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Changes in the serum levels of clusterin in children with sepsis

Stężenie kluśmy u dzieci z posoczną

Žurek Jiří *, Fedora Michal

University Children’s Hospital, Department of Anesthesia and Intensive Care, Czech Republic

A R T I C L E   I N F O

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• posocznica
• sepsis
• śmiertelność

Abstract

Aim: The first aim of this study was to determine levels of clusterin in pediatric patients with systemic inflammatory response syndrome or septic state, comparing these levels with a healthy population. The second objective was to compare levels of clusterin within individual septic conditions, influence of levels of these proteins on mortality. Background: Clusterin is a highly conserved protein which is expressed at increased levels by many cell types in response to a broad variety of stress conditions. Methods: Fifty-seven children aged 0–19 years (30 boys and 27 girls) hospitalized from June 2009 to March 2011, with expected or proven SIRS and septic condition. The degree of severity was evaluated according PELOD Score. Blood tests to determine levels of clusterin were taken throughout the patient meets the criteria of SIRS or sepsis. Control group to determine the serum levels of clusterin has been taken from patients undergoing elective surgery. Results: We found lower concentrations of clusterin in patients with SIRS or septic state, than in the control group. Clusterin cut-off for first day – D1 was 91.04 μg/ml; AUC 0.900; p-value <0.001; for third day – D3 was cut-off 86.73 μg/ml; AUC 0.849; p-value <0.001; for fifth day – D5 cut-off was 105.26 μg/ml; AUC 0.755; p-value <0.001. Effect of clusterin levels on mortality in the dynamics was recorded significant for 5 days in groups non-survivors/survivors, p-value 0.004. Conclusion: We have demonstrated a decrease clusterin levels in pediatric patients with septic state, and its effect on mortality.

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Introduction

Sepsis is the most common cause of death in infants and children in the world [1]. The incidence of pediatric sepsis has been recently estimated to be 0.56/1000 children with the highest incidence in infancy at 5.6/1000; overall mortality is 10.6% [2]. Sepsis is a complex, highly variable, multiple system, clinical process induced by pathogens that causes a deleterious, systemic host response [3]. Organ dysfunction is the final tissue sequelae in response to severe sepsis and the ultimate determinant of survival. It has been amply demonstrated that septic hosts who have progressive multiple organ failure are much more likely to succumb to severe sepsis than those who develop a single or no organ dysfunction in response to sepsis [4, 5].

The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature
Clusterin

The clusterin protein was first discovered more than 25 years ago in rat testis fluid because of its ability to facilitate clustering of a variety of cell types in culture [9]. It is a 75–80-kDa disulfide-linked heterodimeric protein with about 30% of the mass of the molecule comprised of N-linked carbohydrate which is branched, complex, and rich in sialic acid [10].

Clusterin is an enigmatic molecule, implicated in diverse biological processes, and has additionally been associated with opposing functions in regard to apoptosis [11]. Possible protective mechanisms are considered by blockage of the terminal complement cascade (C5b-9) or by protecting against oxidative stress [12, 13]. More recent studies show that clusterin may be a secreted chaperone molecule, inhibiting stress-induced precipitation of a very broad range of structurally divergent protein substrates and binding irreversibly via an ATP-independent mechanism to stressed proteins to form solubilized high molecular weight complexes [14, 15].

The first aim of this study was to determine levels of clusterin in pediatric patients with systemic inflammatory response syndrome (SIRS) or septic state, comparing these levels with a healthy population. The second objective was to compare levels of clusterin within individual septic conditions, and influence of levels of this protein on mortality.

Materials and methods

Prospective observational study occurred during the period from June 2009 to March 2011. The study protocol and informed consent approach were approved by the Ethics committee of the University Hospital, Brno. Parents provided informed consent for their children to participate in this trial. Data were collected and analyzed from fifty-seven consecutive patients with SIRS or septic state who were admitted to the Department of Anesthesia and Intensive Care of the University Children’s Hospital Brno, Czech Republic. The most common sources of infection that led to sepsis were the lungs – bacterial and viral infections, and central nervous system – bacterial infections of the brain. Infections, sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome (MODS) were defined according to commonly used criteria – by International pediatric sepsis consensus conference. The criteria for adult SIRS were modified for pediatric use. Age-specific norms of vital signs and laboratory data were incorporated into the definitions of SIRS. Sepsis was defined as SIRS associated with suspected or proven infection [16]. Patients were categorized into five groups according to their clinical data and to the described definitions: (a) SIRS, (b) sepsis, (c) severe sepsis, (d) septic shock, (e) MODS. In these groups, we compared the difference in the levels of clusterin. The samples from 70 children undergoing elective surgery were used as controls (strabismus surgery, umbilical and inguinal hernia repair), i.e. samples from patients without signs of infection. Blood samples were collected before surgery.

Patient data were recorded at the time of diagnosis of SIRS or sepsis, severe sepsis, septic shock and multiple organ dysfunction syndrome and consisted of age, sex, Pediatric Logistic Organ Dysfunction (PELOD), length of hospitalization [17]. PELOD score is a tool which is used to characterize severity of organ dysfunction in critically ill child. Score which is given to each organ will increase according the severity of organ dysfunction so PELOD score can be used to predict severity of organ dysfunction. The PELOD scoring system consists of physical and laboratory variables representing 6 organs, namely nervous, cardiovascular, renal, respiratory, hematologic, and hepatic system [17]. Value of PELOD 12 was taken as the average of the whole set.

Specimens for the diagnosis of infection were obtained as early as possible.

Complete medical history and clinical examination, laboratory parameters, and disease-specific examinations were evaluated.

Blood samples were obtained from a central venous catheter during the first 12 h after the diagnosis SIRS or septic state, or at the beginning of surgery in the control group. For the evaluation of clusterin dynamics, samples were collected at all times when patients meet the criteria SIRS or septic state.

Samples were allowed to clot at room temperature and were centrifuged at 3000 rpm for 10 min. Separated serum was stored at –80 °C until further analysis. Samples were
measured by enzyme immunoassay for the quantitative measurement (BioVendor, Laboratorní medicína a.s., Brno, Czech Republic). Samples were incubated in microplate wells pre-coated with monoclonal anti-human clusterin antibody. After 60 min incubation and washing, biotin labeled second monoclonal anti-human clusterin antibody was added and incubated with captured clusterin for 60 min. After another washing, streptavidin-HRP conjugate was added. After 30 min incubation and the last washing step, the remaining conjugate was allowed to react with the substrate solution (TMB). The reaction was stopped by addition of acidic solution and absorbance of the resulting yellow product was measured. The absorbance is proportional to the concentration of clusterin.

The laboratory technicians performing the assays were completely blinded to the clinical information.

**Statistical analysis**

Baseline levels of analyzed protein and demographic characteristics were summarized using descriptive statistics (N, mean, standard deviation, median, minimum, maximum). The analysis was performed on logarithmically transformed data to achieve an approximately normal distribution of the evaluated data. The dynamics (kinetics) of the protein levels during the period of SIRS or septic state were analyzed using the analysis of variance (ANOVA). Correlation of values in the patients was performed using a symmetric covariance matrix.

**Table I - Descriptive characteristics of patients, data are presented as median (min-max)**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age (month)</th>
<th>LOS PICU</th>
<th>PELOD score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivors</td>
<td>51</td>
<td>28/23</td>
<td>8 (1-219)</td>
<td>7.5 (1-19)</td>
</tr>
<tr>
<td>Non-survivors</td>
<td>6</td>
<td>2/4</td>
<td>90.5 (3-186)</td>
<td>7.0 (1-41)</td>
</tr>
</tbody>
</table>

LOS PICU – length of stay in the pediatric intensive care unit, PELOD score – pediatric logistic organ dysfunction.

![Fig. 2](image_url)  
**Fig. 2** – Clusterin levels (µg/ml) in the control group and in the group of patients with systemic inflammatory response syndrome or sepsis, severe sepsis, septic shock or multiple organ dysfunction syndrome during a 5-days. AUC – area under curve; SE – sensitivity; SP – specificity; PV+ – positive predictive value; PV− – negative predictive value.
matrix (the type of compound symmetry). Significance of difference in dynamics between analyzed groups is indicated by p-value of group and time interaction effect. ROC analysis was performed to determine the discriminative characteristics of the protein values. ROC curves are shown together with the respective diagnostic characteristics (sensitivity, specificity, positive and negative predictive values).

Results

The study included fifty-seven children patients, whose characteristics are presented in Table I.

Lowered values of clusterin suggest altered clinical condition (systemic inflammation or sepsis). Lowest values can be observed in the most severe clinical condition; SIRS first day D1 – median (min–max) 3.8 (1.1–274.0), sepsis D1 – median (min–max) 97.8 (3.5–335.0), severe sepsis D1 median (min–max) 65.3 (5.8–216.0), septic shock D1 median (min–max) 45.8 (1.8–371.0) (Fig. 1).

Clusterin levels in the control group were compared with a group of patients who were diagnosed with SIRS or sepsis, severe sepsis, septic shock or MODS during a 5-days. Generally, we found lower concentrations of clusterin in patients with SIRS or septic state, than in the control group. Clusterin cut-off for first day – D1 was 91.04 µg/ml; AUC 0.900; p-value <0.001; for third day – D3 was cut-off 86.73 µg/ml; AUC 0.849; p-value <0.001; for fifth day – D5 cut-off was 105.26 µg/ml; AUC 0.755; p-value <0.001 (Fig. 2).

During the evaluation of correlation dependence between clusterin levels and septic state, patients were divided into two groups – SIRS and sepsis vs. severe sepsis+ septic shock + multiple organ dysfunction syndrome (MODS). Higher values were considered to be associated with worse septic condition (as resulted from ROC optimal discrimination, however weak and non-significant). The difference in the dynamics of clusterin levels was recorded significant for 5 days in these groups, p-value 0.031 (Fig. 3).

When the patients were divided into two subgroups (PELOD score <12 and PELOD score >12), the evaluation of clusterin levels according to the degree of severity state showed that that there is no statistically significant difference between the these two groups. The difference in the dynamics of clusterin levels for 5 days was recorded, p-value 0.031. In group of patients with PELOD >12 there is a significant increase of clusterin levels in third days of hospitalization, thus in patients with more severe condition (Fig. 4). Analysis of the control group versus PELOD score >12 showed a significant statistical difference, the cut-off 91.04 µg/ml, AUC 0.939, p-value <0.001 (Fig. 5).

We also assessed the effect of clusterin levels on mortality in patients. There is no statistically significant difference

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**Fig. 3** – Correlation dependence between clusterin levels (µg/ml) and septic states. SIRS – systemic inflammatory response syndrome; MODS – multiple organ dysfunction syndrome; AUC – area under curve; SE – sensitivity; SP – specificity; PV+ – positive predictive value; PV− – negative predictive value; ANOVA – analysis of variance; G. mean – geometric mean; ROC – receiver operating characteristic
Fig. 4 – The evaluation of clusterin levels (μg/ml) and Pediatric Logistic Organ Dysfunction Score. PELOD Score – pediatric logistic organ dysfunction; AUC – area under curve; SE – sensitivity; SP – specificity; PV+ – positive predictive value; PV− – negative predictive value; ANOVA – analysis of variance; G. mean – geometric mean; ROC – receiver operating characteristic

<table>
<thead>
<tr>
<th>Effect</th>
<th>p-value</th>
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<tr>
<td>PELOD</td>
<td>0.287</td>
</tr>
<tr>
<td>Time</td>
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<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>AUC</td>
<td>0.662</td>
</tr>
<tr>
<td>Cut-off</td>
<td>82.1</td>
</tr>
<tr>
<td>p-value</td>
<td>0.227</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Day</th>
<th>N</th>
<th>G. mean (min-max)</th>
<th>PELOD &lt;12</th>
<th>N</th>
<th>G. mean (min-max)</th>
<th>PELOD ≥12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>56.2</td>
<td>46.5 (13.6 - 359.1)</td>
<td>27</td>
<td>41.6</td>
<td>53.2 (19.7 - 195.6)</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>52.8</td>
<td>49.1 (6.0 - 260.5)</td>
<td>24</td>
<td>47.1</td>
<td>48.1 (19.7 - 195.6)</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>51.2</td>
<td>58.2 (4.3 - 193.3)</td>
<td>26</td>
<td>56.2</td>
<td>73.1 (14.3 - 433.4)</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>57.0</td>
<td>63.3 (52.0 - 304.2)</td>
<td>21</td>
<td>74.4</td>
<td>78.6 (27.2 - 248.6)</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>55.2</td>
<td>63.3 (52.0 - 304.2)</td>
<td>22</td>
<td>76.8</td>
<td>75.3 (15.2 - 231.7)</td>
</tr>
</tbody>
</table>

Fig. 5 – Analysis of the patients in control group versus Pediatric Logistic Organ Dysfunction Score. PELOD Score – pediatric logistic organ dysfunction; AUC – area under curve; SE – sensitivity; SP – specificity; PV+ – positive predictive value; PV− – negative predictive value

<table>
<thead>
<tr>
<th>Control x PELOD 12+</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
</tr>
<tr>
<td>Cut-off</td>
</tr>
<tr>
<td>p-value</td>
</tr>
</tbody>
</table>
within groups non-survivors/survivors and clusterin levels, even though they were very borderline significance. The difference in the dynamics of clusterin levels was recorded significant for 5 days in these groups, p-value 0.004. Thus in patients who died clusterin levels increase was very slow over time (Fig. 6).

Discussion

In sepsis, the expected and appropriate inflammatory response to an infectious process becomes amplified leading to organ dysfunction or risk for secondary infection. A continuum exists from a low grade systemic response associated with a self-limited infection to a marked systemic response with solitary or multiorgan dysfunction, i.e., severe sepsis. As a clinical syndrome, sepsis occurs when an infection is associated with the systemic inflammatory response [18].

Many cellular aspects become dysfunctional in sepsis and may be characterized as either excessive activation or depressed function. One of the current areas of active investigation concerning cellular function is the induction of cellular apoptosis or necrosis. The signaling mechanisms and molecules that induce apoptosis are currently being described in great detail by a number of investigators [19, 20].

Clusterin is widely distributed, well conserved, and constitutively secreted glycoprotein that is highly induced in tissues regressing as a consequence of apoptotic cell death. Clusterin gene expression decreases drastically in cells undergoing apoptotic cell death in vitro, but continues to be expressed by morphologically normal cells [21].

In the hypothesis that clusterin may be have as a stress protein we have analyzed its expression in response to SIRS or septic state. This report demonstrates that clusterin expression is down-regulated in response to the above states. We demonstrated lower concentrations of clusterin in patients with SIRS or septic state, than in the control group. We did not find the difference in levels of clusterin between the different states. When evaluating the levels of clusterin and PELOD score, we experienced statistical significance in the dynamics of protein. This we consider very important, because a decrease or increase of the protein indicates the severity of the patient status. We have also demonstrated mortality prediction based on dynamics of clusterin levels. Unfortunately, we can not compare our results with others, because data from the pediatric population and from septic patients are not available. In adult patients with sepsis and septic shock clusterin was highly up-regulated in survivors, with expression factors of 26.5 and 14.9, whereas non-survivors exhibited only up-regulation levels of 3.1 and 5.9 [22].
disease, clusterin concentrations were lower in sepsis patients than in non-sepsis patients. In non-survivors, a modest increase was seen in patients after admission and this was followed by a further decline before death. In survivors, a considerable increase was seen from day 2 to day 6 but no difference was seen between admission and day 2 or between day 6 and week 6. The values found at day 6 and week 6 were comparable to values previously determined in serum samples from healthy blood donors [23].

In the experimental animal study a significant reduction in pulmonary hypertension and edema has been demonstrated due to a protective effect of clusterin in granulocyte induced pulmonary injury [24]. Clusterin expression is also upregulated in rats following traumatic brain injury [25], in seizure [26] and in some neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease [27, 28]. The role of clusterin in brain cell death is contradictory, as both gene-deficiency and overexpression of clusterin inhibit brain damage in mice [29].

Although biomarkers of sepsis are not widely used in research or clinical practice, it is possible to evaluate the utility of approaches that are currently available. The optimal use of biomarkers as surrogates in informing the design of definitive clinical trials presupposes a valid and extensive understanding of the natural history of the biomarker in the population of interest, and how its levels are modified by therapeutic intervention [8]. These data can then be integrated using meta-analytic techniques to evaluate the capacity of a biomarker to predict a clinically important outcome [30]. A methodology for evaluating the level of evidence that a given biomarker might serve as a reliable surrogate outcome measure has recently been proposed, but its utility in the assessment of biomarkers for diseases such as sepsis where mortality is considerable is unknown [31].

In conclusion, we here provide the first clusterin serum analysis of pediatric patients with sepsis and septic shock. We have shown a significant relationship between the levels of clusterin and pediatric patients with septic state. Further studies are required to elucidate the clinical impact of the observed organ-protective properties of clusterin and next studies are needed to examine his potential roles as predictive outcome markers, as well as his precise functional roles in sepsis, or possible therapeutic potential.

Authors’ contributions/Wkład autorów

JZ – study design, data collection and interpretation, literature search, MF – acceptance of final manuscript version.

Financial support/Finansowanie

None declared.

Conflict of interest/Konflikt interesu

None declared.

Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

The own research were conducted according to the Good Clinical Practice guidelines and accepted by local Bioethics Committee, all patients agreed in writing to participation and these researches.

REFERENCES/PIŚMIENNICTWO


