

# The role of Plasminogen Activator Inhibitor 1 in predicting sepsis-associated liver dysfunction: an observational study

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## Research

**Keywords:** sepsis-associated liver dysfunction (SALD), liver failure, plasminogen activator inhibitor 1 (PAI-1), aspartate transaminase (AST), sepsis

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# Abstract

**Background:** Sepsis-associated liver dysfunction (SALD) is associated with poor prognosis and increased mortality in the Intensive Care Unit. Bilirubin is one of the components of Sequential Organ Failure Assessment used in Sepsis-3 criteria. Hyperbilirubinemia is a late and non-specific symptom of liver dysfunction. The aim of the study was to identify plasma biomarkers, which could be used for the early diagnosis of SALD.

**Methods:** A single-centre, prospective observational study was conducted in the ICU of Wroclaw University Hospital. Plasma biomarkers - prothrombin time, INR, antithrombin III, bilirubin, aspartate transaminase (AST), alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, albumin, endothelin-1, hepcidin, plasminogen activator inhibitor-1 (PAI-1), thrombin-antithrombin complex and interferon-gamma inducible protein 10 kDa were analysed. Plasma samples were obtained from eligible septic patients within 24 hours after having developed sepsis/septic shock. Enrolled patients were followed for 14 days for developing SALD and 28 days for overall survival.

**Results:** 79 patients were enrolled in the study, and 24 (30.4%) of them developed SALD. PAI-1 with a cut-off value of 9ng/ml was shown to be a predictor of SALD (AUC=0.727, sensitivity 79.2%; specificity 41.8%; accuracy 53.2%) and of 28-day survival in patients with sepsis/septic shock (log rank test,  $p=0.00091$ ). This was also useful in predicting 28-day survival, in combination with AST (log rank test,  $p=0.00242$ ).

**Conclusions:** Sepsis-associated hyperbilirubinemia is frequent, but bilirubin is a late and non-specific marker of SALD. Measuring PAI-1 serum levels at the onset of sepsis/ septic shock may be useful in predicting the development of SALD. A combination of PAI-1 and AST better predicts the 28-day survival than PAI-1 alone, but due to the low cut-off values of AST, this might not be clinically significant.

## Introduction

Sepsis-associated liver dysfunction (SALD) is associated with poor prognosis and increased mortality in Intensive Care Unit (ICU) patients [1]. It remains a key component of multiple organ dysfunction syndrome (MODS) complicating sepsis.

In sepsis, infection combined with the hyperactivity of the inflammatory response and microcirculatory failure contribute to organ dysfunction. In available reports, due to the lack of homogenous diagnostic criteria, the incidence of SALD varies from 1.1% up to 34.7 % [2]. It may be present as hypoxic hepatitis (HH), sepsis-induced cholestasis and/or coagulopathy [3] and the degree of organ injury may range from mild liver dysfunction to life-threatening liver failure.

According to Sepsis-3 criteria, an acute change in a sequential organ failure assessment (SOFA) score of  $\geq 2$  in response to an infection allows life-threatening organ dysfunction defined as sepsis to be diagnosed [4]. An increase in bilirubin serum concentration can be a signal of developing liver

dysfunction. The elevation of bilirubin concentration in human plasma of >1.9mg/dl gives a SOFA score of 2. Bilirubin itself is a late and non-specific marker of hepatic dysfunction. Hyperbilirubinemia may be caused by hemolysis, cholestasis and hepatic dysfunction of multiple origins: decreased bilirubin transport, uptake and clearance, as well as hepatic ischemia, hepatocellular damage [2].

Acute liver failure (ALF) is defined as an acute liver injury with an onset of hepatic encephalopathy (HE) and an increase in an International Normalised Ratio (INR) of >1.5, in patients with no pre-existing liver disease [5]. In an ICU setting, due to multiple possible causes of impaired consciousness, diagnosing HE is difficult. Sepsis induced HH is a rare cause of ALF, mainly due to organ hypoperfusion. However, an increased blood flow and cardiac output in the primary stages of septic shock might not be enough to compensate for an increased hepatic oxygen demand and also result in the development of HH [2]. Ischemic/hypoxic hepatitis is reported in up to 2.5% of all the ICU patients [6].

We postulated that the definition of SALD requires redefining and creating more specific diagnostic criteria. The aim of the study was to identify plasma biomarkers, which can be used for an early diagnosis of SALD.

## Materials And Methods

A single-centre, prospective observational study was conducted in a 14-bed medicosurgical ICU at Wroclaw University Hospital from September 2015 to April 2019. The study was approved by the Bioethics Committee of the Wroclaw Medical University, informed written consent was obtained from all of the patients or their families.

The study enrolled adult patients admitted to the ICU due to sepsis/ septic shock and patients who developed sepsis/septic shock during their ICU stay. Sepsis/ septic shock was defined according to the Sepsis-3 criteria [4]. Exclusion criteria were: age <18 years old, pregnancy, pre-existing liver disease (cholestatic disorders; genetic, vascular, metabolic liver diseases; viral hepatitis; liver tumours), liver cirrhosis (Child-Pugh class A, B or C), immunosuppression, HIV infection, cancer and xenobiotics intoxication. Patients with missing data were excluded from the study. All patients were treated according to Surviving Sepsis Guidelines 2012 (patients admitted to the ICU before the publication of new definition and guidelines) and Surviving Sepsis Guidelines 2016 [4, 7].

Once the patient was qualified for the study, clinical and demographic data including: age, sex, comorbidity, Acute Physiology and Chronic Health Evaluation (APACHE II) score, SOFA score and origin of sepsis were recorded in the study protocol. The APACHE II and SOFA scores were subsequently recorded on days 1, 3, 5, 7 and 14. Patients were also screened for the development of Disseminated Intravascular Coagulation (DIC) using ISTH Criteria for DIC on the same days [8].

Plasma biomarkers (prothrombin time, INR, antithrombin III, bilirubin, aspartate transaminase -AST, alanine transaminase - ALT, albumin, alkaline phosphatase – ALP, gamma glutamyl transpherase - GGT)

were measured within 24 hours after enrolment in the study (day 1) and on days 3, 5, 7, 14. An abdominal ultrasound was performed in order to exclude mechanical causes of hyperbilirubinemia.

At the same time blood samples were drawn for the analysis of novel biomarkers: endothelin-1 (ET-1), hepcidin, plasminogen activator inhibitor 1 (PAI-1), thrombin-antithrombin (TAT) complex and interferon-gamma inducible protein 10 kDa (IP-10).

Blood samples were collected and centrifuged at 3000 rpm at room temperature in EDTA tubes, consecutively samples were frozen at -28 Celsius degree within 30 minutes of collection.

Levels of human hepcidin, IP-10, PAI-1, ET-1 were measured using quantikine ELISA tests (R&D Systems Inc., Minneapolis, MN, USA). Human TAT complex levels were measured using Assay Max (Assaypro LLC, St. Charles, USA). All assays employ the quantitative sandwich enzyme immunoassay technique. Tests were performed and interpreted according to the manufacturer's instructions.

Taking SOFA scoring into consideration, we defined SALD as an acute elevation of the serum bilirubin level of 2 mg/dl or more, excluding causes of hyperbilirubinemia other than sepsis.

The primary endpoint of the study was the development of SALD, while staying in the ICU. The secondary endpoint was 28-day overall survival.

## **Analysed biomarkers**

### **IP-10**

IP-10 is induced in different cells, i.e. leukocytes (neutrophils, monocytes, macrophages), in response to type 1 and 2 interferons (IFN) and lipopolysaccharide (LPS) stimulation. IP-10 induces apoptosis, cell growth inhibition, chemotaxis and angiostasis [9]. IP-10 activates C-X-C motif chemokine receptor 3 (CXCR3), in response to viral infections, autoimmune diseases, allotransplantation and cancer, which is an important regulator of natural killer (NK), natural killer T (NKT) and T helper (Th)1 lymphocyte trafficking [10]. In the liver, IP-10 is secreted by hepatocytes in areas of lobular inflammation and may be responsible for the development of intrahepatic inflammation. IP-10 is involved in the pathogenesis of hepatitis C and hepatitis B and has recently been shown to play a pivotal role in the pathogenesis of experimental steatohepatitis [11, 12, 13].

### **ET-1**

Elevated ET-1 plasma levels have been reported in patients with septic shock [14]. ET-1 is a vasoconstrictive molecule synthesised by endothelial cells in response to its injury. It also stimulates phagocytosis and chemotaxis of monocytes/macrophages and neutrophils therefore augmenting the inflammatory response [15]. In a murine model, hepatic macrophages were shown to be the primary source of elevated plasma levels of ET-1 [16]. Hepatic stellate cells, sinusoidal endothelial cells and Kupffer cells are the hepatic source of ET-1. ET-1 being a local regulator of hepatic sinusoidal

microcirculation, acts via ET<sub>A</sub>/ ET<sub>B2</sub> receptors on hepatic stellate cells, causing liver sinusoid constriction [17]. Endothelin-mediated microcirculatory failure leads to hepatocellular injury via worsening the oxygen delivery and metabolic dysfunction during sepsis [18].

## **Hepcidin**

Hepcidin is a peptide of mostly hepatic origin involved in iron metabolism. Increased serum hepcidin levels were observed in neoplastic diseases, inflammation and sepsis [19]. Its expression is suppressed by iron deficiency, anaemia, and hypoxia, but induced by iron overload, inflammatory stimuli and LPS [20]. During inflammatory states, hepcidin expression is induced via cytokine IL-6 [21]. The antimicrobial function of hepcidin was shown in several studies, but the mechanism of both antibacterial and antifungal action is not yet well understood [19]. Iron metabolism may be a useful predictor of outcome in patients with liver disease. In the murine N-acetyl-p-amino-phenol (APAP) - induced acute liver failure model, hepcidin was shown to be an independent predictor of mortality [22].

## **TAT**

TAT, a marker of thrombin generation, forms following the neutralisation of thrombin by antithrombin III (AT III) [23]. AT III is synthesised in the liver and is a natural anticoagulant. Its anti-inflammatory function is due to the neutralisation of thrombin, which is responsible for leukocyte rolling and adhesion, but also it depends on blocking the effect of protease activated receptor-1 [24]. TAT can be used as a sensitive parameter of the latent activation of the clotting pathway. The rise of TAT suggests continuous thrombin generation and antithrombin depletion [25]. In patients with liver cirrhosis, elevated TAT levels are also observed in patients with Child-Pugh A. TAT and AT III are thought to be independently associated with the occurrence of liver dysfunction [26].

## **PAI-1**

PAI-1 is a principal inhibitor of fibrinolysis. Elevated PAI-1 is linked to sepsis-induced coagulopathy and the development of disseminated intravascular coagulation (DIC). PAI-1 levels correlate with the severity of MODS in sepsis and DIC [27]. The PAI-1 gene is expressed in the liver, endothelial cells, macrophages, adipose tissue, the heart and kidney [28]. An increase in plasma PAI-1 after LPS-stimulation may be a combined effect of both PAI-1 release from activated platelets and its synthesis associated with PAI-1 gene expression on hepatocytes [29]. Plasma PAI-1 levels are also strongly related to liver steatosis, which supports the thesis of the liver being an important source of circulating PAI-1 [30].

## **Statistical analysis**

Continuous data are presented as median and lower and upper quartiles for non-normally distributed variables or as mean and standard deviation for the normally distributed variables. Statistical differences between the groups were calculated using the non-parametric Mann-Whitney U test. Statistical significance between the frequencies was calculated using the chi-square test. The relation between the

two parameters was assessed using a correlation analysis and the Spearman correlation coefficients were calculated. Survival curves were obtained using the Kaplan-Meier method and were compared using the log rank test. The multivariate analysis was performed using the Cox proportional hazard regression model. The receiver operating characteristic (ROC) curve analysis was performed to calculate the area under the receiver operating characteristic curve. The best cut-off values were calculated to maximise the Youden index. The positive predictive value (PPV), negative predictive value (NPV) and accuracy (true positive+true negative/N) were also calculated. The P value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed using the EPIINFO Ver. 7.2.3.1 software package.

## Results

During the study period 480 patients were admitted to the ICU due to sepsis /septic shock. According to the study criteria 305 patients were excluded and a further 175 were enrolled into the study. During the observation period 88 patients were excluded, due to causes of hyperbilirubinemia other than sepsis. Another 11 patients were excluded due to an observed increased ALP levels caused by factors other than cholestasis. Finally 79 patients were included into the analysis. The flow of patients is shown on Fig. 1. Sepsis was diagnosed in 32 (40.5%) and septic shock in 47 (59.5%) patients.

Patients were severely ill with a mean APACHE II score of 24. The DIC score according to the ISTH criteria were calculated. None of the patients in the study group developed DIC at any time of observation.

Out of the 79 patients, 24 (30.4%) met the criteria of SALD. None of the patients developing SALD required extracorporeal liver support. Median bilirubin in the SALD group was 2.25 (1.1÷4.16)mg/dl; 3.3 (2.1÷5.67)mg/dl; 5.65 (2.25÷6.95)mg/dl; 5.94 (1.9÷7.6)mg/dl; 4.4 (2.2÷5.6)mg/dl at days 1, 3, 5, 7 and 14 respectively. At the time of enrolment in the study, there was no difference in the severity of the disease between the groups. The characteristics of the patients is shown in Table 1.

**Tab. 1. Patients' characteristics at baseline (day 1).**

	All patients (n=79)	Bilirubin <2mg/dl (no-SALD) (n=55)	Bilirubin ≥2mg/dl (SALD) (n=24)	p-value
Age (years)	63,9 ±16,1	67 (60÷74)	61,0 (46,0÷78,0)	0,63
Sex (female/male)	31/48	22(40%)/33(60%)	9(37,5%)/15(62,5%)	0,83
SOFA (min-max)	10 (7÷13)	9(7÷13)	12(9÷14)	0,09
APACHE II	24 (15÷30)	24 (15÷30)	24,5 (15,5÷28,5)	0,98
Sepsis	32	25 (78,1%)	7 (21,9%)	0,18
Septic shock	47	30 (63,8%)	17 (36,2%)	
<b>Source of sepsis</b>				
Abdominal infection (ABD)	32 (40,51%)	23 (41,82%)	9 (37,5%)	0,91
Pulmonary infection (PNEU)	27 (34,18%)	17 (30,91%)	10 (41,67%)	0,5
Soft tissue infection (TISS)	3 (3,8%)	2 (3,64%)	1 (4,17%)	0,99
Neuroinfection (NEUR)	4 (5,06%)	3 (5,45%)	1 (4,17%)	0,99
Bloodstream infection (BSI)	3 (3,8%)	0	3 (12,5%)	0,026
Urinary tract infection (UTI)	6 (7,59%)	6 (10,91%)	0	0,17
UTI, PNEU	1 (1,27%)	1 (1,82%)	0	0,99
NEUR, BSI	1 (1,27%)	1 (1,82%)	0	0,99
PNEU, BSI	2 (2,53%)	2 (3,64%)	0	0,99
<b>Comorbidities</b>				
HTA	37 (46,84%)	26 (47,27%)	11 (45,83%)	0,89
DM	23 (29,11%)	19 (34,55%)	4 (16,67%)	0,18
CCD	3 (3,8%)	1 (1,82%)	2 (8,33%)	0,22
CA	8 (10,13%)	6 (10,91%)	2 (8,33%)	0,99
OBS	8 (10,13%)	5 (9,09%)	3 (12,5%)	0,69
CKD	18 (23,68%)	15 (27,27%)	3 (12,5%)	0,15
CPD	10 (12,66%)	8 (14,55%)	2 (8,33%)	0,72
ARY	16 (20,25%)	11 (22%)	5 (20,83%)	0,99
ALC	10 (12,66%)	7 (12,73%)	3 (12,5%)	0,99
NEUR	4 (5,06%)	4 (7,27%)	0	0,31
OTH	8 (10,13%)	5 (9,09%)	3 (12,5%)	0,69
28-day survival	49(62± 5,5%)	37 (67,3±6,3%)	12 (50,0±10,2%)	0,12

Data are expressed as mean ± SD, median (interquartile range), or No. (%). p-value - comparison of groups with and without SALD. HTA - hypertension arterial, DM - diabetes mellitus, CCD - chronic cardiac disease, CA - cardiac arrest, OBS - obesity, CKD - chronic kidney disease, CPD - chronic pulmonary disease, ARY - arrhythmia, ALC - alcoholic disease, NEUR - chronic neurological disease, OTH - other

There was no statistically significant difference in comorbidities between the groups. None of the patients in our study group had metabolic syndrome. In our study group neither alcohol abuse nor obesity had a statistically significant impact on the development of SALD. Apart from bloodstream infections (BSI), there was no difference in the source of sepsis between the groups. There was no case of BSI in

bilirubin <2mg/dl group. We evaluated the association between plasma biomarkers measured within the first 24h after enrolment in the study. The analysed biomarkers were also investigated as predictors for the 28-day survival. The plasma biomarkers levels at the time of enrolment into the study (day 1) are shown in Table 2. AST and ALP were significantly higher in the SALD group, compared to the no-SALD group (p=0,0234 and p=0.00348 respectively). What is more, a statistically significant difference between the groups was observed in the PCT levels - 3.99ng/ml (1.69÷17.83) vs 28.0ng/ml (3.1÷71.3), p=0.0135. Consequently, we decided to analyse novel biomarkers at baseline.

**Tab. 2. Plasma biomarkers at baseline (day 1).**

	Normal values	plasma All (n=79)	patients Bilirubin <2mg/dl (n=55)	Bilirubin ≥2mg/dl (SALD) (n=24)	p-value
<b>Routinely measured biomarkers</b>					
AST (U/l)	5-34	590,6±1970,8	51 (29÷155)	124 (38÷325)	0,02
ALT (U/l)	0-55	310,9±1109,7	46 (20÷81)	61,5 (27÷102)	0,16
Albumin (g/dl)	3,2-4,6	2,34±0,515	2,35 (2,0÷2,65)	2,3 (1,8÷2,7)	0,85
AT III (%)	80-120	58,8±20,2	58,5 (45÷81,1)	53,3 (41,3÷61,3)	0,08
INR	0,9-1,3	1,45±0,64	1,26 (1,12÷1,45)	1,35 (1,16÷1,83)	0,13
Prothrombin ratio (%)	80-114	73,9±19,8	79,7 (66,6÷87,8)	73,7 (55,8÷86,3)	0,27
APTT (s)	21-30,1	42,7±16,3	36,9 (30,9÷46,7)	44,3 (33,9÷53,6)	0,09
GGT (IU/l)	<40	166,8±342,4	74,0 (20,0÷97,0)	87,0 (20,0÷231,0)	0,427
ALP (U/l)	40-120	137,7±168,0	75,0 (54,0÷148,0)	147,5 (94,0÷206,0)	0,00348
Thrombocytes (/mcl)	150-400	225,6±107,9	224,0 (161,0÷309,0)	206,5 (133,5÷250,5)	0,121
Creatinine (mg/dl)	0,6-1,3	2,00±1,71	1,70 (0,87÷2,45)	1,48 (0,92÷2,04)	0,707
PCT (ng/ml)	0,05 - 0,1	38,6±108,1	3,99 (1,69÷17,83)	28,0 (3,1÷71,3)	0,0135
CRP (mg/dl)	<5	234,5±116,6	239,8 (141,8÷322,9)	247,2 (155,0÷318,0)	0,444
<b>Novel biomarkers</b>					
IP-10 (pg/ml)	47-382	932,7± 1643,5	321,9 (198,7÷796,9)	315,4 (140,7÷961,1)	0,99
ET-1 (pg/ml)	0,58-1,96	2,06±1,60	1,72 (1,19÷2,45)	1,54 (1,06÷2,39)	0,79
PAI-1 (ng/ml)	0,99-16,9	38,3±53,3	13,1 (6,5÷29,9)	51,6 (9,9÷74,9)	0,02
TAT (ng/ml)	0,5-10	21,3±116,6	4,3 (2,6÷6,5)	3,75 (2,83÷5,75)	0,81
Hepcidin (ng/ml)	82,4-56700	221,8±167,1	195 (101,1÷321,9)	158,2 (93,5÷217,9)	0,35

Data are expressed as mean ± SD, median (interquartile range), or No. (%). p-value - comparison of groups with and without SALD

At baseline we observed a statistically significant difference between the groups only for PAI-1. In the group with no SALD the levels of PAI-1 were significantly lower than in patients with SALD. Subsequently, a ROC curve analysis for PAI-1 was conducted (Fig. 2).



The PAI-1 cut-off was calculated for a maximal value of the Youden index (0.21). PAI-1 values of  $\geq 9$  ng/ml can be a predictor of SALD, with a sensitivity of 79.2%, specificity of 41.8% and PPV - 37.3%, NPV - 82.1% (accuracy of 53.2%).

The Spearman correlation was conducted between PAI-1 and CRP, PCT and the platelet count. No statistically significant correlation was observed between PAI-1 and CRP ( $R=-0.21$ ;  $p=0.0787$ ), PCT ( $R=0.21$ ;  $p=0.0687$ ) or the platelet count ( $R=-0.13$ ;  $p=0.265$ ).

We postulated that at baseline bilirubin is not a good predictor of 28-day survival in patients with sepsis/septic shock (log rank test,  $p=0.117$ ). On the contrary, what is shown on Figure 3., PAI-1 seems to be a good predictor of 28-day survival in patients with sepsis/septic shock (log rank test,  $p=0.00091$ ). AST alone with a cut-off value of 70U/l is not a good predictor of survival (log rank test,  $p=0.0797$ ; specificity 65.2%, sensitivity 68.6%, PPV 48.4%, NPV 81.4%, accuracy 67.6%), but in combination with PAI-1 it improves its strength of 28-day ICU survival prediction (log-rank test,  $p=0.00242$ ; specificity 64.7%; sensitivity 73.9%, PPV 48.6%, NPV 84.6%, accuracy 67.6%).

There was no statistically significant difference between the groups in the severity of the disease (APACHE II score 24 vs. 24.5,  $p=0.979$ ). A multivariate analysis with the use of the Cox PH model has shown that only PAI-1 and APACHE II scores  $>21$  were independent predictors of mortality in the SALD group ( $\chi^2=23.2132$ ,  $p=0.00001$ ).

The Spearman correlation was conducted between IP-10, ET-1, PAI-1, hepcidin, TAT on day 1, and bilirubin in consecutive days 3, 5, 7 and 14. A statistically significant positive correlation was observed between PAI-1 on day 1 and bilirubin in the following days: 3, 5, 7 (respectively: day 3.  $R=0.43$ ,  $p=0.00028$ ; day 5.  $R=0.46$ ,  $p=0.00052$ ; day 7.  $R=0.43$ ,  $p=0.00258$ ). A statistically significant negative correlation was observed between hepcidin on day 1 and bilirubin in the following days: 5, 7, and 14. (respectively: day 5.  $R=-0.29$ ,  $p=0.0329$ ; day 7.  $R=-0.32$ ,  $p=0.0244$ , day 14.  $R=-0.60$ ,  $p=0.0603$ ).

The Spearman correlation was also conducted between IP-10, ET-1, PAI-1, hepcidin and TAT on day 1 and APACHE II and SOFA scores. There was a statistically significant positive correlation between PAI-1 and SOFA ( $R=0.26$ ,  $p=0.0198$ ), but no correlation between hepcidin, IP-10, ET-1, TAT and SOFA or APACHE II scores, as well as no correlation between PAI-1 and APACHE II.

Figure 4. shows median novel biomarkers values on days 1, 3, 5, 7 and 14. There is statistically significant difference between the groups in median values of endothelin-1 ( $p=0.0279$ ), PAI-1 ( $p=0.0111$ ) and hepcidin ( $p=0.00194$ ), but no difference in IP-10 ( $p=0.523$ ) and TAT ( $p=0.522$ ) values on day 3. There is a statistically significant difference in PAI-1 ( $p=0.00432$ ) and TAT ( $p=0.0420$ ) values between the groups on day 5, but no difference in IP-10 ( $p=0.0795$ ), endothelin-1 ( $p=0.0761$ ) and hepcidin ( $p=0.0558$ ). The statistically significant difference remains up to day 7, for PAI-1 and TAT with  $p=0.00942$  and  $p=0.0414$  respectively. There is no difference between the groups in median values of biomarkers on day 14.

## Discussion

The results of our study show that almost one third of septic patients had an acute elevation of bilirubin up to 2mg/dl or more. The results are consistent with available reports [1]. Two thirds of the enrolled patients fulfilled our SALD criteria within 24 hours of diagnosing sepsis/ septic shock.

After analysing our study group demographics, we demonstrate that, apart from BSI, the cause of sepsis, or comorbidities did not contribute to SALD development.

Interestingly, there was no difference in the severity of the disease (APACHE II score) or the degree of organ dysfunction (SOFA score) at the time of inclusion in patients who developed SALD and those who did not. This suggests that hyperbilirubinemia may just be a signal of worsening liver function, which contributes to adverse outcomes via independent pathways (not included in APACHE II scoring) or that the serum bilirubin cut-off value of 2mg/dl is too low to identify patients with an adverse outcome. This is reflected in the results of our study. Mild elevations in bilirubin levels, consequently the degree of organ dysfunction, wasn't severe enough to cause a difference in mortality between the groups. What is more, there is a wide range of other causes influencing mortality in sepsis/septic shock, than hyperbilirubinemia itself. This is also confirmed by the fact, that there was no difference in APACHE II scoring between our study groups.

Bilirubin as a single biomarker is a poor factor in distinguishing newly developing organ dysfunction from a pre-existing one. Due to its late increase and low specificity, finding a single cause of hyperbilirubinemia in ICU patients remains a challenge.

Contrary to Patel et al.[31], in our study, bilirubin was shown to be a poor predictor of survival in septic patients.

Our study was the first to evaluate the correlation between PAI-1 and liver function in septic patients. Out of five analysed biomarkers, only PAI-1 at the time of enrolment could be useful to predict the development of SALD. In various studies the liver has been proven to be an important source of circulating PAI-1 [28, 30]. PAI-1 can be a better marker than bilirubin, predicting organ dysfunction, as a correlation between PAI-1 and SOFA scores was shown. The study revealed that the cut-off value for PAI-1 of 9 ng/ml was subsequent with an acute increase in bilirubin levels of 2mg/dl or more. Interestingly, this cut-off value holds within the normal limits (0.99-16.9ng/ml). There were also individuals reaching higher PAI-1 levels than the calculated cut-off value of 9ng/ml in the no-SALD group. PAI-1 was analysed as a predictive marker, meaning that not all the subjects above the cut-off value develop a liver dysfunction. There must be other risk factors influencing the development of SALD, which requires further investigation. Metabolic syndrome, obesity and alcohol intake are known risk factors increasing the probability of acute liver damage [32]. None of the patients in our study group had metabolic syndrome. Alcohol abuse or obesity did not influence the development of SALD.

Interestingly, significantly higher levels of PCT were observed in the SALD group compared to the no-SALD group (3.99 vs 28.0 ng/ml). Even though there was no correlation between PAI-1 and PCT in our study group, it has been shown in an in vitro study on a human hepatocyte model that high concentrations of procalcitonin might impair the hepatocytes function and lead to liver injury [33, 34]. This requires further investigation.

Our results have shown the low specificity of PAI-1 with a high negative predictive value. The low specificity of PAI-1 may be a result of many origins and different factors inducing its synthesis. An increase in plasma PAI-1 after LPS-stimulation may be a combined effect of both the PAI-1 release from activated platelets and its synthesis associated with PAI-1 gene expression on hepatocytes [29]. The results obtained in our study group may indicate that the activated platelets could play a greater role in the increase in PAI-1 levels, than its hepatic source does.

Other authors have investigated PAI-1 as a biomarker of sepsis and DIC. Koyama et al. [35] investigated PAI-1 combined with TAT and protein C as predictors for the development of overt DIC. In our cohort none of the patients developed DIC, so it was impossible to confront these results with ours. The incidence of DIC in septic patients, diagnosed with ISTH criteria, is reported to be approximately 29% [36]. The authors think that the cause of the lack of overt DIC in the study group was a result of the used inclusion criteria resulting in choosing only 79 out of 480 (28.9%) septic patients treated in our ICU. In terms of survival, Koyama's [35] results were compatible with ours, confirming PAI-1 to be a predictor of survival in septic patients. What is more, the PAI-1 and APACHE II score above 21 were shown to be independent predictors of mortality. Higher PAI-1 levels were related to higher SOFA scoring at the onset of sepsis, but there was no reflection in APACHE II scoring at the time of inclusion.

We also evaluated PAI-1 in combination with AST as a predictor of 28-day survival. AST and ALT are the markers of hepatocellular integrity. However AST is a sensitive marker of liver cell injury, its specificity remains questionable. Apart from the liver it can be found in cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocyte and erythrocytes. AST levels usually peak before ALT, especially in ischemic or toxic liver injury. Acinar zone 3 is vulnerable to hypoxic damage and the enzyme is mostly distributed in the peripheral site of the acinus [37]. This was confirmed in our study, as the levels of AST at day 1 were significantly higher in the SALD group. This may also indicate ischemic damage as one of the causes of SALD. Moreover, higher levels of ALP in the SALD group indicate that cholestasis might be another presentation of septic liver dysfunction, especially as the authors have excluded patients with increased levels of ALP due to causes other than sepsis induced cholestasis from analysis.

Low cut-off AST values presented in our study are again proof that liver dysfunction in our study group wasn't severe (sepsis-induced cholestasis usually presents as milder condition than hypoxic hepatitis), and the cut-off bilirubin of 2mg/dl was too low of a value to diagnose SALD with a potential adverse outcome.

Our analysis revealed that a combination of PAI-1 and AST better predicts 28-day survival than PAI-1 alone, but due to the low cut-off values of AST this might not be clinically significant.

The analysis of further days of the observation showed that PAI-1 remained higher during the first week of observation in the group of patients who developed SALD. Higher PAI-1 values observed on day 1 were related to a greater increase in bilirubin levels on days 3, 5 and 7, which confirms a potential usefulness of the marker as a predictor of liver dysfunction.

Lower hepcidin values observed on day 1 were related to a greater increase in bilirubin levels on days 5, 7 and 14. ET-1 and hepcidin on day 3 and TAT on day 5, could be taken into consideration as predictors of SALD, as their difference between the SALD and no-SALD group remained significant, but our study group was too small to conduct further analysis.

The limitations of the study were: it was a single-centre study and the number of patients enrolled into the study was relatively small; although our results (SALD incidence) matched those demonstrated in previous studies [1]. The lack of generally accepted SALD definition remained a challenge.

PAI-1 may be a marker prognosing SALD, but due to its low specificity and high negative predictive value, it has to be analysed in combination with other markers. AST levels at the time of admission might be a good choice. Further studies are needed to find a unique biomarker of SALD, but firstly to broaden knowledge on its pathophysiology and to find a unanimous definition and define clear diagnostic criteria.

## **Conclusions**

Sepsis-associated hyperbilirubinemia is frequent, but bilirubin is a late and non-specific marker of SALD. Measuring PAI-1 serum levels at the onset of sepsis/septic shock may be useful in predicting the development of SALD. A combination of PAI-1 and AST better predicts 28-day survival than PAI-1 alone, but due to the low cut-off values of AST, this might not be clinically significant. Very subtle, but still significant differences and low cut off values of the markers prove the authors' hypothesis that a cut-off value of bilirubin  $\geq 2$ mg/dl is too low, and higher cut-off values of bilirubin or combination of bilirubin with other markers of liver injury, might be needed to distinguish a group of patients with SALD leading to an adverse outcome.

## **Declarations**

### **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

The study was approved by the Bioethics Committee of Wroclaw Medical University (KB 415/2015; KB – 670/2017). Informed consent was obtained from all patients or their families.

### **CONSENT FOR PUBLICATION**

NOT APPLICABLE

### **AVAILABILITY OF DATA AND MATERIALS**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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## AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation was performed by EWN, PL and LL. Data collection and analysis were performed by EWN, PL, JJ, RW and LL. Biomarkers assay was made by MZ. The first draft of the manuscript was written by EWN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## ACKNOWLEDGMENTS

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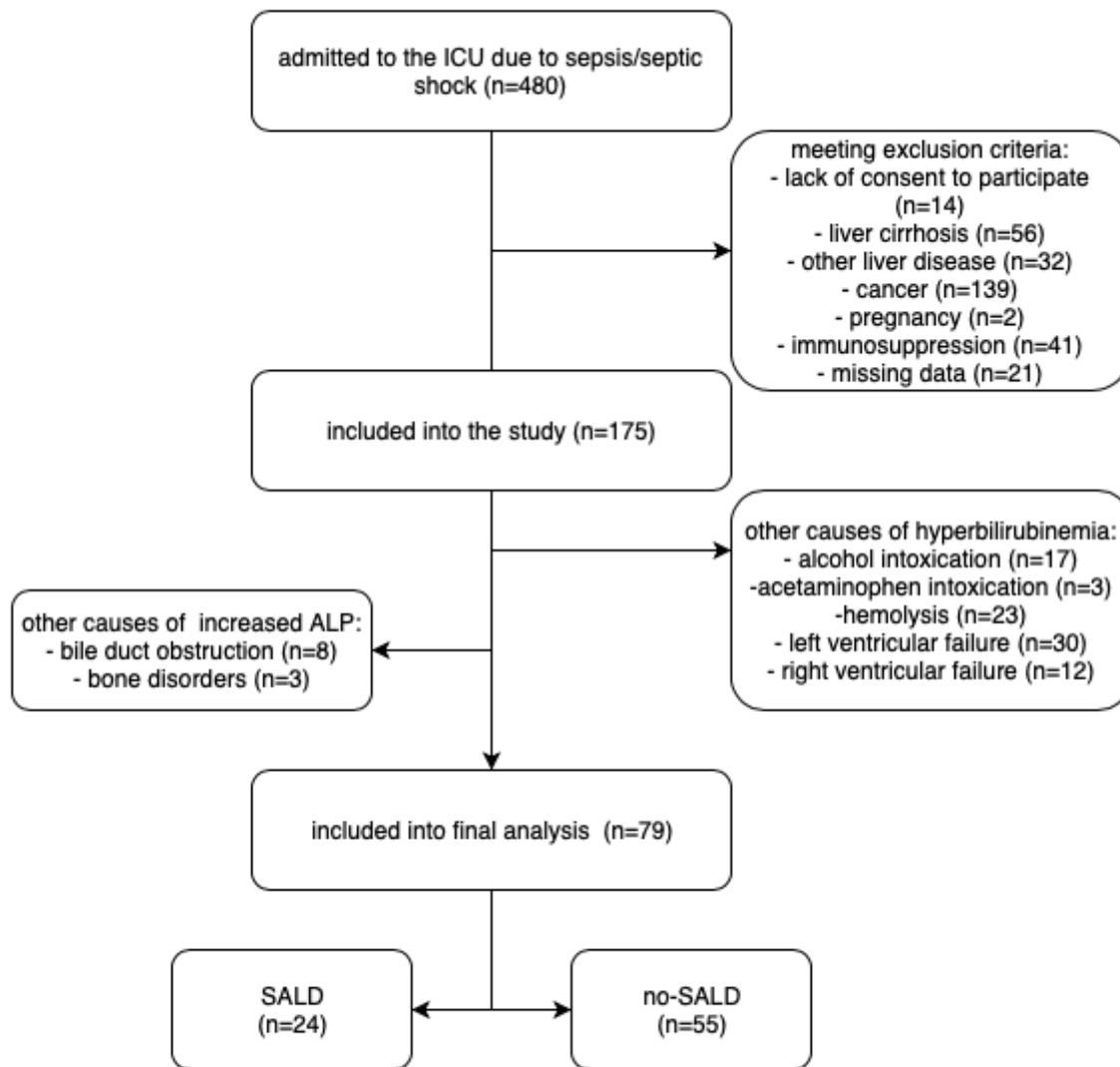
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## Figures





**Figure 1**

Flow of the patients included in the study.

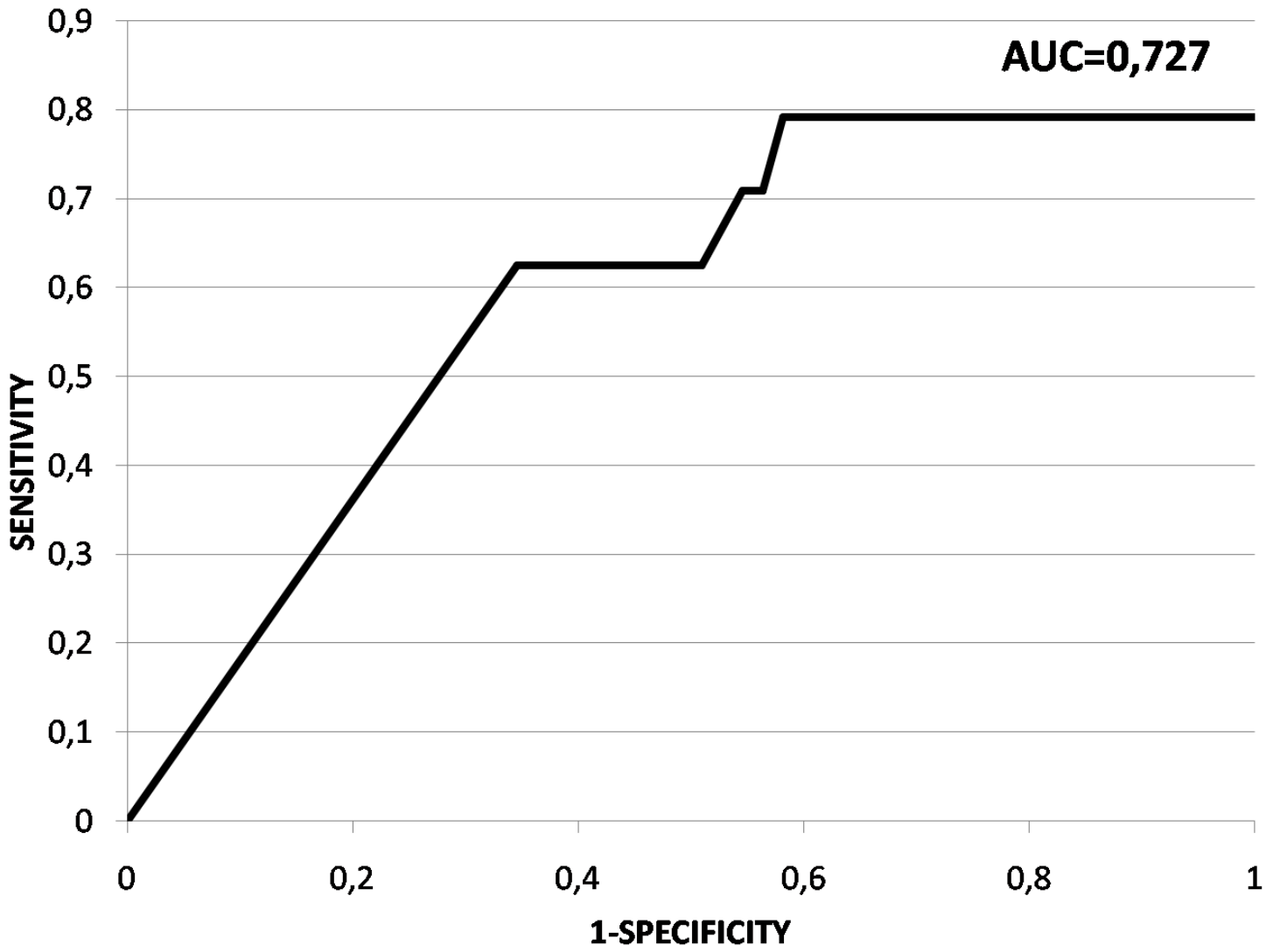
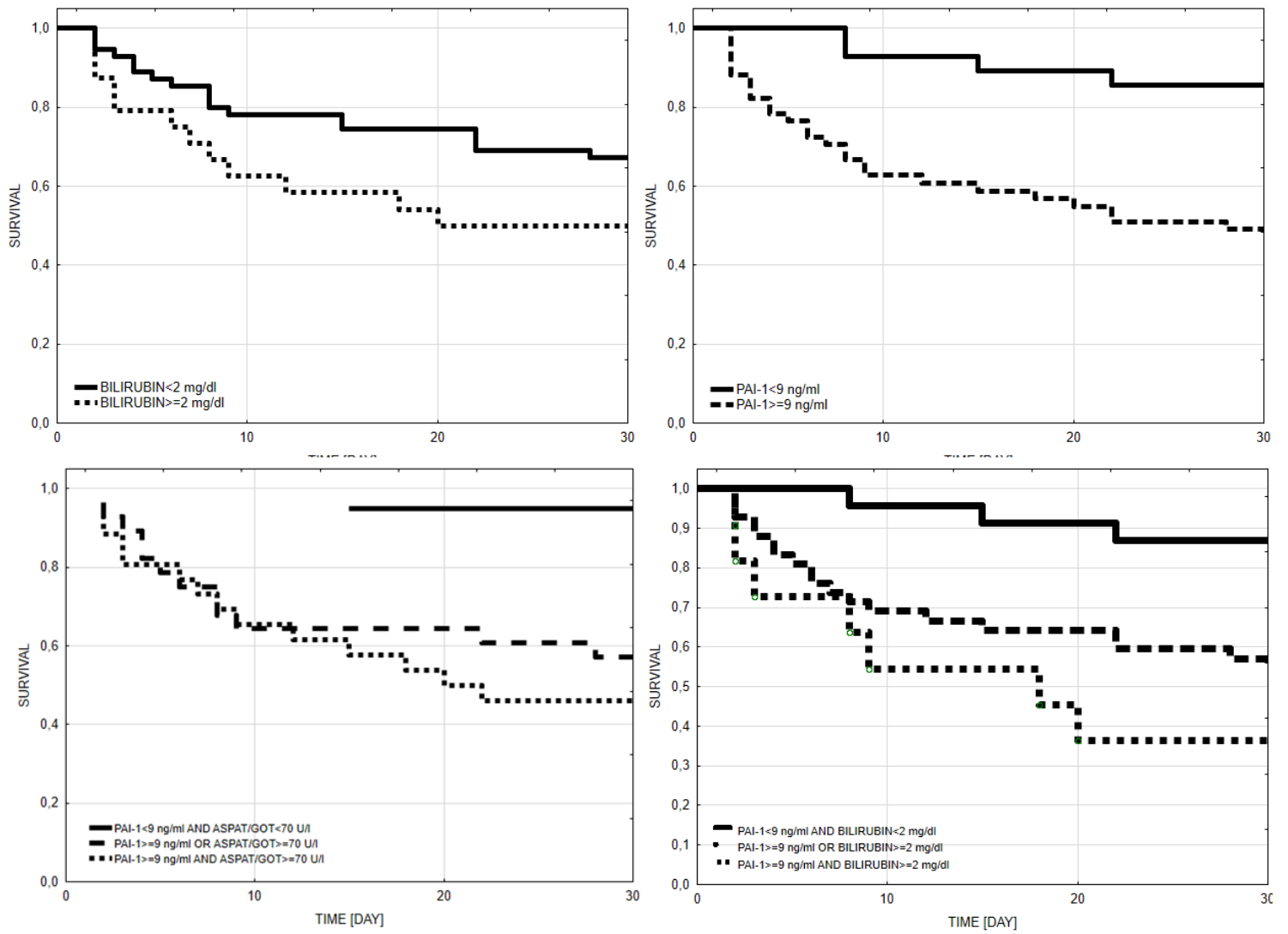


Figure 2

PAI-1 ROC curve for the prediction of SALD.



**Figure 3**

Kaplan-Meier survival curves for bilirubin (Log rank test  $p=0.117$ ), PAI-1 (log rank test,  $p=0.00091$ ), PAI-1+bilirubin (log rank test,  $p=0.00524$ ) and PAI-1+AST (log rank test,  $p=0.00242$ ).

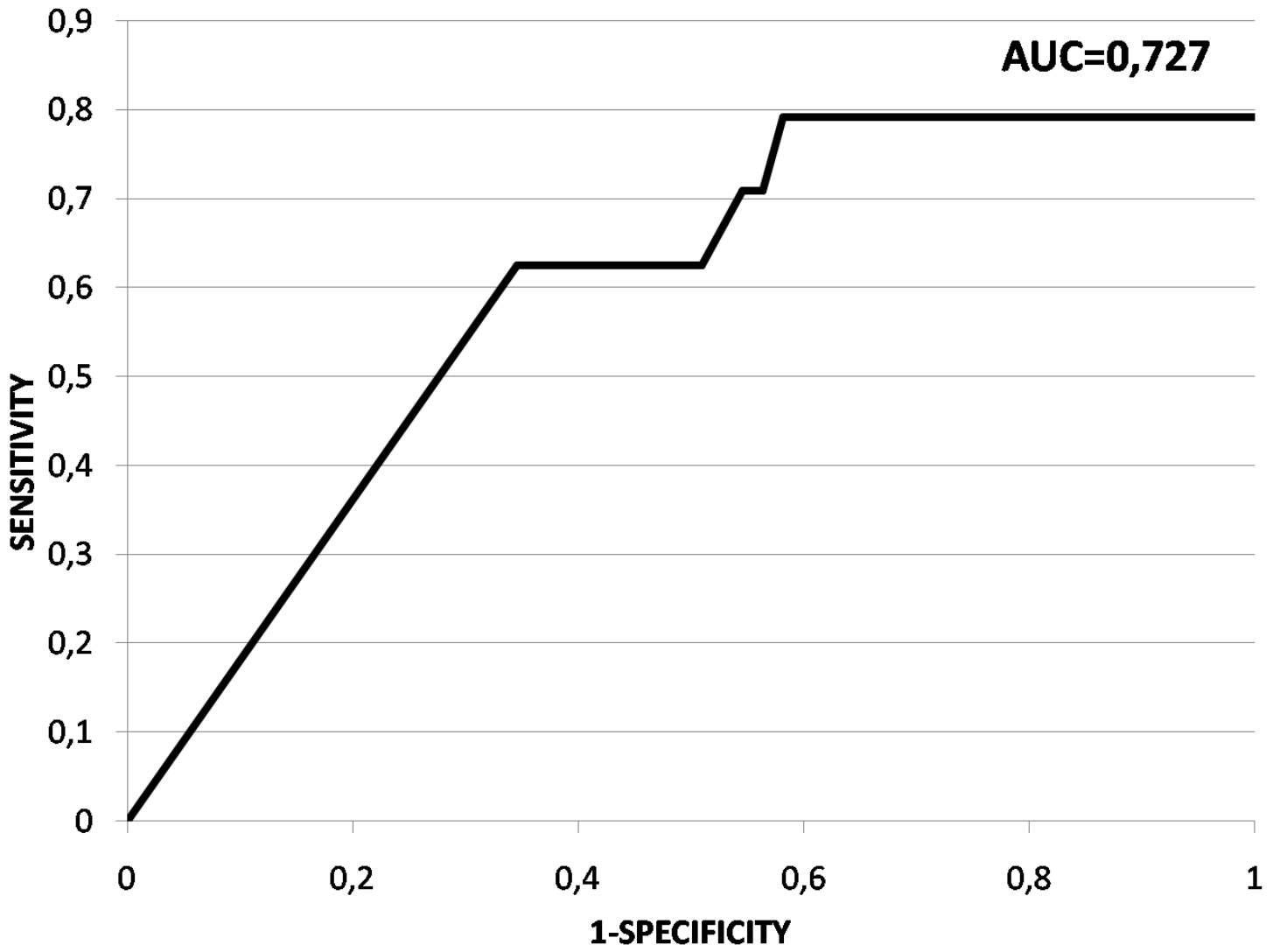


Figure 4

PAI-1 ROC curve for the prediction of SALD.