diameter reduction (ECST-Criteria)) eligible for both methods can be recruited into this trial. Quantification of the stenosis is done by ultrasound, no angiography is necessary to include a patient into the trial. Surgeons as well as the interventionalists have to demonstrate their expertise prior to participation in the trial. Quality committees have been formed for all disciplines (neurologists and ultrasound specialists, vascular surgeons and interventionalists), which define the treatment guidelines and evaluate the personal qualifications of the staff involved. Primary endpoint of SPACE is the occurence of an ipsilateral stroke or death between randomisation and day 30 after treatment. Secondary endpoints include the occurence of any stroke within 2 years, rate of severe restenosis (defined as above 70% in duplex-ultrasound), and technical failure of the intervention. Based on the results of a predefined safety analysis after three years the sample size was re-

calculated as 600 patients per arm. In the meanwhile more than 1.200 patients have been included in 37 centers. Another, mabye the final, interim analysis will be done if the 30days-follow-up data of these 1.200 patients are available. Results are expected in spring 2006 and published presumably at the ESC in Brussels.
It has been prospectively arranged that ICSS, SPACE and EVA-3S will combine their results after completion of initial randomisation and follow-up to conduct combined meta-analysis of their data. To facilitate the meta-analysis, it has been agreed that all three trials will collect identical baseline vascular risk factors, will follow up patients with Doppler ultrasound annually and will collect data on outcome events using the same definitions and measures of disability. The co-operation between the European trials in agreeing to perform this meta-analysis prospectively at an very early stage is a major advance in trial design and will considerably enhance the validity of the meta-analysis.
Until no results from these trials are available, enthusiasm for CAS should be tempered by knowledge of the fact that CEA is a therapy with huge evidence[22]. Although the early results of carotid stenting appear encouraging, there are no sufficient long-term data available, as they are from the carotid surgical trials. Hence, stenting should continue to be seen as an experimental procedure and carried out only in the context of randomised clinical trials.

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HYPOTHESIS ON PHYSIOPATHOLOGICAL RELATIONSHIP BETWEEN MIGRAINE AND PATENT FORAMEN OVALE

Eva MORANDI, Gian Paolo ANZOLA

Key words: patent foramen ovale, right-to-left-shunt, migraine

Migraine is a complex disorder in wich many psychological, environmental, biochemical, neurophysiologic, genetic factors may play a role to trigger the attacks[1,2].

Migraine has long been suspected as a risk factor for stroke. A number of case-control studies[3,4,5,6,7] and a recent meta-analysis have confirmed that compared to non-migraineurs, the RR of suffering a stroke is 1,83 in people with migraine without aura, 2,27 in people with migraine with aura and 8,27 in female migraineurs smoking and taking oral contraceptives.

So far, the mechanism whereby migraine conveys an increased stroke risk has remained largely speculative[6].

Recent evidence indicates that in migraine with aura the prevalence of patent foramen ovale is of the same order of magnitude as that found in cryptogenic stroke[8,9,10], and conversely, the frequency of migraine in patent forame ovale associated cryptogenic stroke is twice than expected[11,12].

However, since both patent foramen ovale and migraine are common conditions, the predictor can be made that patent foramen ovale in patients syntomatic for cerebrovascular disease or migraine would have to cause a larger right-to-left shunt than in patients asymtomatic for both conditions.

Recent evidence indicates that patent foramen closure favourably af-

Eva Morandi, MD
Clinica Neurologica Università di Brescia – Brescia, Italia
Gian Paolo Anzola, MD
Servizio di Neurologia – Ospedale S. Orsola FBF- AfaR
Via Vittorio Emanuele II, 27 – 25100 Brescia, Italia
Telefono: +39 030 29711 – FAX: +39 030 3755269 – gpanzola@numerica.it

fects the course of migraine in term of frequency, intensity, duration of the attack and that reduces the frequency of aura[13,14,15,16,17,18,19,20].
These observations suggest that the link between patent foramen ovale and migraine may be more than coincidental and indeed hint to a causal relationship. A plausible hypothesis is that via the atrial septal defect a venous to arterial passage of activeted platelets or chemical substances may trigger headache by overwhelming the filtering capacity of the lung[21].
Larger shunt might also increase the likelihood of paradoxical embolisation to the brain and hence explain the statistically increased stroke risk associated to migraine[21,22].
The presence of a right-to-left shunt, may be the most potent trigger of migraine attacks in both migraine with and without aura and the main determinant of aura.
Any interpretation of the causal link between patent foramen ovale and migraine needs to take into account the fact that while patent foramen ovale is found in nearly half of the patients with migraine with aura, its frequency in migraine without aura is the same as in non migraineurs[8,9,10].
For migraine with aura a common inheritable trait linking migraine with atrial septal abnormalities has been suggested by Wilmshurst et al. who studied 71 relatives of 20 probands with a significantly sized atrial shunt[14].
When the proband had migraine with aura and an atrial shunt, 15 of the 21 (71.4%) first degree relatives with a significant right to left shunt also had migraine with aura compared with 3 of 14 (21.4%) without a significant shunt ($p<0.02$), suggesting that migraine trait may be inherited in association with atrial shunts, at least in some kinships and that the occurence of atrial shunts is consistent with autosomal dominant inheritance.
Our works support all the hypothesis above mentionated.
We assessed the amount of rigth-to-left shunt with contrast enhanced transcranial doppler in four-hundred twenty patients divided in migraineurs with aura (MA+), without aura (MA-), not-migraineurs and in patients with prior stroke (CVD+) and without prior stroke (CVD-).
Patients with prior stroke had lager shunts than patients without prior stroke (bubble count with ce-TCD= 91 vs 58 respectively). Both migraineurs with and without aura had significantly larger shunts than non-migraineurs (bubbole count = 104, 74 and 46 respectively). Patients with both migraine and CVD+ had larger shunts than mi-

graine patients with CVD- (bubbole count= 123 vs 72 respectively), than no migraine patients with CVD+ (bubble count= 123 vs 55 respectively) and than no migraineurs patients with CVD- (bubble count= 123 vs 38 respectively). (table 1)

	NO-MIGRAINE		MIGRAINE	
	CVD-	CVD+	CVD-	CVD+
N.	100	85	139	96
M/F	40/60	38/47	21/118	18/78
Age (mean ±SD)	48±17	55±14	36±14	42±11
Mean bubble count (SE)	38 (5)	55 (8)	72 (8)	123 (24)

Table 1 – *Age and shunt according to the CVD x migraine condition Age significantly different in all comparisons (p between <0.0001 and =0.037) Mean bubble count in MIGRAINE CVD+ patients significantly larger than in any other group (p between <0.0001 and =0.038).*

The serendipitous observation by Wilmshurst et al.[13] that PFO closure performed to prevent decompression sickness in a cohort of scuba divers had resulted in a dramatic decrease of migraine severity raised considerable interest on the possibile curative effect of a atrial septal repair on migraine. As a consequence, a number of publications reported a reduction in the frequency of attacks after PFO closure in 14-83% of patients[15,16,17,18,23], a decrease of at least 2 point in the MIDAS scale in 40% of patients[17] or a decrease of at least 1 point in a composite migraine severity scale in 59% of patients[19] and a complete solution of aura in 80% of patients undergoing transcatheter closure in comparison with 40% of patients medically treated[20]. Even more impressively, complete cessation of migraine attaccks was reported in a proportion ranging from 29% to 84% of affected people after a follow-up of 6 to 24 months [16,17,18,19,20,23].

We should not forget that, even if transcatheter closure of patent foramen ovale is a safe, effective and minimally invasive procedure, a number of complications have been reported[24].

Among these we reported breathlessness and palpitations, mostly at the 1-month follow-up, which subsequently declined and disappered, major arrhytmia including supraventricular paroxysmal tachycardia,

that in one patient evolved to atrial flutter and required the insertion of a permanent PM and atrial fibrillation in 8% of patients within one month from the procedure with a complete decline at one year follow-up[25] . Not al least is important to stress the risk of cerebral micro-embolism and arrhytmia during the procedure[26].
Therefore transcatheter closure of patent foramen ovale cannot be recommended as a primary prophylaxis in migraineurs patients, many other risk factors must be considered.

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STRESS CARDIOMYOPATHY

Claudio CECONI, Stavroula GAITANI, Antonella BORASO

Key words: Stress cardiomyopathy, Tako-tsubo, apical ballooning syndrome.

Emotional stress can precipitate severe, reversible left ventricular dysfunction in patients without coronary disease.
There have been several reports of patients with profound, reversible left ventricular dysfunction after sudden emotional stress[1-4]. The transient left ventricular (LV) apical ballooning syndrome, also known as Tako-tsubo cardiomyopathy, broken heart syndrome, acute stress cardiomyopathy or ampulla cardiomyopathy, is characterized by an acute and rapidly reversible LV dysfunction without significant stenosis on the coronary artery angiography[1] . It has been firstly described in Japan, but few cases have been recently reported also in Caucasian people[2]. "Tako-tsubo" derives from the Japanese word for a fishing pot with a round bottom and a narrow neck, which is used for trapping octopuses in Japan. This octopus-catcher reminds the LV shape during the acute phase of the syndrome, i.e. mid apical akinesia and hypercontractility of basal segments.
Postmenopausal women seem to be most at risk for developing the syndrome. Nearly all the patients experience acute physical or emotional stressor before the onset of symptoms[3] , although some cases, not preceded by emotional stress, have been described[4].
Not only chest pain but also all other instrumental findings mime a myocardial infarction in the acute phase of the syndrome.
The most common finding on admission ECG is ST-segment elevation or T wave inversion, especially in the precordial leads. It has also been described a transient prolongation of the QT interval that normalizes

Prof. Claudio Ceconi, MD, FESC
Cattedra di Cardiologia – Arcispedale S. Anna
Corso della Giovecca, 203 – 44100 Ferrara – ceconi1@tin.it
Stavroula Gaitani
Cattedra di Cardiologia, Università degli Studi di Ferrara – Ferrara, Italy
Antonella Boraso
Cardiovascular Pathophysiology Center – Fondazione S. Maugeri IRCCS – Gussago (Brescia), Italy

within one or two days[3]. Troponine and creatinine kinase levels are only mildly elevated, disproportionate to the extent of akinesia[5]. Echocardiography shows balloon-like LV wall motion abnormality at the apex and mid-portion with hypercontraction of the basal segments[5]. However, it has been reported a variant type of reversible severe LV wall motion abnormality of the basal segments with hypercontraction at the apex[6].Theremarkable reduced LV ejection fraction, at presentation, improves rapidly over a period of days to weeks[5] . Patients have either no angiographically detectable or non-obstructive coronary disease. Abe and Kondo[7] firstly and recently the Mayo Clinic group[5] proposed diagnostic criteria for this syndrome. As shown in table 1, the proposed diagnostic criteria can be used when there is no evidence of other obvious causes, but with similar apical and mid-ventricular wall-motion abnormalities.
Reported complications associated with this syndrome are: left heart failure with or without pulmonary oedema, cardiogenic shock, dynamic intraventricular obstruction with LV intracavitary pressure gradient generation, mitral regurgitation resulting from chordal tethering as well as systolic anterior motion of the valve apparatus, ventricular arrhythmias, LV mural thrombus formation, LV free-wall rupture and death[5] .

PROPOSED MAYO CRITERIA FOR THE CLINICAL DIAGNOSIS OF THE TRANSIENT LEFT VENTRICULAR APICAL BALLOONING SYNDROME

1. Transient akinesis or dyskinesis of the left ventricular apical and mid-ventricular segments with regional wall-motion abnormalities extending beyond a single epicardial vascular distribution
2. Absence of obstructive coronary disease or angiographic evidence of acute plaque rupture
3. New ECG abnormalities (either ST-segment elevation or T-wave inversion)
4. Absence of
   - Recent significant head trauma
   - Intracranial bleeding
   - Pheochromocytoma
   - Obstructive epicardial coronary artery disease
   - Myocarditis
   - Hypertrophic cardiomyopathy

Bybee KA et al, Ann Intern Med 2004;141:858-865

Tabella 1 – *Proposed Mayo criteria for clinical diagnosis of the transient left ventricular apical ballooning syndrome. All 4 criteria must be met.*

The pathophysiology of this syndrome is still unknown and several substrates have been put forward to explain the underlying pathophysiology, such as raised catecholamine levels. Supraphysiologic levels of plasma catecholamines and stress-related neuropeptides have been reported[3,5,8].These findings suggest activation of the adrenomedullary hormonal system, with marked elevation in plasma epinephrine and metanephrine levels. Enhanced sympathoneural activity is also suggested by the increased plasma levels of dihydroxyphenylalanine, dihydroxyphenylglycol, norepinephrine and normetanephrine, reflecting increased synthesis of norepinephrine, neuronal reuptake and metabolism, spillover and extraneuronal metabolism, respectively. The participation of catecholamines is suggested also by Ueyama et al[8].They reported an experimental study in which left ventriculography of adult female Wistar rats exposed to immobilisation, animal model of emotional stress, showed induction of transient LV apical ballooning, which was normalised by pre-treatment with $\alpha\beta$ adrenoceptor blockade.

Multivessel epicardial coronary spasm has been proposed as an alternative mechanism[9], but it appears to be responsible for onlt few cases of the syndrome. Multivessel coronary vasospasm was rarely found in the patients tested. In addition, it is not clear why multiple vasospasm would invariably afflict the same large apical portion of the LV.

Coronary microvascular function has been shown to be diffusely abnormal when assessed immediately after presentation, by using invasive measurements of coronary flow reserve and TIMI frame count techniques, but it is unclear whether coronary microvascular dysfunction is the primary mechanism involved in the pathogenesis of the syndrome or whether it is an associated secondary phenomenon[10].

Another interesting hypothesis is that an abnormal myocardial functional architecture (such as localised mid-ventricular septal thickening), in the presence of dehydration and/or raised catecholamine levels, leads to the development of a severe transient LV mid-cavity obstruction[11]. This effectively would sub-divide the LV into two functionally different chambers with a marked increase in wall stress in the high pressure distal apical chamber that induces widespread sub-endocardial ischaemia which is unrelated to a specific coronary artery territory.

Moreover, it has also been assessed myocardial metabolism in patients with this syndrome. Fatty acid metabolism resulted more severely impaired than myocardial perfusion, in parallel with apical akinetic region during the early phase. The discrepancy between

myocardial perfusion and fatty acid metabolism improved gradually during follow-up[12]. These and other results suggest that Tako-tsubo LV dysfunction may essentially be stunned myocardium due to impaired multivessel coronary microcirculation. Also, MRI reveals hypoenhancement consistent with the presence of viable myocardium in the LV akinetic regions observed at ventriculography[3]. The hypothesis of myocardial stunning is suggested also by Tissue Doppler assessment in an Italian case proposed by our group (fig.1).

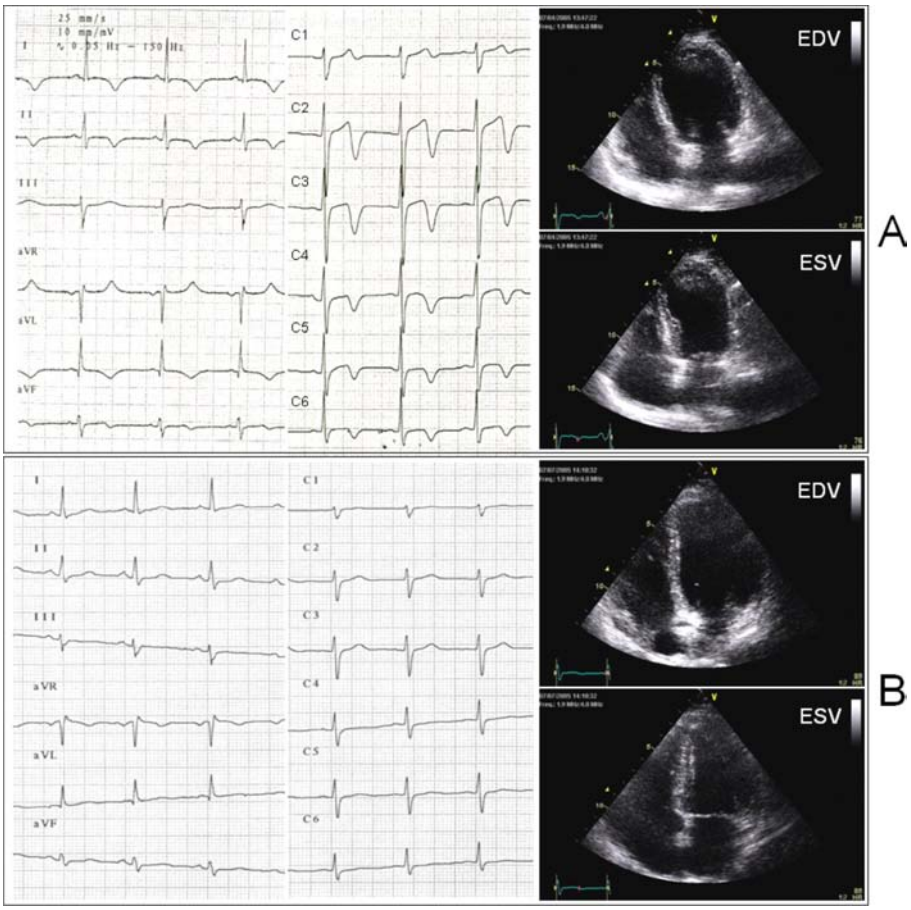

Figure 1 – ***Case Report in a Caucasian Patient:*** *ECG, end-diastolic volume (EDV) and end-systolic volume (ESV) on admission (panel A) and at three months (panel B). In the first ECG, negative T waves in most leads and ST segment elevation in V2 and V3 were evident while ECG at three months became normal. The first ecocardiography showed severe systolic dysfunction and apical ballooning of mid-apical LV that normalised at three months.*

Pathological findings of this syndrome obtained from endomyocardial biopsy have been reported[13], including focal myocytolysis with interstitial fibrosis and no significant inflammatory infiltration or necrosis of adjacent myocytes during the acute phase.
In conclusion several hypotheses have been proposed to explain the aetiology and the underlying pathophysiology of the Tako-tsubo syndrome, but they have not been fully clarified so far.
The prompt recognition of this syndrome is needed because a Tako-tsubo mimicking acute myocardial infarction may inadvertently expose patients to futile administration of fibrinolytic agents. Optimal management is difficult to determine in the acute setting, given the limited data available[5], however, after diagnostic arteriography an appropriate approach to the syndrome seems to involve medical management with β-blockers, angiotensin-converting enzyme inhibitors (in patients with no intracavitary gradient), aspirin and diuretics, as needed. Short-term anticoagulation should be considered to prevent LV thrombus formation. In case a dynamic intraventricular obstruction is demonstrated, administration of β-blockers is suggested to increase diastolic ventricular filling time and LV end-diastolic volume[14], and also phenylephrine to increase afterload with following reduction of the intraventricular gradient[15], β-blockers and phenylephrine would not be recommended for the treatment of dynamic intraventricular obstruction in patients with documented epicardial coronary vasospasm whereas a nondihydropyridine calcium-channel blocker should be considered. Therapy should be continued until LV function recovery.
The Tako-tsubo syndrome is probably not so rare in Caucasian patients, as many diagnoses are likely to be missed or misinterpreted. The establishment of a large scale registry for suspected cases of this cardiomyopathy is highly desirable. The knowledge of this syndrome should be also widened with pathological assessments, molecular analyses and experimental studies.

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ENDOCRINE MANIFESTATIONS DURING SEPSIS

Andrea POLITO, Tarek SHARSHAR, Djillali ANNANE

Summary

During sepsis, patterns of hormonal secretion undergo significant change due to stress-related activation of the hypothalamic-hypophyseal axis. Serum levels of cortisol usually increase in proportion to the severity of the systemic inflammatory response. Around 50% of patients with severe sepsis develop adrenal insufficiency which is associated with poor prognosis and diagnosis on the Synacthen test. In these cases, cortisol treatment for 7 days improves both mortality and morbidity.

The hyperglycaemia present in the initial stages results from insulin resistance as well as secretion of contraregulatory hormones and is associated with poor prognosis. However, the hypoglycaemia typical of a later phase is also associated with poor prognosis. The low T3 and/or T4 Euthyroid syndrome leads to a decrease in the active supply of circulating hormones. Although the basic mechanism is not yet clear, no evidence exists as to the benefits of substitution therapy during septic shock. Vasopressin secretion during shock can also be reduced and studies on the effectiveness of substitution treatment are under way.

The organism has two control systems which guarantee homeostasis of its most important functions: the endocrine system and the autonomic nervous system, described by Sterling and Eyer, and defined by them as "allostasis"[1,2]. In cases of stress, the intensity of the resultant aggression determines whether the integrated response on the part of these two systems is lost or not.

Andrea Polito – Tarek Sharshar
Servizio di Rianimazione Medica – Ospedale Raymond Poincaré, Garches

Prof. Djillali Annane
Sevizio di rianimazione medica – Ospedale Raymond Poincaré (APHP)
Facoltá di Medicina di Parigi Ile de France Ovest (UVSQ)
104 Boulevard Raymond Poincaré, 92380 Garches
Tel: 0033 1 47107787 – Fax: 0033 1 47107783 – djillali.annane@rcp.ap-hp-paris.fr

Adrenal-hypothalamo-hypophyseal axis

1. Glucocorticoid axis

The initial phase of sepsis is characterised by massive inflammatory activity due to recognition on the part of the immune system of gram-positive antigens and bacterial pathogenic components (gram negative endotoxins, peptidoglycans, teichoic acid) which are defined as "pathogens associated with molecular pattern" or PAMP. A series of events is set in motion which concludes with systemic activation of coagulation and pro-inflammatory activity. At the same time, in a bid to re-establish homeostasis, anti-inflammatory and fibrinolytic processes are activated, such that the greater the distance from the origin of the inflammation the greater the anti-inflammatory activity. We can consider three basic stages: the systemic inflammatory response syndrome or SIRS, the compensatory anti-inflammatory response syndrome or CARS and the homeostasis phase or MARS.

Glucocorticoids regulate the function and synthesis of numerous cells and inflammatory proteins like cytokines, nitric oxide, type II cyclo-oxygenase and the phospholipase A_2[4,5].

During sepsis, increases in serum cortisol levels are proportional to the severity of the underlying pathology. At the same time, we see loss of cortisol nictemeral rhythm because of raised levels of production of CRH and ACTH due to circulating pro-inflammatory cytokines resulting from activation of afferent vagus fibres at the site of the aggression and from reduction in negative cortisol control[6].

During sepsis the fraction of free and therefore active cortisol increases in response to various mechanisms including: (i) reduced levels of CBG or cortisol binding globulin, responsible for the weak but high-affinity bond with 90% of plasma cortisol[7] (ii) the protein-transporter complex is broken as a result of the elastase produced by polynucleate neutrophyls which rush to the infected site, (iii) tumor necrosis factor α (TNFα) and interleukines (IL-1, IL-6, IL-2) induce release of vasopressin and CRH. On the other hand, the anti-inflammatory ones, which are the antagonists of the IL-1 (IL-1ra), IL-10, IL-13 receptors, are, in the same way, capable of carrying out regulatory action at the hypothalamic-hypophyseal level. What is more, l'IL-1β, l'NO and other inflammation mediators are present in numerous areas of the brain including the hypothalamus. Finally IL-6 e TNFα adrenal receptors are expressed suggesting there is a cortisol synthesis mechanism under paracrinal/autocrinal control[8,9].

The increase in serum glucocorticoid levels is designed to re-estab-

lish cardiac homeostasis. At the cardiac level they act both on the number and the affinity of β adrenergic receptors for their agonists[10,11]. Although the mechanisms responsible on a vascular level are not very clear, they increase the sensitivity of the catecholamines. The mechanisms which are potentially responsible include the facilitation of agonist-receptor coupling, the increase in intra-cellular calcium and inhibition of the inducible form of NO synthetase (iNO) or of cyclo-oxygenase.

An increase in glucocorticoids like this aims to compartmentalise the inflammatory response and redistribute glucose in the insulin-dependent cells (particularly neurones and inflammatory cells).

Nevertheless, the glucocorticoid response is insufficient in 30-70% of forms of sepsis because of adrenal insufficiency[12-14] or peripheral resistance to glucocorticoids[15]. This happens either because of an irreversible problem with cortisol synthesis through anatomical destruction of the adrenals (Waterhouse Friedrichen syndrome) or the hypophesis (Sheehan syndrome), or because of a reversible problem through enzyme inhibition at three different levels of the axis on the part of inflammatory mediators or drugs (ethomidate, ketoconazolo). It can also happen because of reduced cortical transport or because of a break in the CBP-cortisol link because of anti-elastase activity. The reduction both in the number and the affinity of the receptors to glucocorticoids, as well as excessive conversion of cortisol to inactive cortisone may also account for adrenal insufficiency.

The vitiligo and hyperpigmentation typical of chronic adrenal insufficiency do not appear if the form is rapid onset. All other clinical signs are usually aspecific. In any case, haemodynamic instability, persistent inflammation despite control of the infection, hypereosinophaelia and hypoglycaemia during sepsis must all be capable of causing adrenal insufficiency. Diagnosis is based on plasma cortisol levels below 15μ/dl followed by an increase <9 μg/dl after 250 μg of ACTH (17) (tabella1). On the other hand, base cortisol levels above 34 μg/dl with increases of over 9 μg/dl after ACTH suggests the existence of peripheral resistance to glucocorticoids which, according to some authors[16], indicates the need for substitution therapy according to levels of adrenal insufficiency. (table1).

The administration of 200-300 mg/die of hydrocortisone for 7-11 days and, where necessary, of 9α-fludrocortisone 50 μg/die, reduces the systemic inflammatory situation both short and long-term[18]. However, in cases of renal insufficiency which respond to the ACTH test

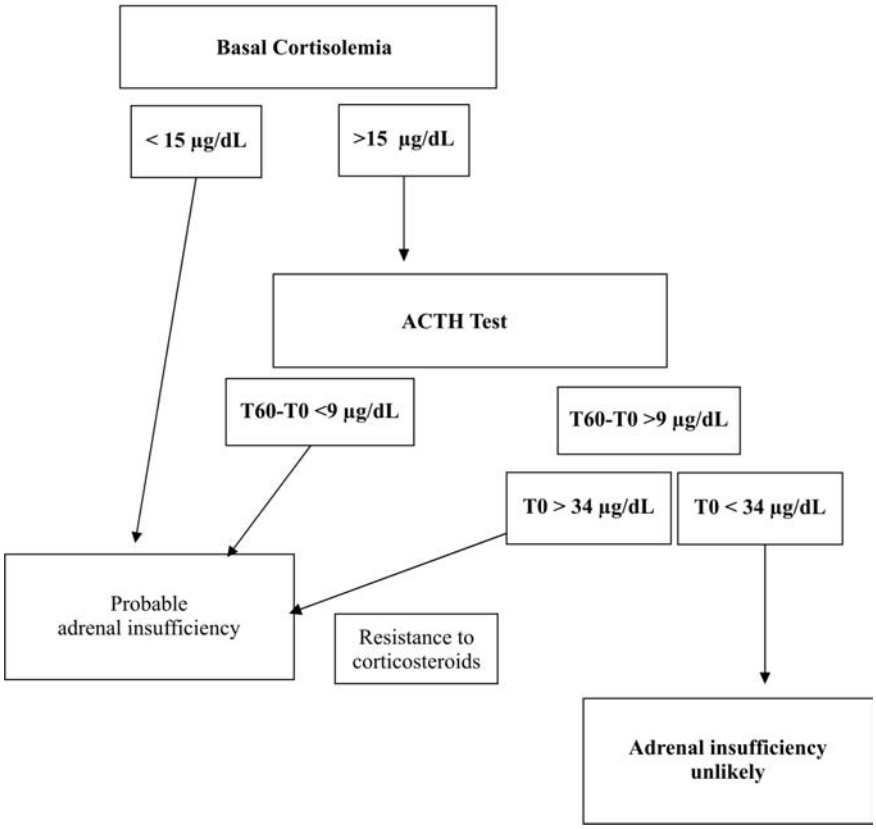

Table 1 – *Decisional algorithm*.

(increases in plasma cortisone of >9 µg/dl)[16], the administration of corticosteroids does not affect either duration of shock or survival rates[22]. In conclusion, where the septic shock depends on catecholamines practical recommendations suggest administering glucocorticoids immediately after the ACTH test. For patients who do not respond, or whose serum cortisol levels are <a 15 µg/dl, the treatment needs to be continued for at least 7 days, whereas the treatment is suspended in patients who respond.
The European study CORTICUS was set up to define the best way to administer hormone therapy and to show that there is a real need for gradual interruption of the therapy if rebound phenomena, like inflammation or haemodynamic deterioration, are to be avoided.

2. Mineralocorticoid axis

Strangely, the function of mineralocorticoids during stress has not

been studied very much, despite the fact that the renin-aldosterone pathway is one of the main regulatory mechanisms for systemic arterial pressure. Hyperreninism-hypoaldosteronism during sepsis seems likely to result from a problem with the angiotensin II activation of the enzyme cascade beginning with angiotensin II. In fact, in these cases, plasma renin and angiotensin are both higher, unlike the aldosterone precursor 18-hydroxicorticosterone, whose values are reduced[19].

3. Glucose-insulin axis

Glucose plays a fundamental role in the regulation of cell metabolism, energy processes, and the synthesis of glycoprotein and nucleic acid. It enters the cells by active transport, either through the sodium-glucose transporter (SGLT protein) or by facilitated transport (GLUT protein)[20].

Glucose metabolism is influenced by insulin by:

- mobilisation of transporters towards target cells in adipose and muscle tissue;
- activation of the hepatic glucokinase gene transcription;
- activation of glycogen-synthetase and inhibition of glycogen-phosphorilase.

Insulin also stimulates growth, cellular differentiation and migration, lipogenesis, glycogenesis and protein synthesis. For this reason it attaches to ubiquitous receptors from the tyrosine-kinase family, like *insulin like growth factor* (IGF-1) and *insulin related receptor* (IRR). Apart from this, it also has a complex role in inflammation, antagonising the TNF[21], the *macrophage migration inhibitory factor* (MIF) and superoxide anions[22]. On the other hand, it slows down production of acute phase proteins, like C reactive protein and haptoglobin. By acting on the adipocytes, it also promotes secretion of leptin which, along with TNF-α, stimulates IL-6, PCR and other agents of acute phase inflammation.

Glucose is a powerful pro-inflammatory mediator[23] capable of inducing production of free radicals IL-8[24] e NF-κB[25].

Hyperglycaemia, especially in the early phases of sepsis, represents an adaptive response to stress[26]. This is why post-infarct hyperglycaemia, in non-diabetic patients, is associated with a worse prognosis compared to that of diabetic patients, or normoglycaemia in non-diabetics [27]. The underlying mechanism may have a dual origin: on the one hand, insulin resistance at the level of the liver and adipocytes, on the other, increased production of contraregulatory hormones (adrenaline, noradrenaline, corticoids, glucagon, GH) and cytokines (IL-1, IL-6, TNF-

α). Intracellular cytokines, particularly TNF-α, determine the production of ceramides which repress the transcription of glucose tranporter 4, indispensable for entry into the cells. Gluconeogenesis, which is not suppressed by glucose perfusion[28], results not controlled by the exogenous uptake of insulin[29,30]. At the same time, peripheral resistance to insulin kicks in, with an increase in its clearance and alteration in the phosphorilisation of its receptors. Insulin resistance in sepsis is directly proportional to the severity of the stress response[31]. The increase in free fatty acids, glycerol and lactates may also be a direct consequence of the insulin. The adipocytes, endocrine cells which secrete adipokines including leptin, influence metabolism and energy supplies. Shortages of these hormones are what characterise insulin resistance. In conclusion, therefore, hyperglycaemia increases the pro-inflammatory response, while insulin has the opposite effect[32].

It is only in the latter stages that hypoglycaemia emerges related to the seriousness of the condition[33]. These variations are linked to the equilibrium between levels of insulin and cortisol[34].

The physiological action of insulin can be restored by continuous infusion of insulin. By maintaining normoglycaemic levels (0.8-1.1 g/l) in surgical reanimation patients through continuous infusion of insulin after glucose loading, mortality rates can be reduced by 40%, infection rates by 46%, acute renal insufficiency by 41% and resulting polyneuropathic events by 44%. There are potentially different levels at which strict glycaemic control can work, including prevention of immune dysfunction, reduction of systemic inflammation and endothelial and mitochondrial function protection. In patients undergoing non-surgical intensive care, normoglycaemic control reduces both morbidity and mortality especially in patients staying for longer periods[35]. The explanation probably lies in a protective effect which needs time for its real effects to show. The reduction of infection rates in surgical patients was not observed in non-surgical patients, probably because the infection was often the reason why the patient was hospitalised. Hypoglycaemia is an independent risk factor and hypoglycaemic episodes related to treatment can be avoided by strict juggling of the doses of insulin and glucose uptake. Given the relationship that exists between the different hormonal pathways and the benefits associated with corticosteroid and insulin replacement therapy, the idea of combining the two treatments for septic shock deserves to be analysed through a prospective controlled study.

The effectiveness of insulin in the acute phase of post-myocardial in-

farction can be attributed to the reduction in TNF and the increase in NO and prostaglandins. These act together to produce powerful vasodilatation and platelet anti-aggregation. Its anti-apoptosic action has also been offered as an explanation of the phenomenon.

Adrenergen axis

Catecholamine, adrenaline, noradrenaline and dopamine are secreted by the adrenal medulla as part of the peripheral sympathetic nervous system. They act on the cardiovascular system and glucolipid metabolism thanks to three types of receptors: α1, α2 e β (table 2). Their synthesis begins with the hydroxylation process of a tyrosine nucleus until dopa is formed. The decarboxylated dopa transforms dopa into dopamine. Successive decarboxylation leads to noradre-

	SITE OF ACTION	MECHANISM
β1	Myocardial fibre	Contraction force I+ Cardiac frequency C+ Dromotropic + Batmotropic +
	Lipolysis	AGL, glycerol
β2	Smooth muscle fibre	Vasodilatation Broncodilatation
	Liver	Gluconeogenesis
α1	Vessels	Vasocostriction
	Bronchi	Broncocostriction
	Skin, eyes and stomach	Contraction
α2	R pre-synaptic SNA	Inhibition noradrenaline release Vasocostriction
Dopamine	Kidneys	Diuretic
	Vessels	Vasocostriction

Table 2 – Adrenergen receptors.

naline. It is only through N-methylisation that noradrenaline can produce adrenaline. While adrenaline accumulates in the medulla, noradrenaline is present in the sympathetic nerve endings. Dopamine is the main catecholamine precursor in the medulla and nerve endings. Small amounts are found in many sites, including the renal tubule. Their half-life is very short, from 10 to 20 seconds for adrenaline. Elimination is by captation, enzymatic inactivation (hepatic or renal methylation), oxidative desamination or renal excration.
Regulation of catecholamine secretion occurs at different levels: hormonal, nervous or by negative feedback from calcium channels. Hormonal regulation recognises cortisol as important in the activation of the enzyme cascade which leads to synthesis. Regulation by the nervous system is based on the pre-gangliar parasympathetic cholinergic system through the splanchnic nerves.
Hypersecretion of adrenaline inducing muscular vasoconstriction and increasing peripheral resistance, like systo-diastolic arterial pressure, aims to guarantee cardiovascular, immune and metabolic homeostasis.
Regional effects of adrenaline vary: absence of vasoconstriction in the cerebral arteries and reduction of renal blood flow with reduction in filtration fraction without interfering with glomerular filtration. At the cardiac level, coronary vasoconstriction is induced, counter-balanced by increased cardiac flow and increased pulmonary arterial pressure. By acting on the β2 o α1 receptors, it leads to gluconeogenesis and hepatic glycogenolysis. The hyperglycaemic effect is independent of insulin and glucagon variations. In normal circumstances, adrenaline concentrations are not sufficient to stimulate hepatic receptors. Catecholamine exacerbates glycogenolysis through the production of lactates without hyperglycaemia simply through the β2 effect. At the level of the kidney, on the other hand, catecholamines are responsible for gluconeogenesis through the calcium effect and dependent AMPc . They are also responsible for control of renal vasomotor tone, glomerular filtration, renin and anti-diuretic hormone secretion, as well as tubular reabsorption of sodium. Adrenalin also stimulates secretion of glucagons (β2 effect) and inhibits that of insulin (α2 effect).

Thyroid axis

The peripheral thyroid hormones are thyrosine or even 3,5,3',5'-tetraiodorironine or T4 which, after peripheral deiodation changes into 3,5,3'-triodotironine T3, which is the biologically active compo-

nent. They are released after stimulus by the hypophyseal TSH which itself is dependent on the release of hypothalamic TRH. They play an extremely important role in the development and growth of the central nervous system. They are capable of exacerbating all the metabolic processes in the organism. At a cardiac level this translates as an increase in the β adrenergen effects which underly chronotropic, batmotropic, inotropic and dromotropic effects. Motility in the intestinal tract accelerates, glucose absorption increases and at the same time gluconeogenesis decreases so that hyperglycaemia results. Lipid catabolism is also increased.

Low T3-T4 syndrome, which can affect fasting patients or those with hypercatabolic pathologies, presents with low levels of T3 which get gradually lower leading to lowering of T4 as well. Anglo-saxons term the condition "euthyroid syndrome", in a bid to stress that there are no clinical signs of hypothyroidism, or "non-thyroidal syndrome" (NTIS) without alluding to the metabolic state of the thyroid hormones. However, T4 values of $<4\ \mu$g/dl are associated with mortality rates of over 50%, while values of $<2\ \mu$g/dl indicate that mortality rates rise to 80%.

The clinico-biological anomalies found are not specific and probably have multiple origins but they are never in direct relation to the thyroidal or hypophyseal localisation of the illness. The condition is often spontaneously reversible as well.

In the first phase of the illness FT3 is low, FT4 normal or slightly increased and TSH normal. 40-70% of patients who are hospitalised because of a general serious illness develop this pathology.

It is only later (24-48 hours) that the illness develops into the low T3-T4 syndrome, and it particularly affects Intensive Care patients. The typical profile includes low FT3-FT4 and normal or reduced TSH but nonetheless measurable (0.05-0.15 μg/dl). At the moment of recovery, TSH can be temporarily increased.

Various hypotheses have been put forward to explain the phenomenon, including the inhibition of T4 transporter protein or insufficient extrathyroid transformation of T4 into T3. This depends on low concentrations or inhibition of type I 5' hepatic deiodase. It is also thought that an alteration in the thyrotropic hypothalamus-hypophyseal retrocontrol might have an effect. There is reduced secretion of TSH[37] or loss of TSH through the nictemeral rhythm[38]. Intrahypophyseal transport is believed to be maintained given that T4 to T3 conversion continues in the hypophesis, whereas it stops peripherally[39].

Pro-inflammatory cytokines IL-1, IL-6, TNFα, INFγ inhibit the thyrotropic centres and/or the expression of hormonal nuclear receptors. In fact, serum concentration of IL-6 is inversely related to T3[40].
Glucocorticoids also participate in the control of TSH such that in Cushing's syndrome, for example, TSH levels are reduced as are those of the thyroid hormones.
Numerous other substances have also been held responsible, including catecholamines where weaning off these leads to a temporary increase in TSH, T3 and T4.
However, even when there is a hypothyroid picture like this, not everyone agrees that substitution therapy is the answer, as they do in cases of adrenal insufficiency. Studies carried out to assess its effectiveness showed zero or even negative effects. In reality, most of these clinical trials investigate thyroid insufficiency alone, without taking accompanying adrenal insufficiency into consideration.

Growth Hormone (GH)

GH operates in two major areas in humans. It affects growth through somatomedine or IGF1, and it also affects glucose and lipid metabolism. GH, together with IGF1, modulates the immune function by inhibiting the production of reactive types of oxygen and pro-inflammatory cytokines. It also has an anabolising effect on protein metabolism, and reduces NO production.
However, high-dose substitution therapy increases morbidity and mortality rates, increasing the time patients spend on mechanical ventilation, in hospital and in Intensive Care. GH esogen mimics the hormonal patterns typical of acromegaly. Arterial pressure goes up as a consequence of tubular sodium reabsorption until hypervolaemia results. Renal haematic flow is thus increased as is glomerular filtration. Hyperincretion of GH is associated with a congestive hyperkinetic cardiomyopathy regardless of whether there is arterial hypertension or not. There may also be GH related insulin resistance.

Vasopressina (AVP) or Antidiuretic Hormone (ADH)

The secretion of antidiuretic hormone is involved in the regulation of plasma osmolarity because of its anti-diuretic effect and systemic arterial vasoconstrictory effects[42], hence its name, vasopressin. It reduces the contractility of the right heart by a direct negative inotropic effect mediated by myocardiac receptors. Vasopressin is secreted by the supraoptic nuclei and hypothalamic paraventricles and is then collected in the posterior hypophyseal lobe. It is released in response

to increased plasma osmolarity, and as a baroreflex when there is a drop in tension or volume. Its two main roles, therefore, are osmotic on the one hand, acting quickly through slight modifications in concentration, and as a baroreceptor on the other, a process which kicks in much more slowly but as a result of much more significant variations. The vasoconstrictor capacity of vasopressin is weaker in healthy individuals compared to what it can do when the sympathetic system is not working.
Reduction in plasma levels of vasopressin as seen in septic shock may depend on increased clearance or a defect in hypophyseal formation as is confirmed by the hypersignal in T1 of IRM in patients in shock[45]. The administration of vasopressin corrects the defect and eliminates the resistance mechanism[46]. In cases like this, we see vasopressin hyperactivity and a reduction in NO production and the effects of noradrenaline on vasopressin production[47,48].
Initially used in the treatment of neurogenic diabetes insipidus and bleeding oesophogeal varices, vasopressin has been more recently proposed for the treatment of cardiac arrest in atrial fibrillation where it has proved more effective than adrenaline. Terlipressin, which is structurally similar to vasopressin, is equally capable or restoring arterial pressure in septic shock patients who are resistant to catecholamine[49]. Various studies have shown that vasopressin, in association with noradrenaline or not, is capable of increasing systolic arterial pressure and diuresis[48]. The negative effects produced include reduction in cardiac flow and an increase in intrahepatic blood flow[16].
Patients in shock can present with a vasopressin deficit related to the haemodynamic instability which is re-established after esogen administration[50]. This is even more obvious in subjects who are resistant to catecholamines. Its action causes an increase in systemic resistance and re-establishes a passive response to catecholamines.

Conclusion

An understanding of the processes underlying the deregulation of the endocrine axes during stress, especially when caused by infection, should improve management of patients with endocrine alterations. However, the response is closely related to the type of stress and does not always present in the same way.

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UNCERTAINTY AND ORGANISATIONAL PRINCIPLES BEHIND RESEARCH FOR INDUSTRIAL CLINICAL DEVELOPMENT OF DRUGS AND INSTITUTIONAL RESEARCH WHAT ARE THE RISKS AND OPPORTUNITIES FOR PATIENTS AND SOCIETY?

Giuseppe RECCHIA

Introduction

Patients, members of the public and doctors are always waiting for more drugs and vaccines capable of dealing with some unresolved or only partially resolved health issues.

The pressing demand for more effective and better-tolerated anti-tumor drugs, for antiviral drugs and vaccines to withstand a possible pandemic, an effective treatment for autism, as well as vaccines to protect against malaria in places where it is endemic, are just some of the areas where pharmaceutical research is focused[2].

Drugs are important for our health, on the other hand, they're also an industrial product and, for this reason, they have to satisfy the requirements by society to guarantee the health of its members as well as nits own social and economic development.

The research and development (RD) process for a new and specific drug involves a high risk level and that is why -in the majority of cases- the pharmaceutical company is asked to accept responsibility where members of the community as well as company shareholders are concerned[3].

Because of the technological complexity involved in the process of research, discovery, development, and production of drugs, the pharmaceutical industry is classified as a high-technology industry, capable of being very innovative playing an important role into the research

Giuseppe Recchia
Vice president medical and regulatory, GlaxoSmithKline
giuseppe.g.recchia@gsk.com

field of a country. The lack of scientific research (1.16% of GNP was invested in RD in 2003, down from 1.19% in 2002) is one of the major structural weaknesses of Italy. Not only we are a long way from European Union ion average of 1.9% but we are even further from 3% stipulated by the Conference of Lisbon considered as a prerequisite for the development of the new economy of knowledge, capable of guaranteeing sustainable economic growth, with newer and better jobs and a greater social cohesion[4].
In this context, the profit of a pharmaceutical company makes can be considered as a recognition of their commitment to research and a reward for the investors.
The uncertainty principle
Over the last few years, various studies have been published whith hypothesis about whether the uncertainty principle (the assumption that it is possible to randomise a patient to a treatment only when we are uncertain about the real benefits and real risks involved, even though it would be reasonable to expect that the newer drug has certain advantages compared to the old one) has been violated when carrying out clinical experiments which have been sponsored by industry[5-8].
According to these authors, research which is sponsored by the pharmaceutical industry would probably produce results much more favourable to the sponsor's product compared to studies which have been financed by other sources. Some of the possible explanations as regards the profit interest and the distortion of the results are outlined below.

- Selective financing on the part of pharmaceutical companies for experiments on drugs which are considered superior to the product chosen for comparison;
- poor-quality research planned and carried out by the industry;
- setting objectives which maximise the chances of getting a positive result which is useful for approval or publication purposes but has no real medical objective;
- experimental design is usually based on consolidated models accepted by the regulatory bodies and makes very little use of *flexible design, adaptive design, traker studie*s;
- the control group is usually chosen to maximise the positive results or to test how big the effect can be (placebo-controlled study) in this way the sample is minimised as well as the time and costs involved in the study, there may even be real distortion (use of terms of comparison which are non-standard in clinical practice or used in

non-standard conditions, for example different dosages);
- selected criteria to get the most suitable subject to be included in the study to prove that there is a difference between A and B but that they are different from the real objective population, without presenting data that enables everyone to determine how representative the sample is;
- definition of the variables which have been used, with the choice of surrogate end-points;
- analyses which exaggerate the effects by biased or repeated representation;
- selective publication of studies with positive results (*publication bias*) and the repetition of studies published on the same subject (*salami slicing*).

The conclusions reached by these studies have been discussed by other authors who questioned the method and the validity of the results[9]. Although I share a few of their reservations, especially regarding the inappropriate patient selection, and the comparison of clinical trials which took place over a period dating from 1966 to 2002, all the raised questions concerning these studies deserve to be investigated more thoroughly, above all, they should remember how different it is in nature and scope research if these are sponsored by industry or by institutions.

RCTs and clinical development of drugs for regulatory purposes

Controlled clinical trials of drugs are a basic tool for the development of knowledge regarding illness and treatment. One of the main applications of this knowledge gained from clinical trials can be found in the development of new drugs (usually by a pharmaceutical company), in the improvement of treatments and in the information reguarding the choice of treatment.

Principles of respecting the integrity of the data and safeguarding the well being of patient are obviously common to all research projects, but the planning and organisation of research can vary significantly depending on its aims.

RCT trials want to distinguish the effect of a drug from the effect of other phenomena like the spontaneous evolution of the illness, the placebo effect and distortion in the observation[10]. The purpose of the RCTs carried out during the clinical development of a new drug by an

industrial sponsor is to provide the regulatory authorities with the necessary documentation to prove the effectiveness, tolerability and quality of the new drug as regards each stipulated point to get the necessary authorisation to sell the drug on the market AIC (Authorisation for entry into Commerce). Once this authorisation has been obtained, the development can continue investigating and analyzing more deeply how valuable the new drug is in terms of helping people it was designed for.

Clinical experimentation is the last and most important stage of RD. It is organised in 4 phases, a chronological sequence at the outset (I,II, III e IV[1]) practically it is often overlapping and it is guided by the requirements of the international regulatory authorities and by the research group which is developing the new compound.

In the second half of the Nineties, they launched the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH to limit the risk of duplicating studies and tests carried out during the development of pharmaceutical products as a result of requirement from various regulatory bodies. This project brought together regulatory bodies from the USA, Europe and Japan, along with experts from the pharmaceutical industry, to discuss scientific and technical aspects of registering pharmaceutical products. ICH formulated a series of recommendations about how to ensure greater consistency in the interpretation and use of the technical guidelines and registration requirements which have been accepted and adopted over the last few years by EMEA and FDA[11].

Organizations like these ones, with their information and guidelines for the clinical development of drugs provide important pointers to obtain AIC. The *European Agency for the Evaluation of Medicinal Products* (EMEA) document entitled ICH Topic 8 General Consideration for Clinical Trials[12] has general references and information about how to structure and conduct RCTs for registration within the EU and there is a set of specific guidelines for each of the major covered points. Simi-

lar documents have been published by the *Food and Drug Administration* (FDA) for the USA[13].
The ICH recommendations accept two different ways of demonstrating that a drug is effective. At first by showing that the experimental drug is superior to a control treatment, secondly that the experimental drug proves to be equivalent to a well-known effective treatment as concern a certain set of criteria. The regulatory bodies accept both of these methods as valid[14] but each one requires a completely different approach. Placebo-controlled studies and active-control equivalence studies (which possibly go some way towards explaining the different results that have been observed depending on the sponsor) are still the subject of debate[15].
The FDA considers the use of placebos inappropriate when delaying or failing the treatment administration could lead to increased mortality rates or irreversible morbidity in the studied population. If not being treated carries no significant risk , however, the FDA says that patient participation in a placebo-controlled study is both appropriate and ethical as long as the patient is well-informed[14].
There is more consensus about using placebos when there is no recognised gold-standard drug available for the pathology in question. The placebo and the new drug are add-on treatments to the best available option or the placebo serves to mask the comparison of two active treatments which are administered differently (double dummy)[16].
There are other situations where the legitimacy of using a placebo has to be assessed on an individual basis by the ethics committee, for example:

- whenever is impossible to predict the clinical effect in case of non treatment (in terms of risk and/or discomfort) in particular in case of slow developing chronic pathologies like slight hypertension, non-insulin dependent diabetes, stable angina or the treatment of moderately severe symptoms (for example sleep disturbances, non-severe pain etc) An emergency drug must be on hand as a rescue treatment in case the patient requests it or the experimenter feels that it is necessary.
- Incidence rates of the event under analysis are very variable within the normal population so the absence of a placebo might mean that the results cannot be evaluated and the study is unethical (for example in the case of post-operative sickness,where using an active drug as the comparison (rather than a placebo) meant that the results were not conclusive in most of the 40 analyzed study [17].

The FDA maintains that stopping the use of placebos in RCTs where an effective treatment is available could have very negative consequences. In fact, since is difficult to interpret equivalent studies in different contexts, RCTs of new drugs that use an active control may not be able to provide persuasive proof as to the effectiveness of the new treatment unless the new drug proves to be superior to the active control[18]. This obviously reduces the number of treatment choices available for the patient and it makes difficult to individualize the treatment. These means that what proves to be the best option for a whole treatment group is not necessarily the treatment of choice for the individual patient[14,18]. The USA agency also points out that it is critical to recognize that a new treatment might be of significant benefit without being more effective than another. It would be difficult, if not impossible, to identify such treatments without studying them in control versus placebo RCTs[18].

Whereas the Food and Drug Administration considers placebo-controlled clinical studies indispensable for the approval of a new drug, other regulatory authorities take the opposite view, and have a very restrictive attitude towards placebos. Since a large number of RCTs conducted in the States are based on regulatory purposes, it is normal to expect that a larger number of the studies undertaken by industrial sponsors are placebo-controlled.

In September 2004, EMEA and FDA set up a pilot programme to give parallel scientific advice to the sponsors regarding clinical development of new drugs with the aim of optimizing development and avoiding repetition of unnecessary research[19].

The clinical development programme for a new drug, which is set in accordance with the regulatory authorities, has to be considered in relation to other similar programmes and to provide useful information and indication in case of future research.

When planning the project, all available information from in vivo pharmacology and experimental medicine studies (phases I and II) as well as previous RCTs are taken into account with a view to define the best way fo future development and o handle the risk of failure appropriately. In my opinion this does not mean that the uncertainty principle is violated nor that of clinical equipoise, since in each case, at the moment of randomization, there could be uncertainty about the benefits and risks of the treatment under study[20] (this means that the patients can only be admitted into a randomized trial if their doctors are unsure about which treatment whould be most appropriate for the condition

of the patient). The results of a recent study performed to find out whether institutional oncological research studies satisfied the uncertainty principle analysed phase 3 studies and showed that 29% of randomisation favoured the experimental treatment as against 3% the standard treatment. In the authors' view, despite such an imbalance between experimental and standard treatment, the uncertainty principle was respected for most of the part[21]. In our opinion, therefore, if we want to understant why certain studies are so different, we would do better to compare the aims behind the research rather than the type of sponsor, since studies which have as princial aim the approval of a new drug are typically sponsored by industry.

Disagreement regarding the development of new drugs is one of the major problems in the RD process. Despite increased spending on RD in recent years the number of requests for AIC received by the FDA decreased considerably and on an international level it is thought that there were 35% fewer studies undertaken in 2003 compared to 2000. DeMasi, using data from the Pharmaceutical Research and Manufacturers of America, estimated that out of every 5,000 compounds which are pharmacologically tested, only five on average reach clinical tests, and out of these, only one gets approval for use on patients[22]. Trying to identify early research and development projects have little chance of success is fundamental for the drug companies and one of the imperatives for maintaining competition in research (DeMasi). The risks regarding the development of a new pharmaceutical product means that it is important to maximise efficiency and minimise risk by proceeding through the sequential phases which enable the effectiveness, tolerability and quality of a product to be established before thinking about its value.

The evolution of any development program nonetheless has the initial aim of demonstrating that any new drug is effective and tolerable, using measures which have been agreed by the regulatory authorities and comply with the guidelines for drugs development. Once this phase of the development is over, often at the same time as AIC is applied for, the development program continues with comparative studies to further define the new drug's therapeutic value, often using clinical success measures like mortality rates or quality of life related to health. Studies like these can be used for regulatory purposes to get money back or increase the amount of money received. Using the documentation which is presented to the regulatory authorities when applying for AIC as a source of information ti judge how good or in-

novative a new drug is, is not an appropriate form of assessment .In general it is not until years after registration that the necessary documentation exist to give us the possibility to make a conclusive assessment. The need to balance and contain risk when developing a new drug means that clinical experimentation conducted by a drugs company with a view of developing a new product is very different from the individual experiments carried out by an institution for different reasons.

Conclusions

Then what are the factors that should be considered when trying to classify research its features maybe even its rules and norms?
The literature we quote considers, incorrectly in our opinion, that characteristic desire of profit of the sponsor actually determines the nature of the study. On the other hand we believe that the characteristics of the sponsor are more easily explained by factors like the aim of the study (for approval or post-registration purposes) which determine the choice of methodology (for example placebo-controlled) and, as a consequence, lead to a different type of result.
An extreme simplification like this one could be dangerous. Discussion regarding the influence of profit interests on research gave the possibility to debate; as a consequence this raised people's awareness about certain distortion like publication bias.
However, this problem needs to be accompanied by reflection and increased commitment from all parties to encourage and obtain real improvement in research.
Confusing problems linked to methodology and organisation and simplifying the answer by blaming the type of sponsor may increase the diffidence in research from the point of view of industrial sponsor and lead to the implicit assumption that the problems could be solved by simply finding a different type of sponsor that whould have to compete with the former one rather than work with them.

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RCTs AND THE UNCERTAINTY PRINCIPLE: A GUIDING PRINCIPLE TOO OFTEN FORGOTTEN? REFLECTIONS ON DESIGN, ANALYSIS AND INFORMED CONSENT

Nicola MAGRINI

This article aims to show how the uncertainty principle is the leading criterion for randomised clinical studies, which are the standard for an effective evaluation of the benefits and risks inherent in any treatment. I will also try to show how this principle is often overlooked, so that the focus is placed on the specific drug rather than on the patient or treatment as a whole and that part of this bias stems from commercial aims. As well as published studies, I use as references sample case studies discussed in recent sessions of the Reggio Emilia Ethics Committee.

A general premise about the uncertainty principle (in superiority studies)

"New experimental treatments for infantile tumours evaluated using phase III randomised studies have the same probability of being superior or inferior to existing standard treatments. Our data show that the results of single randomised trials are equally unpredictable. We maintain that the success or failure of new treatments is consistent with the "uncertainty principle" which forms the basis for involving patients in trials."

This is taken from a study, recently published in the British Medical Journal[1] , where the authors discuss the results they obtained from an analysis of all the randomised studies (also known as RCTs – randomised controlled trials) in paediatric oncohaematology over the last 20 years which were publicly funded (National Cancer Institute).

Nicola Magrini
CeVEAS – Centre for the Evaluation of the Effectiveness of Health Care
Azienda USL, Modena

The perhaps rather non-intuitive aim of this study was to work out if, and to what extent, the studies carried out in this area of great therapeutic progress had respected the fundamental principle upholding the ethics of randomisation, that is, the uncertainty principle, which basically means not knowing, *a priori*, which treatment is better (the standard, the new or the placebo).
The study showed that there is the same degree of probability that a new treatment will prove to be more effective than the old or placebo treatment, as there is that the new treatment will prove less effective than the standard treatment. The progress made with treatment, therefore, is as a result of the "empirical evaluation of new treatment solutions".
Does this data not strike you as strange? Or rather, doesn't go counter to our expectations? If great progress has been made, shouldn't the studies have come out in favour of the new treatments more often?
To make sure we have this clear, let's go back a little bit. Randomized studies are the required standard for evaluating new drugs (before they are put on the market, especially in phase III, when they are compared to the standard reference treatment or placebo within relevant clinical parameters. RCTs can highlight even small differences between the treatments or results which are not glaringly obvious, because they use groups of patients who are similar in terms of all factors determining prognosis, whether these are known to us or not. In these trials, the volunteers are assigned to one or other of the groups completely randomly (by tossing a coin, for example). Members of one group are given the new drug (drug A) and members of the other are given the reference drug (drug B) or a placebo. It is because of the random way in which patients are assigned to the groups that the groups are homogeneous and similar, so we know that all the differences that may (or may not) be observed are due to the treatment under study.
The ethical basis of these trials rests on the fact that, fundamentally, we do not know which treatment might be better or more appropriate for the individual patient: this is what the uncertainty principle means[2].
Vice-versa, if the doctor or patients shows a marked preference for, (or fear of), one of the treatments being used, the uncertainty principle is not being adhered to and the patient should not therefore be included in the trial.
It is because of the uncertainty principle that we are able to interpret The relevance of our work better, i.e. how important are the things we intend to evaluate, and the two principles, uncertainty – relevance, very successfully provide the key to understand what should be

the beginning (ethical base) and the end of an experiment (in terms of the importance of the results obtained).

The question that springs to mind at this point is: to what extent is the uncertainty principle really adhered to in RCTs or how often is it known from the beginning which is better treatment? According to the results of the BMJ study, the answer would seem to be that in one branch of medicine, where great progress has been made, the principle is observed. It is true that the studies they analysed were onces funded and carried out by a major institution, the American National Cancer Institute, but it was nice to see that the studies were really based on the uncertainty principle.
It is questionable, however, whether the same can be said of studies financed by the pharmaceutical industry, which tend to come up with a positive result for the experimental treatment far more often. An analysis of all studies relating to myeloma showed that only non-profit organisations had, overall, observed the uncertainty principle (47% of studies in favour of new treatment vs 53% in favour of standard treatment). In studies financed by the pharmaceutical industry 74% came out favourable to the new treatment[3]. This can only be explained by selective financing of studies which have a much greater chance of being positive[4].

A more difficult case: the uncertainty principle as related to studies of non-inferiority or equivalence

The studies described above may be a lot intuitive and correspond much more to our idea of progress. However, it is much more complex, often much less intuitive, and sometimes even ethically unacceptable when we try to apply the uncertainty principle to non-inferiority studies, where the aim is not to show that the new is better than what is currently available or than a placebo, but rather that the new is not worse within a certain margin which is established beforehand as being clinically relevant. The logic of experimentation is thus overturned and the aim of the study becomes that of refuting the opposite hypothesis.
Equivalence studies, although much rarer than non-inferiority studies, are nonetheless similar. They seek to find a basic equivalence between the new and old treatment (standard) within predefined limits.
It must be said immediately that this approach only makes sense when, within a situation of accepted relative equivalence with a certain amount of uncertainty (non-inferiority), the new drug nonetheless offers some

kind of advantage or margin of superiority. Otherwise, it would be difficult to see what benefit there might be for the individual patient or society in general. The only benefit would be to the market, which allows for competition between different producers offering similar products.
What sense does it make to a patient or an experimenter to rely on a study whose aim is non-inferiority? I think this is a question we need to really ask ourselves and we need to answer on a case by case basis, trying to identify possible secondary benefits which are not simply those of improving the market range.

The relevance (as opposed to futility or irrelevance) of these studies obviously seems questionable when they relate to drugs which are copies of existing ones or similar drugs belonging to the same class; the so-called me-toos[5]. This type of drug, of which there can be a dozen or more in certain categories (6 statin drugs, 13 ACE inhibitors, 12 calcium antagonists, to name but a few), are approved in studies which do not aim to identify any ulterior benefit (because there is no clause in law that says that a new drug within the same therapeutic category has to be superior to existing drugs). It must also be acknowledged that copy drugs these days, compared to the last few decades, are proving to be cheaper than the brand-name drug and are often studied more and this could represent an advantage for the market system by improving the range.

We are not all equal (independent and for-profit): the uncertainty principle and definitions of standard or best therapy

If there is uncertainty, it is between a new treatment and the standard treatment of choice. The selection of an adequate control group is therefore of fundamental importance both for ethical and obvious practical reasons, if the comparison is to be valid. Unfortunately, it happens too often that in the treatment chosen for comparison the drug dose used is less than that recommended in studies to determine the efficacy of the drug and, thus, the level of response, whereas we see suboptimal doses of the experimental drug being used in equivalence studies where the main aim is to see how well the drugs are tolerated. Another, (though much rarer) occurrence is the use of higher doses of the experimental drug in order to get the maximum efficacy.
One example from the literature of this kind of "playing with the doses" can be seen in the studies into the new antidepressants, the selective serotonin recaptation inhibitors (SSRIs) and, in particular,

as regards the brand-name drug of this type, Fluoxetina. When Fluoxetina was studied as an experimental drug and compared with tricyclical antidepressants, it was often tested using higher doses (70% of studies used doses of >40 mg/day), whereas when it was the control drug in trials, where the new SSRI antidepressants were the experimental drug, Fluoxetina was often used at low doses (the >40 mg/day dosage was only used in 27% of studies)[6].
Other examples of poor equivalence are recent often-quoted, much-publicised studies into hypertension (ASCOT and VALUE), where patients in the two groups under study did not have the same blood pressure control, which may go some way to explaining the different results observed[7] but makes it more difficult to explain some of the non-differences observed[8].
In one of the recent sessions of the Ethics Committee we looked at a study which compared two different formulations of Taxolo with different salification levels after the licence had expired, whose aim was to assess the effect of the two drugs. It was surprising to note that the non-inferiority study did not use the same quantity of the drug in the two different treatments (a higher dose was used with the new salification), thus prompting doubts as to observation of the uncertainty principle as well as doubts regarding the study design. Certain conclusions were also drawn which we will later examine.

We are all equal (independent and for-profit): selective publication of study outcomes

Two recent publications, (and others which are in the process of being published), have brought to the attention of the scientific community empirical data, which reveal a big mismatch between results which were originally expected in the study protocol and the results subsequently reported at publication. The extent of this mismatch is what has made people seriously worried about the quality of the available knowledge base, given that the bias introduced by modifying the original parameters begs some very big questions. The empirical evidence of a selection of study outcomes, which are different from those predicted in the protocol ,appears to be similar whether or not the researchers were sponsored by industry or by public funds. The first study[9] examined all clinical studies evaluated over the course of two years by the Copenhagen Ethics Committee and these studies were subsequently re-evaluated once they had been published to see if the type of outcomes presented were those originally predicted. The data

was frankly worrying. More than half of the outcomes relating to effectiveness had been modified and the modifications, substitutions or omissions even related to the main outcome of the study. As far as data relating to safety, and therefore risk, is concerned, the data was modified in about 65% of cases.

In my opinion, it was very comforting to see the same study repeated in Canada[10] on studies financed by government agencies and to see that the results were comparable. This tendency to choose and select, which outcomes to report on, seems to stem from a desire to talk about only the more significant data, as if non-difference was less relevant.
It is understandable that this optimistic attitude towards positive results influences the clinic and the doctor-patient relationship, but it could create distortions in the available evidence base which really ought to report on all the different parameters examined (especially if they were deemed relevant beforehand) whether these were positive, null or negative.
Publication of this data on two databases, which offer free access, (the American NIH and the WHO) has clearly accelerated the initial registration process (which had been talked about for years but had never become operational) for studies which are later to be published usually by the main International medical journals under the auspices of the ICMEJ (International Committee of Medical Journals Editors)[11].

Publication bias, corruption of the available evidence base and solutions to these problems: registration of RCTs

The age-old problem of publication bias, as well as some serious omissions regarding the communication of information, which was known to the drugs producers (selective anti-cox-2 analgesics and antidepressant drugs in children), have speeded up the process of compulsory registration (from 2005) for studies which are to be published in the main International journals[11].
This is a big step forward as far as the transparency and accessibility of the original protocol and tracing studies, which were never published, is concerned. People working in the field complain about the amount of pressure the pharmaceutical industry puts on them in protest against this praiseworthy initiative, which has finally taken shape after so many years of hesitant proposals. This database will mean that publication bias can be quantified much more easily and will

also enable us to see how many of the studies which claim to contribute to the overall data were actually interrupted early.

Uncertainty principle, optimism bias and studies which are prematurely interrupted

In a recent editorial by Sir Iain Chalmers in the Lancet[12], he goes back to these studies and formulates a conclusion about the implications an optimism bias can have, stressing the importance of the uncertainty principle and how it must remain at the heart of any credible scientific approach. Chalmers reminds us that data have been available for two decades which reveal a kind of bias (systematic error) in quotations, where only favourable studies are mentioned. Studies into new treatments tend to quote available positive studies more frequently than negative ones, even when the methodology was equally valid[13]. As far as more important and frequently-quoted studies are concerned, Ioannidis has recently demonstrated that in more than a third of cases, results which were very favourable when they were first published were subsequently modified as regards to the effect of the drug. On occasions, the results were even contradicted or shown to be false (this was particularly true for observational studies)[14]. Chalmers concluded that "an optimistic bias runs the risk of discouraging patients from taking part in clinical studies designed to reduce real and important uncertainty surrounding the effects of treatments and of discouraging the repetition of apparently promising preliminary studies. Until we start to worry about their role, it will always be questionable whether the clinics taking part in clinical trials are really observing the ethical conditions linked to uncertainty".
On the same subject, it may be useful to mention an editorial by Psaty e Rennie[15] in the JAMA, which bitterly criticised the sponsor's decision to suspend a study (CONVINCE[16]) comparing two different therapeutic strategies for hypertension. While acknowledging that the researchers published all available data, it must be remembered that the study, terminated prematurely, provided no useful information which it may well have done had it been continued to the end as stated in the protocol, and decisions like this , which are motivated by commercial reasons, have profound implications for the ethics of research and publications.

Uncertainty principle and informed consent

Consent is actually the informative principle for patients taking part in studies and forms the basis of the knowledge and information that

is to be passed on to the patients themselves or to their next of kin or relatives regarding the decision whether to take part in the trial or not. For example, consent forms used by the National Cancer Institute have a sheet which gives essential and explanatory information (2-3 pages), which aim to sum up the premises on which the study is based, the uncertainty that guides it, and the informatione necessary for a general understanding of the trial.
This NCI form (NCI informed consent template) is clearly ignored by the majority of studies financed by industry, which tend to favour consent forms which are extremely detailed (15 – 30 pages long) and that put the same emphasis on an enormous quantity of details, which are of little relevance to the patient and is often boring and distracting.
This is done for legal reasons in part but, in my opinion, it is also done to make the real explanation less clear.
A study which is currently being carried out into two different basic formulations contains some alarming statements regarding the old method of salification ("can lead to death"), which seem to go well beyond the uncertainty principle and also some confused statements, which are probably worth quoting: "You and your family will receive no financial payment or other forms of compensation for loss of income, pain or suffering or other damages or losses associated with any injuries you may incur as a result of participating in this trial; in any case, by signing this form you are not ceding your civil rights. In agreement with the Italian law, an insurance policy has been taken out, which will cover eventual damages incurred by you as a result of this research". Something in this string of words seems to engender confusion or looks like the result of a poor translation ...

Further reflections

Uncertainty for who? Who is writing and who holds the data and is responsible for analysing and interpreting it

What are we supposed to think when studies are financed by a company which sets out not only the protocol, therefore the objectives and the way the study is to be carried out (including the choice of researchers and the countries it will be run in), but also pays the researchers and has possession of the data which it analyses using its own statisticians, as well as control over how the data is interpreted and, then, these same studies are published in the name of eminent experts in the field? What sort of studies are these, and to what extent do readers and journals feel cheated by this discrepancy between the

people who designed and interpreted the study and those who act as the agents or guarantors for transmitting the information to the scientific community?
This is what Richard Smith wrote in the BMJ[17] some years ago, and, as we can see, the root of the problem is who designs the studies and how to interpret the uncertainty, which the study in question may or may not be able to give an answer to. The need for greater transparency, in terms of how the study was conceived and carried out and in terms of acknowledging any conflict of interest on the part of the authors and the role of the sponsor, is something which has been given more space in the major journals of late. JAMA redefined its criteria for explicitation and set out different standards for studies with an industrial sponsor[18], requiring from them a series of extra specifications compared to independent studies, thus creating a kind of double standard. For example, when it says that "for studies sponsored by industry the declaration (that full access to the study data will be given and responsibility is accepted for the integrity of the data and the accuracy of the data analysis) must be made by a researcher (preferably the chief investigator) who is not being paid by any of the sources of the funding." It also states that it is desirable for the data analysis to be carried out independently of the sponsor.

To finish
Uncertainty principle and financial conflicts of interest and the role of regulatory agencies

A brief mention of the fact that, these days, the "disclosure" formula is increasingly considered over-simplified as a solution to a problem which is as complex as that of economic relations and the relationship between financers and researchers and even between single institutions and universities.
A problem within the system which should also be posed is that of the role of the regulatory authorities. Their role has been seriously weakened over the last 20 years in the USA[19] , prompting urgent requests for their restructuring from many different parties[20,21].
Strong criticism has also been directed at the EMEA especially for its association with the Directorate of Industry and Commerce (as opposed to the National Health) and for the limited access granted to its decisions and available material[22].

Conclusions

There are many different types of distortions in the available evidence base and, maybe there are much more; these are more cumulative, than any fairly good researcher might expect[23]. The tendency to favour the publication of positive studies, the selective publication of the study outcomes (more or less outcomes compared to those originally stated in the protocol) may be neutralised by compulsory registration, in the case of studies which are of major scientific importance. However, registration may have less of an impact (in that it is not binding) on studies whose aim is to get a drug registered and attach greater importance to systematic revision as a way of guaranteeing a better look at the collection of available data.
The role of the regulatory agencies is, for the moment, weighted in favour of safeguarding industrial licences, so that many studies into drugs are designed in a single-drug oriented way, rather than a patient-oriented way, which might show more respect for patient needs and the doctors' need for information.
Moreover, the emphasis given by over-interpretation on their part because of potential conflicts of interest, particularly financial or relating to the system of reprints, conferences and forms of advertising makes the whole system of information weighted in favour of new drugs and more inclined to exaggerate the emphasis and perception of real benefits. Many eminent journals seek to improve and simplify the presentation of the results of studies published (see instructions for authors for the BMJ) by providing both absolute data and the relative measures which are more often included because they highlight differences which are in reality very small and apply to very few patients.

Clinical research, which aims to evaluate new approaches to treatment and care, ought to become an essential component of Health Care and National Health Services to counterbalance the number of studies financed by the commercial sector which too often focus on the single drug.
Finally, in order to ensure that RCTs are not simply a series of technicalities to be applied on an industrial scale, the researchers and doctors involved in the experimentation, together with the Ethics Committees, need to make sure that the uncertainty principle is respected to a large extent and, in addition to this, the principle of relevance (as applied to the objectives) of the study within a context where focus is

firmly kept on the patient, remembering that, primarily, research means having the ability and the courage to dare.

As Drummond Renne said, in his closing speech to the Peer Review Congress in Chicago in September 2005: *"peer review is something similar to democracy ... is democracy at work"*

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TRANSCRANIAL DOPPLER ULTRASONOGRAPHY IN INTENSIVE CARE

Frank A. RASULO, Elena DE PERI

Key words: transcranial Doppler, intracranial pressure, cerebral vasospasm, cerebrovascular self-regulation, critical closing pressure.

Introduction

Transcranial Doppler (TCD) is an innovative, flexible, accessible tool for bedside monitoring of static and dynamic cerebral flow and treatment response. Introduced by Rune Aaslid in 1982[1], it has become indispensable in clinical practice.

Physical principles

TCD is based on the Doppler principle illustrated by Christian Andreas Doppler in 1842, which describes the apparent frequency change of a sound wave caused by relative movement between the observer and the sound source[2,3]. When stimulated by an electric current the Doppler probe emits an ultrasound wave which is reflected by the moving red blood cells it meets: as blood flow approaches the probe, frequency of the reflected wave increases while blood flowing away from the probe produces a lower frequency compared to the original wave. The difference between the frequency of the original signal and the reflected signal is known as the Doppler shift and is directly proportional to blood flow velocity as expressed below:

$$F = \frac{2FtV\cos\theta}{C}$$

here F is the Doppler shift, Ft is the frequency of the wave emitted, V is the actual velocity, C is the velocity of sound in tissue and $cos\theta$ is the cosine of the angle between the insonated vessel and the direction of the ultrasound wave[2,4-5]. Blood flow velocity can be estimated by measuring

Frank A. Rasulo, MD, Medical Researcher
Elena De Peri, MD, Senior Resident
Institute of Anesthesiology and Intensive Care – Spedali Civili University Hospital of Brescia
Piazzale Spedali Civili, 1 – 25125 Brescia, Italy
Tel. +39-030-3995 570/764/563 – www.med.unibs.it/anest/ – rasulo@med.unibs.it – elenadeperi@libero.it

the Doppler shift. The difference between estimated and actual velocity increases with the angle of incidence θ, which means that a better estimate of flow velocity may be obtained by reducing the angle.
TCD employs pulsed wave probes featuring a single piezoelectric crystal which is alternately stimulated to produce a signal and silenced to allow the reflected wave to be read. By varying the time interval between transmission and reception it is possible to modulate the depth of insonation and examine selectively a specific segment of the cerebral vascular tree.
TCD provides information on blood flow in both acoustic and visual form. With the former an audible acoustic signal is produced whose intensity, height and pitch reflect the characteristics of blood flow of the vessel under examination. In visual mode "Fast Fourier Transform" (FFT), also known as spectral analysis, is used to produce a two-dimensional image on the screen. The curve is further elaborated to gain several important diagnostic parameters: peak systolic velocity, telediastolic velocity, mean velocity, Gosling's "pulsatility index" and Pourcelot's "resistance index".

$$P.I.= \frac{FVs - FVd}{FVm} \qquad R.I.= \frac{FVs - FVd}{FVs}$$

Examination technique

The main obstacle to ultrasound penetration of the skull is bone[6]. Low frequencies, 1-2 MHz, reduce the attenuation of the ultrasound wave caused by bone. TCD also provides the advantage of acoustic windows representing specific points of the skull where the bone is thin enough to allow ultrasounds to penetrate[1,2,7].
The **transtemporal window** is sited at the thin portion of the temporal bone, between the external cantus of the eye and the external acoustic meatus, immediately above the zygomatic arch. It is the most frequently used window and allows investigation to be made of the proximal segment (M1) the median cerebral artery (MCA), the A1 segment of the anterior cerebral artery (ACA), the posterior cerebral artery (PCA) and the final segment of the internal carotid artery (ICA). By compressing the carotid it is possible to evaluate the patency of the communicating anterior and posterior arteries. This approach is unsuitable for approximately 10% of patients due to thickened bone or osteoporosis. New substances, ultrasound contrast media, have re-

cently been introduced which allow this approach to be used in these patients as well by amplifying the ultrasound beam reflected.
The **transorbital window** makes it possible to assess the ophthalmic artery, the carotid siphon and the contralateral MCA. The probe is placed above the closed eyelid. This window must not be used in patients who have recently undergone surgery to replace the lens as the effects of ultrasound on artificial lenses are as yet unknown. In addition, the signal power of the Doppler emitted by the transducer must be reduced by 20% to avoid damage to the retina.
The **suboccipital window** is sited posteriorly at the highest portion of the neck. The probe is placed on both sides of the spinal column and must be orientated towards the median; the ultrasound beam penetrates the cranium through the space between the atlas and the base of the skull. This window makes it possible to evaluate the suboccipital and intracranial portion of both vertebral arteries (VA) and the basilar artery (BA).
The **retromandibular window** is not an acoustic cranial window as such, but represents an extracranial approach to assess the distal segment of the extracranial internal carotid (ICA), immediately before it enters the carotid foramen. The probe must be placed at the angle of the mandible and orientated cranially.
The identification of each intracranial vessel is based on the following elements: a) velocity and direction, b) depth of signal capture d) possibility of following the vessel its whole length, e) spatial relationship with other vessels f) response to homolateral and contralateral carotid compression.

Clinical applications

Two recent publications[8,9], specified the main fields of clinical application of TCD, with an assessment of the advantages and limits of the method itself.

Vasospasm

Mean flow velocity (MFV) is directly proportional to flow and inversely proportional to the section of the vessel. Any circumstance which leads to a variation of one of these factors can thus affect mean velocity. Physiological or paraphysiological conditions which modify flow velocity are age, Ht, apCO_2 and metabolic requirement. As for pathological conditions, the main condition affecting flow velocity is the vasospasm, the reduction of the lumen of the vessel following con-

traction of the smooth muscles of the wall.
Vasospasm is a frequent complication of SAH[10-15] and has an incidence of between 30-70%. It has a typical time-span: it is normally absent in the first 48-72 hours after SAH. Onset occurs from day 3 to reach a peak between day 6 and day 12, gradually lessening at 15-20 days. The vasospasm often remains clinically silent and the factors which make it symptomatic are largely unknown[16].
Threshold velocities above which vasospasm comes into place are well defined as regards the MCA, while there is no consensus for the other vessels.
As far as the MCA is concerned, MFVs below 120 cm/s indicate the presence of a slight reduction of the vessel lumen, which cannot show up at angiography. Velocities of between 120 and 200 indicate moderate vasospasm, which leads to a reduction of the lumen of between 25 and 50%, while velocities above 200 indicate serious vasospasm with a reduction of the lumen exceeding 50%.
Nevertheless, an increase in velocity alone is not sufficient to arrive at a diagnosis of vasospasm: a condition of hyperemia also presents with an increase in flow velocity. The Lindegaard Index (LI)[17] has therefore been introduced which is defined by the ratio between the MFV in the MCA and the MFV in the ICA. Thus, an LI <3 indicates hyperemia, between 3 and 6 moderate vasospasm and >6 serious vasospasm.
Other parameters, such as velocity increases >50% in daily serial examination or the presence of an asymmetry (velocity difference exceeding 50%) can aid diagnosis of vasosapsm[18].
TCD studies also correlate well with angiography studies in vasospasm of the BA[19], though less so for the other arteries at the base of the skull[20].

Stenosis of the intracranial arteries

Criteria for diagnosis of a stenosis > 50% of an intracranial vessel with TCD include: a) segmentary acceleration of flow velocity, b) drop in velocity below the stenotic segment, c) asymmetry and d) circumscribed flow disturbances (turbulence and musical murmur)[21-24]. The sensitivity and diagnostic specificity of the TCD is higher in identifying stenosis of the anterior circulation (carotid siphon and MCA in its proximal segment M1), compared with the posterior circulation (VA, BA at segment P1 of the PCA), which presents more anatomic variability and difficulty of insonation. Data to establish diagnostic criteria with TCD for stenosis <50% are insufficient.

Intracranial occlusion

Doppler diagnosis of occlusion in an intracranial vessel is possible when no sign of flow can be found at the normal depth and position, or when its speed is significantly reduced and none of the other vessels can be heard. There may be increases in speed in other intracranial vessels due to activation of the compensatory circles. In patients with acute cerebral ischaemia, the specificity and sensitivity of TCD in diagnosing MCA occlusions is over 90%[25,26]. Repeated or continuous TCD monitoring enables us to follow the evolution of the occlusion and to assess possible recanalisation in the vessel whether that be spontaneous[27,28] or induced by fibrinolysis. Comparative studies with angiographs have shown excellent diagnostic correlation, even when there is partial recanalisation[29,30]. A recent small randomised trial[31], compared 11 patients who underwent thrombolysis with t-PA combined with continuous TCD monitoring, and 14 patients treated with t-PA, without TCD monitoring, all of them with acute MCA occlusion. The patients who were continuously monitored with TCD had higher levels of recanalisation at one hour and better outcome at 90 days compared to those treated with t-PA alone, suggesting that ultrasound may play a role in facilitating the lysis of the thrombus. TCD showed good sensitivity and predictability with carotid siphon, VA and BA[30].

Cerebrovascular autoregulation

Cerebral autoregulation refers to the brain's intrinsic ability to maintain cerebral blood flow (CBF). When this system is compromised, patients are at risk of developing ischaemia and cerebral edema.

Cerebral autoregulation includes *pressure type regulation* which means that changes in CBF are kept to a minimum even when cerebral perfusion pressure varies, as long as the variations are within 50 and 150 mmHg, and *vasomotor reactivity, which is the response of the cerebral circle to changes in apCO_2 e apO_2*.

Pressure regulation can be subdivided into dynamic regulation, involving a rapid response on the part of CBF to changes in arterial pressure or its pulsatile nature, and static regulation, involving a rapid response on the part of CBF to slow changes in average arterial pressure[32] (MAP).

The TCD enables us to assess both components of self-regulation. The static component is measured by observing changes in flow velocity caused by pharmacologically-induced episodes of hypertension and hypotension. In this way the static rate of autoregulation can be measured using the ratio between percentage variation of resis-

tance and percentage variation of MAP. A value of 1 indicates that autoregulation is intact whereas 0 tells us that autoregulation has stopped[33].

The dynamic component of autoregulation can be measured using a method devised by Aaslid known as the "cuff test". Arterial hypotension is induced by placing thigh cuffs on each thigh and tightening them to 50 mmHg above the PAS for 3 minutes and then quickly releasing them. The dynamic rate of regulation, dRoR, whose normal value is 20%/sec is thus calculated, indicating how quickly the velocity of cerebral flow returns to its starting level after the hypotensive stimulus. However, inducing rapid changes in systemic arterial pressure in patients who are already seriously compromised is not a good idea so this limits the application of the cuff test.

A very effective and safe device for measuring cerebral autoregulation is the transient hyperemic response test (THRT)[34,35].

This test is based on the compensatory vasodilatation of the arterioles which occurs after brief compression of the common carotid. The test involves measuring systolic speed of flow in the MCA in base conditions. The common carotid is compressed homolaterally for 10 seconds, which causes a reduction in CPP. If autoregulation is intact, the cerebral arterioles respond to the reduction in CPP through vasodilatation to reduce resistance and keep the cerebral blood flow constant. Once the compression is released we can see that there is a temporary increase in blood flow as CPP acts on a dilatated vascular bed. The increase in flow translates as an increase in velocity of MCA flow. Autoregulation integrity can be checked by calculating the transient hyperaemic response ratio (THRR) which is defined as the ratio between the velocity of systolic flow during the hyperaemic phase (two cycles after the compression release excluding the very first cycle) and the velocity of basic systolic flow (five cycles before compression). The normal THRR range is between 1.105 and 1.29 (average figure 1.2; confidence interval 95%, 1.17-1.24).

THRR is said to be a qualitative autoregulation indicator. The strength and duration of the carotid compression are the two variables which make THRR unreliable as a quantitative indicator. In literature there is disagreement about how long the compression phase needs to be to get the maximum hyperemic response. Some authors say 5 seconds[34] whereas others say 10 seconds[36]. As far as the strength of the compression is concerned, it needs to be sufficient to cause a reduction in cerebral flow of at least 40%[36].

Estimating intracranial pressure

Increased ICP causes variation in CBF speed which translates as changes in the shape of the TCD wave produced[37-38]. Differences in the Doppler wave can be quantified by calculating the pulsatility rate (PR) and resistance rate.

Various authors have demonstrated how PR increases exponentially when there is intracranial hypertension. In cranial trauma patients, raised PR is an indicator predicting worse outcome[39]. In the second half of the Eighties, Klingelhofer[40-41] showed there was good correlation between ICP and the MAP*RI/FVm ratio (where MAP is average arterial pressure and FVm the average velocity of flow). However, many different factors can affect levels of pulsatility and resistance including hemodynamic, respiratory and hemotological factors as well as vascular and tissue compliance. This is why indicators like these cannot be used for early identification of patients at risk for the development of endocranial hypertension.

In 1986, Aaslid[42] applied the Fourier analysis to the Doppler and arterial pressure waves and came up with the following formula for calculating cerebral perfusion pressure:

CPP=AP1xFVm/FV1 where AP1 is the amplitude of the first peak of the AP wave and FV1 is the amplitude of the first peak of the Doppler wave.

More recently Csonyka[43] proposed the following formula based on clinical observation: CPP=MAPxFVd/FVm+14 where FVd is velocity of diastolic flow. In a group of 25 patients with serious cranial trauma, absolute error was less than 10 mmHg in 81% of cases and in 50% of cases it was less than 5. Using this method, a prototype has been put forward which allows for continuous and bilateral CPP measurement (Neuro Q TM Deltex Ltd,Chichester, UK). This technique, based on critical closing pressure, has proved to be fairly reliable in predicting CPP values, but it is not sufficiently precise where ICP is concerned in that it doesn't differentiate between the effects on CPP which are caused by the increase in ICP and those produced by an increase in cerebrovascular resistence, especially in patients with intact autoregulation or in those who have intra or extracranial stenosis.

● Transcranial Doppler and "effective downstream pressure"

There has been a recent return to the concept of "effective downstream pressure (EDP)" and critical closing pressure (CCP). The concept of critical closing pressure was first mentioned in the Fifties by Burton[44] who defined it as the minimum transmural pressure (MAP-

ICP) under which blood flow stops and the vessel collapses. Critical closing pressure corresponds to vasomotor tone[45].
When there is equilibrium (zero flow), transmural pressure is equal to the ratio between the wall tension, which is given by the vasomotor tone, and the radius of the vessel. A reduction in arterial pressure, an increase in ICP or in vasomotor tone can modify the balance between transmural pressure and wall tension leading to collapse of the vessel. The sum of CPP plus ICP gives us EDP. When EDP equals MAP flow is zero. The difference between MAP and EDP is effective cerebral perfusion pressure (eCPP) which CBF depends on.
EDP values can be found by analysing the beat to beat relationship between pressure and cerebral blood flow. If we have MAP and FV for a single cardiac cycle in a single carthesian axis we can calculate a rate of linear regression. Once we have the linear regression rate we can extrapolate the AP value corresponding to zero flow. This value corresponds to EDP.
$APCO_2$ variations have the opposite effect on ICP and CCP. Increased CO_2 when autoregulation is preserved leads to vasodilatation which, on the one hand causes an increase in cerebral blood volume and therefore ICP, and, on the other, leads to a reduction in vascular tone and therefore reduction in CCP. Weyland$_{46}$ questioned what would happen if two Starling resistors were placed in sequence, one at the arteriole level so mainly influenced by CCP, and the other at vein level so influenced mainly by ICP. According to the author, if there is no endocranial hypertension, the eCPP is mainly determined by the arteriole resistor. Traditionally we have always thought of CPP as the difference between MAP and ICP. However, it is also likely that real cerebral perfusion pressure is not determined by intracranial pressure but by CCP. In a study of 70 patients with serious trauma, these showed that in 51% of cases CPP_{ICP} underestimates eCPP by at least 19,8mmHg[47]. However, there is no evidence to suggest that the concept of eCPP is superior to that of CPP in terms of outcome. The mechanism by which the difference between EDP and ICP can be a negative also needs to be clarified. It may be due to vasoparalysis or marked dilatation at rest because of hypercapnea, hypertension or hypoxia. The literature reveals contrasting opinions on this.

Transcranial Doppler and brain death

Brain death is defined as the irreversible cessation of all functions of the whole brain[48]. The clinical criteria are usually considered suffi-

cient to establish a diagnosis of brain death, however, they might not be sufficient in patients who have been on sedatives or when there are ethical or legal controversies. In these circumstances it might be useful to have tests which could confirm a diagnosis of brain death. The use of tests is also recommended in patients with severe facial trauma, in patients with pre-existing alterations in pupillary diameter and in PBCO patients who normally have high apCO_2 levels.
The most frequently-used test is EEG, even though it gives very little information about the brain stem is and it is not always technically possible in ICUs to conduct the test properly. Angiography is more suitable for confirming brain death. However, it remains an invasive test which requires the use of contrast and therefore the patient needs to be taken out of Intensive Care. The same goes for CT and any techniques which use radioisotopes.
TCD is a test which is relatively simple to perform, cheap, and which can be done at the patient's bedside. Many authors have demonstrated the existence of a TCD pattern which is typical of brain death[49]. The increased ICP which leads to arrest of the brain circle, first causes a reduction in diastolic flow velocity which becomes equal to 0 when ICP approximates diastolic arterial pressure. If ICP continues to increase, diastolic flow reappears but in the opposite direction (reverberating or oscillating flow) indicating a retrograde flow during the diastolic phase of the cardiac cycle. When endocranial hypertension is sufficiently high as to cause arrest of cerebral blood flow we observe brief systolic spikes followed by the complete disappearance of the Doppler signal. A reverberating flow and systolic spikes are considered conclusive of cerebral circle arrest because it means that both the anterior and posterior as well as the lateral ones are affected. We cannot exclude the possibility that the acoustic window is not sufficient, so if there is no signal whatsoever in any of the vessels at the base of the cranium this is considered indicative of brain death only when previous examination showed that flow was present. To exclude temporary arrest because of hypotension, systolic arterial pressure must not be less than 70 mmHg and flow patterns need to have been monitored during at least two previous examinations at least 30 minutes apart.
The sensitivity of TCD is 96,5% and its specificity is 100% in the diagnosis of brain death. However, it should be used to complement rather than substitute careful clinical evaluation. It is also important to stress that CNS depressor drugs do not influence the diagnosis of brain death with TCD.

Patent Oval Foramen

Paradoxical embolism through the patent oval foramen (POF) is a possible cause of stroke in young patients. TCD enables us to diagnose the presence of a right-left cardiac shunt with almost 100% concordance if we compare it to a transesophageal ecograph (TEE). 9cc of physiological solution is injected into one of the large forearm veins through a three-way-tap, con 1 cc of air. If there is right-left shunt we can see the gaseous emboli moving in the MCA 5-15 seconds after the injection. If the result of the basic test is negative we can increase the sensitivity of the test by performing it while doing a Valsalva's50 manoeuvre. Extracardiac shunt (eg intrapulmonary) is responsible for false positives.

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INTRACRANIAL PRESSURE MONITORING

Frank A. RASULO, Nicola LATRONICO, Roberto STEFINI

Keywords: Intraparenchymal Catheters, Burr-hole, Intracranial Pressure.

INTRODUCTION

In the normal situation without the presence of pathology, Intracranial Pressure (ICP) may undergo rises without any physiopathological effect on the brain. Abrupt increases in ICP may occur after sneezing, coughing, and straining without any consequences or neurological impairment. However, in the presence of pathology, rises in ICP may become symptomatic and alter brain physiology.
Within the cranium, since the brain is enclosed in a non-expandable case of bone, any change in volume of one component (brain tissue, blood, CSF) will necessitate compensatory changes in volume of one or more of the other components in order for the ICP is to remain constant. This is expressed as the Monroe-Kelly doctrine.
The brain uses compensatory mechanisms in order to maintain ICP constant, which are: shunting CSF to the spinal subarachnoid space, increasing CSF absorption, decreasing CSF production, or by shunting venous blood out of the skull. When these mechanisms are not present or exhausted, there will be a sharp rise in the ICP, leading to herniation of brain tissue downward through the Foramen Magnum. As this happens, blood will cease to flow to the brain and brain tissue hypoxia, ischemia, infarction, necrosis, and death will occur.
ICP is a reflection of the relationship between alterations in craniospinal volume and the ability of the craniospinal axis to accommodate added volume.

Frank A. Rasulo, MD, Medical Researcher
Nicola Latronico, MD, Associate Professor
Institute of Anesthesiology and Intensive Care – Spedali Civili University Hospital of Brescia
Piazzale Spedali Civili, 1 – 25125 Brescia, Italia
Tel. +39-030-3995 570/764/563
www.med.unibs.it/anest/ – rasulo@med.unibs.it – latronic@med.unibs.it

Roberto Stefini, MD, Consultant Neurosurgeon
Institute of Anesthesiology and Intensive Care – Spedali Civili University Hospital of Brescia
Piazzale Spedali Civili, 1 – 25125 Brescia, Italia
Tel. +39-030-3995 587 – roberto.stefini@libero.it

The relationship between volume and pressure in the intracranial space may be expressed as a pressure-volume curve.
Standard physiologic nomenclature defines compliance as the change in volume for a given change in pressure $\Delta V/\Delta P$), reflecting both the viscoelastic properties, or stiffness, of the intracranial content and the functioning of compensatory mechanisms available to reduce ICP at any given point on the curve.
The decision to monitor ICP is usually made on a clinical and imaging basis; the clinical situation must provide indications, and radiologic imaging studies must corroborate the indications and confirm the safety of the proposed monitor placement. Imaging techniques also provide warning of situations, such as mass lesions of the temporal lobes, in which ICP measurement may fail to reflect the progression of pathologic events.
Measuring intracranial pressure enables to determine the interventions necessary to prevent secondary brain injury, which can lead to brain damage and death. If the intracranial pressure increases above 20-25 mmHg, therapeutic interventions, medical and/or surgical, should be initiated. This is because as the ICP increases, it gradually becomes more difficult for the blood to be pumped to the head to perfuse the brain tissue.
Monitoring techniques have grown safer, less expensive, and more sophisticated and our understanding of intracranial pathophysiology has improved. There are several different ways to measure the pressure within the compartment of the skull. These methods may include Subdural, Parenchymal or Intraventricular pressure monitoring. They may be used to monitor the pressure or to drain CSF. Intraparenchymal catheters used to measure ICP are relatively precise and associated with a low complication rate.
A recent study has demonstrated that bedside insertion of a ICP monitor performed by intensive care physicians is a safe procedure, with a complication rate comparable to other series published by neurosurgeons[1-4]. The overall morbidity rate is comparable to, or even lower than, that caused by central vein catheterization. The most modern and common Cranial Access Kits available are disposable intracranial procedural kits which contain the basic items used during each step of the cranial access procedure.

Components

PREPARATION COMPONENTS

– Double Edge Razor, Iodine, Gauze Sponges

CRANIAL ACCESS PREPARATION

– Ruler, Marking Pen, Fenestrated Drape, Tensoplast Barrier

CRANIAL ACCESS

– Xylocaine (1%, 1:200.000 epinephrine), Syringe and 25 gauge Needle, Scalpel,
– Periostium scraper, Drill (hand or batery powered), 2.7 mm Drill Bit w/stop and wrench
– 5.8 mm Drill Bit w/stop and wrench (for ventricolostomy), Bone Wax, Mosquito Forceps.

WOUND CLOSING/DRESSING

– 2.0 Silk Suture, 3.0 Nylon Suture, Needle Holder, Serrated, Adson Forceps, Adson Forceps w/teeth, Suture Scissors

Prep

The skin is shaven and prepped with an iodine-based antiseptic solution. The side preferred for the insertion of the bolt screw is the right side, since the motor cortex is most commonly found on the left, however, this later side can be used if access is not possible on the right.

Landmark for incision and bolt incertion

The landmark for the skin incition called “Kocher’s point” should be found (Figure 1).

Kocher’s point

The bolt should be placed in the mid-pupillary line (2-4 cm or two fingerbreadths lateral to the midline) and 2-3 cm anterior to the coronal suture (fingerbreadth in front of coronal suture, midpupillary line). The coronal suture can sometimes be palpated; however, if that is not possible its location can be estimated by following a line up midway between the lateral canthus and the external auditory meatus.

Drape

Sterile drapes should be placed to define the extent of the surgical field.

Local anesthetic

Skin infiltration using 1/2% lidocaine with 1:200,000 epinephrine from a syringe with a 25-gauge needle.

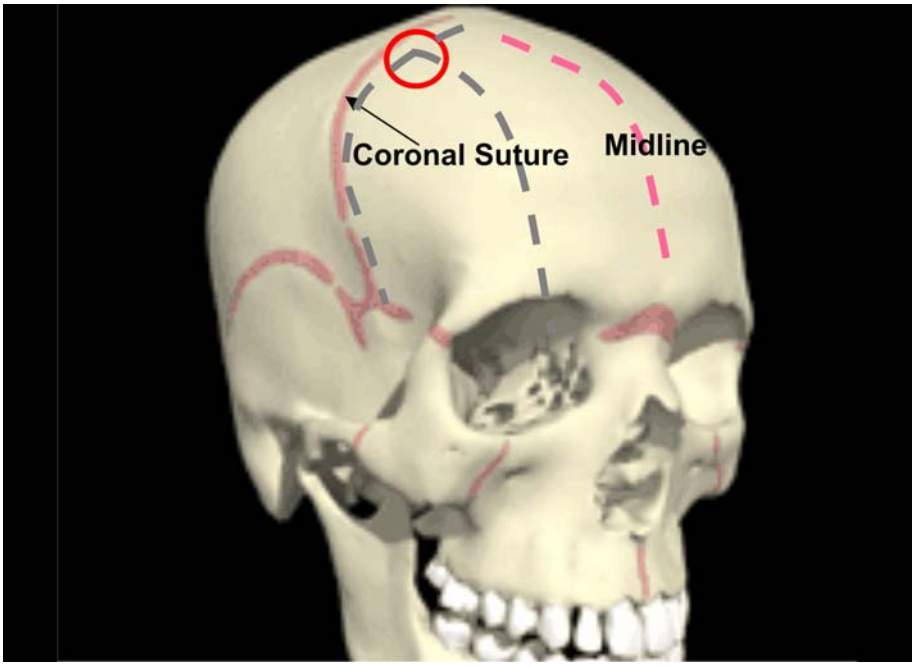

Figure 1 – *Kocher's point: mid-pupillary line (2-4 cm or two fingerbreadths lateral to the midline) and 2-3 cm anterior to the coronal suture (fingerbreadth in front of coronal suture, midpupillary line).*

Surgeon position

The surgeon stands at the head of the bed and should wear a facemask and hat as well as sterile gloves and a gown.

Patient position

Supine with head facing directly forward and held in place with tape. The head of the bed can be elevated as desired.

Sedation/analgesia

The patient should be sufficiently sedated prior to beginning the insertion.

Assistant

Should help hold patient the head of the patient during drilling.The assistant is not sterile during the procedure although he/she may want to put on sterile gloves to help the surgeon in the surgical (draped) field.

Skin incision

The skin and subcutaneous tissues should be incised (antero-posterialy for roughly 2-3 cm in length) until reaching the periosteum. After the incision and retraction of the skin and subcutaneous tissue, the

periosteum should be scraped off with the appropriate tool in order expose the skull.

1. Divaricate with the mosquito forcepts;
2. Select the appropriate drill bit. Use the 2.7mmdrill bit for subdural, intraparenchymal, and bolt procedures, (the 5.8 mm drill bit for ventriculostomy procedures).
3. Place the drill bit into the chuck.

While holding the drill handle in place, tighten the drill bit by turning the chuck counter clockwise. Loosen the drill guide using the appropriate hex wrench. Carefully slide the drill guide towards the tip of the drill bit until the determined skull depth is reached.
Warning: The drill guide will not stop the drill. The guide is designed only to provide the neurosurgeon with a marker for drilling depth. The guide must be adjusted to the proper position prior to drilling. Tighten the drill guide in place with the hex wrench. Begin drilling procedure.

Cranial tissue layers

Several layers of soft and bony tissue must be crossed to get from the skin surface to the intracranial space. The layers, from superficial to deep, are the scalp (skin, connective tissue-dense, aponeurosis, loose connective tissue, pericranium), bone, dura, arachnoid, pia, brain.

Drilling

After stripping away the periosteum the hole is drilled. The hole is then irrigated with sterile saline and an 18G spinal needle is used to open the dura in a cruciate fashion. The drilling procedure must be performed with the drill held within 10º of the perpendicular position to the incision site. Exercise caution when perforating the dura so as to avoid damage to the underlying structures (Figure 2).

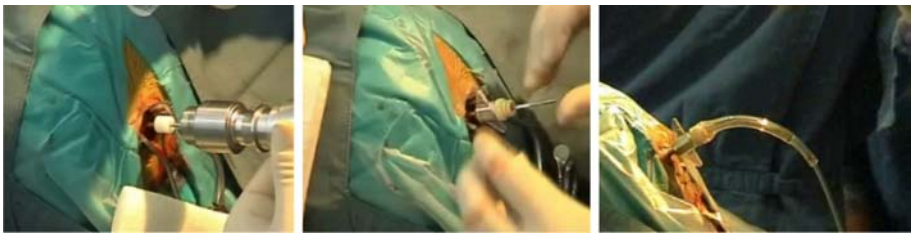

Figure 2

Bolt placement

Following opening of the dura, the Bolt is screwedmanually into the skull. This will be approximately 2-3mm for the neonatal age group, 3-

5 mm for the pediatric age group and 5mm to 1 cm for adults. If desired, the spacer can be used as a guide. The stylet provided in the kit is inserted through the Bolt and dura to clear the passage for theTransducer-Tipped Catheter. Screwing in the Skull Bolt at an angle may result in a fracture of the device. The stylet is then removed after the bolt has been screwed in, after which, the bolt should be filled with saline. It may be important to note if CSF leaks out of the bolt and, if so, at what pressure.

Zeroing the catheter transducer tip

The most commonly used ICP monitoring devices are provided with catheters which have the transducer at one extremity (must remain sterile), and the socket which is to be attached to the monitor itself at the other. The zeroing is performed by simply holding the transducer tip in air while performing the zero on the monitor, afterwhich the transducer is inserted inside the bolt and the screw tightened. The ICP value can then be read. Again, it is important to note the first ICP reading after the catheter is inserted. A few seconds time may be necessary until this first value can be read with accuracy.

Dressing

After the bolt is secured in place, the intracranial pressure bolt site is covered with a sterile Kerlex and the patient is observed in the ICU so as to avoid accidental pulling of the bolt.
Bleeding may occur at the site of the drill hole, originating from the scalp, bone, dural, or cerebral areas.

Precautions

It is essential to maintain strict aseptic technique during craniotomy procedures. Care should be exercised when applying sutures to ventricular catheters and over tightening of bolt screws could result in catheter occlusion or breakage.

Complications

As mentioned previously, complications in literature have been found to be extremely rare, and in some reports they were less in % than those encountered when inserting a central venous line.
The most clinically relevant were: Meningitis, local infection, intracranial haematoma, device failure.

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ELECTROPHYSIOLOGICAL TESTS IN INTENSIVE CARE

Bruno GUARNERI, Jenny AMBROSETTI

Key words: Critical illness polyneuropathy and myopathy, electromyography, electroneurography, nerve conduction.

Introduction

Neuromuscular complications encountered in intensive care can be due to peripheral nerves (polyneuropathies) muscles (myopathies) neuromuscular plate (transmission deficit) or a combination of these[1-7].
Neurophysiopathological tests which enable us to diagnose and maybe differentiate between these causes are essentially electroneurographs (ENG) and electromyographs (EMG). These assess, either directly or indirectly, the integrity of the peripheral system both sensory (centripetal afferent pathways) and motor (lower motor neurones, neuromuscular junction, muscle membrane and contractile substrate).
Electrical activity in the muscle is measured using a special device (an electromyograph) comprising preamplifiers, a computer with special programmes and algorithms for analysing, digitalising, amplifying and filtering the recorded signal and a monitor on which to visualise the on-line trace. The machine also has speakers. These serve to recognise and identify the physiological events being recorded as they make such unusual, individual sounds. An electric stimulator with constant current can be used to stimulate branches of superficial nerves (motor, sensory or mixed) so that electric conduction speed can be studied and the sensory or motor response analysed. The results can be saved on hard disc or on any magnetic or digital support, viewed off-line or even printed.

Bruno Guarneri, MD
Servizio di Neurofisiopatologia – Spedali Civili di Brescia
Piazzale Ospedali Civili, 1 – 25123 Brescia, Italia
Tel. 030-3665568 – bguarneri@tin.it
Jenny Ambrosetti, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Italia

Electroneurography

Electroneurography is the technique that enables us to study electric conduction in sensory and motor nerves. In combination with electromyographs they provide an indispensable diagnostic approach for recognising and localising peripheral nerve pathologies[8].

Methodology and recording techniques

The technique consists in stimulating the nerve using electrodes, normally surface ones, positioned along the nerve trunk or on the area it innervates, and measuring the potentials of the sensory response (along the nerve pathway) or motor response (from the belly of the innervated muscle). This enables us to measure motor and sensory conduction speed and to analyse the parameters of the response potential (size, amplitude and duration)[9].

MOTOR CONDUCTION SPEED

The potentials evoked in muscle are usually measured using surface electrodes (and very occasionally with needle electrodes) positioned on the belly of the muscle (active electrode) or on the muscle tendon (reference or indifferent electrode).

The stimulation electrodes are placed along the nerve at specific anatomical points with the anode 2-3 cm proximal compared to the cathode so that the active electrode is nearer to the recording electrodes.

The negative charge produced by the electric stimulus tends to depolarise the underlying nerve causing an action potential to be fired which spreads in both orthodromic and antidromic directions along the axons.

Square and rectangular wave impulses are delivered, whose duration varies from 0.1 to 1.0 msecs. usually at a frequency of I Hz, and sufficiently intense to excite all the fibres present in the nerve (known as supramaximal level, which may be more than 200 V). The voltage applied to reach supramaximal level obviously depends on the duration of the impulse and tissue resistance.

The muscle potential (M response) obtained from the stimulation of the motor fibres in the mixed nerve, or action potential in the compound muscle (CMAP) reflects electrical activity in both the nerve component and the muscle. In normal conditions the amplitude is quite high (from 5 to over 10 mV) and varies according to the number of underlying fibres excited[8].

– a reduction in CMAP amplitude can show axonal neuropathy or a primary muscular problem. However, even a myelin-type of neu-

ropathy can present with reduced CMAP because of temporal dispersion of the potentials caused by the different conduction speed of the fibres (desynchronisation of the potential) or because of the secondary axonal suffering (tardive);

– motor conduction speed can be obtained by stimulating the nerve trunk at two different points: the difference between the two latents (proximal and distal), measured in msecs, constitutes conduction speed along the segment of the nerve being tested. If the length of the segment tested is measured in mm. we can easily measure motor conduction speed expressed in m/sec. The distal latent alone does not give a real indication of conduction speed along the nerve because this includes synaptic delay due to transmission by the neuromuscular plate (about 0.5 msec)

The most representative motor nerves, and those most frequently studied in Intensive Care are:

n. Peroneal:

– derivation: m. pedidio;
– stimulation points: dorsum of foot (distal), peroneal head (proximal).

n. Tibial:

– derivation: m. abductor hallux;
– stimulation points: internal malleolus (distal), polpliteal fossa (proximal).

n. Median:

– derivation: m. abductor brevis of the thumb;
– stimulation points: wrist (distal), elbow - biceps sulcus (proximal).

n. Ulnar:

– derivation: m. abductor 5° finger;
– stimulation points: wrist (distal), elbow - epitrochlea - olecranon (proximal).

SENSORY CONDUCTION SPEED

Sensory nerves are stimulated by applying electrodes (ring or metal plate) along the pathway of the nerve under investigation.
The sensory active potential (SNAP) is measured by superficial or subcutaneous electrodes positioned away from the stimulation electrodes along the nerve pathway[8,9].
The amplitude of the sensory action potentials is greatly reduced (10-15uV) so it is easy to confuse them with the background noise. To see

them we need to use a special technique called "averaging" (the total and then average of more than one event), which enables us to isolate the evoked response from the background noise which has the same latency (temporal interval) and the same morphology.
Sensory conduction can also be recorded using the orthodromic (direction of centripetal physiological transmission) or antidromic (proximal stimulation and distal derivation) techniques. Sensory Conduction Velocity is calculated in m/sec and is measured by dividing the distance in mm. between the stimulation and registration points by the Latency time.
The most representative sensory nerves and those most frequently used in Intensive Care are:

n. Sural:

- derivation: external submalleolar area;
- stimulation points: extension of triceps tendon at a distance of approx. 13 cm from the derivation electrodes.

n. Tibialis posterior:

- derivation: internal submalleolar area;
- stimulation points: ring electrodes positioned on hallux.

n. Median:

- derivation: wrist;
- stimulation: ring electrodes positioned on 2°-3° fingers.

n. Ulnar:

- derivation: wrist;
- stimulation: ring electrodes on 5° finger.

TABLE SUMMARISING ELECTRONEUROGRAPHICAL PARAMETERS

Motor nerve:

- Motor Conduction Velocity (MCV), measured in m/sec
- Proximal and distant latency (msec)
- Amplitude of action potential (CMAP) measured in mV.

Sensory nerve:

- Sensory Conduction Velocity (VCS), measured in m/sec
- Latency of sensory action potential (msec)
- Amplitude of sensory action potential (SNAP), measured in uV.

From the observation and analysis of the electroneurographical data obtained, 3 major types of alteration were seen:

- *reduced amplitude of CMAP or SNAP, with normal or slightly reduced conduction values:* this is seen when there is a partial lesion of the nerve, neurpraxia or axonotmesis (before distal degeneration sets in) when conduction is blocked in the affected axons or when there is axonal neuropathy with loss of axons and consequent reduction in the motor and/or sensory action potential. Nerve conduction speed is often normal or only slightly reduced because of degeneration of the thicker and therefore faster fibres.
- *normal amplitude and reduced conduction values:* this is found with segmental demyelination, affecting a majority of the nerve fibres, as in some acute inflammatory polyneuropathies, leading to significant reduction in conduction velocity and/or dispersion of the action potential (multiple focal blocks).
  This slowing down of conduction speed, which is so important, is typical of entrapment syndromes which present initially with signs of myelin suffering and only later, as the noxa persists, with signs of axonal suffering.
- *absence of response:* when there is total interruption of electric conduction (axonotmesis, neurotmesis).

Conduction Velocity measurement has the advantage of being a quantitative measure which does not rely on patient collaboration or the examiner's subjective impressions. There are, nonetheless, many possible sources of error, particularly in Intensive Care, and only careful observance of certain rules (cleaning the skin, careful positioning of the electrodes, very precise measurement ...) enables us to avoid errors while carrying out or evaluating the tests[10].
Conduction Velocity is closely linked to body temperature and therefore it is important to control this properly using a lamp if necessary. Johnson and Olsen showed that a 1° change in body temperature corresponds to changes in conduction values of about 5%[11].

F RESPONSE

Conduction in proximal or deep segments of nerves or nerve roots can be studied by recording the "F" wave. If we apply supramaximal stimulus after the "M" response (motor action potential) we can see a lower amplitude second response with a latency of about 25-30 msecs. This is the alpha motorneurone spinal response after antidromic activation (the impulse travels in antidromic direction as far

as the cell body of the the alpha motorneurone and then travels back in orthodromic direction along the same axon)[8,9].
The latency of the "F" wave may be longer in patients with peripheral neuropathy, nerve root lesions, or nerve entrapment. With Guillain-Barrè syndrome, which hits proximal nerve segments first, there may be increased latency in the F wave before there is any evidence of nerve conduction velocity slowing down[12,13].

H REFLEX

Like the F wave, the H reflex can be used to evacuate electric conduction velocity in proximal segments of nerve roots or in peripheral nerves (radiculopathies and peripheral neuropathies). Unlike the "F" response, the "H" reflex, by definition, is a response which is evoked by activation of a monosynaptic spinal pathway (diastaltic arc). It is often used to study the function of the S1 nerve root (stimulation of sciatic-internal popliteal in popliteal fossa and recording of reflex response in soleus muscle)[8].

REPETITIVE STIMULATION

This is the method most widely used to test function in the neuromuscular plate whenever neuromuscular block treatment has been used or when myasthenic or myastheniform pathologies are suspected[8,9].
The most significant transmission defects are:

- defects in transmittor synthesis (hemicholine)
- defects in transmitter release (botulin toxin)
- competitive block (curaro)
- depolarising blocks (decamethone, suxamethone).

The method (Desmedt test) involves stimulating a peripheral nerve, (usually the ulnar nerve at the pulse) and recording the response in the corresponding muscle (5th finger abductor for ulnar nerve). 10 impulses are delivered at supramaximal intensity.
In a healthy subject, repetitive stimulation at low frequencies (usually 3 Hz) does not cause any significant reduction in amplitude of the response potential, whether at rest or under stress.
In patients treated with neuromuscular block, the initial CMAP, which is of practically normal amplitude, tends to reduce in amplitude after only a few repetitive stimuli (decreasing response).
In patients with myasthenia gravis, there is an initial decrease in CMAP (until the 4° or 5° stimulus), followed by a slight increase after continued stimulation (decreasing – increasing response). These al-

terations reflect transmission problems of a post-synaptic nature (reduction in number of receptor sites for acetylcholine).
With Lambert-Eaton Syndrome (myestheniform), where the deficit is at pre-synaptic level, (deficit release of acetylcholine by the synaptic vesicles) repetitive stimulation facilitates transmission and leads to progressive increase in the amplitude of CMAP. This is only demonstrable with repetitive high frequency stimulation (at least 50 Hz).

Electromyography

Electromyography (EMG) is a neurophysiopathological method which, by means of coaxal needle electrodes inserted into the muscle belly, studies electrical activity in the muscle, at rest and during contraction, and gives information about function, structure and the recruitment of motor units to the highest level of activation (interferential pattern)[8,9].

Physiology of the motor unit

The motor unit is made up of a motor nerve fibre and all the muscle cells it innervates; it acts as the contractile functional unit in that all the cells within it contract synchronously when the motor fibre is excited[14,15].
The cell bodies of the motor nerve fibres (alpha-motorneurones) are situated in the ventral horn of the spinal cord. The axon exits via the ventral route and reaches the muscle via the peripheral nerve (usually mixed) and as it gets close to the nerve fibre it gradually loses its myelin sheath.
The force of a muscle contraction depends on the number of motor units recruited, their type, the frequency of firing and the velocity of contraction. The number of motor units varies greatly from one muscle to another.
The muscle fibres in the motor unit are spread out within the muscle, and the fibres innervated by the same cell in the anterior horn are generally not contiguous.
There are two types of motor unit: *those with type I fibres*, which contract slowly for continuous, prolonged activity (energy supplied by oxidative mytochondrial metabolism) and *motor units with type II fibres,* which have rapid conduction and are used for more intense physical effort and rapid movements (energy is mediated by anaerobic glycolosis).

Morphology patterns in electromyograph recordings

Execution and recording according to the standard method requires:

- looking for any spontaneous activity at rest (insertion, fibrillation, positive slow waves, fasciculation potentials, myotone activity,

pseudomyotonic firing)
- analysing individual Motor Unit potentials (morphology, amplitude, duration)
- assessing the voluntary recruitment of motor units to reach maximal force (interferential pattern)

SPONTANEOUS ACTIVITY[8,9]

In a healthy muscle, at rest, there is no electric activity to record (electric silence). However, when the electrode is in an end-plate zone, there may prove to be some activity in isolated fibres or small groups of fibres even in a healthy subject (*end-plate noise or activity*).
The insertion of a needle electrode into a muscle can also cause action potentials of short duration and low amplitude to fire, probably due to mechanical excitation by the electrode (insertion activity).
When there is muscle pathology, either primary or secondary due to denervation, the electric activity lasts for longer at rest and there are also associated polymorphous patterns of spontaneous activity.

- *Fibrillation potentials:* these are small potentials of brief duration (0.5-2 msec) and low amplitude (30-150 uV), of diphasic or triphasic morphology. They probably originate from spontaneous firing of single muscle fibres. The pathogenic mechanism of fibrillation is not very clear. It has been demonstrated, however, that denervated muscle has an abnormal increased sensitivity to acetylcholine.
  Fibrillation potentials are typical of denervated muscle and motor neuron lesions, that is, the connection between the axon and muscle fibre has been broken, but they can also be seen in other types of myopathy.
- *Positive Sharp Waves (PSW)* or Jasper potentials are of greater duration and amplitude compared to fibrillation potentials. Characteristic of their morphology is an initial positive deflection with sharp peak followed by a slow negative wave which can last over 10 msecs. They most likely show firing in structurally-damaged muscle fibres.
- *Fasciculation potentials:* these are characterised by the spontaneous and synergic firing of groups of muscle fibres belonging to the same motor unit. They are big enough to produce a visible contraction though not articular movement. These potentials are usually present in degenerative illnesses of the anterior horn (motor neuron diseases, syringomyelia, acute phase of poliomyelitis) but they can also appear with radiculopathies.

- *Myotonic firing:* high frequency firing of action potentials that can appear spontaneously (spontaneous myotonic activity), be evoked by the movement of the electrode or by percussion of the muscle (mechanical myotonic activity) or follow on from a voluntary contraction during the relaxation phase (post-contraction myotonic activity). These are present in myotonic diseases with or without dystrophy. What is unusual about them is the progressive increase in their frequency of firing (da 10 a 150 Hz), followed by a reduction in amplitude and frequency of the activated potentials, as well as the associated characteristic sound effect of a "warplane dropping bombs".
- *Pseudomyotonic firing:* high-frequency firing but unlike the myotonic type, the amplitude and frequency are constant and both the beginning and end are quite brusque. Also defined as "bizzarre high-frequency firing", these are found with various pathologies like certain forms of neurogenic atrophy (spinal muscular atrophy) and myopathies (poliomyesitis).

MOTOR UNIT POTENTIALS (M.U.P.)

During voluntary activation with minimal effort, individual motor unit potentials can be isolated which are the sum of all the potentials generated by groups of muscle fibres in almost simultaneous contraction. Morphologically they can be monophasic, biphasic, triphasic or polyphasic (with 5 or more phases). Their duration varies from between 2 to 10 msecs. And their amplitude, which varies from 100 uV to 2 mV, can, under pathological conditions, exceed 10 mV.

Both the morphology and size of M.U. are significantly altered by disease processes. With peripheral neuropathy, partial denervation and regeneration phenomena often result and this leads to destructuring of the M.U. causing temporal dispersion, polyphasic morphology and an increase in their duration and amplitude.

If there is a primary muscle problem then we see a loss of muscle fibres but, at least in the beginning, there is no reduction in the number of axons. This means that both the amplitude and duration of the single M.U.s are reduced, the morphology is predominantly polyphasic, closely-serrated and the sound it makes is an unmistakable "dry, crackling noise".

VOLUNTARY CONTRACTION

As the muscle contraction gradually increases, we can see that more and more motor units are recruited until there are so many of them su-

perimposed that it becomes impossible to determine their individual characteristics. This is termed "interference" pattern.
When there are neurogenic problems, there is a progressive reduction in the recruitment until the picture we get of electric activity is the "intermediate" or "single oscillation" type.
With primary myopathies, recruitment is not compromised and so, because of the polyphasia of the M.U. potentials, we get the "interference" type of pattern even during weak voluntary activity (premature recruitment) with the distribution frequency of the electrical activity moving towards the high frequency end of the spectrum.

Limitations of EMG in Intensive Care patients

For Intensive Care patients who are often comatose, sedated and mechanically-ventilated, electrophysiology enables us to diagnose Critical illness Myopathy and Neuropathy but it does not enable us to see the relative significance of the muscle or nerve component. If we apply the standard method, differential diagnosis between myopathy and neuropathy is extremely difficult if not impossible[16,17].
EMGs, by recording spontaneous activity, enable us to identify potentials of acute denervation like fibrillation and PSWs and, although in normal clinical practice activity like this is not usually recorded until between 1 and 3 weeks after the lesion occurs, in Intensive Care it is documented from the moment it first appears[6,18]. However, the presence of spontaneous denervation activity can be seen in both neuropathies and some types of acute myopathies. On the other hand, reduction in CMAP amplitude alone does not enable us to differentiate specifically between motor axonal neuropathy and myopathy.
Analysis of the Motor Unit potential is therefore indispensable as is the study of recruitment patterns, though the latter demands collaboration of the patient which is not usually possible in Intensive Care.

Direct stimulation of the muscle

With the aim of trying to make a differential diagnosis between myopathy (CIM) and neuropathy (CIM) in the critically ill patient, we applied a method devised by Rich[19,20], and then used by other authors[21,22], which enabled us to measure amplitude of the motor action potential (CMAP) by stimulating the nerve (neCMAP) and direct stimulation of the muscle (dmCMAP).
Patients identified with pathological CMAP at the standard electroneurograph, underwent direct muscle stimulation.
This involves the placing of a monopolar subdermic needle electrode

(anode) on the distal third of the tibialis anterior muscle, referred to a monopolar electrode (cathode) positioned 2 cm laterally.
The muscle is stimulated by gradually increasing the intensity of the electric impulse (0.1 msec duration, 0.5 Hz frequency) until we get a palpable muscular twitch (10-100 mA intensity).
Guided by the twitch, the recording electrode, a monopolar subdermic needle electrode (anode) is placed 2-3 cm down from the stimulating electrode and referred to a second monopolar electrode (subdermic or surface) which is placed a few centimetres distal to it. During stimulation, the recording needle electrodes are moved slightly in order to get the maximum amplitude of the muscle action potential (dmCMAP).
By applying supramaximal stimulus to the nerve underlying the muscle area under study, on the other hand, we get the classic compound action potential (neCMAP).
By analysing the amplitude of the two responses from indirect stimulation (of the nerve) and direct stimulation of the muscle under investigation and working out a mathematical ratio between neCMAP and dmCMAP, we get *nerve/muscle ratio* which, according to Rich[20], enables us to deduce the following:

- when the ratio is *greater than or equal to 1*, it suggests either myopathy or a normal clinical picture. Differential diagnosis between these two conditions is easy to make based on the absolute value of the dmCMAP amplitude: whether it is normal or reduced (myopathy: loss of electrically excitable muscle);
- a ratio of *less than 1* with reduced neCMAP shows the presence of neuropathy.

To ensure greater reliability in the results obtained, we thought it better to introduce a modification to Rich's original method. Instead of measuring CMAP amplitude from peak to peak we decided to measure from the isoelectric to the first peak. The reasons for this were:

- neCMAP is usually composed of a normally polymorphic and polyphasic response potential whose components are not homogeneous and temporally dispersed, probably because the recording electrode recruits motor units at the edge of the area of muscle fibre under study.
- the dmCMAP we get is morphologically simplified (bi-triphasic) and probably made up of more homogeneous components.

We therefore felt that it was better to measure amplitude from the isoelectric point to the first peak (morphologically similar and compa-

rable to the response from direct stimulation of the muscle) to minimise possible error introduced by summation (tempero-spatial) of tardive or borderline components recruited by a supramaximal stimulus applied higher up the nerve trunk or, in any case, not directly belonging to the dmCMAP.
To confirm the validity and reliability of direct muscle stimulation, the technique was applied to patients both before and after cururisation thus obtaining stable and reproducible dmCMAP results.
It will be the subject of a future study to apply the technique to healthy subjects in order to get absolute normative data as reference values for direct diagnosis of myopathy without needing to use neCMAP/dmCMAP ratio.

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CRITICAL ILLNESS MYOPATHY AND NEUROPATHY

Elena PELI

Key words: neuropathy, myopathy, pathogenesis, hyperglycemia.

Introduction

Patients in critical condition, during their stay in Intensive Care, often develop muscle weakness which can become paralysis, making it difficult to take them off the ventilator. Only recently has this been recognised as due to an acute polyneuropathy.

The first systematic studies at the beginning of the Eighties indicated that the syndrome was rare. "Over a period of four years, 5 patients developed serious neuropathy within a month of being admitted to Intensive Care"[1]. Over the following 20 years, it was demonstrated that critical illness polyneuropathy (CIP) is a frequent, serious and persistent complication in the more serious patients admitted to Intensive Care. It was also seen that it often, though not always, has a muscular component termed critical illness myopathy (CIM). In a recent review of the literature, we tried to define CIM and CIP[2,3] and we saw that CIM is at least as common, if not more so, than CIP, that CIM is a primary myopathy and not secondary to CIP denervation, and that CIP and CIM can coexist and it is difficult or almost impossible to differentiate between them, as confirmed by the need to resort to new diagnostic techniques in order to do so. The CRIMYNE study (critical illness myopathy and neuropathy), an Italian multicentre study carried out between 1998 and 2001, and coordinated by the GIVITI – Gruppo Italiano per la Valutazione degli Interventi in terapia Intensiva showed that early electromyographical alterations are a sign of primary myopathy, not secondary to denervation which is a sign of CIP. Various terms have been coined over the years to describe these links including polyneuromyopathy[4,5] critical illness myopathy and neuropathy (CRIMYNE)[6,7] and critical illness polyneuropathy and myopathy[8].

Elena Peli, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Spedali Civili
Piazzale Spedali Civili, 1 - 25125 Brescia, Italia
Tel. 030-3995764 – Fax 030-3995779 – elena_peli@yahoo.it

The aim of this paper is to describe the syndrome in terms of its clinical presentation and pathogenesis and to show how it can be recognised.

Critical illness polyneuropathy

CIP is an acute sensory-motor axonal polyneuropathy involving peripheral nerves affecting patients in critical condition.
Its incidence is affected by specific aspects of the patient population involved, the diagnostic criteria used, and when patient assessments are made. The literature gives widely – varying figures – 58% of long-stay Intensive Care patients[9], 70%-80% of patients with sepsis, septic shock or MOF[10-13], 100% of patients with sepsis and coma[6]. It is not clear whether this is because CIP complicates recovery in only the most serious of Intensive Care patients or whether there was a bias in patient selection.
In the excellent De Jonghe et al.[14] study, for example, the patients involved had been on mechanical ventilation for at least a week and were conscious. The study, carried out in 5 French Intensive Care units, lasted 15 months and involved 95 patients. Although the authors say that the patients were not selected, hundreds of patients in critical condition must have passed through Intensive Care in that time. The same is true of all published studies to date. Nor are there any studies in which patients without electrophysiological alteration in peripheral nerves are monitored from when they are admitted into Intensive Care. The methodology used can almost certainly affect the frequency of CIP. Neurological examination is less sensitive than electrophysiological evaluation, leading us to under-estimate the number of CIP cases. On the other hand, electrophysiological assessments can lead us to define as CIP cases which are of little or no clinical significance.
Clinical manifestations of the condition, initially thought to appear at the end of a long stay in Intensive Care, actually appear much earlier[15-18].
Involvement of motor fibres seems prevalent, especially those of the lower limbs which are longer and therefore further away from the cell body. The cranial nerves, especially those responsible for eye movements, are usually spared[16-19].
Histological changes show scarcely-populated nerve bundles with no inflammatory infiltrates or ischaemic necrosis. Residual nerve fibres retain their normal myelin sheath. The histological data serve to explain electrophysiological findings: the action potential recorded, which is the sum of the action potentials of the single fibres making

up the nerve, will have reduced amplitude whereas the conduction speed of the impulse will be normal because myelination is generally preserved[20].

Pathogenesis

A microcirculatory dysfunction has long been considered a possible mechanism responsible for damage to the peripheral nerve[21] and organ perfusion[22]. Bolton suggested that a key factor was the excessive vasodilatation resulting from over-production of nitric oxide during sepsis and septic shock and from the aggregation of cellular elements after activation of the adhesion molecules[21].

As a result, the increased capillary permeability makes it easier for toxic substances to enter. It has recently been proved that E-selectin levels rise in the epithelium of epineural and endoneural vessels in septic patients with CIP[23]. These increased amounts of E-selectin, resulting from the stimulus of the pro-inflammatory cytochines (TNF-α e IL1), encourage leucocytes to bind to the endothelial cells and activated leucocytes to pass into the endoneural space. These latter are ultimately responsible for the tissue damage. Cytokines also increase the permeability of blood vessels enabling more neurotoxic plasma macromolecules to pass into the endoneural space[21]. It should also be noted that raised glycaemia levels are associated with increased incidence of CIP (and with other types of organ insufficiency) so intensive insulin therapy, as well as reducing mortality, also reduces the frequency and duration of CIP[24].

The other hypothesis regarding the pathogenesis is the involvement of intracellular mechanisms which kick in during sepsis or critical illness as a result of inflammation activation or hormonal and systemic metabolic changes.

Fink and coll.[25] show that mitochondrial dysfunction is responsible for energy failure in insufficient organs. The mechanisms deemed to be responsible are: the opening of channels which are normally closed in the mitochondrial membrane (MPTP: mitochondrial permeability transition pore) so that the protonic gradient is lost and ATP production reduced, polymerases (ADP-ribose) are activated leading to reduced cellular NAD (nicotinamide adenine dinucleotide). A cellular energy crisis results as well as irreversible damage to the I mitochondrial complex in the kidney tubule cells as a result of the ischaemic attack. The impossibility of responding to the cell's energy needs through anaerobic glycolisation increases cell damage from hypoxia. The combined effect of vascular and cellular events can cause an en-

ergy deficit in the nerve and therefore a reduction (or absence) in the action potential, as electrophysiological tests show. If the critical situation persists so the energy deficit is prolonged the changes can be seen histologically as well. This can be seen from studies where altered nerve function was identified but nerve structure appeared intact when the biopsy was carried out early whereas both functional and structural changes were observed if the biopsy was carried out later[6]. To support the theory that functional damage is caused by energy deficit during sepsis or septic shock we would do well to remember the Hotchkiss[26] study where the author identified occasional areas of necrosis or apoptosis and very occasionally massive loss of parenchymal tissue in liver and kidney histological preparations taken from dead patients. The absence of any significant histological changes which would be sufficient to explain organ failure suggests that the problem is purely functional and may well result from a breakdown in energy supply.

Diagnosis

Presentation is subtle and vague. Typical symptoms include muscle weakness which may or may not be associated with objective loss of muscle strength. In some cases, the loss of strength is serious and we get tetraparesis or flacid tetraplegia. The difficulty in weaning patients off their ventilators suggests involvement of the neuromuscular respiratory system, more specifically the phrenic nerve, which has never been systematically studied in the literature.

Electrophysiological tests enable us to supplement clinical findings. Electroneurographs (ENG) show that the action potential of both sensory and motor nerves is of reduced amplitude yet conduction speed is normal. This enables us to diagnose axonal polyneuropathy. This electrophysiological picture is different from the one seen with Guillain-Barré sindrome (GBS), where the demyelination typical of polyneuropathies is identified on the ENG by a reduction in impulse conduction speed. Even if this is a useful way of differentiating between the two conditions, it is the clinical history which really aids differential diagnosis. GBS patients rarely end up in Intensive Care whereas CIP is a frequent complication in patients who are in Intensive Care for a whole range of pathologies.

Critical illness myopathy

CIM is an acute primary myopathy which affects patients in critical condition.

The real incidence of CIM is unknown. As with CIP, the particular pa-

tients studied, the diagnostic criteria used and when the readings are taken all affect the figures. Despite limitations linked to the large patient sample and questionable diagnostic criteria, Lefaucheur and coll. recently claimed that no less than 83% of patients on ventilation for 7 days and with muscular weakness acquired during their stay in Intensive Care had myopathy[3].

CIM presents with a continuum of signs and symptoms ranging from a silent presentation where changes are only detectable through electrophysiological tests[6,12,13,27], to cases where there is muscular weakness or even total paralysis[28]. Any muscle can be involved, including respiratory muscles, which obviously has a significant effect in terms of weaning and prolonged stay in the Intensive Care[15,29].

In 1997, Leijten[30] suggested there was a connection between CIM and CIP. In 1998, we defined[31] CIM as a term which could describe various myopathies, including those which were purely functional with normal histology (acute quadriplegic myopathy) as well as those with atrophy or necrosis. The necrosis may be visible only under an electron microscope (thick filament myopathy) or may be seen using a simple optic microscope revealing isolated, widespread or massive areas of necrosis (acute necrotizing myopathy). In 2000 other authors tried to redefine the term CIM and laid down a series of criteria for diagnosis based on electromyographical, histological and biochemical tests[32]. Some of the criteria were questionable[2], for example, the main EMG criteria require patient collaboration and cannot be used on unconscious patients; the loss of myosine filaments on histological examination leaves out of count the purely functional forms; plasma CK levels can be normal (and they often are) if there is no, or very little, necrosis; the sensory action potential could be altered in any case given that CIP and CIM can coexist. This is important when remembering that CIM is a primary myopathy not one which is secondary to denervation. Histological examination often shows signs of both: primary myopathy (necrosis) and secondary (groups of fibres which follow nerve regeneration)[6], thus demonstrating the coexistence of both CIM and CIP.

Pathogenesis

Muscular damage which develops in critically ill patients forms part of a hypercatabolic clinical picture typical of SIRS (Systemic Inflammatory Response Syndrome). SIRS, which is due to infection or trauma, leads to significant loss in muscle proteins despite optimal

protein and calorie supply[33].
Cytokines are responsible for the mechanisms leading to muscle wasting as well as TNF and Interleucine-1 in the early stages. These directly induce muscular proteolysis and, in combination with reduced levels of anabolising hormones (insulin), and increased levels of catabolising ones (cortisol, glucagon and catycolamines) which are typical of the stress-response, lead to significant mobilisation of amino acids[30].
What ultimately carry out the proteolysis are the intracellular proteolytic enzymes, ubiquitin-proteosome system, the calpain system and the lysosomial and non-lysosomial systems[34-36]. Di Giovanni et al.[37], in a recent study of patients with CIM and muscular atrophy showed that while activation of the ubiquitin ligates is common to both neurogenic and muscular atrophy, activation of the transforming active growth factor complex (TGF-beta)/MAPK was only present in patients with CIM. The TGF-β/MAPK cascade remains unchanged in forms of inflammatory neuropathy associated with necrosis like dermatomyositis, poliomyositis and inclusion body myopathy, and may therefore be a feature of CIM. Activation of the TGF-beta/MAPK leads to loss of myofilaments and apoptosis.
Over the last few years a lot of progress has been made in terms of explaining how a muscle whose structure is normal can become unexcitable. Using denervated muscle fibres taken from rats and exposing them to high doses of corticosteroids, Rich et al. showed that the overall excitability of a muscle depends on a combination of factors: reduction in the membrane resting potential of the denervated muscle (from a normal value of -85mV to -60 mV), loss of the normal down-regulation in membrane chlorine transportation[38] and a shift in the inactivation potential of the rapid voltage-dependant sodium channels to more negative values (-11mV)[39,40]. This shift towards hyper-polarisation of the inactivation potential of the rapid voltage-dependent sodium channels plays a fundamental role in reducing the excitability of both the denervated muscle fibres and in the denervated fibres treated with steroids, even though the role of the steroids seems to predominate[40]. The density of the embryonic form of the sodium channel $Na_v1.5$ is greater in Rich's experimental model[38], but this does not justify the shift in the inactivation potential. In fact, the inactivation potential in both the adult forms of the sodium channel $Na_v1.4$ and $Na_v1.5$ tends towards more negative values. The limitations with this experiment were the high levels of steroids used, which is not normal

in clinical practice[42]. Cases of paralysis followed by rapid recovery have been documented in patients who had not received any steroids, suggesting that other risk factors may be responsible for changes to the membrane resting potential and sodium channels.
Mitochondrial dysfunction, on the other hand, is another possible cause of functional damage to the muscle. Brealey and coll.[43] showed, through their muscle biopsies on septic patients with MOF, that there is an increase in tissue oxygen tension, and therefore they again put forward the theory that the problem is at the level of the cell which is unable to utilise the oxygen (tissue dysoxia or cytopathic hypoxia). There is correlation between the seriousness of the sepsis and mitochondrial dysfunction, ATP and glutation depletion and nitric oxide production in skeletal muscle. Reduced activity in I complex of the respiratory chain is probably triggered by the nitric acid and facilitated by the depletion of cellular antioxidants like glutation. Damage or inhibition to the I complex is therefore what is responsible for the mitochondria being able to produce less ATP. The bioenergetic deficit is therefore an important mechanism in the pathogenesis of muscular damage caused by sepsis and one which is very similar to that caused by peripheral nerve insufficiency. How this bioenergetic deficit on the one hand, and changes in the resting potential of the muscle transmembrane and altered sodium channels on the other, can be correlated is not yet known.
The bioenergetic hypothesis was confirmed by studies using magnetic resonance spectroscopy which showed that patients with neuropathy associated with acute pathology had a reduction in the phosphocreatine/inorganic phosphate ratio, an indicator of cellular energy reserves[10].

Diagnosis

The diagnosis of CIM and differential diagnosis between CIM and CIP are often not possible in Intensive Care patients for various reasons.
First of all, the clinical picture is not applicable because the patient cannot collaborate either because they are sedated or have a CNS pathology.
Secondly the EMG readings can only be diagnostic if the patient is capable of voluntary use of motor units, which is often impossible because they are unconscious or because their muscle weakness is too advanced.
Thirdly, routine electrophysiological studies reveal non-specific changes: abnormal spontaneous muscle activity (fibrillation poten-

tials and positive sharp waves) and reduced amplitude of the combined muscular action potential. The fact that the amplitude of the sensory action potential remains unchanged is not typical of CIM as forms of CIM exist which are purely motor [6,42] . What is more, CIP and CIM can coexist as already mentioned.
Lastly, a muscle biopsy, which is the gold standard for CIM diagnosis as it can differentiate between levels of muscular compromise[31], is not capable of recognising the purely functional forms and it is also an invasive test.
This is why many authors have suggested innovative techniques to allow for differential diagnosis between CIM and CIP.
Direct muscle stimulation (DMS)[44,45] involves stimulation and recording on the muscle. The muscle can thus be stimulated bypassing the nerve and neuromuscular junction. In cases of CIP (or when neuromuscular transmission is blocked pharmacologically) the action potential can still be shown by DMS. The action potential would obviously be absent if the muscle was unexcitable. The muscles which are most often tested using this method are tibialis anterior, biceps brachialis and vastus laterale. Limitations of this technique are that it depends very much on the person carrying out the procedure and requires a lot of experience.
Lefaucheur and coll.[3] diagnosed myopathies in critically ill patients using DMS in combination with EMG standards and recordings of plasma CK levels.
The myosin/actin ratio in small fragments of muscle can be worked out using a rapid electrophoretic method (24 hours). The normal myosin/actin value is 1.37 (DS 0.21), but this goes down to 0,37 (DS 0.17) in patients with CIM[46].

Risk factors

There are many factors contributing to the development of CIP and CIM: sepsis, Multiple Organ Dysfunction Syndrome (MODS), Multiple Organ Failure (MOF), being female, corticosteroid use, serious asthma, hydro-electrolyte changes, malnutrition and immobilisation[2]. In conclusion, critical illness, in the sense of insufficiency of at least one organ on the SOFA score, is fundamental to the development of CIP and CIM.
In De Jonghe's[47] systematic review, the authors concluded that there was no evidence in the clinical studies they analysed to suggest a causal relationship between sepsis and CIP or CIM. In the prospective

multicentre cohort study carried out by the same group[14], sepsis is not associated with the paresis acquired in Intensive Care.
Hyperglycaemia is an undisputed risk factor for CIP[24], and normalisation of the serum lipid profile[48], has positive effects on morbidity and mortality rates as well as controlling glycaemia levels.

Conclusions

Involvement of nerves and muscles in critically ill patients occurs frequently and quickly in Intensive Care.
The only treatment currently available is intensive control of the glycaemia and intensive insulin therapy.
Only the implementation of electrophysiological techniques like ENG, EMG and DMS in Intensive Care can allow for the diagnosis of CIP and CIM, as well as for differential diagnosis between the two and an indication of how frequently they co-exist.

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OUTCOME OF CRITICAL ILLNESS POLYNEUROPATHY AND CRITICAL ILLNESS MYOPATHY

Marco BOTTERI, Elisa SEGHELINI, Serena ANDREOLETTI

Key words: critical illness polyneuropathy, critical illness myopathy, outcome.

Introduction

Critical illness polyneuropathy (CIP) and critical illness myopathy (CIM) are dysfunctions of muscles and peripheral nerves which can present in Intensive Care patients and complicate their stay not only during the acute phase but also during the rehabilitation period. Development of these conditions is often associated with the appearance of sepsis and multiple organ failure (MODS)[1,2].

Polyneuropathy presents as an acute condition, and is characterised by axonal generation in both motor and sensory peripheral nerves. The myopathy process is also acute, and its histological presentation varies from mild atrophy to overt necrosis. In Intensive Care patients, the clinical presentations of CIP and CIM are very similar and the conditions usually coexist making it very difficult to differentiate between them. Incidence of the conditions varies according to the diagnostic criteria used and the patients studied[3,4].

Given the frequency with which neuromuscular pathologies like these are diagnosed, clinical studies have been carried out over the last few years not only to reach a better definition of CIP and CIM from the point of view of etiopathogenesis, but also to better understand to what extent they affect the prognosis of Intensive Care patients and

Marco Botteri, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Spedali Civili
Piazzale Ospedali Civili, 1 – 25125 Brescia, Italia
Tel. +39(030)3995764 – FAX +39(030)3995570 – marcobotteri@alice.it

Elisa Seghelini, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Italia

Serena Andreoletti, MD
Servizio di Anestesia e Rianimazione – Ospedale di Vallecamonica, Esine, Italia

interfere with a return to normality for those patients who have been discharged.
To put it bluntly, does having CIP and/or CIM increase mortality and morbidity rates in critically ill patients? And, for those who survive, is it going to make things more difficult during the rehabilitation period and mean that functional recovery (both sensory and motor) is going to be worse?
The aim of this paper is to summarize the evidence currently available in the literature regarding the effect that having CIP and/or CIM might have on patient outcome both in the short (during their stay in Intensive Care) and long term (after they have been discharged).

In intensive care

Do CIP and CIM affect ventilation, length of stay in Intensive Care and risk of readmission as well as mortality rates?

When an Intensive Care patient gets CIP and CIM it can affect the peripheral nervous system throughout the whole body. The upper and lower limbs are those most frequently involved but even respiratory nerves and muscles can be affected. Obviously, when they are, this interferes with weaning the patient off the ventilator and therefore means that the patient is on mechanical ventilation for longer. In fact, if we can exclude other probable causes of difficulty, like pulmonary and cardiac pathologies, the reason why patients have to stay on ventilation for longer may well be because they have developed neuromuscular pathologies like CIP and/or CIM, especially if the patient has developed sepsis in the meantime[14].
Some studies highlight this possible link. In 1995 and 1996 two studies were published which established a link between difficulty in weaning patients and prolonged mechanical ventilation with the development of critical illness polyneurophaty and electrophysiological anomalies in the respiratory musculature and phrenic nerve[15,16]. Similar conclusions were reached over the following few years[9,17]. It wasn't until 2005, however, that Garnacho-Montero showed conclusively, for the first time, that patients who develop CIP take longer to wean off mechanical ventilation compared to those who don't[18]. Again in 2005, Bercker, in a retrospective study of patients who had survived ARDS, showed that patients with CIP all spent longer mechanical ventilation[19].
That is more, if CIP or CIM persist when the patient is discharged from Intensive Care, it can lead to the development of acute respiratory in-

sufficiency which presents during the patient's stay in the department they were transferred to. If this happens, the patient has to return to Intensive Care to get adequate ventilatory support until they are able to breathe autonomously again. Acute respiratory failure like this, however, can be so sudden and so acute that the patient dies before adequate help can be given[11].

The development of CIP and CIM can also affect how long a patient has to stay in Intensive Care. In his 2001 study, Garnacho-Montero found that patients who developed CIP had to stay longer than those who did not (53 vs 32 days)[20]. These results were not confirmed by De Jonghe in 2002 however: although out of his sample of 95 patients the ones who stayed longer in Intensive Care were also the ones who presented with neuromuscular changes, the data did not appear to be of statistical significance[17]. In 2005 both Garnacho-Montero, with his 64 patients with signs of sepsis, and Bercker, with his 50 patients with ARDS, found that patients who developed neuromuscular alterations had to stay significantly longer in ICU than those in the control group[18,19].

A few studies link death in Intensive Care or hospital with the development of CIM/CIP. Leijten found that mortality rates in Intensive Care were significantly higher in patients with CIP than in those without[3]. The two patient groups appear suitably similar as regards the seriousness of their condition and the development of sepsis and MODS. What is missing, however, is a multivariate statistical analysis to eliminate the possible effect that other factors may have had on the data. In his 2001 study, Garnacho-Montero, found that although mortality rates in Intensive Care were not significantly different, they were much higher in hospitals in patients with CIP[20]. In this case, however, the patient group was a small, select group and they had significant disturbances with other physiological variables (serious hyperglycaemia, serious hyperosmolarity, serious acidemia, septic shock) which makes it very difficult to generalise the data. In his later study (2005) the same author published data which basically confirmed his previous findings[18]. In this case, however, the prime objectives of the study were different, and so no information was given about the other physiological variables in the patients. This study also concludes that mortality rates are higher in Intensive Care in patients with CIP compared to those without, but the figures are not sufficiently high as to be statistically significant.

Long-term outcome

Do CIP and CIM affect a person's ability to carry out normal daily activity once they are over the "acute phase"?

As well as affecting their stay in Intensive Care, developing CIP and CIM can also affect patients once they have been discharged because clinical signs like generalised muscle weakness, and reduced strength, especially in the lower limbs, ataxia, sensory deficit and the machine-identified electrophysiological disturbances can persist for a long time. In patients who have been transferred from Intensive Care, the symptoms can be so serious that patients are not able to go about their normal daily business independently. Various studies clearly show that this type of handicapping symptomology can last a long time.

In his 1995 cohort study, Leijten studied patients during a follow-up period of up to one year and assessed mortality and morbidity. CIP was diagnosed while in Intensive Care based on electrophysiological test results. Patients with CIP weeks after beeing discharged from Intenssive Care still had deficit, and functional recovery appeared slow[3].

Zifko U. A. (2000) assessed long-term outcome after an average of 17 months (range 13-24 months) in 26 Intensive Care patients with CIP based on electrophysiological alterations. In clinical tests only 2 (15% of the total) patients had normal results, while in ENG tests all patients had at least one pathological nerve. Quality of life a year after being discharged appeared low, with persistent motor and sensory deficit[21].

DeJonghe, in his study published in 2002, assessed, at a distance, patients who had developed neuromuscular alterations during their stay in Intensive Care. He observed reasonable recovery of strength within nine months of being discharged in all patients[17].

Fletcher and coll. in 2003 assessed 22 Intensive Care patients with sepsis and MOF. These patients were tested after an average of 42.5 months using ENG and EMG and also clinically to assess muscular strength (MRC), sensory deficit, level of handicap (Barthel Index) and any medical history compatible with the development of CIP. At follow-up, all patients had had clear signs of extreme muscle weakness after being discharged from ICU[22].

Over the last few years, the concept of quality of life has emerged as a long-term outcome measure. The long period of rehabilitation which necessarily follows a stay in Intensive Care has become the subject of

study in a bid to provide global assessment of how appropriate the treatment was.
Although we can assume that persistent CIP and CIM have an impact on the patient's physical and psychological recovery, there is not much data in the literature.
Herridge, in 2003, assessed ICU patients who had survived ARDS at 3, 6 and 12 months after discharge. He showed that although parameters of respiratory function had returned to normal the major disability impacting heavily on the patients' lives was caused by muscular weakness mainly due to extra-pulmonary causes[23].
Similar results were obtained in Davidson's 1999 study in which patients with ARDS, a year after being discharged, had low quality of life scores regardless of the fact that respiratory problems had been resolved in the majority of cases. In both studies the authors hypothesise that the persistence of CIP and/or CIM could explain why quality of life scores were so low, but the patients were not measured electrophysiologically.
Van der Schaaf in 2004 studied 16 patients diagnosed with sepsis, MODS and CIP. After six months, muscle strength in the four limbs was good in all patients while six patients still suffered sensory deficit; many of them had difficulty going for a walk, coping with stairs and picking up objects. Their quality of life was consequently low. At the 12-month follow-up all patients were improving but all defined quality of life as unsatisfactory[25].
Currently only the two above-mentioned studies by Zifko and Van Der Shaaf analyse quality of life during follow-up in patients discharged from ICU with diagnosed CIP and CIM.
The impact CIP and CIM have on quality of life six months after discharge from ICU recently formed the subject of a thesis written for specialisation exams at the University of Brescia[26]. Patients with persistent CIP and/or CIM were compared with the control group whose electrophysiological readings had normalised. From analysis of the Short Form General Health Survey (SF-36)[27] the persistence of CIP and/or CIM emerges as one of the factors determining quality of life, especially as regards patient health and the limitations this imposed on their working or social lives. If we combine electrophysiological test results with functional assessment scores on the Medical Research Council Score (MRC) we get further evidence of the negative effect this syndrome has on quality of life. The worst quality of life scores were recorded for patients who combined anatomical-struc-

tural alterations with significant neuromuscular deficit. As we can see from the evidence in the literature, in fact, there are patients with an instrument-diagnosed CIP/CIM who do not have clinical neuromuscular signs. During rehabilitation, the MRC could be a valuable tool for assessing the real impact of CIP and CIM on patients' everyday lives.

Is it a pathology with a benign evolution?

Neuromuscular deficit, therefore, even if it lasts quite a long time, does tend to get progressively better. Generally speaking, the upper limbs tend to recover more quickly, followed by the respiratory apparatus and finally the lower limb[7,22,28]. This is understandable given that we are dealing with a distal axonal lesion: the longer the nerve, the longer it will take to repair the nerve tissue[28].

Do CIP and CIM develop in the same way?

Since differential diagnosis between CIP and CIM is difficult in ICU there are not many studies in the literature which look at the possibility of differentiating outcome on the basis of electrophysiological diagnosis. From the limited data published, it would seem that the final clinical picture in patients affected by neuropathy can be confirmed only years after the diagnosis has been made, even when those symptoms are handicapping[3,21,22]. This makes us think that CIP is linked with a worse outcome. In patients with CIM, recovery seems earlier and faster[22,29]. However, the data also shows that patients suffering from myopathy do not always recover completely and the prognosis can be worse in patients diagnosed with myopathy[30].

Summarising the numbers

In a recent review of the literature regarding patient outcome in CIP and CIM acquired in ICU[31] all existing data from studies on this subject were analysed. The review found a total of 36 studies which covered the topic, and then only in part. The total number of patients with CIP and/or CIM where outcome was assessed was 263, with an average follow-up period of 3-6 months and a very variable range (from 2 days to 8 years). The most frequent diagnosis was CIP (on the basis of electrophysiological data) whereas CIM was only diagnosed when autopsies or biopsies were also done. In 68.4% of these patients, functional recovery was complete, whereas 28.1% still had severe disability (tetraparesis, tetraplegia, paraplegia). Moderate disability was fre-

quent and sufficiently bad to cause difficulty, either real or perceived, in carrying out normal daily activity.
Overall mortality rates in patients with CIP and/or CIM indicated in this literature review were 23.8%. The authors point out that many factors influence the validity and interpretation of this data regarding mortality, especially since mortality rates in some studies refer to Intensive Care whereas in others they refer to the follow-up period, and the length of time that had elapsed since discharge from ICU varied from study to study.

Conclusions

It is possible to draw certain conclusions regarding outcome in patients with CIP and CIM based on the data in the literature, but most of these are not well-supported because of the small number of studies dealing with the topic. Length of stay in Intensive Care and hospital is longer in patients with CIP and CIM but the literature on this is very limited and so not very conclusive. Data regarding the possible influence of CIP and CIM on mortality rates in ICU and hospital is even more flimsy. There is more evidence, however, to show that the development of neuromuscular alterations while in Intensive Care increases morbidity and, in particular, makes it more difficult to wean the patient off mechanical ventilation thus prolonging the period of time they are on it.
There is very little literature on long-term outcome, especially as regards mortality after discharge from hospital. Long-term effects can last a long time and the clinical picture is one where the patient still has real or perceived handicaps. There is very little literature regarding differences in long-term outcome as regards differential diagnosis between CIP and CIM and what there is is inconclusive. Future studies which carefully assess the development of these neuromuscular pathologies over time in an adequate number of patients will definitely help to bridge the gap.

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NEUROMUSCULAR RESPIRATORY SYSTEM INVOLVEMENT IN CRITICAL ILLNESS POLYNEUROMYOPATHY

Indrit SHEHU, Elena MALPETTI

Key words: neuropathy, phrenic nerve, neuromuscular insufficiency, weaning.

Introduction

Critical Illnes Polyneuropathy (CIP) and Myopathy (CIM) identify neuromuscular insufficiency along a continuum of clinical situations ranging from a silent presentation to total paralysis of the limbs, weakness of the respiratory muscles, and difficulty weaning the patient off mechanical ventilation.

Although neuromuscular involvement of the limbs is very familiar to us, the same cannot be said of the neuromuscular respiratory system and very little is known about its effect on the duration of mechanical ventilation nor the weaning-off process.

A detailed clinical assessment of the neuromuscular respiratory system in critically-ill patients is difficult and this is why electrophysiological tests are so important. Electric and electromagnetic stimulation of the phrenic nerve (in combination with needle electromyography of the diaphragm) have been used to successfully determine the precise cause of neuromuscular respiratory insufficiency (alterations in the central drive, axonal neuropathy or demyelination of the phrenic nerve, primary myopathy of the diaphragm) especially in Intensive Care.

A precise diagnosis is important in terms of trying to decide the best ventilation support, avoiding neuromuscular blocks and steroids wherever possible, preventing and treating sepsis and multiple organ failure (MOF), structuring a suitable rehabilitation programme for

Indrit Shehu, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Spedali Civili
Piazzale Spedali Civili, 1 – 25125 Brescia, Italia
Tel. 030-3995764 – Fax 030-3995779 – indrit1972@interfree.it
Elena Malpetti, MD
Istituto di Anestesia e Rianimazione – Università degli Studi di Brescia, Spedali Civili

weak respiratory muscles and limbs, and in terms of guiding future research[1].

Physiological and pathophysiological signs in respiration

Respiration, the efficient alternation of inhalation and exhalation, relies on the correct functioning of various anatomical structures: cerebral cortex, brain stem, descending nerve pathways, phrenic and intercostal nerves and respiratory muscles as well as the thoracic cage and airways.

Although they represent only 3% of total body weight, the respiratory muscles constitute a powerful muscular pump capable of varying ventilation levels depending on physiological and pathological conditions. The most important of the inspiratory muscles is the diaphragm which alone moves 50% of air volume during normal breathing. Innervation is from phrenic nerves (motor component) which originate at C3-C5 level which provide innervation for the two hemidiaphragms ipsilaterally. The biochemical and functional characteristics of the diaphragm are similar to those of any skeletal muscle. Contraction of the diaphragm generates negative intrapleural pressure (measured as oesophageal pressure, Pes) and positive abdominal pressure (measured as gastric pressure, Pga) The resultant transdiaphragmatic pressure (Pdi, Pdi=Pga-Pes) has been used to show the tension generated by the diaphragm[2,3]. The intercostal muscles are capable of maintaining respiration in the absence of any diaphragm activity when the subject is in erect position[4].

Respiratory muscle fatigue, caused by exercise and reversible with rest, is different from respiratory muscle weakness. The latter condition, which often occurs in neuromuscular illnesses, refers to the inability to generate adequate levels of pressure or inspiratory and expiratory air flow[5]. The compliance of the thoraco-pulmonary system is also reduced in neuromuscular illnesses, which leads to an increase in mechanical load on the respiratory muscles. This imbalance between muscle load and capacity of respiratory muscles causes muscle fatigue and then respiratory insufficiency. When the muscle weakness is only slight or moderate, there is an increase in central respiratory drive which causes alveolar hyperventilation with a reduction in CO_2 partial pressure, whereas the alveolar-arterial oxygen gradient can remain normal. When serious respiratory muscle weakness develops, carbon di-

oxide retention occurs, eventually leading to obvious respiratory insufficiency.
Clinically, weakness of the diaphragm and other respiratory muscles presents with reduction in vital capacity and total pulmonary capacity, reduction in maximum inspiratory pressure (MIP) and with a paradoxical abdominal respiration whereby the abdomen moves inwardly during inspiration. A reduction of 25% or more in vital capacity from the erect to the supine position is a good indicator of diaphragm weakness[6].
Weakness of the expiratory muscles (abdominals and internal intercostals) causes inadequate clearance of the airways with retention of secretion which leads to an increase in airways resistance and changes in the mechanics of respiration and also facilitates infections making atelectasia more likely. There is also a reduction in maximum expiratory pressure (MEP) and the ability to cough, though these factors alone are not considered sufficient to warrant dependence on mechanical ventilation[7].

Pathogenesis and risk factors

CIP and CIM develop as a consequence of sepsis and/or MOF and are probably linked to an increase in pro-inflammatory cytokines induced by the underlying pathology[8,9]. However, cases have also been seen in patients on mechanical ventilation without any evidence of sepsis or MOF[10].
The contractility of the diaphragm can alter with myopathies or other pathologies of the skeletal muscle with loss of muscle mass and an increase in the muscular protein degradation[11]. In sepsis the failure of the contractility of the diaphragm contributes to the onset of respiratory insufficiency which can be a significant cause of death in these patients[12]. A French study in 2002 carried out on animals showed how diaphragm contractility reduces during sepsis and what an important role is played by 02 free radicals and nitric oxide[13].
A prolonged stay in Intensive Care Unit (ICU)[14] and a long period on me chanical ventilation before the diagnosis of CIP[15] are important risk factors for the development of neuromuscular alterations. Recent data suggests that mechanical ventilation can damage the respiratory muscles[16]. Studies on animals showed that the maximum force developed by the diaphragm when on mechanical ventilation is reduced by 50% compared to the group of animals breathing normally[17,18]. There is very little data available on humans. In a retrospective study of 13 children ventilated for at least twelve days before

death, most of the diaphragm fibres had atrophied and the muscle appeared smaller compared to that of 26 children who died after mechanical ventilation of seven days or less[19].

Clinical features

When the neuromuscular respiratory system is involved in CIP it is difficult to wean the patient off mechanical ventilation. The problem is only identified after days or even weeks because of the use of sedatives and also because of the septic encephalopathy which often develops[20]. Another major feature of CIP is muscular weakness. The patient complains of feeling unwell and fatigued but, unlike patients with pulmonary parenchymal pathologies or airways obstruction, they do not have cyanosis nor maybe even dyspnea. Breathing is rapid and superficial, and cardiac rate is often increased because of accompanying autonomic involvement. Other clinical features indicating a neuromuscular component at the root of the respiratory insufficiency are paradoxical respiration or the absence of abdominal distension during inspiration, more difficulty breathing when lying down compared to a sitting position, and weak or absent cough. Signs like these are non-specific and difficult to interpret in critically-ill patients who are on mechanical ventilation, and lung function tests like measuring vital capacity or maximum inspiration and expiration pressure are difficult to perform in ICU[21]. This is because they rely heavily on patient motivation and collaboration which critically-ill patients cannot offer. As a result, it has often been questioned whether these tests are actually capable of identifying whether it is involvement of the neuromuscular respiratory system which is responsible for the difficulty in weaning the patient off mechanical ventilation[22,23]. Conversely, twitch transdiaphragmatic pressure (Pdi tw) in response to bilateral stimulation of the phrenic nerve can assess diaphragm contractility regardless of patient collaboration[24,25]. However, an esophageal and gastric balloon need to be positioned in order to measure Pdi tw, which limits its clinical application. Measuring airways pressure in the end part of the endotracheal tube in response to stimulation of the phrenic nerve (Paw tw) may be a non-invasive alternative to Pdi tw. However, is still needs to be proved that Pdi tw is significantly altered in patients with CIP and can therefore act as a useful measure when deciding when to wean a patient off the ventilator. Other muscle groups, including abdominals and laryngo.pharyrngeals, contribute to the success of extubation, guaranteeing an effective cough, clearance of tracheo-

bronchial secretions and protection from inhalation of material from the alimentary canal. Involvement of these muscles in patients with CIP has never been studied.

Electrophysiological features

Electrophysiological tests are the only way to identify the nature and level of the lesion responsible for the neuromuscular respiratory insufficiency.

Transcortical magnetic stimulation (TMS) of the cerebral motor cortex recording the diaphragm via surface electrodes is a promising method for investigating central respiratory drive[26]. It can differentiate between a central or peripheral cause of the neuromuscular respiratory insufficiency by revealing alterations in the motor potentials evoked.

Electroneurography (ENG) of the phrenic nerve is a technique which involves stimulating the nerve directly at the posterior edge of the sternocleidomastoid muscle and recording the compound motor action potential at the level of the diaphragm via surface electrodes positioned on the thorax (the first on the sternum 5cm away from xyphoid process and the second 16cm away from the first on the costal margin on the same side)[27]. This enables us to record any increase in the latent period, which indicates a reduction in conduction speed which in turn indicates a demyelinating neuropathy or a reduction in the amplitude of the action potential typical of axonal neuropathies. Repetitive stimulation of the nerve together with recording of the action potential of the diaphragm, assesses the neuromuscular junction which is useful when neuromuscular block drugs are being used[28]. Given the anatomical proximity of the site of stimulation of the phrenic nerve at the neck to the brachial plexus it is obviously important not to stimulate this plexus at the same time[29]. This is particularly important when magnetic stimulation is used on the phrenic nerve[30], a technique introduced in 1989 by Similowski and colleagues[31], which consists in stimulating the nerve at cervical level through a bobbin which creates a magnetic field. This technique is easy to use and less painful for the patient but it is not selective, cannot be used repeatedly and is expensive.

Electromyography (EMG) of the diaphragm consists in recording muscle activity through the insertion of needles to detect different situations. Alteration or absence of muscle firing during inspiration suggests the loss of central respiratory drive. In the case of critical illness polyneuropathy, needle EMG of the diaphragm can show fibrillation

potentials and positive sharp waves as an indication of denervation and the MUPs (motor unit potentials) will fire in decreased numbers. EMGs of the thoracic wall muscles can also show signs of denervation like the diaphragm. Whenever the patient presents with an anomalous motor potential, whether that be after direct stimulus of the phrenic nerve or after transcortical magnetic stimulation, a diaphragm EMG is always useful in determining whether the alteration is secondary to myopathy of the diaphragm, to neuropathy in the phrenic nerve, or a combination of both.

Acute neuromuscular respiratory insufficiency

Neuromuscular respiratory insufficiency is caused by a pathological process which may involve motor neurones, peripheral nerves, neuromuscular transmission or respiratory muscles.
There are multiple causes[32-35] (Table I and II). In ICU, once central, cardiac or pulmonary causes have been ruled out, prolonged dependence on mechanical ventilation is most likely due to acute neuromuscular problems and can be considered a type of acute neuromuscular insufficiency acquired in ICU[36].
Difficulty in weaning patients off the ventilator is of historical significance because it was this which led to the identification and classification of Critical Illness Polyneuropathy in the early Eighties[37]. Despite this, it is only recently that CIP has been effectively demonstrated as being responsible for making the weaning process longer. Various studies[15,38-42], but not all[43,44] have shown a direct correlation between CIP and length of time on mechanical ventilation, probably because there are multiple factors determining the duration of ventilation, not least being the patient's general condition and the reason for them being in ICU in the first place[45]. Two recent studies assessed the role of possible confounders, and found that CIP is an independent risk factor in terms of weaning failure[42,46]. Curiously, neither of the studies assessed alteration in the phrenic nerve or diaphragm but focused only on lower limbs.
The De Jonghe et al.[46] study in 95 critically-ill patients on ventilation for more than 7 days showed two variables which are independently linked to duration of ventilation: a diagnosis of COPD and the presence of ICU-acquired paresis, defined as having an MRC score of <48 for 7 or more days after regaining consciousness. Garnacho-Montero et al.[42], who investigated 64 patients with severe sepsis or septic shock on mechanical ventilation for more than 7 days, showed that

Lower Motor Neurones	SLA Poliomyelitis Werdig-Hoffmann syndrome Post-polio syndrome
Peripheral Nerve (Acute symmetrical peripheral neuropathy or multiple mono-neuropathy)	CIP Drugs: *nitrofurantoin, isoniazide, vincristin, taxol, cisplatin, inverse transferase inhibitors* Metabolic: *diabetes, porphyria, thyrosinemia, uremia* Nutritional deficit: *alcohol, thiamine* Vasculitis: *polyarteritis nodosa, LES, Churg-Strauss* Toxins: *heavy metals (arsenic, thallium, lead, gold), organophosphorus, esacarburi, molluscs (saxatoxin)* Infections: *diphtheritis, Lyme disease* Tick paralysis Sarcoidosis Paraneoplastic Lymphoma Guillain-Barré syndrome (acute inflammatory demyelinating polyradiculopathy*; acute sensory-motor neuropathy; acute motor axonal neuropathy)*
Neuromuscular transmission	Myasthenia gravis Botulism Lambert-Eaton syndrome Hypermagnesemia Snake, scorpion, spider Poisoning from fish, shellfish, crabs
Muscle	Acquired alterations: *rabdomiolissi, hypokalemia, hypophosphatemia, toxic myopathies, dermatomyesitis, poliomyositis* Genetically-determined alterations: *X-linked dystrophies, myotonic dystrophy, maltase acid deficit, mitochondrial myopathy, periodic hypokalemic paralysis*

Table 1 – *Possible causes of neuromuscular insufficiency.*

1. Compromise at cervical levels C-3, C-4 and C-5 as a result of trauma, neoplasia, syringomyelia
2. Compression of nerve root at cervical level due to herniated disc or trauma
3. Degeneration of I° or II° motorneurone or both cervical segments in lateral amyotrophic sclerosis.
4. Compressive or traumatic paralysis of phrenic nerve due to damage somewhere along its path:
   - Application of ice during heart surgery;
   - Insulation during liver transplant or oesophagus or larynx surgery;
   - As a result of mediastinal pathologies: pericarditis, empyema, adenocarcinoma;
   - Spread of oesophageal or laryngeal neoplasias
5. Acute or chronic polyneuropathies (for example critical illness phrenic neuropathy; Guillain-Barré syndrome).
6. Neuromuscular transmission disturbances which present after neuromuscular respiratory insufficiency.
7. Acute primary myopathy of diaphragm (for example critical illness diaphragm myopathy).

Tabella 2 – *Possible causes of phrenic neuropathy and/or myopathy of diaphragm.*

CIP prolongs mechanical ventilation and is an independent factor in failure to wean patients from it. Out of the 34 patients (53%) diagnosed with CIP at the beginning of the weaning period, the average duration of weaning was significantly longer as compared with patients without CIP (15 vs. 2 days, $p < 0{,}001$). What is more, 14 out of the 34 patients (41,2%) with CIP required return to intubation as compared to only 4 out of the 30 patients (13.3%) without CIP. Given that the neuromuscular respiratory system was not investigated in either of the two studies, the question still remains as to whether CIP prolongs mechanical ventilation or whether prolonged mechanical ventilation encourages the development of CIP. De Jonghe's study in fact showed that length of time on mechanical ventilation is an independent predictor of ICU-acquired paresis[46]. We are still left with the basic question, however, of which came first, the chicken or the egg.
Even though it is normally difficult to determine the extent of neuromuscular respiratory system involvement in patients with CIP, the extent to which CIP interferes with the weaning process and its role in prolonging mechanical ventilation are important issues. Only a few

studies conducted electrophysiological tests on respiratory muscles in ICU patients,[14,47-50].Zochodne et al.[47], in 1987, showed during autopsy that there was axonal degeneration of the phrenic and intercostal nerves and denervation atrophy of the respiratory muscles in 9 of the patients with CIP. Witt et al.[14], in 1991, showed significant correlation between reduction in the compound action potential of the diaphragm and the seriousness of CIP. Nonetheless, it was only in patients with serious CIP of the limbs that this was associated with alterations in the diaphragm. The other patients may well have had either type of alteration in isolation. Spitzer et al.[48] in 1992 carried out a prospective study on 21 patients on prolonged ventilation at a fairly advanced stage of their condition. 62% of the patients showed signs of neuropathy, mainly axonal. The intercostal muscle EMG revealed pathology in 6 out of the 10 patients studied (60%). This has important implications for the risk of respiratory insufficiency and difficulty weaning patients off the ventilator. Quantitive analysis of the motor unit potentials of 57% of the patients showed that half these people had myopathic processes going on. This highlights how high the rates of myopathy are compared to what was previously thought, and how an accompanying neuropathy of the phrenic nerve may explain why patients cannot be weaned off the ventilator. Maher et al.[49] in 1995 studied 40 ICU patients over 3 years where a neurological cause was suspected as being responsible for the difficulty in weaning them from the ventilator. Both limbs and phrenic nerve were assessed with ENG and the intercostal muscles and diaphragm with EMG. Incidence of CIP was high (25 patients or 62.5%) and more than half of them (20 patients) had bilateral neuropathy of the phrenic nerve (only 6 of them did not have clinical presentations which would explain the neuropathy at the record-taking stage). The bilateral neuropathy was not observed in the absence of peripheral neuropathy (except in one case of sub-diaphrgmatic abscess). Out of 21 patients with moderate electroneurographical alterations in their lower limbs, only 6 (28.6%) had diaphragm involvement, whereas 9 (90%) of the10 patients with serious electroneurographical alterations in their lower limbs had diaphragm involvement. These patients also showed correlation between the seriousness of the CIP and duration of mechanical ventilation. Ventilation was much longer with serious forms of CIP (136 days), compared to patients with mild or moderate forms (52 days). Zifko et al.[50] in 1998 identified electroneuromyographical alterations in 48 (77%) of the 62 patients with CIP. Patients with a reduction in the amplitude of the

compound muscle action potential in the diaphragm generally stayed on ventilation longer than those patients where the amplitude was normal, though the difference was not statistically significant (an average of 62 vs 55 days respectively). These results show that electrophysiological alterations in the diaphragm are common in patients with ENMG alterations of the limbs, especially where the alterations are severe.

As far as evolution is concerned, Latronico et al.[51] in 1999 described 5 patients who developed neuromuscular insufficency secondary to neuromyopathy acquired in ICU. The patients, all with a history of sepsis, MOF and acute pulmonary damage, had been discharged from Intensive Care once the symptomology that accounted for their admission had been resolved. In 4 out of 5 of the patients, a diagnosis of neuromyopathy was made during the days immediately after their tranfer from ICU. In one of these patients orotracheal intubation was necessary and return to ICU . The other cases resolved after respiratory physiokinesiotherapy. In these patients measurement of vital capacity, maximum inspiratory pressure and maximum expiratory pressure allowed for an assessment of the clinical evolution, since these are reliable indicators of the degree of muscular weakness, however they are not specific enough for diagnostic purposes.

Conclusions

Showing the existence of respiratory muscle dysfunction in critically ill patients is important for confirming or excluding the diagnosis of neuromuscular respiratory insufficiency. The phrenic nerve stimulation technique is not invasive, can easily be repeated and can be implemented in ICU.

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