Biochemical and neurophysiological parameters of acute brain injury

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Chapter 1

Introduction
The two most common conditions that result in acute brain injury are stroke and head injury. Despite the fact that considerable progress has been made in the diagnosis and treatment of these disorders, many unsolved problems remain. In the field of stroke, new neuroimaging techniques play an important role in diagnosis and management. Major therapeutic improvements have been achieved with thrombolytic therapy and improved standard care on stroke units. However, clearly effective neuroprotective agents are not yet available and a long list of promising substances have failed to show any efficacy in human stroke subjects, despite spectacular preclinical results. A similar situation exists for head injury, where standard care and neuroimaging procedures have greatly improved. Furthermore, with the introduction of cerebral pressure and perfusion monitoring, a major step forward has been achieved in decreasing secondary injury to the brain. As is the case for stroke, specific neuroprotective drugs are not yet available.

A major question is why clinical trials with neuroprotective drugs have failed to show any efficacy so far. There are several possible explanations why neuroprotective trials have been unable to prove an effect. The effects of neuroprotective agents on brain injury are time dependent, and treatment has often been initiated much later than in successful experimental models. Insufficient doses of the drugs and slow availability of the drug at the target area may be other explanations. Too small sample sizes in trials and imbalance of prognostically important baseline variables are examples of shortcomings in trial methodology. Furthermore, clinical trials have often been limited by insensitive outcome measures, failure to target specific pathophysiological subtypes, and in stroke patients, failure to target the ischemic penumbra.

New strategies in trial design are needed to compensate for these shortcomings. New parameters are needed to assess more accurately several aspects of the pathophysiology and consequences of acute brain injury. It is therefore necessary to re-evaluate and optimize all phases of acute brain injury care. These include diagnostic classification, selection of eligible patients for treatment strategies, adequate monitoring of neuronal function and response to treatment, estimation of prognosis, assessment of outcome and rehabilitation strategies. Correct diagnosis is often not difficult if a clear history can be obtained from the patient or relatives, and when clinical signs and symptoms match the impression that is obtained from the history. For example, it is fairly straightforward to conclude towards a large hemispheric ischemic stroke in the elderly patient, who has a history of hypertension, diabetes and smoking, and who presents in the emergency department with an acute onset global aphasia, a right sided hemiparesis and hemianopia. Computed Tomography (CT) scanning may show some early signs of cortical infarction such as effacement of sulci and decreased gray/white matter differentiation, but even when no abnormality is seen on CT most clinicians will find the combination of age, risk factors and neurological signs sufficient for the diagnosis of ischemic stroke. In the absence of clear risk factors, or when an adequate history is not possible, or when other signs such as nystagmus or myclonus are present (indicating focal
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seizures), doubt will arise, and the clinician will turn to other diagnostic modalities, such as Magnetic Resonance Imaging (MRI) and Electroencephalography (EEG). These may reveal other causes for the acute onset focal neurological deficit such as tumor, focal epilepsy, migraine, metabolic disturbances or even psychiatric disease. While these procedures will usually yield the correct diagnosis, the process is time consuming and may interfere with adequate therapy, such as thrombolysis. Much would be gained if a simple and easy applicable diagnostic test existed, preferably with very high specificity, so that more time is available to proceed with proper acute treatment.

Selection of patients most likely to benefit from acute treatment. This issue is best illustrated by thrombolytic therapy for ischemic stroke. While early thrombolysis trials included many patients quite indiscriminately, later trials used increasingly more complicated inclusion criteria to select patients, which resulted in better success, but also in very low inclusion rates. This trade-off between wide and easy applicability and time consuming state of the art diagnosis is the field where many new diagnostic procedures try to fit in. A good example is Transcranial Doppler (TCD) which can be used easily to assess neurovascular status in stroke patients, and is more readily available and applicable than for example MRI scanning. The decision to proceed with therapeutic thrombolysis may depend on the finding of a Middle Cerebral Artery (MCA) main stem occlusion, which can be demonstrated with TCD in most instances. On the other hand, the opposite situation may arise when normal TCD signals are found in the presence of a large hemispheric stroke, which may indicate spontaneous reperfusion and a less aggressive treatment strategy. Despite such useful techniques, more detailed information on vessel occlusion status and the amount of reversible and irreversible neuronal damage is needed to further improve patient selection and therapy results.

Adequate monitoring of neuronal function and response to treatment is of vital importance in stroke, but also in head injury. In stroke, monitoring of occlusion status is necessary for establishing the result of thrombolytic therapy. In addition, intensive monitoring of several vital parameters such as blood pressure, blood oxygenation levels, cardiac rhythm, blood glucose levels and body temperature are now part of routine practice in stroke care units. Keeping these variables within normal limits may improve outcome in these patients, which underlines the importance of adequate monitoring.

In severe head injury, intracranial pressure monitoring has proved to be valuable in early detection of complications such as secondary intracranial hemorrhage, and has provided further insight into the dynamics of developing secondary intracerebral edema. However, deep anesthesia is often necessary, and this precludes clinical assessment of the patient. Furthermore, intracranial pressure parameters do not directly reflect neuronal function or damage. Neuronal damage may be assessed by repeated imaging studies, but in the severe head injury patients this is often impractical.

Estimation of prognosis remains very difficult in an acute setting, for both stroke and head injury patients. If irreversibility of neuronal damage could be determined in an early stage, this may help to further separate patient groups who may benefit from therapy from those in
which therapy is expected to fail. Furthermore, it may help to identify the patients whose cerebral damage is beyond any reasonable chance of recovery. While new imaging techniques are being used for this purpose, they are hampered by logistical problems. Here again, the need for an easily usable parameter of neuronal damage and function is clear.  

In the post-acute phase, recovery of neuronal function is usually assessed by clinical neurological examination. This will reveal major motor and sensory deficits, and a superficial impression of cognitive function is obtained. For more detailed analysis of cognitive function, neuropsychological investigation is often used. This is especially useful for quantifying subtle cognitive deficits, which are not evident in normal situations, but may interfere with functioning during work, for example when under time pressure, or when performing multiple tasks simultaneously. However, more severe disturbances may also need correct evaluation, and this may not always be possible, because patients are unable to complete several tests. An extra parameter that quantifies basic cognitive resources would be useful to determine which patients should be referred for neuropsychological testing, and could also be used for monitoring recovery or cognitive rehabilitation therapy.

In this thesis, two diagnostic modalities are presented, which are both aimed at improving several aspects of diagnosis and prognosis of acute brain injury. These are biochemical serum markers of brain injury and the event related P300 potential.
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Biochemical serum markers

Nothing would be easier than to assess aspects of stroke and head injury using simple blood tests. Such serum markers will have to be very specific for brain damage, and very sensitive for even small amounts of brain damage. For such a test to be clinically useful, fast dynamics are required for correct early diagnosis and for monitoring, slower dynamics are needed if diagnosis must be made in the subacute phase. Ideally, some aspect of the test indicates the degree of reversibility, which may be used for selection of patients who are eligible for acute therapy, and for estimation of prognosis. Further requirements are pharmacokinetics that are time-locked to the brain injury and low age and sex related variability \(^{12,13}\). It is a priori unlikely that one simple test will have all these desirable test properties. Therefore, several biological markers of brain damage are analyzed in this thesis.

*S-100:*

S-100 is a calcium binding protein, which is mainly present in glial cells and Schwann cells, but also in other cells, such as melanocytes, adipocytes, chondrocytes and epidermal Langerhans cells \(^{14-17}\). S-100 is also present in certain tumors such as schwannoma, glioma, melanoma and neuroblastoma \(^{18,19}\). The presence of the S-100 beta isomer (S-100B) is restricted to glial cells and Schwann cells. Therefore, brain specificity is probably quite high. Recently however, its specificity for brain tissue has been questioned, mainly by the finding that in multi-traumatized patients and in cardiac surgery patients S-100B can be raised while there is no evidence for brain damage \(^{20-22}\). Its function is not fully understood, but a variety of findings suggest both beneficial functions (induction of reactive synaptogenesis and plasticity processes) and detrimental functions (induction of neuronal cell death) \(^{23}\). It is eliminated by urinary excretion and its biological half-life is below 30 minutes. There is no significant variation with age or sex. Increased concentrations of S-100B have been found in cerebrospinal fluid and serum of both stroke and head injury patients \(^{24,25}\).

*Neuron Specific Enolase (NSE):*

NSE is a glycolytic enzyme of the cytoplasm of neurons and the cells of the Amine Precursor Uptake and Decarboxylation (APUD) system (neuroendocrine cells). It is also present in substantial amounts in red blood cells and platelets. It can be found in neuroendocrine tumors such as neuroblastoma and small cell lung carcinoma. Brain specificity is therefore probably not very high. Beside its role in glycolysis it also increases neuronal chloride levels during onset of neuronal activity. Its biological half-life is between 24 and 48 hours, which indicates slow elimination from the blood. There is no significant variation with age or sex. Increased concentrations of NSE have been found in cerebrospinal fluid and serum of both stroke and head injury patients \(^{24,25}\).
**N-Acetyl-Aspartate: (NAA)**

N-Acetyl-Aspartate (NAA) is a small amino acid (0.1 kD) present in high concentrations in the brain. It is synthesized and stored in neurons, but catabolism is regulated by oligodendrocytes. Although its exact function is largely unknown, synthesis is directly coupled to glucose metabolism, and it may have a role in osmoregulatory mechanisms. In proton nuclear magnetic resonance spectroscopy (MRS) studies, NAA can be detected and is regarded as a marker of neuronal function and integrity. NAA can be detected in serum, urine, cerebrospinal fluid and amnion fluid and is used for diagnosis of an inborn error of metabolism called Canavans disease. Its biological half-life is not known, but is possibly quite short given its small molecular size. There is no significant variation with sex, but there seems to be a difference in serum NAA content of children and adults, with adults having lower values. This may be due to a relative abundance in the child's brain of O2A progenitor cells, which are precursor cells for both neurons and glia that contain twice as much NAA as normal neurons. NAA is released into the serum after stroke, but this has not been studied extensively yet.

**Event related Potentials (P300)**

Electrophysiological techniques are frequently used to obtain information about the functioning of the human brain. Standard EEG and evoked potentials have been used in a clinical setting for many years to aid in the diagnosis of a variety of disorders that affect the brain. Cognitive event related brain potentials (ERPs) are not used routinely in standard practice, despite a considerable amount of research that demonstrates significant differences between patient groups and normal subjects. This is probably due to large variability, which limits their diagnostic properties and clinical applicability. Lack of standardization of recording and analysis procedures, a large influence of subject biological variables and substantial inherent variability all contribute to large ERP variability. If variability could be reduced, this could improve the distinction between normal subjects and patient groups, and renewed efforts to examine the true diagnostic possibilities of ERPs would be justified.

The most frequently studied ERP in a clinical setting is the P300 potential, a large positive wave that arises when a subject has to respond to an infrequently occurring stimulus embedded in a series of standard stimuli. The earlier part of this potential, termed P3A, is thought to reflect an orienting response to sudden alterations in the sensory environment. The later part, termed P3B, is associated with further higher order stimulus evaluation and the start of memory processes. P300 latency is often thought of as a parameter for stimulus evaluation time, while its amplitude is thought to reflect the amount of attentional resources activated. In standard recordings, these components are usually not visible as separate waves. Separating these components is one way to try to reduce some of the inherent variability of P300. With advancing technology such as Positron Emission Tomography (PET), functional MRI, Magnet Encephalography (MEG) and multichannel EEG, several strategies have become
available to separate these components, but most existing studies have focused on finding the intracranial generators of P300, and have paid little attention to diagnostic applicability. In this thesis, a new analysis strategy of P300 is presented and assessed in head injury patients, a group in which cognitive complaints are frequently encountered in the post-acute phase.

Outline of the thesis

In this thesis, two diagnostic modalities are discussed, which are both aimed at improving several aspects of diagnosis and prognosis of acute brain injury. These are biochemical serum markers of brain injury and the event related P300 potential. In Chapter 2, the temporal profile of a serum parameter of brain damage (S-100) after acute head injury, ischemic stroke and Transient Ischemic Attacks (TIA) is assessed. The profiles were compared between the different conditions and were correlated with clinical outcome scales. In Chapter 3, the monitoring potential of serum markers during experimental neuroprotective therapy is assessed in ischemic stroke patients. The possible role as a surrogate outcome parameter is also evaluated. While slow dynamics of serum markers were studied in the first two chapters, Chapter 4 focuses on fast dynamics to see if serum markers can be used to detect reperfusion in ischemic stroke patients who are treated with thrombolytic therapy. A relatively new serum marker N-Acetyl-Aspartate (NAA) is introduced for this purpose. In Chapter 5, a new method for analyzing the event related P300 potential is introduced, which consists of a source analysis strategy, which includes topographic mapping. Variability of latency and amplitude parameters is evaluated in a series of normal control subjects and the source analysis method is compared with the conventional analysis method. In Chapter 6, an unexpected finding is presented that was found after analysis of a head injury group with both the conventional method and the source analysis method. The results have important consequences for the neurophysiological interpretation of P300 results in head injury patients. In Chapter 7, the diagnostic properties of conventional P300 analysis are compared with source analysis in a group of head injury patients. The implications for use of P300 testing in a clinical setting and its place in the diagnosis of cognitive disturbances after head injury are discussed. In Chapter 8, the results of this thesis are summarized.
References


