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Immunomonitoring in patients with early moderate and severe head trauma

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Abstract

Background: *Trauma patients with severe and moderate central nervous system (CNS) injuries are at risk of immunologically derived complications (infection, "2nd-hit injuries"). The aim of this study is to evaluate the phagocytic arm of the immune system in patients with isolated CNS injuries during a period of time which is critical for the incidence of infections. The phagocytic arm is analyzed to optimize the timing of definitive surgery and to introduce the immunomodulation therapy and prophylactic measures.*

Material and methods: *The study group consisted of 7 men and 2 women from 18 to 79 years of age who had sustained an acute, isolated CNS injury as a result of mechanical trauma. Brain damage was severe or moderate in all of the cases [≤ 11 Glasgow Coma Scale (GCS) pts]. All patients were studied on the 3rd, 6th and 9th day after trauma. Eleven healthy age- and sex-matched volunteers served as controls.*

Results: *A statistically significantly lower individual cellular phagocytic activity (number of bacteria per cell) was observed in the CNS-injured patients on the 3rd, 6th and 9th day. The percentages of granulocytes showing phagocytosis in CNS-injured patients on the 3rd and 9th day were also significantly lower. A statistically significantly lower enzymatic activity of granulocytes was observed on the 9th day in CNS-injured patients.*

Conclusions: *A significant deficiency of the phagocytic arm was observed during a period of time which is critical for "2nd-hit injuries". That deficiency played a role in the high rate of infections and multiple organ failure in numerous patients described in the literature.*

Key words: *severe and moderate CNS injury, immunomonitoring, flow cytometry, phagocytosis, oxidative burst, infection.*

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Introduction

Severe trauma is associated with an early over-activation of an innate immune response followed by immunosuppression [1, 2]. It is reported that the patients with a severe central nervous system (CNS) injury also showed a profound acute phase systemic response [3]. The next phase of this response is CNS injury-induced immunodepression (CIDS) [4]. Up until now we do not know the exact timeline of these two phases. Over a period of months following the injury the immune response returns to normal [5]. The process of pathogen elimination by granulocytes constitutes a vital, primary event in the host defense against bacterial or fungal infections. It is known that the development of infection depends on two key factors: decreased host defense, and colonization by pathogenic micro-organisms. The peak incidence of the infections is observed at 5-11 days after brain trauma [6]. This might suggest a decreased host defense in that period of time. The decreased host defense is also an essential component to trigger the "second-hit" mechanism. Despite the observed immunological deficiency, the period of time between the 3rd and 9th days is critical as a window of opportunity for definitive surgery [7].

The aim of this study is to characterize the changing functional behavior of the granulocytes after the 3rd day until the 9th day post severe and moderate CNS injury, using flow cytometric analysis of phagocytosis and "oxidative burst" activities.

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Material and methods

Patient population

Patients admitted to the Department of Trauma Surgery at the Medical University of Gdańsk following accidental trauma, were included in the prospective clinical study, which started on 1 November 2005 and lasted until 1 May 2006.

The study group consisted of 9 brain injury patients (7 men and 2 women) aged 18 to 79 years who sustained a moderate or severe CNS injury (GCS not higher than 11 pts), and who were admitted to the Department of Trauma Surgery or Intensive Care Unit (ICU) at the Medical University of Gdańsk hospital. The injuries were caused by mechanical trauma in all cases. Patients with coexisting trauma were excluded.

All patients were clinically evaluated on admission within half an hour since the accident and all scored 11 points or less on the GCS (moderate or severe brain damage). Seven patients were artificially ventilated, five patients had an operation on the day of admission (craniotomy, hematoma evacuation), all patients received parenteral fluids. The nutritional condition of all patients was appropriate and has been maintained by administration of adequate parenteral solutions. Basic clinical data of included patients are summarized in Table 1.

The control group consisted of 11 qualified healthy subjects (3 females, 8 males) aged 17 to 82 years with no history of craniocerebral trauma and unknown immunodeficiencies.

The project was approved by the Local Ethics Committee of the Medical University of Gdańsk (MUG).

Methods

We have measured four parameters: the function of granulocytes – the intensity of oxidative reactions; number of bacteria per cell; phagocytic activity – number of granulocytes that show phagocytosis; and the number of granulocytes that have produced reactive oxygen metabolites.

The intensity of oxidative reaction and the number of granulocytes that have produced reactive oxygen metabolites were determined by the Bursttest™ (Orpagen) and then measured by means of flow cytometry. The number of bacteria per cell and the percentage of granulocytes that show phagocytosis were measured by the Phagotest™ (Orpagen) and also by means of flow cytometry.

To evaluate the phagocytic activity of the granulocytes in the peripheral blood sample we have incubated and heparinized this blood sample with the labeled bacteria (*Escherichia coli*) at 37°C. At the same time the control sample has remained on ice at 0°C. The reaction in the first sample was stopped by both placing it on ice and adding a quenching solution. The quenching solution allows us to differentiate the phagocytizing and non-phagocytizing cells. Then after two washing steps we have removed the remaining erythrocytes. Afterwards, the DNA staining solution was added to exclude artifacts. At the end, the acquisition gate was set at the proper fluorescence histogram to exclude the bacteria and platelet aggregates.

To assess the oxygen-dependent processes (the percentage of cells that have produced reactive oxygen metabolites and their mean fluorescence activity) we have tested the patient's and control's group blood samples by means of the Bursttest™. The heparinized blood samples were incubated with the following stimuli: *E. coli* bacteria, PMA and fMLP. The fourth sample without stimulus served as a control. As a result of stimulation, granulocytes produced reactive oxygen metabolites, which was observed by oxidation of dihydrorhodamine DHR 123. The process was stopped by addition of the lysing solution. Then, the DNA staining solution was added. The number (percentage) of cells producing reactive oxygen metabolites as well as their mean enzymatic activity was assessed by means of the flow cytometry.

Table 1. Age, clinical features, and course of stay in hospital of 11 patients with moderate or severe CNS injury

Patients (initials)	Age (years)	GCS pts	Infection (day 3)	Infection (day 6)	Infection (day 9)	Neurosurgical procedure (type)	ICU days	GCS (outcome)
MDZ (m)	27	11	Staph. sp	NIP	NIP	CHE	5	15
HO (m)	61	11	Rt lobar pneumo (LP)	Rt LP	Rt LP	none	0	15
HM (m)	41	10	Staph. sp	Staph sp	Staph sp	none	0	14
SC (f)	79	9	NIP	NIP	NIP	CHE	over 9	14
FP (m)	63	11	NIP	NIP	NIP	none	7	15
MR (m)	23	10	NIP	Staph sp	NIP	CHE	9	14
HS (m)	36	8	NIP	Acinet sp	Rt LP	CHE	over 9	13
AK (f)	18	5	NIP	Acinet sp	Acinet sp	none	over 9	14
HR (m)	39	11	NIP	Acinet sp	Acinet sp	CHE	over 9	14

NIP – no infection proven; CHE – craniotomy, hematoma evacuation; f – female, m – male

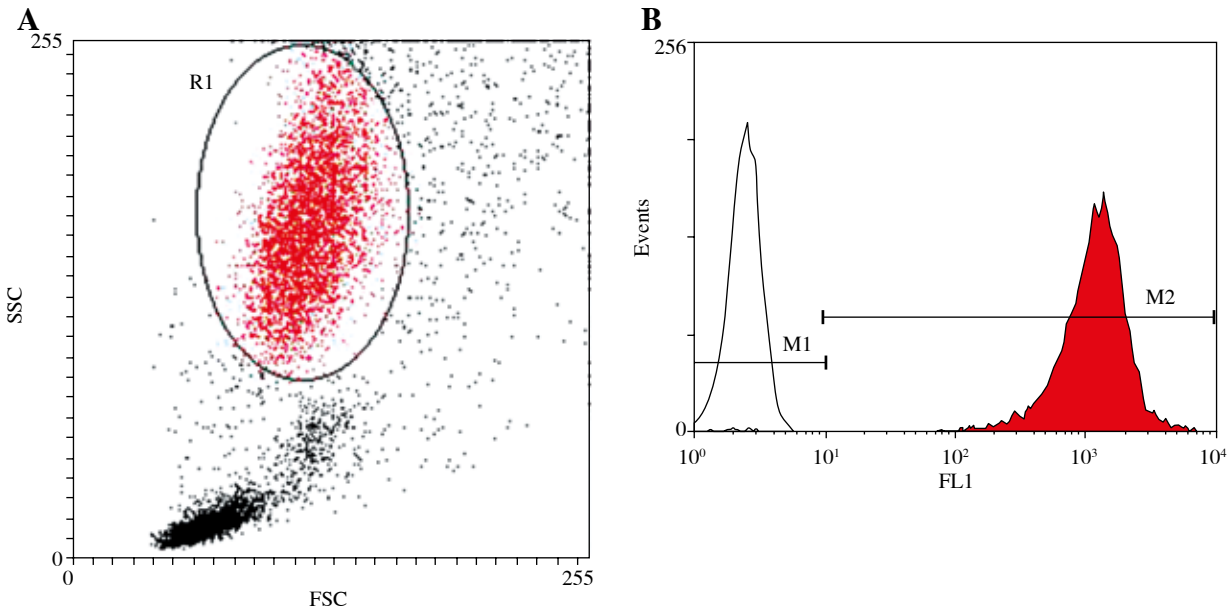

Fig. 1. A) Typical dot plots FSC/SSC histograms of the phagocytosis test (incubation time of 10 min at 37°C). Gate set on granulocytes; **B)** Typical dot plots FL1 histograms of the phagocytosis test (incubation time of 10 min at 37°C). Gate set on granulocytes

Fluorescence data from both tests were obtained using the FACScan (Becton Dickinson, USA) cytometer. The minimum number of 10,000-15,000 leukocytes per sample was collected.

The percentage of cells having performed phagocytosis were analyzed as well as their mean fluorescence intensity (number of ingested bacteria). For that purpose the relevant leukocyte cluster was gated in the software program in the scatter diagram (lin FSC vs. lin SSC) and its green fluorescence histogram (FL1) was analyzed (see Fig. 1A, B).

The Friedman ANOVA nonparametric test measures analysis of variance by ranks. This test was performed to evaluate the differences between compared groups. The *U* Mann-Whitney test was used in the analysis of data.

Results

A statistically significantly lower individual cellular phagocytic activity (number of bacteria per cell) was observed in CNS-injured patients on the 3rd, 6th and 9th day when compared to the control with the strongest decrease seen on the third day post-injury (Fig. 2).

The percentage of granulocytes showing phagocytosis in CNS-injured patients on the 3rd and 9th day was significantly lower when compared to the controls (Fig. 3).

A statistically significantly lower enzymatic activity of granulocytes associated with reactive oxygen metabolites production was observed on the 3rd, 6th and 9th day in CNS-injured patients when compared to the controls.

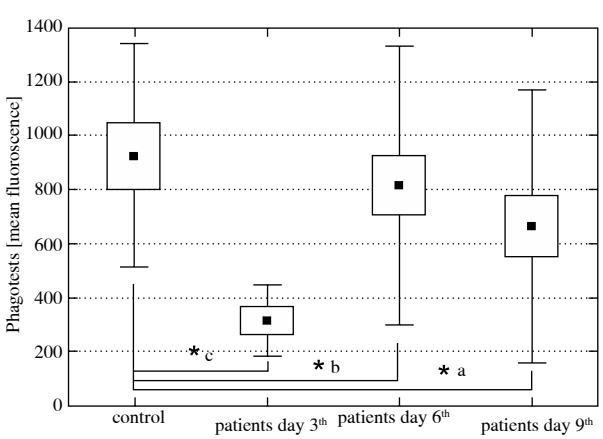

Fig. 2. Phagocytic activity (number of bacteria per cell) in CNS-injured patients on the 3rd, 6th and 9th day

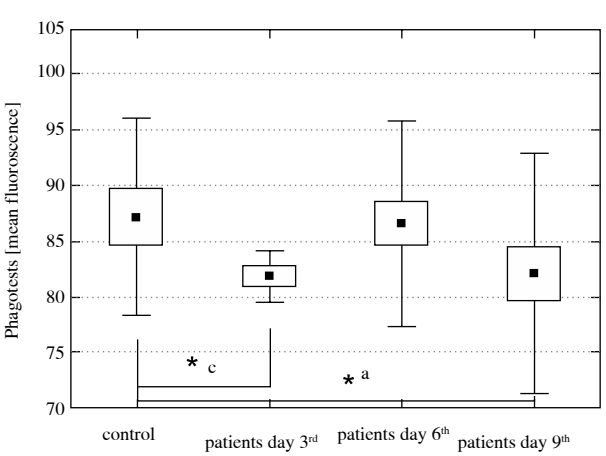

Fig. 3. The percentage of granulocytes showing phagocytosis in CNS-injured patients on the 3rd, 6th and 9th day

However, for this test, the results mostly deviating from those observed for healthy controls occurred on the last (9th) day of observation (Fig. 4).

When comparing patients with and without infection there was no significant difference observed except a statistically significantly lower percentage of cells that have produced reactive oxygen metabolites on the 6th day in a group of infected patients (not shown).

Discussion

Central nervous system injury activates an inflammatory response with an intensity depending on the severity of the primary trauma. The primary role of cerebral inflammation post-injury is not a defense against microorganism infection but a reaction to tissue injury, which consequently may have a profound effect on systemic immune responses [9]. Soon after trauma, pro-inflammatory cytokines and complement components lead to recruitment of peripheral inflammatory cells (granulocytes) [10]. Central nervous system injury has been reported to induce various immunological abnormalities, including peripheral granulocytes hyperactivation under the influence of proinflammatory mediators in the first 12 h post-trauma [11-13]. Thereafter, they enter a post-excitatory phase wherein they become reduced in number and functionally deficient, placing the patient at a high risk of the "second-hit" injury [11, 12]. Nevertheless, after various kinds of CNS injury, the balance between the brain-mediated pro- and anti-inflammatory response is remarkably shifted to a predominance of anti-inflammatory cytokines, producing immunodepression with an increased risk of infection and the "second hit" injuries [14]. A rapid interleukin 10 (IL-10) release after sympathetic activation (in a rat model) may represent a common pathway for imwmunodepression induced by CNS injury [15].

However, the concept of a predominance of anti-inflammatory cytokines is not fully proven, yet in CNS-injured patients, cytokines such as IL-6, IL-8 are released at much higher levels than anti-inflammatory cytokines [16].

The process of pathogen elimination by granulocytes constitutes an essential, primary event in the host defense against bacterial or fungal infections. This process can be divided into several major stages: chemotaxis (migration of phagocytes to inflammatory sites), attachment of particles to the cell surface of phagocytes, ingestion (phagocytosis) and intracellular killing by oxygen-dependent (oxidative burst) and oxygen-independent mechanisms [17, 18].

The activity of the phagocytic arm of the immune system early after CNS injury seems to be crucial for the appearance of infection and second-hit complications [19-22].

The oxidative burst in circulating granulocytes depends on the underlying insult. The measurement of the degree of the oxidative burst in granulocytes is a predictive factor used to distinguish between survivors and non-survivors.

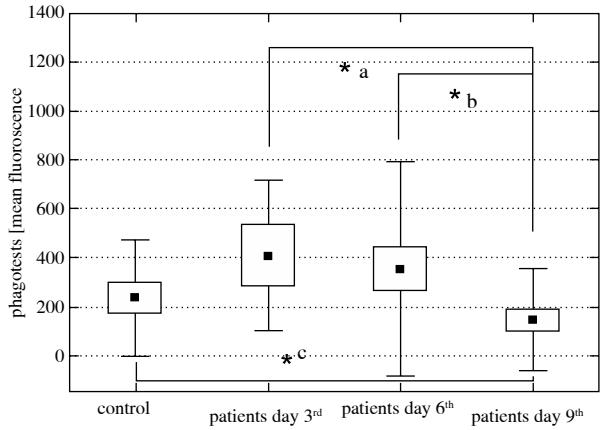

Fig. 4. The enzymatic activity of granulocytes associated with reactive oxidative metabolites production observed on the 3rd, 6th and 9th day in CNS-injured patients

This measurement is completed on the 3rd day after admission to the ICU [23]. There are studies that try to establish an anti-inflammatory marker (IL-10 and IL-13) but the phagocytic arm seems to be more relevant to the assessment of the clinical status of the patients [24].

The severe brain injury used to be reported as a factor that precipitate significant deficiencies in the immune system within 72 h of the occurrence of the injury (among others: decrease in superoxide generation, oxidative burst) but immune system deficiencies measured months after the occurrence were found normal [5, 25, 26].

In this study, during the observation period, between the 3rd and 9th day after CNS injury most of the measured parameters were statistically significantly decreased. According to that, the recovery of the phagocytic arm would probably happen after the 9th day post CNS injury.

The change in the functional behavior of the granulocytes could be an independent risk factor that could trigger the "second-hit" injury and infection [27]. During the phases of hyperinflammation and immunosuppression, severely injured patients are highly susceptible to sustain so-called second-hit insults. Therefore, the ideal timing for the definitive surgery is somewhere between these two immunological phases. The empirically assigned time-window is about 5-10 days after trauma [7].

It seems that CNS-injured patients are at risk of further immunological disturbances and at a high risk of infection during the observed period of time. The decision-making process should be individualized in accordance with the clinical status of the patient, and all more exacerbating procedures should be performed at a later date.

It is well known that a decrease in the oxidative burst of phagocytic cells is associated with bacterial or fungal infections [28]. The higher incidence of an early-onset pneumonia was found in patients with neurotrauma [29, 30].

However, infections are not only specific to CNS-injured patients, but common in all patients treated at the ICU. Especially, in patients with prolonged intubation since such intubation usually increases the risk of lung infection. Infections that start on the 3rd day after injury are presumed to be nosocomial [31]. The clinical and immunological parameters related to pneumonia were taken under revision and compared, showing that the levels of chosen cytokines (IL-6 and IL-10) could not confirm the diagnosis of pneumonia earlier than the clinical parameters [32].

In our study, the infection incidence was the highest (78% of the patients) on the 6th day after the CNS trauma. At that time, we noticed in the infection group, a statistically significantly lower percentage of cells that have produced reactive oxygen metabolites compared to the non-infection group.

We have to take into consideration that usually, patients with a relatively low GCS are most likely to be ventilated and sedated, as a result of which they would have reduced mobility, receive IV fluids, and have altered calorific intake. All of the above constitute multiple cofactors that might influence the results and cannot be excluded.

Several important issues should be addressed in the future studies. Firstly, the mechanisms responsible for the immunodepression in CNS-injured patients require further exploration. Secondly, future studies should be conducted on larger more homogeneous groups of carefully selected patients. It is important to select appropriate patients for immunotherapy. Finally, the specific cytokines or growth factors that have the greatest therapeutic impact should be identified and the patient populations to derive the greatest benefit should be defined [25].

Conclusions

1. On the 3rd, 6th and 9th day, 4 parameters are measured. Each time these are analyzed, at least 1 out of 4 parameters of the phagocytic arm is significantly lower when comparing to the control group.
2. In a vulnerable phase when the patient's immunological defense is hypoactive and uncontrolled, the techniques and timing used in trauma surgery should be appropriately adjusted to limit the systemic response. An individually adjusted surgical approach and immune monitoring are important in order to avoid the "second-hit" injuries, mainly infectious complications.

The authors declare no conflict of interest.

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