The Role of BCL2 Protein and Tumour Protein p53 in Septic Cardiomyopathy

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A decreased left ventricular ejection fraction (LVEF) was observed in patients suffering from septic shock with normalization of systolic function after 10 days. Similar courses of reversible myocardial dysfunction due to the systemic inflammatory response syndrome were also encountered in other critical illnesses. Since the pathological and histological mechanisms are not fully understood, the present study tries to understand the septic cardiomyopathy related to the apoptotic pathway. Thestudy included a number of 29 cases of adults that died of septic shock being analysed for BCL2 and p53 expression rates of myocardial tissue. This is the first time the expression of BCL2 protein, p53 tumour protein were evaluated in septic shock and septic cardiomyopathy of humans. There was a strong link between the increased expression of BCL2 and of p53 protein in cardiac muscle cells in the studied group (p=0.0300). The study showed a significant correlation between markedly increased values and poor outcome.

Keywords: septic cardiomyopathy, apoptosis, BCL2, p53

Sepsis represents a major public health concern [1,2]. Sepsis-induced myocardial dysfunction is present in up to 40% of patients with sepsis and can increase mortality rates by up to 70% [3,4]. Therefore, it represents an important factor concerning morbidity and mortality, especially in patients with septic shock. Since the pathological and histological mechanisms are not fully understood, further research is necessary.

To better understand the correlation between the systemic inflammatory response and reversible myocardial dysfunction in sepsis the levels of BCL2 protein, tumour protein p53 and CD14 surface protein (results not presented in this article) in patients that died from septic shock were investigated. BCL2 (B-cell lymphoma 2) is a regulator protein that is encoded by the BCL2 gene. It influences cell death by inducing or inhibiting apoptosis [5,6]. The name derives from its first description as the second protein translocation discovered to be responsible for follicular lymphomas. It is located on the mitochondrial surface and promotes cellular survival by inhibiting pro-apoptotic proteins. In sepsis besides the extensive apoptosis of immune cells, an increase of cell death has been described in cardiomyocytes [7-9]. In murine models, genetic manipulation of cell death showed marked effects in sepsis [10]. This could suggest a new therapeutic approach by blocking apoptosis [11].

Contrary to these findings, a transgenic over-expression of BCL2 in T- and B-cells, intestinal epithelium and in myeloid cells improved the survival rate in septic mice [12,13]. Furthermore, the presence of extracellular BCL-2 proteins showed reduced myocardial ischemia-reperfusion damage and decreased hypertrophy and fibrosis in pressure-overload heart failure in another mural trial [14,15].

P53 is an isoform of a protein encoded by homologous genes in various organisms. It prevents cancer formation as a tumour suppressor and conserves gene stability by preventing genome mutation [16]. Polymorphism of the p53 gene is associated with a significantly increased risk for developing cervical, breast, lung and renal cell carcinoma [17-20]. In addition to its role in malignancy, p53 seems to play a central role in lymphocyte and neutrophil mediated immune response to infection. In sepsis p53-dependent and -independent pathways of cellular apoptosis is present. A murine model showed that mice with p53 expression lymphocytes had a different apoptotic response in splenocytes and thymocytes. This suggests a cell-type specific cellular response or death-inducing signal [21]. Another study suggests that an increase of p53 in T lymphocytes might be responsible for inhibiting the cell proliferation and enhancing apoptosis and immune dysfunction of T cells during sepsis or endotoxin challenge [22]. The neutrophil response plays an important role in fulminant bacterial sepsis, but a prolonged life span can lead to organ/tissue damage.

If cellular stress occurs, the p53 tumour protein progress may block the evolution of the cell cycle for damage repair or may induce apoptosis by upregulation of the BCL2 (BH3 only) gene PUMA (p53 upregulated modulator of apoptosis) [23-26]. In the absence of the p53/PUMA pathway in animals and primary cell cultures, a significantly increased resistance to cell death due to DNA damage was demonstrated [27-29]. The p53/PUMA apoptotic pathway regulates the neutrophil lifespan. Therefore, it ensures an adequate bacterial clearance as well as balancing the innate immune response to infection and the survival to DNA damage [30].

Sepsis-induced myocardial dysfunction was first observed in 1984. A decreased left ventricular ejection fraction (LVEF) was observed in patients suffering from septic shock with normalization of systolic function after 10 days [31]. Similar courses of reversible myocardial

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dysfunction due to the systemic inflammatory response syndrome were also found in other critical illnesses [32-34].

The aim of this study is to find out any significance of the BCL2 and p53 mediated pathways in the septic cardiomyopathy. On the second hand the role played by apoptosis cascade in the septic shock, was analysed.

Experimental part

Material and methods

The hearts of 29 consecutive cases of adults that died of septic shock were analysed for BCL2 and p53 expression rates of myocardial tissue. Exclusion criteria were: known heart insufficiency and malignant diseases. The cardiac exclusion criteria were cardiomyopathies with moderate to high decreased left ventricular ejection fraction and moderate to severe valvular disease. The malignant exclusion criteria were solitary tumours and haematological malignancies.

The control group consisted of 10 consecutive new-born patients that died in the same period without any heart or malignant diseases. The cases have been taken from the County Clinical Emergency Hospital of Oradea and Municipal Clinical Hospital of Oradea (both located in Oradea, Romania), during the period January 2018 - December 2018. The pathological diagnoses were established in the pathology department of the hospitals.

Tissue specimens were fixed in 10% buffered formalin (*p*H 7.4) between 24 hours and 72 hours, paraffin-embedded according to standard procedures. Immunohistochemical analysis were performed on 4 µm thick sections using Ventana Benchmark GX (Ventana Medical Systems Inc., Tucson, AZ, USA) automated staining instrument. Following the manufacturer's instructions, the slides were deparaffinized using EZ prep solution (Ventana Medical Systems, Inc.), incubated with monoclonal antibodies, developed using the Opti View DAB detection kit (Ventana Medical Systems, Inc.) and counterstained with haematoxylin and blueing [7,19-25]. For BCL2 staining, cells were incubated for 20 minutes with anti-bcl-2 monoclonal primary antibody (CONFIRM, 124, mouse, IgG1, cytoplasmic, IVD) in accordance with the manufacture protocol [35]. For p53 staining, sections were incubated with anti-p53 primary monoclonal antibody (CONFIRM, DO-7, Mouse, Nuclear, IgG1/K, IVD) in accordance with the manufacture protocol. For each run, a positive control slide (colon adenocarcinoma) was performed [36]. For each positive / negative case, the control of the slides was done.

The interpretation was performed using the hot-spot technique. The hot spot areas refer to areas with high expression. In the study, patients were divided into 3 groups, namely <10% (low expression), 10-50% (medium expression) and >50% (high expression) [37]. For data storage and statistical calculations, the statistical software MedCalc® version 12.5.0.0 (MedCalc® Software, Mariakerke, Belgium) was used. The results of the statistical test are represented by the probability of the *null* hypothesis (p), a value under 0.05 proves a statistically significant difference between study groups. Some of the results are described through graphical figures using the same statistical software or with the of Microsoft® Excel® 2010 (Microsoft® Corporation, USA).

Results and discussions

The patients in the study group had a significantly higher expression of BCL2 compared to the control group ($p<0.0001$). Most of the cases were in the low expression group (25 cases) and 4 cases belonged to the medium expression group (Figure 1).

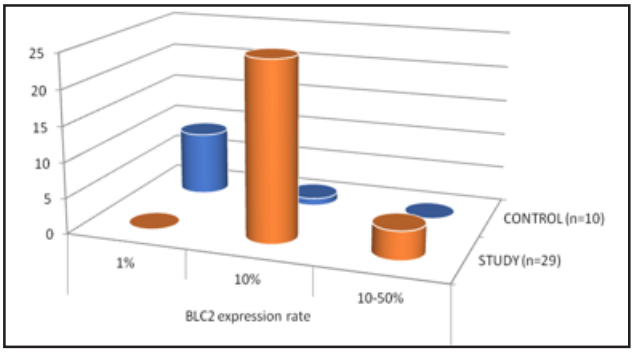

Fig.1. Distribution of BCL2 in the study and control group

There was no statistically significant difference in gender ($p=0.5066$), provenience ($p=0.7510$), infection site ($p=0.5077$) or age ($p=0.8010$). Nevertheless, older patients tended to be present in the 10-50% group. When the age is associated to the expression group it is revealed that the average age is lower for the <10% group (57.92 ± 19.7) and higher for the 10-50% group (62.25 ± 13.7).

The expression rate of p53 was significantly increased in the study group. In the control group the expression rate never exceeded 1% ($p<0.0001$). It can be noticed that, from the total number of patients included in the study, 10 cases belong to the low expression rate, 12 cases are in the medium expression rate, and 7 cases belong to the high expression rate group (Figure 2).The cases from the rural area were associated with a higher expression rate of p53 ($p=0.0130$). There was a tendency for higher p53 expression rates to be associated with increasing age in the study group, without reaching statistical significance ($p=0.2110$) (Figure 3).

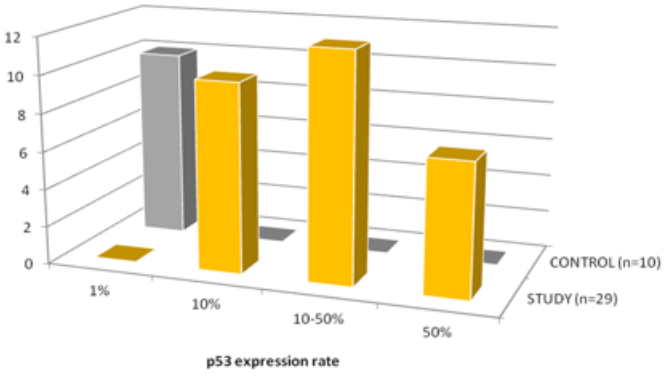

Fig.2. Distribution of p53 in the study and the control group

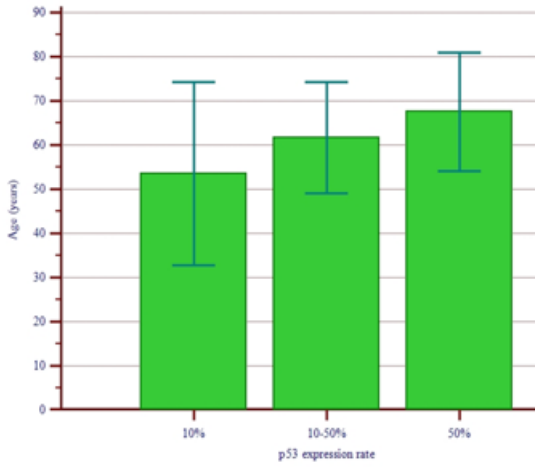

Fig.3. Age distribution at different p53 expression rates of the study group

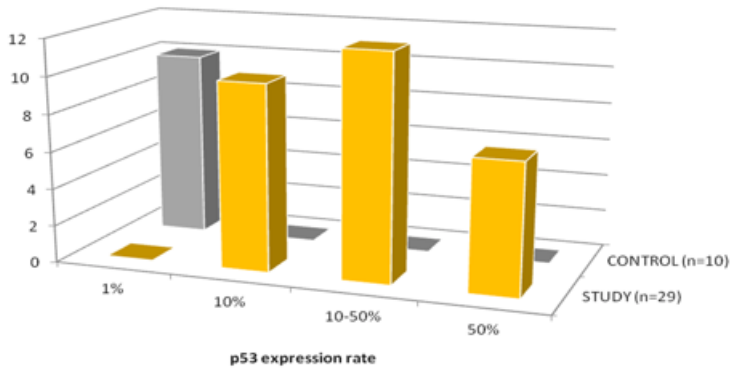

Fig.4. Comparison between BCL2 and p53 levels in the study group

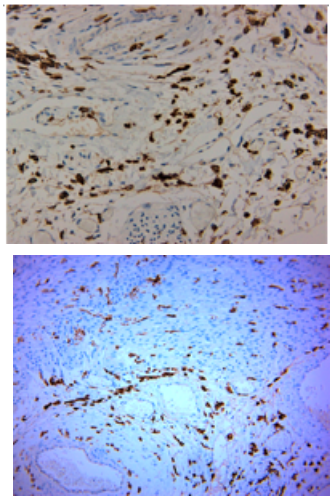

Fig.5.a. Immunohistochemistry: BCL2 overexpression; b. p53 expression

There was no statistically significant difference between gender (p=0.5006) and infection site (p=0.5805).A strong connection was observed between the increased expression of BCL2 and of p53 protein in cardiac muscle cells, in the study group (p=0.0300).The correlation between BCL2 and p53 expression in the study group revealed that when one is high, the other expression is usually low (Figure 4). Figure 5a points out many cardiac cells with brown cytoplasmic BCL2 expression, and figure 5b shows the nuclear expression for p53. All data obtained by us in the study group have been compared with control group (the heart tissue collected from new-born who died in the same period without any heart or malignant diseases).

Septic patients developing myocardial dysfunction have a significantly higher mortality than those without cardiovascular impairment [3,4]. In sepsis, the massive drop of after load due to the decrease of systemic vascular resistance (SVR) can be compensated partially by an extremely high cardiac output (up to 20L/min). This can be inadequate due to decreased myocardial contractility, impaired response to fluid therapy and ventricular dilation. Along with other organs (central nervous system, kidneys, lungs, etc.) the function of the heart is decreased in severe sepsis and septic shock. Additionally, autonomic dysfunction reduced heart rate variability and impaired baroreflex and chemoreflex sensitivity have a contribution [38]. The restriction of cardiac performance must be correlated with the markedly reduced SVR [39].

Often cardiac troponins are increased as a sign of septic cardiomyopathy. However, increased troponin I, can also be present in sepsis with no evidence of myocardial dysfunction. There is no decrease in perfusion, since coronary arteries are dilated in accordance with systemic vasodilatation, with a high coronary blood flow [4,40]. Cardiac depression can be caused directly by microorganisms (endotoxins, exotoxin, etc.) or indirectly by inflammatory mediators [41]. This results in left and/or right ventricular dysfunction, disturbances in heart rate regulation with arrhythmias as well as disturbed heart rate variability. There is a complex mechanism of inflammatory pathways causing impaired myocardial depression in septic shock [42-44].

Although there has been remarkable progress in the intensive treatment care, over the last decades no real breakthrough in the outcome of patients with septic shock has been achieved. The measures performed include many standardised procedures, i.e. early and invasive regulated volume substitution, early antibiotic treatment, adjusted catecholamine treatment, protective ventilation, glucocorticoid administration and even cytokine filtration as well as extra-corporal life support.

In some animal trials, a better outcome with immune modulation of the discussed pathways has been proven. Further investigation in human clinical medicine is necessary to prove the significance of the BCL2 and p53 mediated pathways. It is possible that this could lead to new treatment strategies that could improve the clinical outcome with regards to mortality and morbidity of patients with septic shock.

BCL2 has pro- and anti-apoptotic properties. The previous data was controversial, whether increased values lead to an increased amount of apoptosis or lead to a protection from apoptosis [45]. The data obtained suggest an association between increased values of BCL2 and death from septic shock. It would be interesting to see how the values of BCL2, p53 are in survivors of septic shock of comparable severity. This would prove the association between increased expression rates and poor outcome in septic shock. Myocardial biopsies cannot be obtained in critical ill patients. Furthermore, it would be highly advisable to perform a study that compares our data with levels of BCL2 and p53 that could be found in cases of patients died in politraumas and those deceased as a consequence of septic shock or septic states in the context of violent deaths.

Overall, there was a tendency for higher values corresponding with increasing age, but without statistical significance. Not significantly increased values with increasing age could correlate the increasing mortality with increasing age of the patients.

Conclusions

This is an original study about the expression of BCL2 protein and p53 tumour protein that were evaluated in septic shock and septic cardiomyopathy of humans. The research showed a significant correlation between markedly increased values and poor outcome. A statistically significant increase of BCL2 protein / p53 protein was found in the study group. There was a strong link between BCL2 and p53 values. However, sepsis-induced cardiac dysfunction is seen to be completely reversible in survivors of septic shock. Therefore, a lack of improvement of the cardiac function can be seen as a predictor of poor prognosis. This could be due to an increased, irreversible apoptosis of cardiomyocytes. So far, the significance of increased values of BCL2 and p53 in sepsis and septic cardiomyopathy was mainly investigated in murine trials. In these investigations however, they were associated with an increased mortality.

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