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High postoperative serum levels of surfactant type B as novel prognostic markers for congenital heart surgery

Níveis séricos pós-operatórios tipo surfactante B elevados como novos marcadores prognósticos para cirurgia cardíaca congênita

Onur Isık[1], MD; Olcay Murat Disli[2]; Tolga Bas[2], MD; Hakan Aydın[1], MD; Murat Koç[1], MD; Ali Kutsal[1]

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Abstract

Objective: **Congenital heart diseases are observed in 5 to 8 of every 1000 live births. The presence of a valuable biomarker during the surgical periods may aid the clinician in a more accurate prognosis during treatment.**

Methods: **For this reason, surfactant protein B plasma levels may help to evaluate patients with cardiac problems diminishing the alveolocapillary membrane stability. In this study, plasma levels of this biomarker were measured in the preoperative and postoperative periods. This study was conducted to detect the differences between pulmonary hypertensive and normotensive patients. The differences before and after cardiopulmonary bypass were examined.**

Results: **The differences in cardiopulmonary bypass time, cross-clamp time , inotropic support dose, and duration of intensive care of patients with and without pulmonary hypertensive were found to be statistically significant ($P<0.05$). The results revealed that this pathophysiological state was related to other variables that were studied. We believe that the differences in preoperative and postoperative SPB levels could be attributed to alveolocapillary membrane damage and alveolar surfactant dysfunction. We found that this pathophysiological condition was significantly associated with postoperative parameters.**

Conclusion: **The findings of the current study showed that surfactant protein B was present in the blood of patients with a congenital heart disease during the preoperative period. Long by-pass times may exert damage to the alveolocapillary membrane in patients with pulmonary hypertension and preoperative heart failure, and it is recommended to keep the option of surfactant therapy in mind during the postoperative course at the intensive care unit before preparing the patients for extubation.**

Descriptors: **Heart Surgery. Biological Markers. Pulmonary Surfactant-Associated Protein B. Heart Defects, Congenital.**

Resumo

Objetivo: **As cardiopatias congênitas são observadas em 5 a 8 em cada 1.000 nascidos vivos. A presença de um biomarcador importante durante os períodos cirúrgicos pode auxiliar o clínico a um prognóstico mais preciso durante o tratamento.**

Métodos: **Por esta razão, os níveis plasmáticos de proteína B do surfactante podem ajudar a avaliar os pacientes com problemas cardíacos, diminuindo a estabilidade da membrana alvéolo-capilar. Neste estudo, os níveis plasmáticos deste biomarcador foram medidos nos períodos pré-operatório e pós-operatório. Este estudo foi realizado para detectar as dife-**

[1]Dr. Sami Ulus Maternity and Children's Research and Education Hospital, Department of Cardiovascular Surgery, Ankara, Turkey.
[2] Inonu University, Turgut Ozal Medical Center, Department of Cardiovascular Surgery, Malatya, Turkey.

This study was carried out at Dr. Sami Ulus Maternity and Children's Research and Education Hospital, Ankara, Turkey; Department of Cardiovascular Surgery, Dr. Sami Ulus Maternity and Children's Research and Education Hospital, Altindag Ankara, Turkey.

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Correspondence address:
Onur Isık
Dr. Sami Ulus Maternity and Children's Research and Education Hospital
Department of Cardiovascular Surgery
Babur street no 14 - Altindag Ankara, Turkey
E-mail: dr.onur.aras@gmail.com

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Abbreviations, Acronyms & Symbols	
CPB	cardiopulmonary bypass
DMV	duration of mechanical ventilation
PH	pulmonary hypertension
SPA	Surfactant protein A
SPB	surfactant protein B
SPC	surfactant protein C
SPD	surfactant protein D
XCT	cross-clamp time

renças entre pacientes hipertensos e normotensos em nível pulmonar. As diferenças antes e depois da circulação extracorpórea foram examinadas.

Resultados: **As diferenças no tempo de circulação extracorpórea, tempo de pinçamento, a dose de drogas vasoativas, e a duração da terapia intensiva de pacientes com e sem hipertensão pulmonar foram estatisticamente significativas (*P*<0,05). Os resultados revelaram que este estado fisiopatológico foi relacionado a outras variáveis que foram estudadas. Acreditamos que as diferenças nos níveis de SPB pré-operatório e pós-operatório pode ser atribuída a danos na membrana alvéolo-capilar e disfunção do surfactante alveolar. Descobrimos que esta condição fisiopatológica foi significativamente associada com parâmetros pós-operatórios.**

Conclusão: **Os resultados do estudo mostraram que a proteína B surfactante estava presente no sangue de pacientes com doença cardíaca congênita no pré-operatório. Longos tempos de circulação extracorpórea podem exercer danos na membrana alvéolo-capilar em pacientes com hipertensão pulmonar e insuficiência cardíaca pré-operatória, e recomenda-se manter a opção de terapia com surfactante em mente durante o período pós-operatório na unidade de terapia intensiva antes de preparar os pacientes para a extubação.**

Descritores: **Cirurgia Torácica. Marcadores Biológicos. Proteína B Associada a Surfactante Pulmonar. Cardiopatias Congênitas.**

INTRODUCTION

Cardiac surgery has been a rapidly developing scientific area that has experienced many innovations in the second half of the last century. The rapid advances in surgery have created a need for markers to guide intensive care follow-up[1].

The discovery of a biomarker that will guide the postoperative intensive care follow-up, aid in anticipating complications, shape treatment, and facilitate decision-making for the duration of intensive care follow-up has great benefits. Furthermore, the biomarker's ability to provide results in a short time and reliability is beneficial, as well.

Surfactant protein B (SPB) can be used for the aforementioned purposes; since it is not present in the peripheral blood of healthy individuals, but rather enters into the peripheral bloodstream as a result of a pressure imbalance in the pulmonary alveolocapillary membrane due to heart failure[1].

SPB is used as a biological marker to determine the degree of pulmonary dysfunction in adult patients with heart failure and the detection of the response to medical treatment. However, the research on the use of SPB in congenital cardiac surgery has not been sufficient yet[2].

This study examined the levels of SPB protein in the peripheral blood of patients with congenital heart disease undergoing open heart surgery, in the preoperative and postoperative periods. The study further investigated their relationships with mortality and morbidity by comparing these values with routine variables obtained in intensive care follow-ups.

METHODS

Ethics approval was obtained from Clinical Research Ethics Review Board of Ankara University Faculty of Medicine (Date: 16/10/2012, Number: 70), and written informed consent was obtained from the patients' families. Thirty patients who were admitted to our clinic for open heart surgery due to congenital heart disease, who were diagnosed using catheter angiography in 2011, and who had undergone surgery by the same surgeon were included in the study. The detailed description of the patients' congenital heart diseases and the performed surgeries are presented in Table 1.

Table 1. Patient diagnose and performed surgery.

Diagnose	Procedure	n (%)
Sec ASD	Primary Closure	5 (16)
P. VSD	Patch Closure	8 (27)
PAPVC	Interatrial baffle reconstruction	2 (7)
Aortic Rg.	Mechanical AVR	2 (7)
AVSD	Australian Technique	2 (7)
TOF	Total Correction (transannular patch)	2 (7)
TGA	Arterial Switch	5 (16)
T. Arteriozus	Total Correction (12 mm valved tube graft)	3 (10)
ALCAPA	Detachment reatachment	1 (3)

ASD: Secundum type atrial septal defect, P. VSD: Perimembranous type ventricular septal defect, PAPVC: Partial abnormal pulmonary venous connection, AVSD: Atrio ventricular septal defect, TOF: Tetralogy of fallot, T. Arteriozus: Truncus arteriozus, ALCAPA: Abnormal left coronary artery from pulmonary artery)

All patients with congenital heart disease were examined in two groups; patients with (group 1, n_1=13) or without (group 2, n_2=17) pulmonary hypertension. Pulmonary hypertension was defined as systolic pulmonary artery pressure higher than 50% of the systolic systemic pressure[3].

Patients were also assessed regarding inotropic medication they received, total cardiopulmonary bypass (CPB)

and aortic cross-clamp time (XCT), duration of mechanical ventilation and pressure support ventilation, liver and kidney functions in the postoperative period, intensive care time, and discharge time.

All patients underwent median sternotomy and aorto-bicaval cannulation. During CPB, hypothermia (up to 28-32°C) was used with respect to the patient's cardiac pathology. Diastolic cardiac arrest was achieved using topical cold and antegrade hypothermic crystalloid cardioplegia.

After induction of anesthesia, blood samples were taken from all patients to investigate the BNP and the surfactant protein B. Then, the samples were centrifuged at 1000 rpm at room temperature (22°C) for 15 minutes. Separated plasma was placed in a polypropylene tube and stored at -20°C. Tests for hemodynamic data, arterial blood gas samples, and samples for BNP and surfactant protein B values were repeated in the first hour after the surgery. In addition, patients' inotropic treatment needs after surgery, CPB time, cross-clamp (XCT), duration of mechanical ventilation (DMV), and pressure support in providing the appropriate tidal volume were recorded.

The collected plasma samples were studied using a Human Pulmonary Surfactant Associated Protein B ELISA kit (Cusabio Biotech Co. Ltd. Wuhan, PR China).

Measurement of Surfactant Protein B

Microtiter surfaces that were pre-coated with a specific antibody against surfactant protein B were used in this kit. Then, the samples were properly placed on a micro-surface together with avidin-conjugated horseradish peroxidase, which was prepared with antibodies specific to biotin-conjugated SPB, and were later incubated. Next, a tetramethylbenzidine substrate solution was added to each sample. Color change was observed only in the samples containing SPB, i.e. samples containing biotin-conjugated antibodies and enzyme-conjugated avidin.

For the samples terminating with the enzyme substrate reaction, the samples were spectrophotometrically measured at a wavelength of 450 nm after the addition of sulfuric acid solution. The SPB concentration in the samples was calculated by a comparison of the standard slope of normal values.

Statistical Analysis

Based on post-hoc power analysis, a sample size of 13 achieved 99% power to detect a mean of paired differences of 0.50 with an estimated standard deviation of differences of 0.40 and with a significance level (alpha) of 0.05 using a two-tailed paired t-test. The quantitative variables were represented as median (min-max). Kolmogorov Smirnov test was used as a goodness of fit test to assess the normality of the sample distribution. Mann-Whitney U-test and Wilcoxon test, where appropriate, were used for the comparison of all quantitative variables. Spearman's rho correlation coefficient was used in the correlation analyses. *P* values <0.05 were considered as statistically significant. SPSS for Windows 21.0 statistical software was used for data analysis.

RESULTS

Patients were divided into two groups according to pulmonary artery pressure, measured with catheter angiography in the preoperative examination. 13 patients were included in the group of patients with pulmonary hypertension (PH), whereas 17 patients were included in the group of patients without PH.

Table 2 presents the comparison of patients with and without PH in terms of CPB time, XCT time, duration of mechanical ventilation, duration of intensive care, and discharge time of patients. The differences in CPB time, XCT, inotropic support dose, and duration of intensive care of patients with and without PH were found to be statistically significant ($P<0.05$), whilst duration of mechanical ventilation, pressure support ventilation and discharge time were not statistically significant ($P>0.05$).

Table 3 demonstrates SPB serum levels before and after surgery. In patients with and without PH, the differences between the SPB blood values in the preoperative and postoperative periods were observed to be statistically significant ($P<0.05$).

The levels of pre-SPB and post-SPB between the groups were not found to be significant ($P>0.05$).

Table 4 defines the BNP levels before and after surgery. The increases in BNP values, which were simultaneously studied with SPB, were found to be significant ($P<0.05$). Whereas the levels of pre-BNP and post-BNP between the groups were not statistically significant ($P>0.05$).

When two groups were formed of patients who died in the postoperative period and who were discharged, the increases of percentage in SPB levels of these patients were observed to be statistically significant ($P<0.05$).

Table 2. Descriptive statistics of patients with and without PH.

Variable	Without PH (n_1=17) Median (Min - Max)	With PH, (n_2=13) Median (Min - Max)	*P* value
CPB Time, min	106 (53 - 486)	68 (20 - 190)	0.025
XCC Time, min	60 (34 - 97)	41 (10 - 110)	0.016
Duration of mechanical ventilation, min	5 (1-16)	7 (3-384)	0.11
Duration of intensive care, min	1 (1-2)	1 (1-17)	0.043
Discharge time, min	11 (7-370)	7 (6-14)	0.12

Table 3. SPB serum levels before and after surgery.

Variable	Without PH (n_1=17) Median (Min - Max)	With PH, (n_2=13) Median (Min - Max)	*P* value
Pre-SPB serum levels	2.95 (2.42 – 4.31)	3.18 (2.46 - 3.74)	0.29
Post-SPB serum levels	3.48 (2.96 – 5.35)	3.51 (3.00 – 4.23)	0.80
P value	<0.001	0.002	

Table 4. BNP levels before and after surgery.

Variable	Without PH (n1=17) Median (Min - Max)	With PH, (n2=13) Median (Min - Max)	*P* value
Pre-BNP levels	129 (44-18208)	170 (32-1250)	0.39
Post-BNP levels	161 (68-35000)	900 (54-18000)	0.32
P value	<0.001	0.001	

Significant increases were found in SPB levels of the patient group requiring prolonged mechanical ventilation and pressure support ventilation in the preoperative and postoperative periods ($P<0.05$).

Positive correlations were found between CPB time and XCT time (Spearman's rho=0.92, $P<0.001$) and hospital stay duration (Spearman's rho=0.53, $P=0.02$). Positive correlations were detected between mechanical ventilation time and hospital stay duration (Spearman's rho=0.63, $P=0.005$) and discharge time (Spearman's rho=0.49, $P=0.04$). Similarly, discharge time was positively correlated with duration of intensive care (Spearman's rho=0.70, $P=0.001$) and discharge time (Spearman's rho=0.49, $P=0.04$).

DISCUSSION

Lung epithelial cells provide a large surface area allowing for gas exchange between capillary vessels. The greatest problem of such a large and hydrated area is the collapse of the alveolar surfaces in the expiratory phase of respiration. This problem is solved by a lipid-rich surface generated by pulmonary surfactant protein on the epithelial cells, separating alveolar gas and liquids. Pulmonary surfactant phospholipids decrease the surface tension to negligible levels by forming mono- and multi-layers[4].

There are four types of surfactant protein in the alveoli, namely: Surfactant protein B (SPA), Surfactant derived protein B (SPB), surfactant protein C (SPC) and surfactant protein C (SPD). Although the chemical structures of these proteins are different, their tasks within the alveoli overlap each other. The functions of these proteins are to prevent alveolar collapse at the end of expiration, to create a large surface area for gas exchange, and to perform immunoregulation against external aggressors[5-7].

Among these proteins, SPB is different in terms of its entrance into the blood after the pressure changes in the respiratory and circulatory physiopathology by passing through the alveolocapillary membrane due to its molecular size. The half-life of this protein in the blood is short about 3 to 12 minutes. Although SPB is not the smallest of the surfactant proteins in the alveoli, it is the smallest surfactant that can be measured in the bloodstream. At the same time, alveolar concentration of SPB also changes after these transformations[8,9].

Because of these characteristics, SPB has become the subject of research in acute and chronic lung injuries, heart failures, and congenital lung diseases.

Dyspnea is a major finding resulting from pulmonary edema that occurs with congestive heart failure. Based on this fact, De Pasquale et al.[8] used SPB blood levels as a biomarker of lung function and status in follow-ups of patients with heart failure. Since SPB blood levels have a tendency to increase in all pulmonary diseases, this marker was used only in the follow-up of lung status in patients with congestive heart failure and damaged alveolocapillary membranes. They explained that during patient follow-ups, SPB levels were increased in decompensation periods of heart failure, and that compensation had a low course of progression with medical treatment. The researchers described SPB as a useful biomarker for anticipation of the future progression of the patients with congestive heart failure in the clinical follow-ups.

West et al.[10] showed the structural fragility of the alveolocapillary barrier under high pulmonary pressure and designated this situation as 'stress failure'. Pascual-Figal et al.[11] compared the SPB blood levels of patients with heart failure to SPB blood levels of normal, healthy people and found that blood levels of this protein were significantly higher in patients with heart failure. De Pasquale et al.[12] categorized the patients in their research according to the NYHA functional classification and 6-minute walk test, and they found the differences in SPB levels in blood samples of these patients to be in accordance with the functional groups. Magri et al.[13] monitored patients with congestive heart failure and explained that SPB values

were increased in the peripheral blood samples of patients with heart failure, and also that these values were directly related to the damage of gas diffusion in the lungs. Researchers also reported that SPB blood levels correlated with the degree of heart failure. The results of this study were similar. SPB levels were higher in the pulmonary hypertension group and this subgroup's clinical condition was worse than the group without pulmonary hypertension.

Pulmonary dysfunction after cardiac surgery is a significant morbidity despite advancing heart-lung pump technology and developing intensive care conditions. This dysfunction includes a wide range of consequences from severe ARDS tables to more moderate extubation delays or mild respiratory failures. Anesthesia, cardiopulmonary bypass, and surgical trauma are included together in the etiology of this dysfunction[14,15]. Tennenberg et al.[16] in their study aimed at enlightening how lung physiology changed after CPB, asserted that lung function was impaired. They related the impairment of lung function with the decrease in lung volume, the decrease in carbon monoxide transfer, and the increase in alveolar-arterial oxygen pressure gradient. They suggested that these changes were not caused by the increase in pulmonary endothelial permeability.

Cox et al.[17] investigated the cause of postoperative pulmonary dysfunction occurring after CPB and considered that cardiopulmonary bypass triggered the dysfunction through leukocyte and complement activation. They indicated that this system could result in the deterioration of gas exchange in the postoperative period, increased alveolar-arterial oxygen gradient, and decreased pulmonary compliance; these factors were reflected as atelectasis and as an increase in respiratory workload depending on the clinical status of the patient. Although the researchers explained the mechanism of action as such, they did not report any differences between the control group and patients without any pulmonary disease in the preoperative period and with good myocardial performance in terms of lung dysfunction[15,16].

Following total correction of the primary congenital heart disease, the patients without pulmonary hypertension made a good recovery and had an uneventful postoperative course. We believe that the literature findings which were discussed in the preceding paragraph are the underlying reasons for this good outcome. On the other hand, patients in the pulmonary hypertension group had various respiratory system complications, as well as longer discharge times. Griese et al.[18] argued that CPB caused acute lung injury in various degrees and asserted that complex congenital heart patients under the age of one year were the most risky candidates of this group. They explained the mechanism of this injury as the mediators released from activated platelets following the contact of blood with non-physiological surfaces, the activation of the complement system, the activation of the kallikrein-kinin system, and the development of interstitial edema in the pulmonary capillary endothelium following the sequestration of activated PMN (polymorphonuclear) cells into pulmonary alveoli.

They suggested that this edema directly impaired the intra-alveolar surfactant metabolism. In order to demonstrate this, the researchers took alveolar lavage samples from infants with complex congenital heart disease with long CPB times and with poor general conditions in the postoperative period; they assessed the samples in terms of surfactant activity and found functional disorders afterwards[18].

Preoperative SPB blood levels of all patients included in the study were high due to alveolocapillary membrane damage that was caused by the hypoxia or the left to right shunt of various degrees, which developed as secondary to the congenital heart anomaly. SPB values, measured in all patients within the preoperative and postoperative periods, had a tendency to increase due to alveolocapillary membrane damage, resulting both from the immunological and acute pressure changes, which took place during and after CPB. The fact that there was an increase in SPB in the blood, which was higher in patients with pulmonary hypertension before and after surgery, suggested that high pulmonary blood pressure damaged alveolocapillary membrane more severely in this patient group and these patients suffered greater pulmonary damage from CPB.

Higher pressure required to obtain normal tidal volume in mechanical ventilation in patients with higher SPB levels in the postoperative period suggested that this was accordant with the fact that these patients were more complex cardiac patients, they required longer CPB times, consequently suffering from greater alveolar surfactant dysfunction, and had more severe alveolocapillary membrane damage.

Study Limitations

The current study had certain limitations, including small sample size, variability in age and primary pathologies in the subgroups; however, the groups were divided according to the pulmonary artery pressures.

CONCLUSION

SPB is a protein that is normally undetectable in peripheral circulation, but it enters into the bloodstream when the structure of the alveolocapillary membrane is disrupted for any reason. In the current study, SPB was present in the blood in patients with congenital heart disease (pulmonary hypertension, heart failure, hypoxic pathophysiology) in the preoperative period. We believe that the significant differences in preoperative and postoperative surfactant type B levels could be attributed to alveolocapillary membrane damage and alveolar surfactant dysfunction. We found that this pathophysiological condition was significantly associated with several postoperative parameters; however, there was no positive correlation between the given parameters.

Long by-pass times may exert damage to the alveolocapillary membrane in patients with pulmonary hypertension and preoperative heart failure, and it is recommended to keep the option of surfactant therapy in mind during the postoperative course at the intensive care unit before preparing the patients for extubation.

The findings of the current study should be reproduced and validated on a larger sample population to be able to use SPB as an indicator of mortality and morbidity in the intensive care follow-ups of patients who underwent open heart surgery due to congenital heart disease. Overall, this study showed that surfactant protein B plasma levels may provide useful as a prognostic marker for all phases of pediatric cardiac surgeries.

Authors' roles & responsibilities	
OI	Primary responsibility for protocol development, outcome assessment, preliminary data analysis and writing the manuscript
OMD	Participated in the development of the protocol and preliminary data analysis and writing the manuscript
TB	Participated in the outcome assessment
HA	Participated in the development of the protocol
MK	Participated in the writing the manuscript
AK	Participated in the development of the protocol

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