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Acta Medica Marisiensis 2016;62(3):368-372 DOI: 10.1515/amma-2015-0099

CASE REPORT

Viability Changes in Leucocytes in a Critical Trauma Patient: Monocyte, Lymphocyte, Granulocyte Response to the Acute Phase: Case Report

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Trauma affects the activity of the innate immune system. The objective of this case report is to present the case that prompted us to analyse all the peripheral white blood cell lines. A 19 year old male patient was admitted to the Intensive Care Clinic with severe head trauma. The final diagnosis was set to be severe cerebral trauma with subarachnoid hemorrhage, right frontal and temporal cerebral contusions, diffuse cerebral edema, left parietal and temporal fracture, sphenoid hemosinus and right sided lung contusions. **Material and Method**: Whole blood was immediatly analyzed by flow cytometry for leukocytes. Apoptosis was detected with Annexin V, necrotic cells were stained with propidium iodide. Samples were drawn three consecutive days. **Results**: Lymphocytes, monocytes and granulocytes all showed marked increase in viability and decrease in necrosis during the biological monitoring in correlation with a positive clinical outcome. The most important changes were noted in the monocyte population. **Discussion**: Although we started out monitoring neutrophil viability and death, this particular case prompted us not to overlook other leucocyte populations. **Conclusion**: The apparent positive relationship between this patient's positive clinical outcome and cellular viability and death changes is promising but they warrant further study.

Keywords: trauma, monocytes, lymphocytes, granulocytes, viability

Received: 07 August 2015/ Accepted: 03 September 2015

Introduction

Trauma in its acute phase affects the activity of the innate immune system, causing the depression of cell-mediated immunity and is one of the main causes of systemic inflammatory response syndrome [1].

Peripheral granulocytes consist of three major cell types, neutrophils, basophiles and eosinophils, each with its own role in immune activity. Due to the much larger neutrophil population, granulocyte counts are often referred to as neutrophil counts. Monocytes are the largest of leucocytes with multiple roles in the immune response and main role in the innate immune system. Lymphocytes are part of the leucocyte group, consisting of three main types: T cells, B cells and natural killer cells [2,3].

Apoptosis is programmed cell death, which occurs during the normal life cycle of each cell. Necrosis is the pathological cell death, which occurs to certain internal or external noxious stimuli [3,4].

Objective

The objective was to present a case, part of a larger ongoing study focused on neutrophil viability in trauma patients, which prompted us to analyse other leukocyte types as well.

Material and Method

We enrolled critical trauma patients in a pilot study in order to determine cellular viability and death modifications with the approval of the Târgu Mureş Emergency Clinical County Hospital's Ethics Committee and consent of a relative. Our initial goal was to study neutrophil viability and death changes, but during the analysis of our partial results we encountered several modifications in other cell lines as well, so we decided to expand the goal of our study. The decision to present this case is the result of set observation.

An amount of 2 ml of whole blood was sampled using K3-EDTA containing tubes and analyzed in the Târgu Mureş Emergency Clinical County Hospital's Central Laboratory using flow cytometry.

Staining for cell surface immunofluorescence: Sample preparation and analysis were performed within a maximum of 5 minutes of venipuncture. This time frame was set to exclude ex vivo cell death. For flow cytometric immunophenotyping 100 µl of whole blood were incubated for 15 minutes at room temperature in the dark with saturating concentrations of fluorochrome-conjugated Annexin V-FITC from Becton Dickinson (Apoptosis Detection Kit II® BD Biosciences) and Propidium Iodide (BD Pharmingen™). After incubation, the blood samples were treated for 10 minutes at room temperature in the dark, with 2 ml erythrocyte lysis solution from BD (1 x FACS

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lysing solution), followed by two washes (5 minutes each, 500 g) with 2 ml of BD Cell wash solution.

Data acquisition and analysis: with the use of CaliBRITE beads and FACSComp software (BD Biosciences, San Jose, California, USA) the fluorescence compensation and the photomultiplier tube voltages were set and the instrument sensitivity was tested. Cell Quest software on a BD FACS Calibur™ flow cytometer (BD Biosciences, San Jose, California, USA) was used for acquisition and analysis. The cellular light-scatter signals and two fluorescence signals were analysed in list mode at channel resolution of 1024, with forward scatter as a trigger parameter. Using a forward scatter threshold, all events are acquired with at least $100*10^3$ peripheral blood mononuclear cells per sample by setting an acquisition gate on these cells in forward scatter/side scatter dot plot. Data are displayed as two color dot plots and histogram statistics to determine cell surface markers, as specific fluorescence intensity.

Samples were drawn in the first 24 hours after admission to the intensive care, after 48 hours and after 72 hours. This protocol was designed this way to ensure detection in real time of different types of cell deaths and changes in their viability. Intermediate forms consist of both apoptotic and necrotic cell forms.

Results

We present the case of a 19 year old male patient, admitted to the Intensive Care Clinic with severe head trauma after an accident through crushing with a heavy object. He was found unconscious by the Emergency Department's medical team on site, with a Glasgow Coma Score of 4 points. Tracheal intubation and mechanical ventilation was performed on site. After computer tomographic examination, the final diagnosis was set to be severe cerebral trauma with subarachnoid hemorrhage, right frontal and temporal cerebral contusions diffuse cerebral edema, left parietal and temporal fracture, sphenoid hemosinus and right sided lung contusions.

Upon admission to the Intensive Care Clinic, the patient was intubated and mechanically ventilated in bi-level positive airway pressure mode, with a fraction of inspired oxygen of 60%, and a peripheral oxygen saturation of 100%, hemodynamically stable with a blood pressure of 120/90 mmHg, heart rate of 71 beats/minute. His neurological status could not be assessed due to residual sedation with propofol from the emergency department. Blood gas values showed a slight hyperventilation with an arterial partial pressure of oxygen of 194 mmHg, for which the ventilatory frequency was reduced. Electrolytes were in the normal range, but a moderate anemia was present with hemoglobin levels at 11 g/dl and hematocrit at 35%. He received prophylactic antibiotic treatment with ceftriaxone, H_2 receptor blockers for prevention of stress ulcers, furosemide and mannitol to enhance diuresis and help reduce cerebral edema. He was weaned from mechanical ventilation 4 hours after admission, with a spontaneous respiratory rate of 18 breaths/minute using 100% oxygen via face mask, a Glasgow Coma Score of 9 points and a FOUR score of 12 points, hemodynamically stable.

Leucocyte viability and death modifications were recorded in three consecutive days accordingly. (Table I, Figure 1-2)

The first blood sampling for flow cytometry was performed 12 hours after admission, considered to be the first day of the acute phase of trauma. The complete blood count showed a number of total white blood cells in the normal range, but lypmhopenia, with $0.6*10^3/\mu l$ was present. Monocytes were in the normal range. Neutrophylia was present with $7.3*10^3/\mu l$.

The patient's clinical status had improved, on the second day he did not need further mechanical ventilation, he was hemodynamically stable but his neurological status was stagnating. His blood gas values and electrolytes were in the normal range, and the anemia showed spontaneous improvement.

The second blood sample for flow cytometric analysis was drawn at the same hour as in the first day, thus allowing a period of twenty four hours between samplings. The number of total white blood cells was still in the normal range and lymphopenia was persisting at $0.9*10^3/\mu l$. Lymphocytes showed an increase in viability with no apoptotic forms present. The most remarkable change was noted in the monocyte population. These increased their viability and monocyte necrosis decreased. Neutrophylia had increased to $9.6*10^3/\mu l$ with an increase in neutrophil viability but no apoptotic cell forms were present. Neutrophil necrosis decreased as well.

On the third the patient's clinical status was deemed not critical and he was transferred out of the Intensive Care Clinic. He was stable from a hemodynamic and respiratory point of view, but still no further improvement in neurological status.

Blood was sampled on the third day at the same time as in the first and second day, thus allowing a period of fourty eight respectively twenty four hours between samplings. The decision to perform the third sampling, although the patient's status was no longer considered critical, was made due to the observation of rapid cellular viability and death modifications noted on the first two days. By the third day

Table I. Modifications of leukocyte viability in the first 24 hours after admission to the intensive care, after 48 hours and 72 hours, respectively

Day	Leucocytes	Viable	Apoptotic	Necrotic
Day 1	Lymphocytes	3.3%	0	96.5%
	Monocytes	4.2%	0.7%	89.4%
	Neutrophils	0.2%	0	98.9%
Day 2	Lymphocytes	8.2%	0	91.6%
	Monocytes	29.8%	0.5%	68.2%
	Neutrophils	1.2%	0	97.3%
Day 3	Lymphocytes	10.8%	0.05%	88.7%
	Monocytes	76.9%	3.1%	19.1%
	Neutrophils	7.4%	0.3%	88.5%

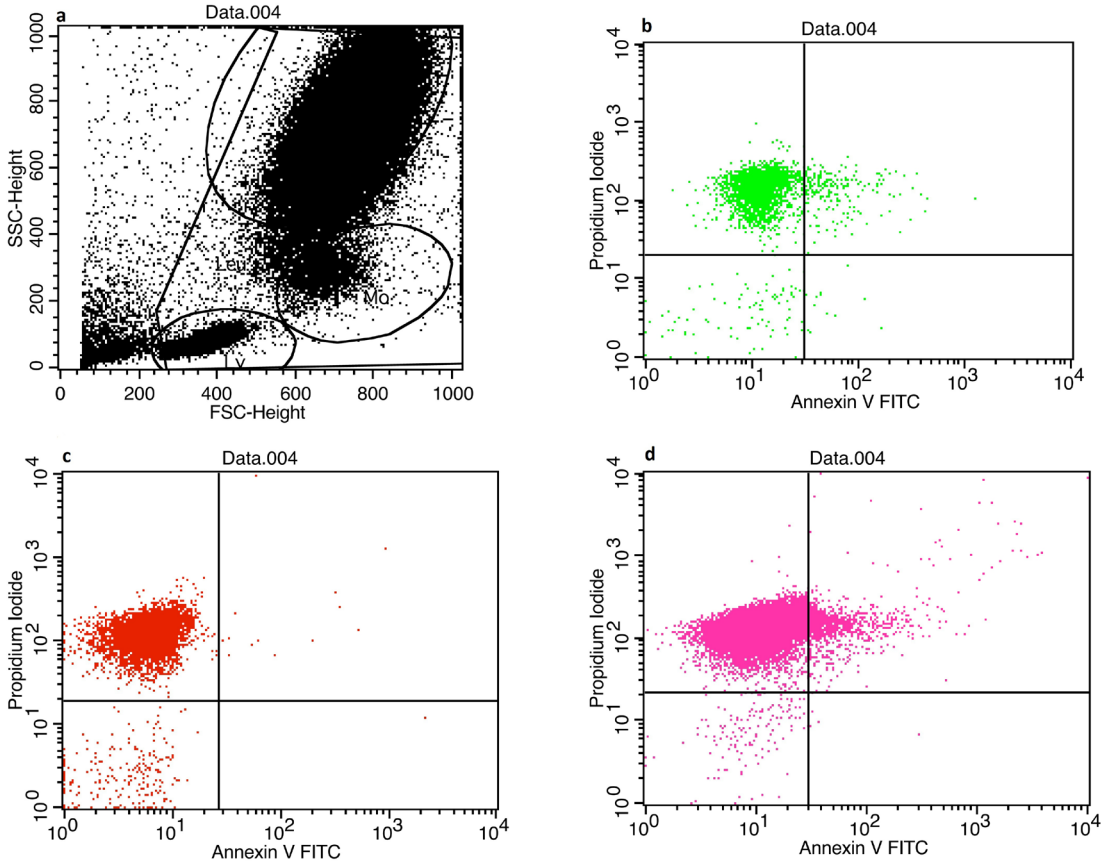

Fig. 1. Results displayed by the flow cytometer on the first day of biological monitoring (a: Forward scatter FSC / Side scatter SSC dot plot for white blood cell gating, and two color dot plots to determine fluorescence intensity of the apoptosis/necrosis markers for b: Monocytes, c: Lymphocytes, d: Neutrophiles).

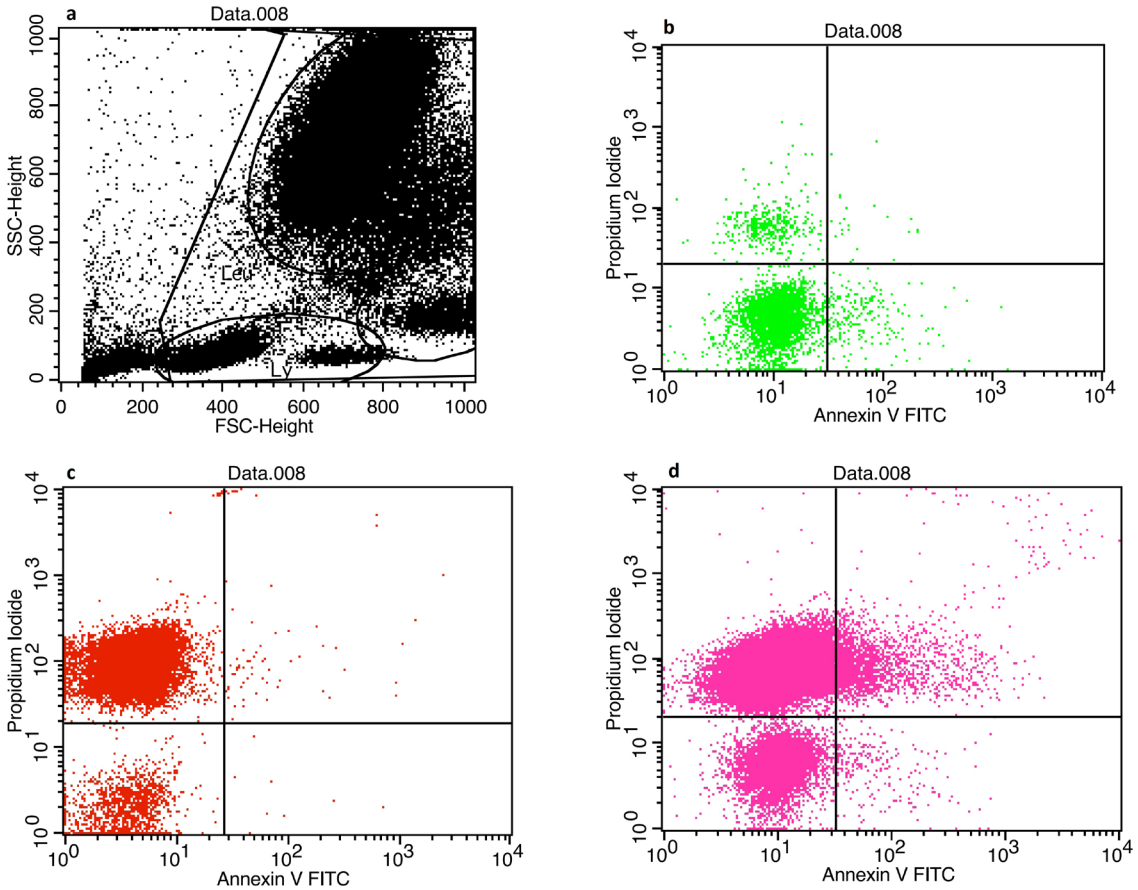

Fig. 2. Results displayed by the flow cytometer on the third day of biological monitorig (a: FSC/SSC dot plot for white blood cell gating, and two color dot plots to determine fluorescence intensity of the apoptosis/necrosis markers for b: Monocytes, c: Lymphocytes, d: Neutrophiles).

lymphopenia and neutrophylia resolved spontaneously. Lymphocyte viability increased remarkably and apoptotic cells were present. A rapid decrease of necrotic cells was noted. Monocytes showed the most rapid increase in viability and apoptotic forms. Neutrophil viability increased as well and apoptotic forms were present.

Computer tomography examination was repeated, which showed the resolution of the cerebral edema but described the presence of diffuse axonal brain injury, explaining the stagnation in the neurological status.

The patient was transferred out of the Târgu Mureş Emergency Clinical County Hospital on the seventh day after admission to another medical facility to ensure adequate recovery. Upon transfer the patient had a Glasgow Coma Score of 13 points with a FOUR score of 14 points and a positive clinical and biological outcome.

Discussion

Although we started out with taking in to consideration only neutrophil viability and death changes in an ongoing study, this particular case prompted us no to overlook other peripheral white blood cell populations. Lymphocyte depletion in the circulating blood is a normal phenomenon during the acute phase of trauma, due to their role in the immune reactions. Delogu et al. designed a study researching the role of lymphocyte apoptosis in response to postoperative trauma using the 7-amino-actinomycin D method, as well as for Fas and Fas ligand, interleukin 1–converting enzyme p20/caspase-1, Bcl-2, and p35 expression. They concluded that in the early postoperative period, surgical trauma induces an intracellular perturbation on peripheral lymphocytes, resulting in both up-regulation of death-signaling factors and down-regulation of survival-signaling factors [5].

In our case report this phenomenon was observed by the lymphopenia described in the early stages and by the high levels of lymphocyte necrosis in the first day, then the rapid decline in necrosis and increase in viability by the third day of monitoring.

The greatest values of apoptosis and parallel increase in viability were noted in the monocyte population. Efstathopoulos et al. conducted an experimental study using white New Zealand rabbits subjected to multiple traumas. Their results revealed the existence of early apoptosis of blood monocytes, a phenomenon accompanied by apoptosis of blood lymphocytes [6].

Sawai et al. stated that the use of dual staining with Annexin V and propidium iodide did not discriminate between primary necrotic and post-apoptotic secondary necrotic cells. Primary necrotic cells can show Annexin V positive and propidium iodide negative staining before they become positive with propidium iodide staining. They introduced necrostatin-1 to differentiate primary and post-apoptotic secondary necrosis in human monocytic leukemia cell lines and concluded that caution would be needed to regard Annexin V positive and propidium iodide negative staining as only apoptotic cell death [7].

Thus the rapid increase in monocyte apoptosis on the third day should be interpreted with caution. However, the increase in Annexin V positive monocytes, may it be apoptotic cell forms or early propidium iodide negative necrotic cell forms, was accompanied by a much larger increase in viability and decrease in propidium iodide positive cell forms.

Spolarics et al. analysed the effects of major trauma on the cytokine-producing activity of monocytes and CD4+ T lymphocytes in a group of 12 patients. They concluded that major trauma results in an early and marked decrease in monocyte cytokine-producing activity and the degree of alterations in monocyte and T-cell responses on day 2 post injury correlates with the development of adverse clinical outcomes and the subsequent duration of the inflammatory response [8].

In our case report both the lymphocyte and monocyte population showed a rapid increase in viability by the third day post-injury with substantial decline in necrotic cells in a possible correlation with the clinical outcome.

The particularity of our case consisted in a young trauma patient's recovery to a positive clinical outcome that was predicted by rapid changes in all white blood cell population's viability.

The most important limitation of this study is related to the sampling protocol. Whole blood samples can not be stored neither at room temperature conditions nor below these temperatures due to false readings of ex vivo necrosis. The cost related issues, expensive detection kits, staining solutions and adjacent laboratory equipment are also notable obstacles.

Conclusions

The apparent positive relationship between this patient's clinical outcome and cellular viability and death modifications is promising. But however encouraging these results may seem, they warrant further study, revising the initial design and including the analysis of the lymphocyte and monocyte population adjacent to the initial neutrophil cell line. This study is ongoing to fulfill this purpose.

Acknowledgement

This paper is supported by the Sectoral Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/159/1.5/S/133377/.

Conflicts of interest

The authors report no conflicts of interest.

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