1. INTRODUCTION

Biomarkers

The ideal biochemical serum marker. In 1983, Bakay and Ward suggested that an ideal serum marker should have high specificity for the brain, a rapid appearance in serum, high sensitivity for brain injury, and be released only after irreversible destruction of brain tissue in a time-locked sequence with the injury. The age- and sex-related variability should be low to ensure a predictable relationship between the serum concentration and the amount of brain injury. Furthermore, reliable assays for immediate analyses should be available. Finally, and most importantly, it should show clinical relevance. These prerequisites have remained the most important properties of a biochemical serum marker of acute cerebral damage.

In clinical practice a biomarker can serve several objectives. There are a large number of studies assessing the value of biomarkers in the prognosis of (i) infection, (ii) acute focal brain injury (stroke, TBI) and (iii) global hypoxic brain damage due to cardiac arrest. However, biomarkers can provide additional information to the clinical evaluation, i.e. in the diagnosis of infection, minor head injury, or TIA/minor stroke, when neuroimaging is not sensitive enough. Furthermore, they are suitable for risk stratification and assessment of the response to certain therapies (antibiotics, immunmodulators, fibrinolytics, therapeutic hypothermia etc.). Biomarkers are not static but dynamic, presenting marked changes in response to different stimuli. Consequently, serial measurements could be more informative than a single one. Additionally, there is a continuous search for novel markers to better predict the outcome of patients with acute cerebral insults.

Markers of brain damage:

Neuron Specific Enolase (NSE): Enolases are glycolytic enzymes. NSE is located in the cytoplasm of neurons and is probably involved in increasing neuronal chloride levels during the onset of neural activity. In clinical practice, NSE is predominantly used as a marker for tumors of the amine precursor uptake and degradation system, such as small-cell lung cancer, neuroblastoma, and melanoma.

S100B protein: These proteins comprise a group of small Ca\(^{2+}\)-binding modulator proteins with a molecular mass of about 21 kDa. Members of this protein family have been implicated in the Ca\(^{2+}\)-dependent regulation of a variety of intracellular activities. The isoforms αβ and ββ are predominantly present in astroglial cells of the CNS and commonly referred to as the brain-specific S100B protein. Serum S100 levels >0.2 µg/L are considered pathologic. It is reasonable to hypothesise that S100B accumulates in the extracellular space after astrocyte death or due to increased release by activated astrocytes, or after cellular disintegration of the damaged parenchyma.

Markers of inflammation:

C-reactive protein (CRP): This acute phase protein is produced by the liver and by adipocytes. CRP rises in the sera whenever an inflammatory process is present and characteristically, its concentration depends only on the intensity of the stimulus and on the rate of synthesis. Recently, it was shown that elevated CRP levels independently predict the risk of future stroke and transient ischemic attack in the elderly. After acute stroke, a single CRP concentration measured within 72 hours was found to be an independent predictor of survival. There is an association between increased levels of CRP measured within 24 hours after ischemic stroke and unfavorable outcome, however an accompanying infection could not be ruled out if blood samples were taken within 24 hours after symptom onset. Others found that CRP concentration at discharge was better related to outcome than earlier measurements. Importantly, a CRP increase between 12 and 24 hours after symptom onset predicts an
unfavorable outcome and is associated with an increased incidence of cerebrovascular and cardiovascular events.

**Procalcitonin (PCT):** Procalcitonin is a prohormone physiologically transformed into calcitonin by the medullary cells of the thyroid. However, the origin of this substance can be extrathyroidal. High PCT serum levels were found in thyroidectomized septic patients, while calcitonin was undetectable. PCT, a marker of septicemia and infection severity has also been proposed as an indicator of systemic inflammatory response in noninfectious situations. There are only few studies related to PCT in stroke, although inflammatory response is a principal early component in the pathophysiology of stroke.

**Leukocyte antisedimentation rate (LAR):** We have recently established a simple test to examine systemic activation of leukocytes by measuring upward floating in a tube during one hour of gravity sedimentation. The LAR indicates the percentage of leukocytes crossing the middle line of the blood column upwards during 1 hour of sedimentation. In short, Westergreen blood sedimentation rate technique was modified for measuring leukocyte motion during gravity sedimentation of the whole blood. After one hour sedimentation, the leukocyte counts of upper and lower half sections were measured with an automatic cell counter (Coulter Counter CBC5, Coulter Electronics Ltd, Luton, UK) and results were expressed as a rate.

**Role of leukocytes in acute ischemic stroke:** Leukocytes accumulate in the region of cerebral ischemia in the early stage of stroke, within hours. The neurological outcome was shown to be worse and the infarct larger in patients with severe polymorphonuclear leukocyte (PMNL) accumulation. A significant correlation between stroke volume or stroke severity and an acute increase of white blood cell (WBC) in the peripheral blood have been reported. Increased aggregation of peripheral leukocytes in the absence of WBC elevation was also reported in major stroke. Increased in vitro adhesive properties, activation of leukocytes indicated by increased plasma oxidation of adrenaline to adrenochrome and higher plasma levels of cytokines and proteases have been shown in ischemic stroke and TIA. While these studies addressed the detrimental effect of leukocyte activation, it may also play a major role in defense against pathogens, and participate in combating infections. A deficient activation of leukocytes may thus contribute to an increased susceptibility to post-stroke infections.

**N-terminal –prohormone of brain derived natriuretic peptid (NT-proBNP):** Natriuretic peptides play an important role in the regulation of cardiovascular homeostasis and fluid volume. Increased plasma levels of natriuretic peptide hormones have been identified as predictors of cardiac dysfunction and death in many critical care settings, including congestive heart failure, myocardial infarction and septic shock. The prohormone brain natriuretic peptide (pro-BNP) is produced by ventricular myocytes in response to wall stress. In the circulation the biologically active hormone is separated from the N-terminal part of the prohormone termed NT-proBNP. It promotes natriuresis and diuresis, acts as a vasodilator and aldosteron antagonist, and also inhibits the endothelin release and smooth cell proliferation. Because BNP- or NT-proBNP-guided therapy of heart failure could reduce the occurrence of cardiac events and thereby decrease mortality, the marker itself seems to be an early predictor of prognosis and myocardial dysfunction in patients with septic shock. Investigation of their levels and prognostic roles in patients after cardio-pulmonary resuscitation (CPR) with or without sepsis is of great interest.

**II. Acute brain injuries examined in the Thesis**

**Transient Ischemic Attack (TIA):** It is an acute ischemia with the signs and symptoms of stroke, disappearing within a short period of time (less than 24 hours). Also called a mini-stroke, a TIA is due to a temporary ischemia to the brain and symptoms typically last for 2 to 30 minutes. Patients with TIA have a 25% greater risk of stroke within 90 days.
Stroke: In addition to being the third leading cause of death, many survivors of stroke have to adjust to a life with varying degrees of disability. While direct neurological deficits cause early deaths, infectious complications, particularly pneumonia and urinary tract infections, prevail in the postacute phase of stroke contributing to the poor outcome, which may suggest early alteration of immune responses. Recently, a loss of T cells in the peripheral blood of patients with acute ischemic stroke within 12 hours from onset of stroke symptoms, was revealed, which gradually normalized.

Perioperative cognitive dysfunction (POCD): Advances in anaesthetic and surgical techniques have led to the assumption that postoperative cognitive decline is currently less common than previously. The benefits of such technological advances, however, may have been offset by inclusion of older patients with more comorbidity. The neuro-behavioural outcomes range from the well-documented incidence of stroke to the less well-delineated postoperative delirium, cognitive difficulties (such as memory loss and visuospatial deficits), and depression. Prolonged postoperative cognitive dysfunction (POCD) is reported to occur frequently after cardiac surgery. However, it is rarely assessed in routine clinical practice and receives little attention after non-cardiac major surgery. The incidence of cognitive dysfunction has been reported to be between 1% and 60% depending on the type of operation.

Post-cardiac arrest syndrome (PCAS): Among cardiac arrest survivors, brain injury is the major determinant of functional outcome and quality of life. A major goal is to identify patients destined for poor neurological outcomes. The failure of injured structures to recover leads to a spectrum of neurological dysfunction including the inability to perform tasks independently, persistent vegetative state, coma, and brain death. The need for protracted high-intensity care of neurologically devastated survivors presents an immense burden to healthcare systems and society in general. To limit this burden, clinical factors and diagnostic tests are needed to prognosticate functional outcome. Beside others, biochemical markers have been used to prognosticate functional outcome after cardiac arrest. Numerous studies showed varying thresholds of NSE < from 30 to 65 µg/L associated with poor outcome and mortality. In cardiac arrest survivors, S100B exhibited similarly high specificity but low sensitivity. Interestingly, the non brain-originated PCT was found in successfully resuscitated out-of-hospital cardiac arrest victims as sensitive and specific as the brain originated S100B regarding the neurological outcome. In postresuscitation care, there is a great need for further biomarkers or their combination in order to distinguish the acute phase response due to tissue injury and the infectious complications.

2. AIM OF THE STUDIES

1. The innate immune response in patients with acute ischemic stroke complicated by post-stroke infections

Cerebrovascular diseases of acute onset, including ischemic stroke, are associated with diverse immune repsonses. The high incidence of infections in stroke patients is likely to be a result of an impaired immune function. Therefore, we investigated different biomarkers in patients within hours after onset of acute ischemic stroke and TIA to characterize the innate immune response and its relation to post-stroke infection and functional outcome. Furthermore, the aim of our study was to evaluate the most reliable markers in identifying a subset of stroke patients, which later develop infections.

Therefore, we addressed:

1. the relation of leukocyte activation to post-stroke infections;
2. whether post-stroke leukocyte activation depends on the duration of ischemia and the extent of infarct;
3. whether a simple bed-side tests to measure leukocyte activation could predict outcome and susceptibility for post-stroke infections;
4. serial measurement of leukocyte activation in the sera to compare acute ischemic stroke and TIA;
5. serial measurement of leukocyte activation in patients with or without post-stroke infections;
6. the relationship between leukocyte activation and functional outcome;
7. plasma hsCRP of patients with acute ischemic stroke compared to TIA and healthy controls;
8. plasma hsCRP as marker of stroke in first-ever or recurrent stroke subpopulations;
9. association between plasma hsCRP levels and NIHSS indicating the severity of symptoms,
10. association between the inflammatory aspect of atherogenesis represented by hsCRP and inflammation mediated by leukocytes;
11. serial measurement of hsCRP comparing patients with or without post-stroke infections;
12. the relationship between hsCRP and functional outcome in acute ischemic stroke;
13. the best cut-off value of hsCRP in prediction of infectious complications after acute ischemic stroke;
14. functional alterations in cytokine production and cytotoxicity of innate lymphocyte subsets capable of shaping adaptive T cell responses in the acute phase of ischemic stroke.

II. Measurement of brain derived biomarkers in the sera of cardiac patients with accidentally provoked transient neurological signs by intravenous dipyridamole (DP) stress test

Intravenously administered DP can induce short lasting and reversible minor neurologic deficit in high-risk vascular patients, that can be detected by using brain SPECT imaging providing a very early assessment of patients at risk of threatening cerebrovascular events. The aim of this prospective study therefore was to determine:
15. if DP-stress impacts biomarkers (S100B, NSE, hsCRP);
16. the frequency of transient neurological signs (TNS) during DP-stress;
17. if induced biomarkers and TNS could predict manifest cerebrovascular events in 7 years;
18. the best predictor of upcoming cerebrovascular events in patients with coronary artery disease.

III. Measurement of biomarkers of endothel and platelet activation in the sera of patients with non-cardiac surgery

Cardiac operations are frequently complicated by postoperative cognitive decline. Less common and less studied is postoperative cognitive decline after noncardiac surgery. Therefore, we examined:
19. the incidence of POCD after lung surgery at the Department of Surgery, University of Pécs, Hungary;
20. if MMSE were suitable for determination of POCD;
21. if biomarkers reflecting immuno-endothelial dysfunctions could be related to ischemia and neurocognitive alterations after lung surgery;
22. the best molecular predictors of susceptibility for POCD before major surgeries.
IV. Measurement of biomarkers in the sera of successfully resuscitated cardiac arrest victims

Increased plasma levels of natriuretic peptide hormones have been identified as predictors of cardiac dysfunction and death in many critical care settings. To date no studies were addressed to investigate the relationship between NT-proBNP and PCT in the serum of patients successfully resuscitated after in-hospital cardiac arrest with or without concomitant infection or sepsis. Therefore, we performed a serial analysis of S100B, PCT and NT-proBNP in the first 72 post-resuscitation hours in cardiac arrest victims to examine the role of these markers in prediction of septic complication:

23. differences in plasma levels of PCT, S100B and NT-proBNP in patients resuscitated due to either ventricular fibrillation or asystole/pulsless electrical activity;
24. differences in plasma levels of PCT, S100B and NT-proBNP in cardiac arrest victims with or without septic complication;
25. relationship between the measured biomarkers and clinical outcome.

3. EXPERIMENTS

I. THE INNATE IMMUNE RESPONSE IN PATIENTS WITH ACUTE ISCHEMIC STROKE COMPLICATED BY POST-STROKE INFECTIONS

1. Deficient leukocyte antisedimentation is related to post-stroke infections and outcome:

Patients with stroke are more susceptible to infections suggesting possible deficiencies of early immune responses, particularly of leukocytes. Here, we examined whether post-ischemic activation of leukocytes is related to duration of ischemia and extent of infarct. We also addressed, if dysregulated leukocyte activation might be related to post-stroke infection and influence outcome. We used leukocyte antisedimentation rate (LAR) to detect activation of leukocytes and correlated LAR with clinical and laboratory parameters. An additional aim was to test simple bedside investigations in predicting outcome and susceptibility for post-stroke infections early.

LAR, a simple test to detect activation of leukocytes was serially examined and correlated with blood level of S100β related to extent of infarct as well as to procalcitonin indicating infection and outcome in patients with acute ischemic events. Venous blood samples were taken from 61 healthy volunteers and 49 patients with acute ischemic events: 38 patients with acute ischemic stroke, and 11 patients with transient ischemic attack (TIA) where symptoms disappear in 24 hours and cranial CT scan does not indicate infarct. Sampling was done within 6 hours, at 24 and 72 hours after onset of symptoms. LAR was significantly higher in acute ischemic events within 6 hours after onset of stroke regardless of post-stroke infections. In addition, elevation of LAR was delayed and attenuated in TIA in contrast to definite stroke. We also observed a positive correlation between LAR and S100β at 72 hours after the onset of ischemic stroke both indicating that the extent of tissue injury correlates with the magnitude of innate immune responses. Importantly, a deficiency in early elevation of LAR was associated with post-stroke infections and a poor outcome measured by Glasgow Outcome Scale.

We conclude that an early activation of leukocytes indicated by elevation in LAR is characteristic of acute ischemic cerebrovascular events. A delayed and ameliorated leukocyte activation represented by LAR is characteristic to TIA in contrast to definite stroke. Our data
suggest that acute activation of leukocytes, which has been regarded detrimental so far, serves also to prevent post-stroke infections. Our data imply that concept about the post-ischemic role of leukocytes should be changed and dissected: recruitment of leukocytes in the CNS may be damaging but should be separated from the systemic activation, which may prevent post-stroke infections. A disregulated early immune response or deficient leukocyte activation may result in an increased susceptibility to infections in patients with stroke.

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2. Relationship between C-reactive protein and early activation of leukocytes indicated by leukocyte antisedimentation rate (LAR) in patients with acute cerebrovascular events

A non-specific inflammatory response occurs after stroke and the degree of inflammation is predictive of outcome. The purpose of this study was to determine the relationship between the acute phase reactant C-reactive protein (CRP) and leukocyte antisedimentation rate (LAR) as a specific test to detect early activation of leukocytes. Here, we examined relationship among LAR, astroglia specific S100B indicating the extent of brain tissue damage and hsCRP in 49 patients within hours after onset of acute ischemic stroke (AIS) and transient ischemic attack (TIA) to characterize the innate immune response and its relation to the outcome.

Serum levels of hsCRP on admission was significantly higher in patients with acute ischemic stroke (AIS) compared to healthy subjects and were higher in patients with recurrent to first ever ischemic stroke. Increased basal levels of hsCRP also correlated with extent of infarct reflected by S100B levels in sera, but did not correlate with post-stroke infections. However, a higher rate of infection was observed among patients, in whom hsCRP was elevated at 72 hours but LAR did not increase. Therefore, such late elevation of hsCRP may indicate pre-clinical infections due to deficient leukocyte activation. Indeed, our data indicate that early (within 6 hours) and late (after 72 hours) changes in serum levels of hsCRP may reflect different pathological processes in stroke.

We can conclude that simple tests like LAR and hsCRP may help in predicting outcome and high risk of infectious complications.

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3. Impaired function of innate T lymphocytes and NK cells in the acute phase of ischemic stroke

While direct neurological deficits cause early deaths, infectious complications prevail in the postacute phase of stroke contributing to the poor outcome. The increased susceptibility to infections after stroke may suggest early alteration of immune responses, thus immunodepression induced by stroke has been proposed. The few animal and human studies all addressed the rapid changes in the adaptive arm of the immune system, mainly T cells. We analyzed rapid changes in immunological functions of cells of the innate immunity or lymphocytes bridging the innate and the adaptive arms of the immune system, all capable of shaping subsequent immune responses through rapid production of cytokines and/or cytotoxicity. The analyzed cell subsets were Vδ2 T cells, CD3+CD56+ natural killer T (NKT)-like cells and CD3-CD56+ NK cells. Their frequencies, cytokine production, intracellular perforin, surface Fas ligand (FasL) expression, and NK cytotoxicity were measured in 28 patients’ peripheral blood obtained within 6 hours and also after 72 hours of ischemic stroke. The paired samples were compared both with each other and with 20 healthy controls.

Percentages of Vδ2, NKT-like and NK cells at 6 and 72 hours after stroke were constant and similar to percentages in healthy subjects. In contrast, pro-inflammatory
intracellular IFN-γ expression by Vδ2 T cells, NKT-like cells and NK cells and IFN-γ production by isolated NK cells in culture was low at 6 hours and reached the level of healthy subjects by 72 hours after stroke. Anti-inflammatory IL-4, IL-5 and IL-10 production of NKT-like and NK cells was not altered. Intracellular perforin expression by Vδ2 T cells, NKT-like cells and NK cells, and NK cytotoxicity were low at 6 hours and reached the level of healthy subjects by 72 hours. The increase in IFN-γ production by Vδ2 T cells strongly correlated with decrease in NIHSS scores indicating clinical improvement.

According to our results, pro-inflammatory and cytotoxic but not anti-inflammatory responses of NK, NKT-like and Vδ2 T cells become acutely deficient in ischemic stroke, which may contribute to an increased susceptibility to infections. The rate of increase in pro-inflammatory cytokine IFN-γ correlates with the rate of improvement.

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4. Immune responses and neuroimmune modulation in the pathogenesis of acute ischemic stroke and post-stroke infections

Acute-onset cerebrovascular diseases are connected to a number of immunological changes. Here, we summarized and reviewed immune responses participating in the evolution of atherosclerotic plaques and post-stroke local immune responses in the injured CNS as well as in the systemic circulation. Ischemic injury of the CNS alters the balanced neuroimmune modulation resulting in CIDS, the central nervous system injury-induced immune deficiency syndrome. Due to the immunodepression and reduced pro-inflammatory immune responses, the susceptibility for infection is increased; indeed, post-stroke infection plays a major role in stroke-related mortality. On the other hand, CIDS may protect against damaging autoimmune responses elicited by exposed CNS antigens. Investigation of immune responses related to ischemic stroke may result in novel therapies indicated by an increasing number of experimental and clinical trials altering post-stroke immune responses and preventing infections.

II. MEASUREMENT OF BRAIN DERIVED BIOMARKERS IN THE SERA OF CARDIAC PATIENTS WITH ACCIDENTALLY PROVOKED TIA BY INTRAVENOUS DIPYRIDAMOLE (DP) STRESS TEST

1. Dipyridamole stress test in the early evaluation of cerebral circulatory disorders?

The diagnosis of transient ischaemic attack (TIA) is mainly based on the clinical symptoms presented by the patient. Professionals in nuclear medicine have made a successful attempt to detect TIA by single photon emission tomography (SPECT) imaging. Acetazolamide stress test was used to improve the sensitivity of the examination till now. The aim of this study was to analyse the relationship between transient neurological symptoms provoked by dipyridamol (DP) test and the change in regional cerebral blood flow indicated by brain SPECT imaging. Patients with ischaemic heart disease were stressed by intravenously administered DP and considerably higher incidence (23%) of DP induced TIA was found than reported previously in the literature. The so called „TIA positive” patients were re-examined later on using the combination of dipyridamole stress test and brain SPECT imaging which was compared to baseline brain SPECT at rest. Perfusion abnormalities revealed by brain SPECT were topographically consistent with the neurological deficits.

We conclude that DP stress test provides an opportunity to provoke TIA safely, determining the high-risk patients threatened by cerebrovascular event. Combined with brain
SPECT it may have an important role in the neurologic diagnostic evaluation, management of cardiac patients prior to major surgery and the prevention of stroke.

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2. “Cerebrovascular stressing”: drug-induced S100B elevation and transient neurological signs predict ischemic cerebrovascular events

This study was primarily conducted to compare the profile of serum S100B in the very early stage (within 6 hours after onset of symptoms) between stroke, spontaneous TIA and cardiac patients with and without dipyridamole (DP) induced transient neurological signs (TNS). Furthermore, we investigated the association between the clinical status (symptoms and signs provoked by DP), peak S100B levels and the risk of a future cerebrovascular event in patients with coronary artery disease who were stressed with DP. We found that DP itself did not influence the serum level of hsCRP, which is a good predictor of manifest stroke/TIA. However, DP had an impact on S100B protein release by presumably changing permeability of the BBB. Moreover, the median serum S100B level in cardiac patients with DP-induced TNS was very similar to spontaneous stroke/TIA and significantly differed from cardiac patients without DP-induced TNS.

Results of 7 years follow-up: Manifest ischemic events occurred in 48% of patients (TIA: 15/37, stroke: 3/37) in seven years. Importantly, new cerebrovascular events occurred in patients with DP-induced TNS twice as often as in patients without DP-induced TNS (64% vs. 30%, p=0.05). All ischemic events (TIA and stroke, n=18) and TIA (n=15) were more common among patients with DP induced elevation of S100B measured at T60 seven years earlier. When adjusted for age, gender and comorbidities in a multivariate analysis, DP-induced S100B above the cut-off value (S100B ≥ 0.11ng/ml) indicated 70-times increased risk for manifest ischemic cerebrovascular events within 7 years.

As the DP stress test is basically performed by cardiologists, symptoms of TIA are often unrecognized. Therefore, S100B can be a surrogate marker to select (cardio)vascular patients with high-risk for cerebrovascular events, who can have the most benefit from early therapy. SPECT brain scan combined with DP test (S100B and neurological signs) might be an important diagnostic tool in the early recognition of silent cerebral circulatory disorders, stroke prevention and in the preoperative assessment of patients scheduled for cardiac surgery. In this context, for the analogy of “coronary stress” this method can serve as “cerebrovascular stress”.

III. MEASUREMENT OF BIOMARKERS OF ENDOTHELIAL ACTIVATION IN THE SERA OF PATIENTS WITH NON-CARDIAC SURGERY

1. Increased levels of baseline biomarkers reflecting platelet and endothelial activation predict early cognitive dysfunction after lung surgery

The reported high incidence of short term postoperative cognitive dysfunction (POCD) (33-83%) after cardiac surgery is mainly related to cardio-pulmonary bypass; however POCD is also frequent and potentially serious after elective lung surgery, where deleterious effect of pump is excluded.

The goal of this study was to examine biomarkers reflecting immuno-endothelial dysfunctions, which could be related to ischemia and neurocognitive alterations, in the early recognition of POCD after lung surgery: soluble P-selectin (sP-selectin), soluble vascular cell adhesion molecule-1 (sVCAM-1), monocyte chemoattractant protein-1 (MCP-1), soluble CD40 ligand (sCD40L), tissue plasminogen activator (tPA), interleukin-6 (IL-6), interleukin-
8 (IL-8), S100B and high-sensitivity C-reactive protein (hsCRP). We investigated 35 patients with non-small cell lung cancer, who underwent elective lung surgery. Patients with neuropsychiatric event in the past medical history, pre-existing cognitive dysfunction (defined as MMSE score below 24), and certain types of neoplasm resulting per se elevation in serum level of S100B (e.g. malignant melanoma) were excluded. Biomarkers were measured preoperatively and on the second postoperative day. Early neurocognitive deficit was assessed on admission by mini-mental status examination (MMSE) and on the second postoperative day (MMSE48). POCD was defined as a decrease in MMSE score >3 points from baseline. Nine patients had POCD within 48 hours (25.7%). Baseline scores of MMSE showed no significant difference in patients with or without POCD; however MMSE48 was significantly lower in patients with POCD, and the rate of decline in MMSE was significantly higher in the POCD group.

In summary, we found that molecules reflecting platelet and endothelium activation (sCD40L, sP-selectin, sVCAM-1, MCP-1) were increased preoperatively in patients developing POCD and remained elevated at least for 48 hours after lung surgery. We may hypothesize that the endothelium and platelets are more sensitive to immunological responses against tumors in a subpopulation of patients and become activated. Such pre-operative activation of platelets and endothelium can result in an increased activation/transmigration of leukocytes, disruption of blood-brain barrier and can also participate in brain ischemia resulting in POCD. In addition, these molecules may be used as potential biomarkers predicting susceptibility for POCD before major surgeries. Patients known to be at such an increased risk preoperatively may benefit from closer monitoring and earlier medical intervention or being offered non-surgical oncological treatment if the operative risk is considered to be unacceptable.

IV. MEASUREMENT OF BIOMARKERS IN THE SERA OF SUCCESSFULLY RESUSCITATED CARDIAC ARREST VICTIMS

1. Prognostic role of serum S100β and procalcitonin in patients after cardio-pulmonary resuscitation

The prognostic value of S100β considering the neurological outcome in patients after cardio-pulmonary resuscitation (CPR) is already well documented. The non-SIRS related changes in PCT levels indicating brain cell damage can provide additional information. The aim of this pilot study was to reveal any difference between survivals and non-survivals in serum S100β and PCT levels after CPR regardless of the type of cardiac arrest.

A total of 20 victims after either in or out-of-hospital successful resuscitation were investigated. CPR and postresuscitation treatment were carried out according to the current advanced life support (ALS) protocol of the European Resuscitation Council. Physical neurological examination was performed and serum S100β and PCT were serially measured on admission to the ICU (t₀), after 24 hrs (t₂₄) and 72 hrs (t₇₂), respectively. Biomarkers were compared in 9 survivors and 11 non-survivors. In accordance with other papers, serum S100β level was higher in non-survivals indicating more severe anoxic brain tissue injury due to cardiac arrest, however the difference between the two groups was not significant (p=0.06). Interestingly, PCT serum level was very similar among survivors and non-survivors at any timepoints. Importantly, a significant negative correlation was found between PCT levels measured at t₂₄ and the time of anoxia (p<0.01), however such association was not observed between S100β and time of anoxia.

To decide whether these biomarkers have any impact on the prognosis of patients after CPR, we are going to expand the study with larger number of patients.
2. NT-proBNP as early marker of septic complications in patients after cardiopulmonary resuscitation

In the postresuscitation phase, there is a whole-body ischaemia/reperfusion syndrome, which leads to alteration in the immuno-inflammatory profile and results in potentially serious infectious complications. Interestingly, the non brain-originated PCT investigated in out-of-hospital cardiac arrest was similarly sensitive and specific to the CNS-derived S100B, regarding neurological outcome. Moreover, there is a strict association between outcome and septic complications. Recent studies also suggested that NT-proBNP may be an early prognostic factor of myocardial dysfunction in patients with septic shock. Here, we examined whether NT-proBNP and/or PCT are useful markers in recognition of patients after CPR at risk of sepsis in the early post-resuscitation phase.

After excluding preexisting conditions known to increase NT-proBNP levels, such as chronic heart insufficiency and renal insufficiency, a total of 35 patients were included into the study. Twenty-five patients suffered from asystole/pulseless electrical activity (ASYS/PEA) and 10 patients from ventricular fibrillation (VF). In the ASYS/PEA group, 28-day survival was significantly decreased compared to the VF group. Defined upon the criteria of the ACCP/SCCM consensus conference, patients were further divided into septic (S) and non-septic (NS) groups. In the ASYS/PEA group, 8 patients were septic and 17 non-septic. Our study confirmed that NT-proBNP levels were elevated in patients with sepsis even after cardiopulmonary resuscitation. Interestingly, we found similarly elevated natriuretic peptide both in patients with severe sepsis (ASYS/PEA-S) and ischaemia induced myocardial dysfunction (VF), despite the marked pathophysiological differences in the circulatory response. The elevated NT-proBNP level in the serum of successfully resuscitated patients after VF is not surprising, as severe ischemia always proceeds VF similarly to acute coronary occlusion, where the prompt elevation of serum NT-proBNP has been already reported. Obviously, serum procalcitonin levels were also significantly higher in patients with sepsis; however, we also observed an increase in patients, where cardiac arrest was presumably forestalled by significant hypoxic period independently from infection.

It seems that on ICU admission, serum NT-proBNP was similar to the inflammatory markers such as procalcitonin in septic patients, who underwent cardiopulmonary resuscitation, therefore it might be useful in early recognition of post-arrest septic complications.

4. SUMMARY OF THESES

1. Activation of leukocytes represented by elevation in LAR happens within hours after onset of ischemic stroke.
2. Although LAR was elevated in TIA compared to healthy subjects, it was delayed and ameliorated compared to definitive ischemic stroke.
3. Decreased activation of leukocytes reflected by a deficient elevation of LAR may predispose to post-stroke infections and predict worse outcome.
4. Concept about uniformly harmful post-ischemic role of leukocytes should be changed and dissected: recruitment of leukocytes in the ischemic brain may be damaging by amplifying brain injury. However, systemic activation of leukocytes plays an important role in preventing post-stroke infections.
5. A positive correlation exists between LAR and S100β on the 3rd post-stroke day indicating a relationship between innate immune responses and the volume of infarct.

6. CRP on admission was significantly higher in patients with acute ischemic stroke (especially in recurrent stroke), however not in TIA patients, demonstrating that CRP is an indicator of atherothrombosis and an independent predictor of increased cardiovascular risk in apparently non-infected patients.

7. CRP has a predictive value for poor prognosis (GOS) and more severe symptoms (NIHSS) irrespective of post-stroke infections.

8. Early elevation of hsCRP also correlated with serum levels of S100B at 72 hours, supporting the hypothesis that magnitude of tissue injury may have an impact on acute phase reactants.

9. We also observed a late increase of hsCRP in patients with a tendency of decreased leukocyte activation, indicating a higher rate of infection. Such a late elevation of hsCRP by 72 hours thus may be an early pre-clinical sign of infections due to deficient leukocyte activation. Indeed, our data indicate that early (within 6 hours) and late (after 72 hours) changes in serum levels of hsCRP may reflect different pathological processes in stroke.

10. The percentages of particular innate lymphocytes Vδ2, NKT-like and NK cells do not change in the acute phase of ischemic stroke in contrast to published decrease of conventional αβT cells.

11. In contrast to the percentages, an acute functional deficiency of innate T lymphocytes and NK cells occurred in the acute phase of ischemic stroke, within 6 hours: pro-inflammatory cytokine production, expression of perforin and NK cytotoxicity were decreased, while there was no change in production of Th2 cytokines and Th2-related ICOS expression. We may hypothesize that such early deficiency or its disregulated normalization may substantially influence susceptibility to infections, similar to animal models of cerebral ischemia.

12. Normalization of IFN-γ production by γδT cells correlated with rate of clinical improvement, suggesting that pro-inflammatory cytokines might participate in neuroregeneration in stroke, similar to spinal cord injury.

13. The stress agent dipyridamole (DP) can provoke transient neurological signs (TNS) in cardiac patients indicating subclinical cerebrovascular disorder. Additionally, DP stress combined with brain SPECT imaging is suitable for an early visualisation of cerebral circulatory disorders.

14. Investigating the association between peak S100B and the risk of future cerebrovascular events in cardiac patients after DP-stress, we found that both elevation of S100B and TNS were more common among patients who experienced manifest stroke/TIA within seven years (75% and 67%, respectively). In multivariate analysis, only DP-induced elevation of S100B was a good independent predictor of stroke and TIA after adjusting for confounding factors: the risk of such events within seven years was 70-times higher compared to patients without such elevation.

15. Molecules reflecting platelet and endothelium activation (sCD40L, sP-selectin, sVCAM-1, MCP-1) are increased preoperatively in patients developing POCD and remain elevated at least for 48 hours after lung surgery. These molecules may be potential biomarkers predicting susceptibility for POCD before major surgeries: such patients may benefit from closer monitoring and earlier medical intervention or being offered non-surgical oncological treatment if the operative risk is considered to be unacceptable.

16. Prior pulseless electric activity (PEA) arrest, patients often have long, undetected hypoxic periods triggering the release of inflammatory markers such as PCT.
Contrary, significantly lower inflammatory response can be detected in patients in ventricular fibrillation arrest, accompanied by better survival regardless of anoxic time.

**17.** NT-proBNP is elevated after successful cardiopulmonary resuscitation in patients who became septic, and its kinetics is similar to inflammatory markers such as procalcitonin; therefore it might be useful in early recognition of post-arrest septic complications.

### 5. BIBLIOGRAPHY

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AWARDED LECTURES


Környey Emlékérem


CHAPTER

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