Opis

Lista lucărilor din portofoliu de lucrări ştiinţifice considerate relevante de către candidat (maxim 10 lucrări), elaborate în domeniul de doctorat vizat

1. **Adela Golea**, Christiana Dumulesc, Andrei Stărică, Sorana D. Bolboacă, Raluca Tat. *Amplitude spectrum area as an indicator of effective return of spontaneous circulation in prehospital resuscitation—experience from a single regional center in Romania.* Signa Vitae. 2024; 20(10): 47-55. **Q3, IF 1 (prim autor) – pg. 2-10**
2. Sonia Luka, **Adela Golea**, Raluca Mihaela Tat, Eugenia Maria Lupan Muresan, George Teo Voicescu, Stefan Cristian Vesa, Daniela Ionescu. *Biomarkers as Predictors of Mortality in Sepsis and Septic Shock for Patients Admitted to Emergency Department: Who Is the Winner? A Prospective Study.* J. Clin. Med. 2024. 13(19): 5678 (1-12). **Q1, IF 3 (autor correspondent) – pg.11-22**
3. Monica Puticiu, Mihai-Bujor Grecu, Luciana Teodora Rotaru, Mihai Alexandru Butoi, Gabriela Vancu, Mihaela Corlade-Andrei *, Diana Cimpoesu *, Raluca Mihaela Tat, **Adela Golea**. *Exploring Burnout, Work Addiction, and Stress-Related Growth among Prehospital Emergency Personnel.* Behavioral Sciences 2024; 14(9): 851 (1-14). **Q2, IF 2,5 (ultim autor) – pg.23-36**
4. Eugenia-Maria Muresan, **Adela Golea**, Ştefan Cristian Vesa, Manuela Lenghel, Csaba Csutak, Lacramioara Perju-Dumbravă. *Emergency department point-of-care biomarkers and day 90 functional outcome in spontaneous intracerebral hemorrhage: A single-center pilot study.* Experimental and Therapeutic Medicine 2022; 36(3): 1534-1543. **Q3, IF 2,7 (autor cu drepturi egale) – pg.37-44**
5. Muresan EM, **Golea A**, Vesa SC, Givan I, Perju-Dumbrava L. *Admission Emergency Department Point-of-care Biomarkers for Prediction of Early Mortality in Spontaneous Intracerebral Hemorrhage.* IN VIVO 2022; 36(3): 1534-1543. **Q3, FI=2,3 (autor cu drepturi egale) – pg.45-54**
6. Tat RM, **Golea A**, Vesa ŞC, Răhaian R, Ionescu D. *Resistin and cardiac arrest – A prospective study.* JOURNAL OF CLINICAL MEDICINE 2020; 9(1): 57 (1-12). **Q1, IF 4,24 (autor cu drepturi egale) – pg.55-66**
7. Raluca Mihaela Tat, **Adela Golea**, Ştefan Cristian Vesa, Daniela Ionescu. *Resistin—Can it be a new early marker for prognosis in patients who survive after a cardiac arrest? A pilot study.* PloS one 2019; 14 (1): e0210666. **Q2, IF 3,24 (autori cu drepturi egale) – pg.67-78**
8. AD Farcas, CL Vonica, **A. Golea**. *Non-alcoholic fatty liver disease, bulb carotid intima-media thickness and obesity phenotypes: results of a prospective observational study.* Medical ultrasonography 2017; 19 (3): 265-271. **Q3, IF 1,55 (ultim autor) – pg.79-85**
9. **Adela Golea**, Adriana Rusu, Christiana Dumulesc, Cornelia Bala. *"Investigation of biomarkers variation post-return of spontaneous circulation following an out-of-hospital cardiac arrest".* Revista Română de Medicină de Laborator 2017; 25 (3): 245-253. **Q4, IF 0,14 (prim autor) – pg.86-95**
10. **Adela C. Golea**, Eugenia G.P. Mureşan, Sorin S. Lăcan, Mihaela Gh. Pasc, Sorana D. Bolboacă. *Ultrasonography as an integrated tool in clinical decision-making in the Emergency Department.* Medical ultrasonography 2016; 18(4): 419-424. **Q3, IF 1,16 (prim autor) – pg.96-101**

Situaţie poziţie autor: Prim autor – 3 articole/Ultim autor – 2 articole/Autor corespondent – 1 articol/ Autor cu drepturi egale – 4 articole

Submitted: 05 January, 2024 Accepted: 06 May, 2024 Published: 08 October, 2024 DOI:10.22514/sv.2024.125

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Signa Vitae

ORIGINAL RESEARCH

Amplitude spectrum area as an indicator of effective return of spontaneous circulation in prehospital resuscitation—experience from a single regional center in Romania

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Abstract

Analysis of electrocardiography (ECG) signals recorded during cardiopulmonary resuscitation showed that it could be effectively used to predict successful defibrillation. The amplitude spectrum area (AMSA) was not affected by chest compression and showed potential as a monitoring parameter for defibrillators. This retrospective observational study aimed to evaluate AMSA values during out-of-hospital cardiac arrest (OHCA) due to ventricular fibrillation (VF) and to identify the optimal AMSA value indicating a higher chance of return of spontaneous circulation (ROSC) maintained until Emergency Department (ED) admission. Additionally, we examined factors influencing AMSA and ROSC in our emergency medical services (EMS) system. To achieve these, we analyzed the AMSA values of each patient with OHCA and VF using ECGs recorded before each manual defibrillation. Patient data were collected from the EMS database, prospectively gathered from 01 July 2014, to 30 April 2015. The cohort of 46 patients was divided into two groups: Group 1, admitted to the ED with ROSC (n = 25), and Group 2, who died at the scene (n = 21). Successful defibrillation resulted in ROSC for 21 patients (45.65%). Statistically significant higher AMSA values ($p < 0.0029$) were observed in Group 1 (30.77 $\pm$ 13.20 mV-Hz) compared to Group 2 (23.21 $\pm$ 10.73 mV-Hz). AMSA values of 27.6 mV-Hz were associated with a specificity of 73.33% for ROSC after manual defibrillation. In Group 1, 64% of patients had a shorter time to start advanced life support (ALS) of less than 5 minutes ($p = 0.0798$). Additionally, a significantly lower dose of adrenaline was observed in Group 1 ($p < 0.0001$). Fewer defibrillation attempts were required in Group 1 compared to Group 2 ($p = 0.0872$). In conclusion, a delay in the initiation of ALS (>5 minutes) and delayed manual defibrillation attempts are associated with lower AMSA values and reduced defibrillation efficiency.

Keywords

Amplitude spectrum area (AMSA); Ventricular fibrillation (VF); Defibrillation; Out-of-hospital cardiac arrest (OHCA); Return of spontaneous circulation (ROSC)

1. Introduction

Annually, over 55 out of 100,000 people suffer cardiac arrest in Europe [1]. The European Resuscitation Council introduced the Registry of Cardiac Arrest (EuReCa), which is composed of 29 countries that cooperate to provide data on out-of-hospital cardiac arrest (OHCA). The recorded data show an incidence of OHCA between 67 and 170 per 100,000 inhabitants, with a low rate of bystander cardiopulmonary resuscitation (CPR) (average 58%, range 13%–83%) and insufficient use of automated external defibrillators (AEDs) (average 28%, range 3.8%–59%) [2]. In 20–30% of cardiac arrest cases, ventricular fibrillation (VF) is the initial registered arrhythmia [3]. Current resuscitation guidelines recommend defibrillation as the initial therapy for VF [4]. However, repetitive unsuccessful defibrillation attempts may cause myocardial injury, ranging from a reversible decrease in myocardial contraction to irreversible myocardial necrosis [5–7]. When a shock is not immediately available, CPR prior to defibrillation increases coronary artery perfusion, thus improving the chances of successful defibrillation and return of spontaneous circulation (ROSC) [8]. Most of the time, CPR is unable to generate adequate coronary blood flow, necessitating the use of a vasopressor agent, such as adrenaline [8]. A predictive indicator for successful defibrillation, such as the amplitude spectrum area (AMSA), reflecting the myocardial energy state, could be very useful in deciding

when to stop CPR for defibrillation [9].

The interpretation of the electrocardiogram (ECG) can be challenging due to the effects of chest compression, making it necessary to interpret the ECG without this interference. According to the literature, AMSA has been reported as an ECG parameter unaffected by chest compression [10, 11]. The analysis of AMSA during resuscitation has proven valuable in predicting the success of defibrillation [12–14]. However, AMSA analysis is not yet sufficiently reliable to be adopted as an indicator and, therefore, is not integrated into defibrillator software.

Several studies have analyzed short strips of VF, ranging from 2.05 to 5 seconds, with threshold values ranging from 10 to 27.6 mV-Hz reported in the scientific literature [8, 9, 13–15]. However, AMSA analysis is not yet sufficiently reliable to be adopted as an indicator because the reported performances are heterogeneous and, therefore, are not integrated into defibrillator software (Table 1) [13, 15–18].

Moreover, Ruggeri *et al.* [18] highlighted that AMSA values below 6.5 mV-Hz are ineffective in 86% of cases. Comorbidities, drug therapy, bystander CPR, time to start advanced life support (ALS), and adrenaline dosages during CPR have all been demonstrated to influence AMSA [12, 18, 19]. However, no single factor affecting AMSA has yet been identified.

This study aims to evaluate the AMSA values of VF in OHCA and determine the optimal value to predict the return and maintenance of spontaneous circulation until admission to the Emergency Department (ED). Furthermore, we also aimed to identify factors that affect AMSA and ROSC, considering the specific characteristics of the emergency medical service (EMS) system in Romania.

2. Materials and methods

The prehospital emergency medical system in the studied region is operated by three types of prehospital ambulance teams with different resuscitation competencies: (1) SMURD (Mobile Emergency Service for Resuscitation and Extrication) team with higher competencies, capable of performing basic life support (BLS), manual defibrillation, and ALS; (2) Nurses' team with medium competences, capable of performing BLS and defibrillation using AEDs, and sometimes ALS under regional hospital guidance in remote areas until SMURD teams arrive; and (3) Fire department paramedics team with low competences, capable of performing BLS and using AED.

In Romania, CPR training (BLS and defibrillation with AED) for laypersons is limited compared to other European countries, despite sustained efforts by EMS workers to emphasize the importance of early resuscitation. Dispatchers are able to guide laypeople to deliver CPR over the phone in only a small number of cases, and even then, they often face reluctance from the public to start CPR. Given these conditions, for this study, we analyzed data from the SMURD teams, which handle more than 85% of OHCA cases in the region. These teams are standardized and use the same manual defibrillator device, the Corpuls 3 (GS Elektromedizinesche Geräte G. Stemple GmbH, Kaufering, Bavaria, Germany).

2.1 Study design

We conducted a retrospective, observational cohort analysis using a database of OHCA patients from 01 July 2014, to 30 April 2015. Data from the register were prospectively collected during this interval primarily to gather post-ROSC biomarker information. The study included all consecutive patients with OHCA, older than 18 years, who experienced VF either as the initial ECG rhythm (iVF) or developed VF during prehospital resuscitation (noted as no-iVF). The eligible population with OHCA comprised patients diagnosed with cardiac arrest by emergency physicians working on the prehospital EMS of Cluj County, specifically the SMURD teams. The SMURD team comprised an emergency physician, a nurse, and two paramedics. The teams had standardized equipment, including a manual Corpuls 3 defibrillator with patches, and they followed the European Resuscitation Council Guidelines protocol for cardiac arrest.

We excluded patients younger than 18 years, those with known end-stage oncological diseases (information obtained from the patient's family or medical records on site), patients with polytrauma (defined as having at least two injuries, one of which is life-threatening), those with medium or severe head trauma according to the Miller scale, with severe thoracic trauma (defined as rib fractures associated with pneumothorax or hemothorax), and patients with proximal limb amputation. Additionally, we excluded patients with incomplete ECG recordings and ECG strips under 10 seconds from the analysis.

Patients were declared in cardiac arrest if they were found unconscious and without spontaneous breathing, following

TABLE 1. Amplitude spectrum area cutoff and associated performances (data from the literature).

Authors	No. of ECG VF traces	ECG trace duration analyzed (seconds)	AMSA value (mV-Hz)	Se (%)	Sp (%)	NPV (%)	PPV (%)
Nakagawa *et al.* [17] (2012)	n/a	n/a	24.2	n/a	n/a	n/a	n/a
Ristagno *et al.* [15] (2013)	1260	2.0	17.0	n/a	97	n/a	n/a
Indik *et al.* [16] (2014)	n/a	4.1	24.5	n/a	n/a	n/a	n/a
Ristagno *et al.* [13] (2015)	1381	2.0	15.5	36	97	76	84
Ruggeri *et al.* [18] (2023)	2447	2.0–5.0	15.5	n/a	n/a	84	77

No.: number; ECG: electrocardiography; VF: ventricular fibrillation; AMSA: amplitude spectrum area; Se: sensitivity; Sp: specificity; NPV: negative predictive value; PPV: positive predictive value; n/a: not available.

resuscitation guidelines. The initiation of chest compressions, attachment of defibrillation patches for rapid rhythm assessment, and support of ventilation were immediately and synchronously performed by the members of the SMURD resuscitation team, according to their assigned roles and professional training. Resuscitation medication was provided in accordance with the identified cardiac arrest rhythm, following the recommendations of the resuscitation guidelines.

ROSC was defined as the return of a sustained cardiac rhythm that could allow blood circulation throughout the body after cardiac arrest, according to resuscitation guidelines. Death at the scene was declared when a life-sustaining heart rhythm was not achieved despite following the entire ALS protocol, including asystole lasting more than 20 minutes, and after all potential reversible causes of cardiac arrest had been identified and corrected, in accordance with resuscitation guidelines.

2.2 Source of raw data

The patient's ECG strip was continuously recorded during CPR and collected on the Corpuls 3 defibrillator card. The defibrillators recorded patient data, which was later extracted, transferred, and analyzed. The ECG strips did not contain any information that could identify individual patients. The prehospital teams collected the following data for each patient: age, sex, urban or rural residence, initial cardiac arrest ECG rhythm (iVF/no-iVF), time to ALS (time from the call being received by the dispatch center to the ambulance arriving at the scene and starting resuscitation), bystander CPR, acute myocardial infarction (AMI), history of cardiovascular disease (CVD), number of defibrillations and dosages of adrenaline during resuscitation.

2.3 Electrocardiogram analysis

The ECG strips recorded by the manual Corpuls 3 defibrillator were set at a speed of 25 cm/s on the second standard bipolar limb lead (DII) and exported to MATLAB (MathWorks, Natick, MA, USA). We analyzed periods of VF during the 10 seconds before each shock delivery, which is the time interval from the detection of a shockable rhythm to the actual delivery of the shock.

Each period was visualized using the memory card of the Corpuls 3 defibrillator, extracted as *.jpg files (Fig. 1), and converted from the time domain to the frequency domain using Fourier Transformation. The AMSA was calculated using the MATLAB platform. AMSA represents the sum of the products of individual amplitudes and their frequencies, according to the formula: $AMSA = \Sigma\ A_i \times F_i$, where A_i represents the amplitude and F_i is the frequency. The AMSA values were analyzed individually and divided into two groups: "success", defined as being admitted to the ED with ROSC after defibrillation (Group 1), and "failure", defined as prehospital death (Group 2). No filter for artifacts was applied, based on the findings of Young *et al.* [10], which indicated no significant changes in sensitivity or specificity when using a lower cutoff frequency to filter chest compression artifacts.

2.4 Statistical analysis

An independent sample *t*-test was used to compare normally distributed raw data and summarized as means and standard deviations. The Mann-Whitney test was used to compare groups that did not follow a normal distribution and reported as medians and IQR (Interquartile Range defined as [Q1 to Q3], where Q1 represents the first quartile and Q3 the third quartile). Categorical data are shown as absolute and relative frequencies, and comparisons between groups were performed using the Chi-Squared test or Fisher's exact test, depending on the theoretical frequencies.

Regression analysis was conducted to identify factors associated with ROSC. Independent variables with a *p*-value of up to 0.10 were included in the univariable regression analysis using the Wald stepwise methodology. All variables identified in the univariable model with at least a tendency toward statistical significance were included in the multivariable regression analysis.

Sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (Acc) were calculated for different AMSA thresholds. The criteria used to establish the cutoff AMSA values included the minimum AMSA value in Group 1, the arithmetic mean of AMSA in Group 2, the AMSA third quartile in Group 2, and the 90th percentile of AMSA in Group 1.

Statistical analysis was performed using the Statistica program (v8, StatSoft, Tulsa, OK, USA) and JASP (Jeffreys's Amazing Statistics Program, v.v0.18.30., Amsterdam, Netherlands). A significance level of 5% was applied, with *p*-values < 0.05 considered statistically significant.

3. Results

3.1 Characteristics of the patients with OHCA

Of the 73 cases retrieved, 46 met the inclusion criteria and were included for study analysis. The results showed that only 25 (14.20%) defibrillation attempts of 176 "10-second ECG strips" recorded during this study were successful. In 21 cases, defibrillation attempts resulted in ROSC (11.93%), and in 4 cases, a rhythm compatible with life, pulseless electrical activity (PEA), was followed by ROSC (Fig. 2).

The patients' age ranged from 26 to 87 years, with a mean of 58.59 ± 14.19 years. No statistically significant differences were observed between the study groups, except for the incidence of acute myocardial infarction and doses of adrenaline administered during resuscitation (Table 2). Most of the patients were men from urban settings, and half of the patients admitted to the ED had a diagnosis of myocardial infarction. Only 34.78% of patients received bystander assistance for OHCA before the arrival of the ambulance team.

3.2 AMSA and defibrillation efficiency

The number of VF sequences (AMSA values calculated) for each patient recorded during 10 seconds varied from one to eight (median = 3, IQR = [1 to 4]) in Group 1, and from two to thirteen (median = 4, IQR = [2 to 7]) in Group 2 (Mann-

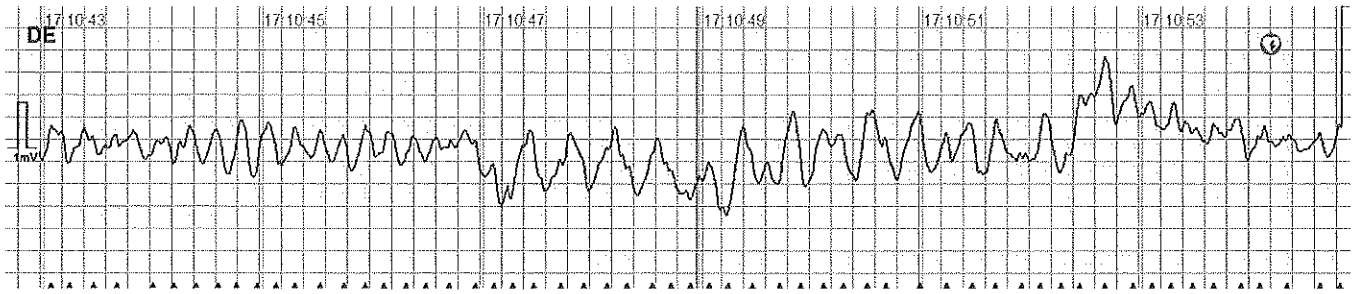

FIGURE 1. Illustration of 10-second ventricular fibrillation strips used for amplitude spectrum area analysis. DE: Deutschland (screen device inscription).

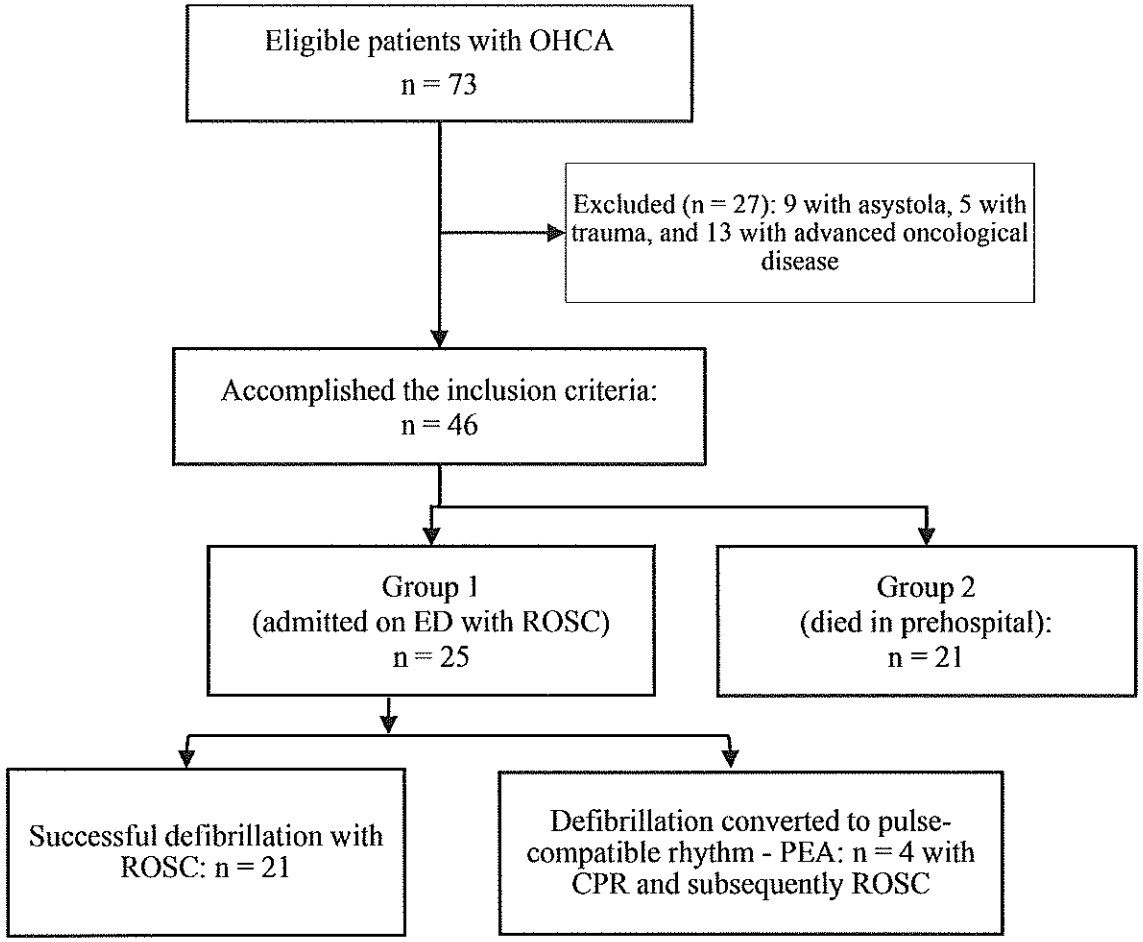

FIGURE 2. Flow diagram summarizing the out-of-hospital outcome: from subjects with out-of-hospital cardiac arrest (OHCA) identification to the selection of patients included in the study. n: sample size; ED: emergency department; ROSC: return of spontaneous circulation; PEA: pulseless electrical activity; CPR: cardiopulmonary resuscitation.

Whitney test: $p = 0.160$). The AMSA values were significantly higher ($p = 0.003$, Fig. 3) in Group 1, with a mean of 30.77 ± 13.20 mV-Hz (ranging from 13.60 mV-Hz to 72.49 mV-Hz), compared with Group 2, which had a mean of 23.21 ± 10.73 mV-Hz (ranging from 7.43 mV-Hz to 68.85 mV-Hz).

Further analysis showed that the values of the last AMSA (mV-Hz) were statistically significantly higher in Group 1 (median = 29.99, IQR= [18.99 to 33.85]) than in Group 2 (median = 15.59, IQR= [10.77 to 25.01]) (Mann-Whitney test: $p = 0.007$). As expected, although different performances for various AMSA cutoff values were observed, all demonstrated limited abilities in identifying ROSC (Table 3).

3.3 Possible confounders and clinical outcome

Data analysis showed that the time to ALS and the presence of bystanders influenced the evolution of VF and its parameters, including the AMSA value, thereby reducing the chances of survival. A shorter ALS time (≤5 minutes) was more common in Group 1, but the comparison showed only a tendency towards statistical significance when compared to Group 2 (Table 4).

Although the number of initial bystander CPR instances was low (approximately one-third in each group), the absence of bystanders had a statistically significant negative impact on

TABLE 2. Baseline characteristics of the patients included in the study.

Variable	Group 1 (n = 25)	Group 2 (n = 21)	*p*-value
Age, yr[1]	57.28 ± 15.18	60.14 ± 13.11	0.5016
Male[2]	19 (76.0)	18 (85.7)	0.4777[3]
Urban[2]	21 (84.0)	16 (76.2)	0.7114[3]
iVF[2]	15 (60.0)	7 (33.3)	0.0713
Time to ALS, min[4]	5 [4 to 6]	7 [4 to 12]	0.0723
Bystander CPR[2]	9 (36.0)	7 (33.3)	0.8500
AMI[2]	13 (52.0)	1 (4.8)	<0.0001
History of CVD[2]	16 (64.0)	17 (81.0)	0.1878
Hypertension[2]	14 (56.0)	16 (76.1)	0.1521
Ischemic cardiomyopathy[2]	11 (44.0)	12 (57.1)	0.3745
Congestive heart failure[2,3]	3 (12.0)	6 (28.6)	0.1133
Other cardiovascular comorbidities[2]	0	5 (23.8)	n/a
Defibrillation attempts[4]	3 [1 to 4]	4 [2 to 7]	0.0872
Adrenaline during resuscitation, mg[4]	4 [3 to 7]	13 [10 to 15]	<0.0001

[1]*m ± SD, where m: mean and SD: standard deviation, comparison between groups with student t-test;* [2]*no. (%), where no.: count and %: percentage, comparison between groups with Chi-squared test or* [3]*Fisher's exact test;* [4]*median [Q1 to Q3], where Q1: 25th percentile; Q3: 75th percentile, comparison between groups with Mann-Whitney test. n: sample size; yr: year; iVF: initial ventricular fibrillation rhythm of cardiac arrest; ALS: advanced life support; min: minute; CPR: cardiopulmonary resuscitation; AMI: acute myocardial infarction; CVD: cardiovascular disease; n/a: not applicable; mg: milligram.*

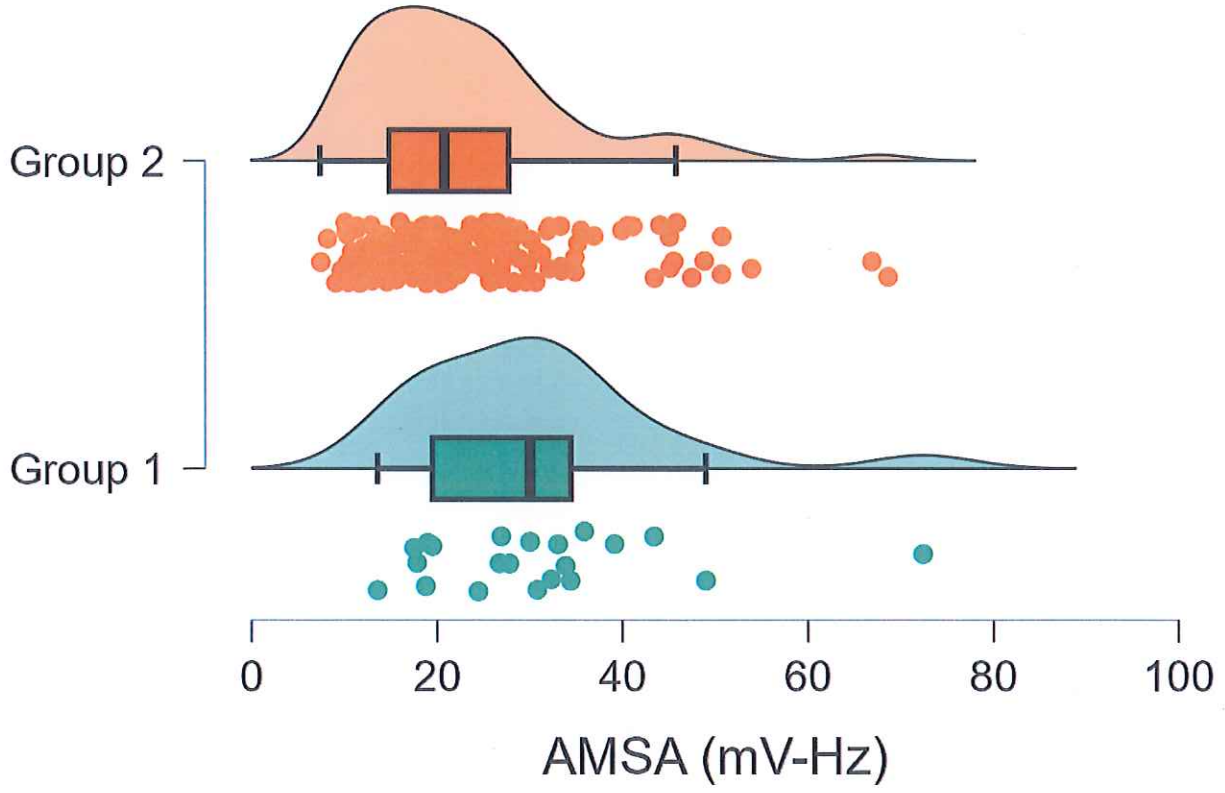

FIGURE 3. AMSA values in Group 1 compared with Group 2: success *vs.* failure in ROSC. The value of median gives the line in the box, the lower and upper box are the values of first and respectively third quartile, while the whiskers are the minimum and maximum values. The ° show raw data. AMSA: amplitude spectrum area; ROSC: return of spontaneous circulation.

TABLE 3. Analysis of AMSA values performances for successful ROSC.

AMSA (mV-Hz) cutoff	Se (%)	Sp (%)	PPV (%)	NPV (%)	Acc (%)
13.6	95.2	25.8	14.8	2.4	34.0
23.2	84.6	57.8	15.0	2.3	60.0
27.6	57.1	73.3	23.0	7.5	71.3
39.0	20.0	89.2	6.2	3.1	86.3

AMSA: amplitude spectrum area; ROSC: return of spontaneous circulation; Se: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value; Acc: accuracy.

TABLE 4. Distribution of potential confounders in the context of clinical outcome in each group.

Characteristic	All (n = 46)	Group 1 (n = 25)	Group 2 (n = 21)	*p*-value
ALS ≤5 minutes	24 (52.2)	16 (64.0)	8 (38.1)	0.0798[1]
Bystander CPR	16 (34.8)	9 (36.0)	7 (33.3)	0.8500[1]
ALS ≤5 minutes and bystander CPR				
ALS ≤5 & CPR	10 (21.7)	5 (20.0)	5 (23.8)	0.0599[2]
ALS ≤5 & no bystander CPR	14 (30.4)	11 (44.0)	3 (14.3)	
ALS >5 & CPR	6 (13.0)	4 (16.0)	2 (9.5)	
ALS >5 & no bystander CPR	16 (34.8)	5 (20.0)	11 (52.4)	
History of CVD and iVF				
No history of CVD & iVF	6 (13.0)	3 (12.0)	3 (14.3)	0.0055[2]
History of CVD & iVF	16 (34.8)	12 (48.0)	4 (19.0)	
History of CVD & no-iVF	17 (37.0)	4 (16.0)	13 (61.9)	
No history of CVD & no-iVF	7 (15.2)	6 (24.0)	1 (4.8)	

Data are expresses as number (%); n: sample size; ALS: advanced life support; CPR: cardiopulmonary resuscitation; CVD: cardiovascular disease; iVF: initial ventricular fibrillation rhythm of cardiac arrest; no-iVF: ventricular fibrillation is not the first rhythm of cardiac arrest; [1]Chi-squared test; [2]Fisher's exact test.

the time to ALS, with longer times observed in Group 2. The probability of success increased when ALS was administered early and bystanders were present (Table 4).

Most patients in our study had a history of cardiovascular disease (CVD) (Table 2). A statistically significant association was observed between CVD and initial VF (iVF), with most patients in Group 1 having both CVD and VF as the initial rhythm, while Group 2 had CVD but no initial VF (Table 4).

The significance obtained by different factors in univariable logistic regression models was not necessarily retained in the multivariable model, except for adrenaline (Table 5). The combination of variables in the multivariable model showed an overall accuracy of 89.1%, with a sensitivity of 88% and a specificity of 90.5%.

4. Discussion

In this study, we identified several barriers that hindered achieving successful ROSC in prehospital settings and ED admission after OHCA with initial VF, such as a low incidence of bystander CPR (Tables 2 and 4), prolonged time to ALS team arrival (greater than 5 minutes), and low AMSA values (the lowest successful defibrillation AMSA value being 13.6 mV-Hz). The specificity and accuracy of AMSA values for successful defibrillation using the first manual defibrillation by the ALS team were acceptable, reaching up to 70% at an AMSA value of 27.6 mV-Hz.

4.1 Study findings

Previous studies have reported that myocardial defibrillation produces electrical injury and alters the energy state of the myocardium, potentially creating ectopic sites that could trigger life-threatening arrhythmias [5–7, 9]. The use of a manual defibrillator and the continuous registration of cardiac rhythm using patches is a particularity of our study, distinguishing it from previous studies using AED defibrillators [12–15]. We measured AMSA over 10 seconds, in contrast to earlier studies that used shorter periods (2–5 seconds) [13, 15], aligning with more recent studies reporting periods between 8 and 11 seconds [12, 14, 18]. A 10-second period offers a practical "on the field" approach for establishing AMSA values in a prehospital population. However, the specific conditions of our study may impact AMSA values and their effectiveness in predicting ROSC. Severe myocardial ischemia and delayed resuscitation are known to modify AMSA values by decreasing the amplitude of VF waves, thereby reducing the AMSA value. The discrepancies observed in sensitivity and specificity between our present study and current literature could be explained by the difference in the length of analysis

TABLE 5. Univariate and multivariate regression analysis for ROSC as the outcome.

	Univariable models			Multivariable models		
	Estimate (SE)	OR [95% CI]	*p*	Estimate (SE)	OR [95%CI]	*p*
Intercept				2.728 (1.729)		0.115
Age, yr	−0.015 (0.021)	0.985 [0.945 to 1.028]	0.493			
Sex (M)	−0.639 (0.780)	0.528 [0.114 to 2.432]	0.413			
iVF (yes)	1.099 (0.617)	3.000 [0.895 to 10.058]	0.075	0.595 (1.080)	1.813 [0.218 to 15.052]	0.582
Time to ALS, min	−0.101 (0.066)	0.904 [0.795 to 1.028]	0.124			
Adrenaline, mg	−0.480 (0.138)	0.619 [0.472 to 0.811]	<0.001	−0.451 (0.148)	0.637 [0.477 to 0.851]	0.002
AMI (yes)	3.076 (1.100)	21.667 [2.508 to 187.159]	0.005	2.468 (1.378)	11.803 [0.792 to 175.854]	0.073
Last AMSA (mV-Hz)	0.066 (0.030)	1.068 [1.007 to 1.132]	0.028	0.016 (0.044)	1.016 [0.932 to 1.108]	0.716

ROSC: return of spontaneous circulation; yr: year; M: masculine; iVF: initial ventricular fibrillation rhythm of cardiac arrest; ALS: advanced life support; min: minute; mg: milligram; AMI: acute myocardial infarction; AMSA: amplitude spectrum area; OR: Odds Ratio; CI: Confidence Interval; SE: Standard Error.

(10 seconds *vs.* 2–5 seconds) and the differences in digitization and calculation methods used to evaluate AMSA values.

When using a longer analysis period of 10 seconds, chest compressions may artifact the rhythm just before defibrillation, potentially leading to an overestimation of AMSA [13, 14, 20]. The literature reports various techniques to reduce or remove chest compression artifacts from ECG analysis [13, 14, 21, 22], and some studies have identified deep-learning strategies for sliding ECG analysis during resuscitation with AEDs [23]. Corpuls 3 can be used to effectively reduce the influence of chest compressions on the VF signal, allowing for analysis without the need for chest compression corrections.

The AMSA value analysis using manual defibrillation revealed higher specificity and accuracy at 27.6 mV-Hz compared to the 15.5 mV-Hz using AEDs in different health system organizations reported in literature [13, 15, 16]. A consistent finding was the decreased number of defibrillations (Table 2) in patients with higher AMSA values and positive short-term outcomes. AMSA values are influenced by factors such as early and high-quality chest compressions, bystander CPR response, and medication [10, 24]. In this context, the regional EMS system and health policies play an essential role in improving short-term outcomes (ROSC and ED admission) by organizing public access defibrillation programs, dispatcher-assisted CPR training, and promoting comprehensive bystander response [25, 26]. Due to the low incidence of bystander CPR (approximately one-third in both groups), the study could not demonstrate the well-documented benefit of bystander CPR on ROSC (Table 2). However, we observed a significantly lower incidence of bystander CPR and an increased time to start ALS (>5 minutes) in the prehospital death group (Group 2), which thereby highlights the role of EMS teams in providing early defibrillation and achieving ROSC, even when bystander CPR is not provided [4, 26]. Insufficient education and awareness of the Romanian population regarding CPR initiation and AED use reduces short-term outcomes, which depend directly on early defibrillation provided by EMS teams (Table 2). Implementing bystander CPR training in Romanian schools, in accordance with the 2021 European Resuscitation Council Guidelines, could improve outcomes for OHCA and VF patients as the training would increase the likelihood of early defibrillation, resulting in higher AMSA values and thereby enhancing the chances for effective defibrillation, ROSC and ED admission.

AMSA reflects the energy state of the myocardium and is dependent on coronary perfusion. Adrenaline administration combined with chest compression could improve AMSA values [8]. In this study, the observed lower dosage of adrenaline in Group 1 compared to Group 2 (Table 2) suggests an association between high AMSA values and a reduced need for vasopressors, which may decrease negative effects after ROSC [4]. Univariable regression analysis for ROSC as an outcome of successful defibrillation identified several significant predictors, including adrenaline ($p < 0.001$), AMI ($p < 0.005$), and AMSA value ($p < 0.028$) (Table 5).

Ristagno *et al.* [13] reported a higher incidence of successful defibrillation in patients with a history of CVD and an initial VF rhythm. This study confirms that patients with an initial VF rhythm had a higher number of sustained ROSC and were more likely to be admitted to the ED compared to those with a different initial cardiac arrest rhythm (Table 4). Additionally, in cases of CVD, we observed a 32% decrease in success rates when the time to start ALS exceeded 5 minutes. The data confirm that initiating ALS (which includes the first defibrillation attempts in our health system) within the first 5 minutes for patients with VF increases the success of defibrillation, thereby improving the ROSC rate and ED admission (Table 4).

As observed in previous studies, acute myocardial infarction is a significant cause of cardiac arrest [2, 4, 27]. In this study, the results demonstrate a significantly higher ROSC rate and ED admission among VF patients with acute myocardial infarction (52% in Group 1, Table 2), suggesting that effective

defibrillation of VF in patients with acute myocardial infarction could be more likely to result in ROSC [27–29].

4.2 Study limitations

This study had several limitations that should be clarified. First, it was conducted at a single center, using prospectively recorded data over a short period of 10 months; therefore, the number of patients and analyzed ECG tracks was limited. In addition, the reported results reflect only the evaluated cohort and should be validated in larger cohorts. Moreover, the small cohort size might have affected the cutoff AMSA value's performance, limiting its clinical relevance. However, the results suggest potential utility in establishing a clinically useful cutoff, supporting the need to extend the study to a larger cohort. Second, we did not use any methods to filter ECG artifacts, relying on the defibrillator technology to minimize the influence of chest compressions on the ECG rhythm. Third, due to the limited number of patients, we could not stratify the performances according to factors that could have influenced AMSA changes. Thus, analyzing indicators related to myocardial dysfunction (*i.e.*, the number of defibrillation attempts and adrenaline dosages), individual factors (*i.e.*, presence of CVD), and out-of-hospital conditions (*i.e.*, bystander CPR response, public access defibrillator availability, and time to ALS) could provide key information for defining computer-assisted models to evaluate AMSA efficiency for defibrillation. Further research could develop defibrillator algorithm software to report AMSA values predictive of successful defibrillation.

5. Conclusions

This study demonstrated high specificity (90.5%) for predicting ROSC and ED admission and that an AMSA value up to 27.6 mV-Hz could be significantly associated with sustained ROSC and ED admission in OHCA cases when using a manual defibrillator (specificity, 73.3%). In addition, the results show the negative impact of current deficiencies, such as limited access to public defibrillators and a low incidence of bystander CPR, on short-term outcomes, which ultimately adversely affect VF waves and decrease AMSA values. A prolonged time from the initiation of ALS (>5 minutes) combined with delayed manual defibrillation attempts reduces AMSA values and shock efficiency.

Moreover, the study results could be extrapolated to in-hospital resuscitation. Larger cohort studies and evaluation of potential confounding factors could help develop a prognostic score for hospital admission by analyzing the reported out-of-hospital factors, such as bystander CPR, public access to AEDs, time to start ALS, AMSA value, adrenaline dosages, and the presence of CVD.

ABBREVIATIONS

Acc, accuracy; AED, automated external defibrillator; ALS, advanced life support; AMI, acute myocardial infarction; AMSA, amplitude spectral area; BLS, basic life support; CI, Confidence Interval; CPR, cardiopulmonary resuscitation; CVD, cardiovascular diseases; DII, the second standard bipolar limb lead; ECG, electrocardiography; ED, Emergency Department; EMS, emergency medical services; n, sample size; n/a, not applicable; No., number; NPV, negative predictive value; OHCA, out-of-hospital cardiac arrest; OR, Odds Ratio; PEA, pulseless electrical activity; PPV, positive predictive value; ROSC, return of spontaneous circulation; SD, standard deviation; Se, sensitivity; SMURD, Mobile Emergency Service for Resuscitation and Extrication; Sp, specificity; VF, ventricular fibrillation; iVF, initial ventricular fibrillation rhythm of cardiac arrest; no-iVF, ventricular fibrillation is not the first rhythm of cardiac arrest.

AVAILABILITY OF DATA AND MATERIALS

The raw data are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

AG, CD—designed the research study. SDB, RT—verify the study protocol and give advice. CD, AS, RT—performed the research. AG—provided help and advice of the research. CD, SDB—analyzed the initial data. AG, RT—discuss the results of the initial data. AG, CD, SDB—wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Ethics Committee of the Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, Romania (No. 107/28.02.2014) and was conducted following the principles of the Declaration of Helsinki. In accordance with Directive 2001/20/EC, an adapted informed consent was applied. The study was observational and did not involve any changes to the resuscitation protocol. The absence of consent did not affect patients' healthcare rights. The data were used in accordance with national regulations on patient confidentiality (Order of the Romanian Health Ministry 46/2003).

ACKNOWLEDGMENT

The authors thank Filip Cristian for the specialized consulting in MATLAB and all paramedics and the nurses for the help in data collection.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Adela Golea, Christiana Dumulesc, Andrei Stărică, Sorana D. Bolboacă, Raluca Tat. Amplitude spectrum area as an indicator of effective return of spontaneous circulation in prehospital resuscitation—experience from a single regional center in Romania. Signa Vitae. 2024; 20(10): 47-55. doi: 10.22514/sv.2024.125.

Journal of
Clinical Medicine

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Article

Biomarkers as Predictors of Mortality in Sepsis and Septic Shock for Patients Admitted to Emergency Department: Who Is the Winner? A Prospective Study

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Citation: Luka, S.; Golea, A.; Tat, R.M.; Lupan Mureșan, E.M.; Voicescu, G.T.; Vesa, Ș.C.; Ionescu, D. Biomarkers as Predictors of Mortality in Sepsis and Septic Shock for Patients Admitted to Emergency Department: Who Is the Winner? A Prospective Study. *J. Clin. Med.* **2024**, *13*, 5678. https://doi.org/10.3390/jcm13195678

Academic Editor: Toshiaki Iba

Received: 28 August 2024
Revised: 15 September 2024
Accepted: 22 September 2024
Published: 24 September 2024

Abstract: Background/Objectives: Sepsis and septic shock remain significant contributors to high early mortality rates among patients admitted to the emergency department (ED). The objective of this study was to identify among newer biomarkers those with the highest sensitivity in early mortality prediction. **Methods**: This prospective, unicentric, observational study enrolled 47 adult patients admitted to the ED between November 2020 and December 2022. This study monitored the kinetics of the older and newer biomarkers, including azurocidin (AZU1), soluble triggering receptor expressed on myeloid cells (sTREM), soluble urokinase-type plasminogen activator receptor (suPAR), high-sensitivity C-reactive protein (hsCRP), procalcitonin (PCT), and interleukin-6 (IL-6), and their capacity in predicting mortality. **Results**: SuPAR showed the most significant predictive utility for early prognosis of mortality in the ED, with an area under the curve (AUC) of 0.813 (95% CI: 0.672 to 0.912), a cutoff value > 8168 ng/mL, sensitivity of 75%, and specificity of 81.48% ($p < 0.001$). IL-6 and PCT showed comparable prognostic accuracy, whereas hsCRP and AZU1 demonstrated lower predictive performance. **Conclusions**: In our study, suPAR, IL-6, and PCT showed good predictive value for short-term mortality in sepsis and septic shock patients.

Keywords: biomarkers; prognostic; mortality; sepsis; suPAR

1. Introduction

Despite the advances in modern medicine, sepsis and septic shock remain two critical conditions that represent significant challenges for EDs worldwide. These conditions often result in substantial patient morbidity and high mortality, particularly affecting vulnerable populations such as the elderly, immunosuppressed patients, and those with multiple comorbidities [1]. The management of sepsis and septic shock frequently requires a multidisciplinary approach, which involves the contributions of several medical specialties

along with the establishment of immediate care and life support measures in the ED and, last but not least, often requires hospitalization in intensive care units (ICUs) for prolonged hospitalization periods [2]. Additionally, sepsis and septic shock represent a significant healthcare logistics challenge due to their expensive treatment costs, the need for further investigation, and long hospital stays [3].

Sepsis is a complex condition that is difficult to diagnose due to its diverse causative agents, variable disease progression, and the varied stages at which patients present to the emergency department (ED). These challenges may delay targeted treatments, adversely affecting patient outcomes [4]. While clinical signs are often non-specific, biomarkers offer potential for earlier sepsis detection and treatment initiation. However, accurately identifying the infection type and distinguishing infection-related inflammation remain difficult in many ED cases [5].

Multiple alert systems, scores, and interventions are now available for early detection of sepsis. However, there is currently no golden standard method that may reliably predict outcomes for all patients and guide therapy from early stages of diagnosis. Since EDs are the primary assessment points of these patients, early recognition and initiation of appropriate therapeutic measures based on the identification of highly sensitive and specific biomarkers, either alone or in combination with novel prognostic scores, could potentially improve long-term outcomes in the future [4].

High-sensitivity C-reactive protein (hsCRP), procalcitonin (PCT), soluble urokinase-type plasminogen activator receptor (suPAR), soluble triggering receptor expressed on myeloid cells (sTREM), interleukin-6 (IL-6), and azurocidin (AZU 1) are biomarkers commonly used to assess sepsis severity and predict patient outcomes.

While hsPCR [5–7] indicates systemic inflammation, PCT [8–10] aids in early diagnosis, risk stratification, and antibiotic management. Among the newer biomarkers, suPAR [11–13] correlates with immune activation, while sTREM [12,14,15] plays a significant role in innate immunity.

IL-6 [9,16,17] is widely used for assessing inflammatory activity, and AZU1 [18–20] is a novel biomarker used for sepsis prognosis.

Our study investigated these biomarkers, measured at three critical time points: at admission and first and second days post-admission, to assess their potential to predict short-term mortality with the highest accuracy. We chose this approach considering that patients are admitted to the ED at different stages of sepsis with varying degrees of severity, making the initial measurement of these biomarkers potentially unreliable.

2. Materials and Methods

2.1. Study Design and Setting

This observational, prospective, longitudinal and analytical study was carried out in Cluj-Napoca County Emergency Clinical Hospital's ED, a tertiary hospital with academic activity and 1500 beds. This study included consecutive adult patients with sepsis or septic shock who were admitted to the ED between 1 November 2020, and 1 December 2022. Given that this study was conducted during the COVID-19 pandemic, recruitment of patients followed government regulations and local medical protocols regarding access to hospitalized patients.

This study adhered to the ethical principles and standards of clinical practice as stipulated in the Declaration of Helsinki [21] as well as EU legislation [22]. Patients above 18 years old were prospectively recruited and provided written informed consent themselves or via their legal representative within the first hour of arrival in the ED. This study was approved by the Ethics Committee of Iuliu Haţieganu University of Medicine and Pharmacy (Approval No. 139/30 March 2020) and the ECs of participating hospitals: Cluj Emergency County Hospital (Approval No. 5416/10 25 February 2020), Infectious Diseases Cluj-Napoca (Approval No. 6010/14 April 2021), and Clinical Institute of Urology and Renal Transplantation Cluj-Napoca (Approval No. 03/02 February 2021).

2.2. Study Design

Adult patients (over 18 years old and under 90 years) admitted to the ED for sepsis or septic shock, as defined by The Third International Consensus Definitions for Sepsis and Septic Shock—Sepsis 3, "Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection and an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more. Septic shock can be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia" [23], were enrolled within one hour of presentation and after obtaining informed consent.

This is a planned sub-study focused on the dynamics of biomarkers in patients who survived for at least three days following presentation to the ED.

Exclusion criteria: patients who survived less than 48 h after admission, patients under 18 or over 90 years of age, patients with neoplastic disease, patients with other acute illnesses more severe than sepsis (other types of shock, cardiac arrest, the need of urgent surgery), pregnant women, incomplete data, or those who refused to participate in this study.

Upon arrival in the ED (T_0) and after informed consent, each patient underwent a prospective evaluation, which included clinical assessment, vital signs monitoring, laboratory tests, and routine blood work. The same assessment was repeated at 24 h (T_{24}) and 48 h (T_{48}). A panel of six biomarkers (IL-6, hsPCR, PCT, AZU1, suPAR, and sTREM) was examined following admission at three time points (T_0, T_{24}, T_{48}). Patients were monitored for 28 days following their referral to the ED, until either hospital discharge or death, if these events occurred within a 28-day period. Patients' status was evaluated by a follow-up phone call at 28 days. Patients were categorized into two groups based on their outcomes at 28 days: survivors and non-survivors.

The primary objective was to evaluate the dynamics of this new set of biomarkers during the 48 h in sepsis or septic shock patients diagnosed in the ED. The secondary objective was to assess and compare their ability to predict 28-day mortality in these patients.

Medical management in the ED during this study was not based on the results of the novel inflammatory markers described in this manuscript but according to standard practice. Patients were admitted to the hospital and treated according to standard protocols, which included fluids, vasopressors, and wide spectrum antibiotics, in compliance with Surviving Sepsis Campaign recommendations [1].

Patients in our study were transferred from the ED to the ICU within an average of 6 h. Those with a mean arterial pressure (MAP) below 65 mmHg were treated in the ED with norepinephrine and fluid resuscitation. If hypotension persisted despite reaching a maximum noradrenaline dose of 0.5–0.7 μg/kg/min as recommended by protocols, vasopressin was administered in the ICU as an adjunct vasoconstrictor. For patients experiencing cardiac dysfunction due to sepsis or pre-existing heart disease, dobutamine was also associated.

2.3. Data Collection

The collected data included baseline demographic information, medical history, clinical evaluation, signs and symptoms, vital signs, laboratory testing (cultures and imaging), type of oxygenotherapy, medication and fluids administered during the ED stay and hospital stay, and survival status at 28 days. The laboratory tests included routine blood tests such as complete blood count (CBC), arterial blood gases (ABG), liver function, kidney function, coagulation, and blood cultures.

2.4. Sample Collection and Biomarker Assays

Blood samples for biomarker analysis were collected by ED nurses during the first hour of patient presentation, with informed consent. The samples were drawn using 5 mL serum separator tubes containing a clot activator and left to remain for 30 min at room temperature. Then they were centrifuged at 1000× *g* for 15 min. Plasma was transferred

to a 1 mL Eppendorf tube and kept at −80 °C until assay. The samples were processed at the Cluj County Hospital laboratory. Hemolyzed samples were redrawn. The following Enzyme-Linked Immunosorbent Assay (ELISA) kits were used for biomarker analysis (BioVendor—Laboratorni medicina a.s., Karasek 1767/162100 Brno, Czech Republic). Biomarkers were evaluated using the Sandwich-ELISA immunoassay method. Heidolph Shaker Titramax 100 (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany), ELISA Spectrophotometer (LabSystems Multiskan Plus LabSystems, Helsinki, Finland), and Autoanalyzer ELISA Personal Lab (ADALTIS, Rome, Italy) were among the analytical tools utilized. Measurements were made in accordance with the manufacturer's instructions.

2.5. Statistical Analysis

Statistical analysis was performed using MedCalc® Statistical Software version 22.021 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; accessed on 1 June 2024). The sample size was determined based on an initial small study group (n = 5 patients in each group). IL-6 mean values were 249.6 pg/mL in the survival group and 653.9 pg/mL in the non-survival group. A sample size of 16 patients per group was calculated in order to achieve a power of 80% and a level of significance of 5%. A 25% increase in sample size was added to compensate for eventual incomplete data and dropouts. We calculated sample size based on IL-6 levels considering that this is the most used biomarker in sepsis.

Quantitative data normality was assessed using the Shapiro–Wilk test. Quantitative data were expressed as median and (25th–75th) percentiles for non-normally distributed data or means ± standard deviation for normally distributed data. Qualitative data were characterized by frequency and percentage. For the biomarkers and score, we calculated the area under the curve (AUC) using the trapezoidal method with measurements from 0 to 24 h and 0 to 48 h. The differences between groups were verified with Mann–Whitney (for non-normally distributed data), Student *t*-test (normally distributed data), or chi-square test. Receiver operating characteristic (ROC) analysis was performed to determine a cutoff value for the association of several quantitative variables and mortality. The Youden index was used to determine the ideal cutoff threshold, ensuring an optimal balance of sensitivity and specificity. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

During the study period, 488 patients with sepsis or septic shock were referred to the ED. Of these, 47 patients met the eligibility criteria and were included in this study (Figure 1).

The overall 28-day survival rate was 57.44%. The median age of patients in our study was 71.5 years for survivors and 74.5 years for non-survivors.

85% of non-survivors and 55% of survivors had septic shock within the first 48 h of admission. Norepinephrine was the preferred vasopressor for all these patients.

Patients with altered mental status, as indicated by a lower GCS score, were associated with an increased mortality rate. As expected, the clinical prediction scores (SOFA, APACHE II, SAPS II) effectively discriminated between survivors and non-survivors.

Other predictors of 28-day mortality included tachycardia with a ventricular rate exceeding 110 bpm, lactate levels higher than 2 mmol/L, FiO_2 above 0.4%, and a low PaO_2/FiO_2 ratio (<195) (Table 1). Additional standard laboratory tests are listed in Supplementary Materials Table S1.

Potential explanations for the high percentage of patients not having fever include Gram-negative infections in 32 patients and fungal infections in 12 patients.

As shown in Table 2, significant variations during the specified time intervals were registered for IL-6, suPAR, PCT, and hsCRP.

Table 1. A comparison between survivors and non-survivors of baseline characteristics, scores, and comorbidities.

Parameters	Time	Survivors (n = 27)	Non-Survivors (n = 20)	*p*
Age (years) †	T_0	71.5 (63.25–81)	74.5 (64.25–82.5)	0.45
BMI n (%) †	T_0	27.56 (23.43–33.53)	25.29 (22.05–30.52)	0.16
Vital signs and physiological parameters				
GCS †	T_0	15 (13–15)	11.5 (13–15)	**0.004**
	T_{24}	15 (12–15)	10.5 (4.5–2.5)	**<0.001**
	T_{48}	14 (12–15)	8 (4–10)	**<0.001**
Respiratory rate (resp/min) †	T_0	27 (24–30.75)	30 (26–34.75)	0.20
	T_{24}	25 (22.5–30)	25.5 (16–32.75)	0.64
	T_{48}	25 (23–28)	22 (18–30)	0.18
Heart Rate (beats/min) †	T_0	108 (92.5–112.75)	116 (107.75–126)	0.11
	T_{24}	101 (72.5–110)	107 (88.25–121.25)	0.05
	T_{48}	102 (70–114)	110 (105–128)	**0.005**
Glycemia (mg/dl) †	T_0	186 (117.5–235.5)	121 (99.25–190.5)	0.02
	T_{24}	118.50 (105–183.25)	115.50 (79.25–210.25)	0.30
	T_{48}	133 (106–170)	122 (80–215.70)	0.87
MAP (mmHg) ††	T_0	68.2 ± 15.5	60.6 ± 9.9	0.06
	T_{24}	76.8 ± 16	73.8 ± 14.9	0.50
	T_{48}	75.5 ± 14.8	67.6 ± 9.7	0.04
Lactate (mmol/L) †	T_0	2 (1.52–3.27)	2.85 (1.72–5.6)	0.15
	T_{24}	1.7 (1.10–2.22)	2.5 (1.55–3.40)	0.19
	T_{48}	1.60 (1–2)	2 (1.8–6)	**0.003**
SaO_2 (%) †	T_0	94.5 (92–97)	92.5 (86–96)	0.40
	T_{24}	96 (94.25–97)	96 (92.75–98.5)	0.81
	T_{48}	95 (94–98)	96 (90–99)	0.54
FiO_2 (%) †	T_0	0.21 (0.21–0.55)	0.6 (0.21–0.87)	0.24
	T_{24}	0.21 (21–40)	0.45 (40–71)	**0.001**
	T_{48}	0.21 (21–40)	0.40 (35–60)	**<0.001**
PaO_2/FiO_2 †	T_0	390.70 (315.87–459.75)	267.55 (109.28–429.82)	0.11
	T_{24}	371 (291.25–433.25)	176 (102.14–241.25)	**<0.001**
	T_{48}	333 (225–423)	195 (108–288)	**<0.001**
Temperature (°C) †	T_0	37.65 (36.32–38.8)	37.40 (36.6–38.15)	0.22
	T_{24}	37 (36.42–37.87)	37 (36.15–37.2)	0.79
	T_{48}	36 (36–37.5)	36.5 (36–37)	0.67
Scores				
SOFA †	T_0	5 (2.25–9.75)	9.5 (5.50–12.75)	0.06
	T_{24}	6 (3–9)	11 (8–14.25)	**0.001**
	T_{48}	5 (2–7)	10 (7.25–14)	**<0.001**
APACHE II ††	T_0	18.8 ± 5.6	26 ± 7.7	**0.01**
	T_{24}	14.6 ± 5.1	24.9 ± 7.1	**<0.001**
	T_{48}	13.1 ± 6.8	24.4 ± 8	**<0.001**
SAPS II †	T_0	46 (39.25–55.50)	59.5 (56–78)	0.001
	T_{24}	44 (35.5–49)	63.5 (48–79)	**0.002**
	T_{48}	39 (33–51)	62 (41–84)	**0.01**
Comorbidities, n (%)				
Cardiovascular disease	T_0	24 (82.8)	15 (88.2)	1.00
Diabetes	T_0	18 (62.1)	9 (52.9)	0.76
Chronic kidney disease	T_0	7 (24.1)	3 (17.6)	0.88
Chronic lung disease	T_0	9 (31)	4 (23.5)	0.83
Obesity	T_0	13 (44.8)	13 (76.5)	0.25
Neuropsychiatry	T_0	11 (37.9)	11 (64.7)	0.14

Legend: † median (IQR), IQR: interquartile range, †† mean ± standard deviation, BMI: body mass index, GCS: Glasgow Coma Scale, MAP: mean arterial pressure, SaO_2-oxygen saturation, PaO_2/FiO_2-ratio of arterial oxygen partial pressure to fractional inspired oxygen, SOFA-Sequential Organ Failure Assessment, APACHE II: Acute Physiology and Chronic Health Evaluation II, SAPS II and III: Simplified Acute Physiology Score II and III.

Table 2. Mean and median serum levels of biomarkers measured over the first two days after arrival in the ED.

Biomarker (Plasma Levels)	Time	Survival Group (n = 29)	Non-Survival Group (n = 17)	*p*
IL-6 (pg/mL) †	T_0	406.50 (91.22–535.07)	441.60 (304.90–791.05)	0.11
	T_{24}	129.85 (67.70–369.82)	402.10 (245.95–669.60)	**0.003**
	T_{48}	75.60 (40.87–213.12)	238.70 (117.95–531.55)	**0.001**
suPAR (ng/mL) ††	T_0	7343.8 ± 1971.1	8512.1 ± 1848.4	0.04
	T_{24}	6556.3 ± 1809	8641.8 ± 1765.3	**<0.001**
	T_{48}	6405.4 ± 2020.7	8318.9 ± 2449.1	**0.005**
PCT (pg/mL) †	T_0	13.85 (2.87–31.17)	23.10 (7.95–58.15)	0.13
	T_{24}	9.95 (3.67–38.82)	21.7 (8.30–81.35)	0.11
	T_{48}	6 (1.75–19.72)	15.6 (6.45–71.05)	**0.01**
hsCRP (pg/mL) †	T_0	26.05 (15.20–29.67)	18.40 (16.50–22.40)	0.11
	T_{24}	17.90 (14.6 –23.7)	21.5 (15.61–26.72)	0.52
	T_{48}	22.80 (20.32–28.85)	17 (14.10–21.75)	**0.01**
sTREM-1 (pg/mL) †	T_0	264.75 (89.80–741.50)	224.50 (119.6–813.90)	0.50
	T_{24}	229.75 (110.47–474.72)	341.60 (77.90–555.70)	0.57
	T_{48}	175.95 (63.72–467.10)	184.70 (65.90–551.60)	0.69
AZU1 (ng/mL) †	T_0	8.30 (7.55–9.07)	7.30 (7.00–8.60)	0.09
	T_{24}	7.80 (6.82–8.57)	7.60 (7.10–9.50)	0.82
	T_{48}	7.95 (6.95–9.12)	7.60 (6.90–9.15)	0.63

Legend: † median (IQR), IQR: interquartile range, †† mean ± standard deviation, IL-6: interleukin-6, suPAR: soluble urokinase plasminogen activator, PCT: procalcitonin, hsCRP: high-sensitivity C-reactive protein, sTREM-1: soluble triggering receptor expressed on myeloid cells-1, AZU-1: azurocidin 1.

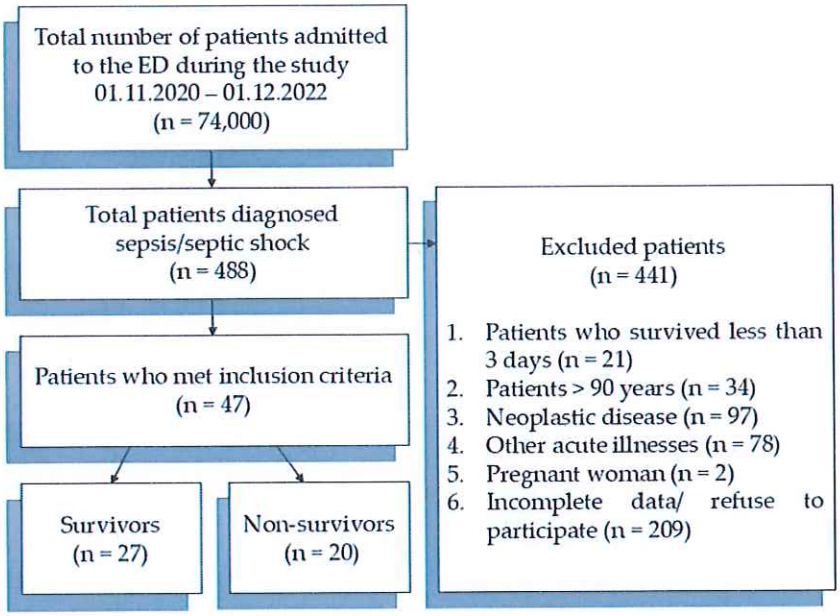

Figure 1. STROBE diagram of patient inclusion/exclusion criteria.

During the first two days of observation, 20 patients did not survive. The median IL-6 level in these non-survivors was 707.85 pg/mL (IQR 365.5–864.57), significantly higher than those reported for the patients included in this study. Additionally, the mean suPAR level in these non-survivors was 8348.6 pg/mL (SD ± 2195.35), which was consistent with the values observed in this study.

The ability to predict 28-day mortality of selected biomarkers based on ROC analysis and their cutoff levels are shown in Tables 3 and 4.

Table 3. AUC and cutoff values of tested biomarkers.

Biomarkers	Time	AUC (95% CI)	Cutoff Values	Se (95% CI)	Sp (95% CI)	p
IL-6 (pg/mL)	T_0	0.630 (0.476–0.766)	>246.6	80 (56.3–94.3)	48.15 (28.7–68.1)	0.11
	T_{24}	0.698 (0.547–0.823)	>109	90 (68.3–98.8)	44.44 (25.5–64.7)	**0.009**
	T_{48}	0.720 (0.570–0.841)	>96.6	80 (56.3–94.3)	62.96 (42.4–80.6)	**0.004**
suPAR (ng/mL)	T_0	0.695 (0.544–0.821)	>7434	85 (62.1–96.8)	59.26 (38.8–77.6)	0.01
	T_{24}	0.813 (0.672–0.912)	>8168	75 (50.9–91.3)	81.48 (61.9–93.7)	**<0.001**
	T_{48}	0.731 (0.581–0.849)	>8465	50 (27.2–72.8)	88.89 (70.8–97.6)	**0.002**
PCT (pg/mL)	T_0	0.595 (0.442–0.736)	>19.8	50 (27.2–72.8)	70.37 (49.8–86.2)	0.26
	T_{24}	0.662 (0.509–0.793)	>10	75 (50.9–91.3)	59.26 (38.8–77.6)	0.04
	T_{48}	0.706 (0.556–0.830)	>2.4	95 (75.1–99.9)	40.74 (22.4–61.2)	**0.006**
hsCRP (pg/mL)	T_0	0.591 (0.438–0.732)	>24.9	80 (56.3–94.3)	48.15 (28.7–68.1)	0.59
	T_{24}	0.551 (0.399–0.696)	>18	30 (11.9–54.3)	92.59 (75.7–99.1)	0.55
	T_{48}	0.551 (0.517–0.800)	>18.1	60 (36.1–80.9)	81.48 (61.9–93.7)	**0.04**
sTREM-1 (pg/mL)	T_0	0.554 (0.402–0.699)	>189	70 (45.7–88.1)	51.85 (31.9–71.3)	0.53
	T_{24}	0.509 (0.359–0.658)	>429.8	40 (19.1–63.9)	74.07 (53.7–88.9)	0.91
	T_{48}	0.504 (0.354–0.653)	>70.7	35 (15.4–59.2)	77.78 (57.7–91.4)	0.96
AZU1 (ng/mL)	T_0	0.608 (0.455–0.747)	>7.3	45 (23.1–68.5)	81.48 (61.9–93.7)	0.20
	T_{24}	0.507 (0.358–0.656)	>9	35 (15.4–59.2)	88.89 (70.8–97.6)	0.93
	T_{48}	0.520 (0.368–0.670)	>7.8	60 (36.1–80.9)	53.85 (33.4–73.4)	0.82

Legend: AUC: area under the curve, CI: confidence interval, Se: sensitivity, Sp: specificity, IL-6: interleukin-6, suPAR: soluble urokinase plasminogen activator, PCT: procalcitonin, hsCRP: high-sensitivity C-reactive protein, sTREM-1: soluble triggering receptor expressed on myeloid cells-1, AZU1: azurocidin 1; receiver operating characteristic (ROC) analysis.

At the initial assessment (T_0), suPAR demonstrated the highest sensitivity at 85%, followed by IL-6 and hsCRP, both at 80%. At T_{24}, the sensitivity of IL-6 increased to 90%, while at T_{48}, PCT achieved the highest sensitivity at 95%.

In terms of specificity, hsCRP achieved the highest value at T_{24} with 92.59%. At T_0, AZU1 had a specificity of 81.48%. At T_{24}, AZU1 reached 88.89%, followed by suPAR at 81.48%. By T_{48}, suPAR specificity increased to 88.89%, while hsCRP specificity decreased to 81.48%.

Among the biomarkers, suPAR proved to be the most reliable predictor of 28-day mortality, AUC = 0.81 ($p < 0.001$), at T_{24}. However, at T_{48}, the AUC of suPAR decreased to 0.73 ($p = 0.002$), with similar performances observed for IL-6 (AUC = 0.72, $p = 0.004$) and PCT (AUC = 0.70, $p = 0.006$).

The AUC obtained with three consecutive measurements at T_0, T_{24}, and T_{48} did not surpass the AUC from a single biomarker evaluation. The highest AUCs were observed with suPAR (0.733, $p = 0.002$), IL-6 (0.730, $p = 0.002$), and PCT (0.700, $p = 0.009$) (Figure 2).

Table 4. AUC and cutoff values of biomarkers T_0–T_{48}.

	Time	AUC (95% CI)	Cutoff Values	Se (95% CI)	Sp (95% CI)	*p*
Biomarkers						
AUC for IL-6 (pgxh/mL)	T_0–T_{48}	0.730 (0.580–0.849)	>180	80 (56.3–94.3)	62.96 (42.4–80.6)	0.002
AUC for suPAR (ngxh/mL)	T_0–T_{48}	0.733 (0.584–0.852)	>13,558	50 (27.2–72.8)	88.89 (70.8–97.6)	0.002
AUC for PCT (pgxh/mL)	T_0–T_{48}	0.700 (0.549–0.825)	>10.03	80 (56.3–94.3)	59.26 (38.8–77.6)	0.009
AUC for hsCRP (pgxh/mL)	T_0–T_{48}	0.670 (0.518–0.800)	>30	60 (36.0–80.9)	81.48 (61.9–93.7)	0.04
AUC for sTREM-1 (pgxh/mL)	T_0–T_{48}	0.504 (0.354–0.653)	>119.63	35 (15.4–59.2)	77.78 (57.7–91.4)	0.96
AUC for AZU1 (ngxh/mL)	T_0–T_{48}	0.506 (0.356–0.655)	>12.23	50 (27.2–72.8)	62.96 (42.4–80.6)	0.94

Legend: AUC: area under the curve, CI: confidence interval, Se: sensitivity, Sp: specificity, IL-6: interleukin-6, suPAR: soluble urokinase plasminogen activator, PCT: procalcitonin, hsCRP: high-sensitivity C-reactive protein, sTREM-1: soluble triggering receptor expressed on myeloid cells-1, AZU1: azurocidin 1; receiver operating characteristic (ROC) analysis.

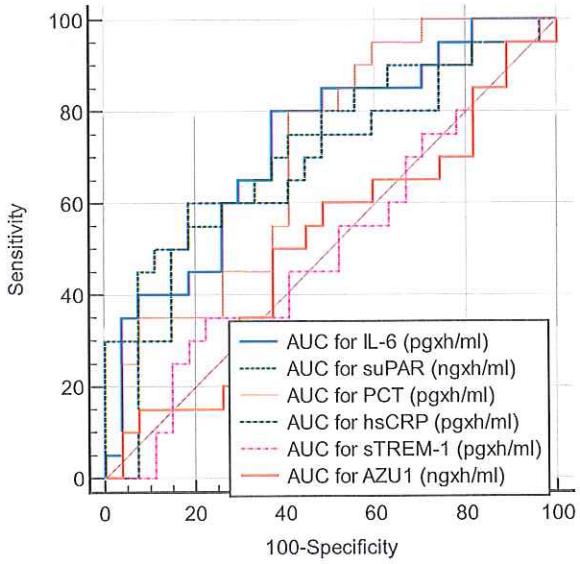

Figure 2. AUCs for study biomarkers.

In terms of sensitivity, both PCT and IL-6 achieved 80%. For specificity, suPAR reached 88.89%, followed by hsCRP at 81.48%.

4. Discussion

The incidence of sepsis is increasing, particularly in an aging population, with more severe cases seen in the ED due to rising bacterial resistance and the presence of co-morbidities [24]. Sepsis is associated with high morbidity and mortality, making timely management and early intervention critical [1]. Given the 42% mortality rate and the median ages of survivors (71.5 years) and non-survivors (74.5 years), age appears to be a significant factor in mortality risk [25–27]. The high death rate in this study group may be explained by the combination of advanced age, comorbidities, prolonged inflammation, and the fact that 70% of non-survivors experienced septic shock [25,28].

Biomarkers play an important role in early diagnosis, guiding treatment decisions, and improving prognosis estimation, thereby supporting effective resource allocation [3,29]. This has led to ongoing research to identify new modern biomarkers, reliable ones which in the future can prove their usefulness in real life, at the patient's bedside, as close as possible to the concept of the "ideal biomarker".

In this study, we assessed six different biomarkers, suPAR, IL-6, PCT, hsCRP, sTREM, and AZU1, from the point of view of variability over time and their ability to predict mortality at 28 days.

suPAR is recognized as a proinflammatory marker with a significant role in immune system activation. Elevated suPAR levels have been linked to increased disease severity and higher readmission rates in intensive care settings [30]. In our study, suPAR was an independent predictor of 28-day mortality, achieving the highest AUC of 0.813 at T_{24}, with a sensitivity of 75%, specificity of 81.48%, and was statistically significant.

Similar findings to our results were reported in a systematic review and meta-analysis conducted by Huang et al. [13] which found that suPAR had a sensitivity of 74% and specificity of 70% for predicting mortality in sepsis. Another study by Nasr El-Din et al. [12] reported an AUC of 0.998 (95% CI, 0.92–1) for suPAR, measured on day seven in septic patients. Given these results, along with suPAR's remarkable stability over time (estimated to have a half-life of over 19 h and extending up to 7–10 days) [31], suPAR may be a promising biomarker for predicting early mortality in sepsis patients, even beyond the first week of hospitalization [11–13].

IL-6 is a key proinflammatory cytokine extensively studied for its role as an independent predictor of sepsis mortality [5,16,32,33]. It plays a critical role in the innate immune response by enhancing monocyte and neutrophil sensitivity and boosting NK cell cytotoxicity [15,32]. In healthy adults, IL-6 concentrations typically range from 0 to 43.5 pg/mL [34], but in sepsis or septic shock, levels can exceed 3500 pg/mL [32]. In our study, IL-6 levels were significantly higher in non-survivors (441.6 pg/mL) and were identified as an independent predictor of 28-day mortality, consistent with previous findings [7,33]. The best mortality prediction values for IL-6 in our study were observed at T_{48}, where the AUC reached 0.72. At this time point, sensitivity was 80% and specificity was 62.96%, with a statistically significant *p*-value. It was reported in the literature that IL6R blockade may improve outcome and decrease mortality in sepsis. The use of tocilizumab has proved efficacy in managing cytokine storms during COVID-19 [35–37]. There are also studies for other inflammatory conditions, including acute pancreatitis, where targeting the IL-6 pathway may reduce excessive inflammatory response [38].

PCT is one of the most widely used biomarkers in clinical practice, particularly for early diagnostic, prognostic, and guiding antibiotic cessation [1]. Primarily responsible for regulating blood calcium levels, it is produced by thyroid C-cells. In healthy individuals, PCT is present in very low concentrations (<0.05 ng/mL) in the blood, but its levels can increase significantly in response to cytokine stimulation [32]. In our study, the most accurate outcome prediction was observed at T_{48}, with an AUC of 0.706. The optimal cutoff value was >2.4, yielding a significant sensitivity of 94% and a specificity of 40.74%, higher than reported in previous studies [8,32].

hs-CRP is an acute-phase protein, and it is well-known as a reliable marker of inflammation. It has been demonstrated to exhibit a substantial increase in concentration within 6–8 h following infection [39], making it a useful tool for the timely diagnosis of sepsis [32]. Although in our study hsCRP demonstrated high specificity, with values of 92.59% at T_{24} and 81.48% at T_{48}, its effectiveness in predicting mortality is limited. In comparison, a study by Ling et al. [5] found that although hs-CRP shows high sensitivity for sepsis diagnosis, its utility in predicting mortality is less robust compared to PCT.

To our knowledge, this study is the first to evaluate these novel specific biomarkers within the first two days after a sepsis or septic shock diagnosis in predicting mortality. However, our study has some limitations. This is a monocentric study, which limits the generalizability of the findings. This study was conducted in a single center, and the results may not be representative of broader populations or different healthcare settings. Second, this study was conducted during the COVID-19 pandemic, which imposed several constraints. Our access to patients in hospitals was often restricted, and patient participation was further hindered by their reluctance and fear of exposure to the virus. This resulted in delayed presentations to the ED. These factors reduced the potential sample size, limiting

the robustness of our conclusions. However, our sample size is similar to other studies on this topic. It is also possible that if we had included all patients, the predictive value of biomarkers may have been changed. We did not intend in our study to corelate suPAR levels or other biomarkers levels with bacterial strain, despite the fact that there are some publications on this.

5. Conclusions

SuPAR may be a promising novel biomarker for predicting short-term mortality in sepsis. IL-6 and PCT are effective predictive biomarkers for 28-day mortality in patients with sepsis and septic shock initially admitted to the ED. To validate these results and better understand the broader applicability of these biomarkers, further large-scale, multicentric studies are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm13195678/s1, Table S1: Standard Blood Tests.

Author Contributions: Conceptualization, S.L. and D.I.; data curation, Ș.C.V.; formal analysis, S.L., A.G. and Ș.C.V.; funding acquisition, S.L.; investigation, S.L., R.M.T., E.M.L.M. and G.T.V.; methodology, S.L., A.G. and D.I.; project administration, S.L.; software, Ș.C.V.; supervision, A.G. and D.I.; validation, Ș.C.V.; visualization, Ș.C.V. and D.I.; writing—original draft, S.L. and R.M.T.; writing—review and editing, S.L., A.G., R.M.T., E.M.L.M., G.T.V., Ș.C.V. and D.I. All authors have read and agreed to the published version of the manuscript.

Funding: This study received partial funding from the "Iuliu Haţieganu" University of Medicine and Pharmacy in Cluj-Napoca through doctoral research project grants (no. 2461/42/17 January 2020 and no. 1033/40/13 January 2021). The allocated grant funds were used to purchase biomarker kits and laboratory materials. No additional external funding was provided for this study.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki for human subject research, and approval was granted by the Ethics Committee of the Iuliu Haţieganu University of Medicine and Pharmacy (Approval No. 139/30 March 2020) and EC of participating hospitals: Cluj Emergency County Hospital (Approval No. 5416/10 25 February 2020), Infectious Diseases Cluj-Napoca (Approval No. 6010/14 April 2021), and Clinical Institute of Urology and Renal Transplantation Cluj-Napoca (Approval No. 03/02 February 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Article

Exploring Burnout, Work Addiction, and Stress-Related Growth among Prehospital Emergency Personnel

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Abstract: Burnout and stress-related issues are significant concerns among medical personnel involved in emergency situations due to the high demands of their work. A cross-sectional descriptive and comparative study was conducted on 266 prehospital emergency personnel across five Romanian counties, comprising 41 physicians, 74 nurses, and 151 paramedics. Data were collected through an online form, including demographic and professional characteristics, and five validated scales. This study revealed moderate (49.3%) to high (25.9%) burnout rates, with 35% showing signs of work addiction. Despite these challenges, the personnel demonstrated high levels of stress-related growth (61.2%), strong self-discipline (74.1%), and low to moderate responsive distress (100%). Physicians exhibited higher work addiction and job satisfaction, whereas paramedics faced higher burnout, self-discipline, and distress levels. Nurses showed lower burnout and self-discipline levels. These findings highlight the prevalence of burnout and work addiction among emergency medical personnel, while also underscoring the presence of protective factors like higher self-discipline, good level of stress-related growth, and low to moderate responsive distress. The distinct differences in experiences among physicians, nurses, and paramedics emphasize the need for tailored strategies to address these issues within each group.

Keywords: emergency personnel; burnout; work addiction; responsive distress; self-discipline; stress-related growth

Citation: Puticiu, M.; Grecu, M.-B.; Rotaru, L.T.; Butoi, M.A.; Vancu, G.; Corlade-Andrei, M.; Cimpoesu, D.; Tat, R.M.; Golea, A. Exploring Burnout, Work Addiction, and Stress-Related Growth among Prehospital Emergency Personnel. *Behav. Sci.* **2024**, *14*, 851. https://doi.org/10.3390/bs14090851

Academic Editors: Lei Wang and Marialaura Di Tella

Received: 1 August 2024
Revised: 11 September 2024
Accepted: 19 September 2024
Published: 22 September 2024

1. Introduction

Emergency medicine (EM) is a medical specialty focused on the immediate diagnosis and treatment of illnesses or injuries. EM medical care operates in two modes: prehospital and in-hospital interventions [1]. Our study specifically focuses on prehospital EM personnel.

EM medical care is characterized by heavy workloads, rapid transitions, time pressure situations, uncertainty, often danger, and a necessity for high efficiency [2]. Ambulance EM teams are the first to attend the patients, requiring them to make quick and often life-saving decisions outside the hospital environment, with limited resources and under significant time pressure. EM personnel are often exposed to intense stressful situations which can affect not only the quality of the medical services, but also their own health and personal lives [3].

Burnout is a syndrome characterized by three major dimensions: exhaustion, disengagement, and a sense of ineffectiveness regarding one's work [4]. The term burnout, coined by Herbert Freudenberger, is described as a state of mental and physical exhaustion caused by the challenges of professional life [5]. Occupational burnout among EM personnel is a significant concern due to the highly stressful conditions. This can lead to medical errors, interpersonal conflict, fatigue, employee turnover, increased absenteeism, and mental health problems [2,6,7].

A 2020 meta-analysis [8] shows that 40% of EM physicians exhibit high levels of emotional exhaustion, 41% show high levels of depersonalization, and 35% experience low levels of personal accomplishment, as rated by the Maslach Burnout Inventory. Somville et al. [9] argue that burnout levels among EM physicians have increased in the last decade, with prevalence rates ranging between 43% and 54%, and Colville et al. [10] report similarly high levels of burnout among EM healthcare workers, ranging from 30% to 60%, with a greater risk for physicians than nurses. Liu et al. [2] found a global burnout rate of 33.4% in a sample of 2299 EM personnel (physicians and nurses) from Chengdu, China and a burnout rate of 36.94% for physicians and 31.11% for nurses.

Baier et al. [11] reported burnout rates between 19% and 40% among 1101 German prehospital EM health workers. In Riyadh, 63% of Saudi physicians experienced emotional exhaustion, and 40% reported depersonalization/disengagement [12]. A study of 327 EM physicians in India found a burnout rate of 28.7% [13].

Yao Xiuyu et al. [14] studied 256 EM nurses from several hospitals in Beijing, China, finding that 31.6% experienced severe burnout. Rodriguez-Rey et al. [15] reported a 57% burnout rate and 72.8% low perceived well-being among Spanish pediatric critical care personnel. In Japan, Morikawa et al. [3] found that among EM physicians, 8.9% reported high burnout, 16.1% experienced severe emotional exhaustion, 19.8% had high depersonalization, and 67% scored low on personal accomplishment.

A previous study on Romanian EM personnel [16] involving 184 participants (physicians, nurses, carers, paramedics, stretcher-bearers, registrars) found that 30.2% are at risk of burnout, which is linked to low work satisfaction and factors like social support and feedback. EM personnel are exposed to intense stress due to their work, increasing the risk of emotional distress, work–life imbalance, and burnout. Burnout rates in EM physicians are higher than in general physicians and significantly exceed those in the general population [8,10,17].

Work addiction, initially described by Oates as an uncontrollable need to work, is now defined as an irresistible drive to work excessively and/or compulsively [18]. It involves working beyond job requirements and becoming obsessively engaged in work. Work addiction is a behavioral addiction (non-substance) with harmful consequences, distinct from a hard worker who enjoys their work [19]. It is linked to poor health, low well-being, and work–family conflicts [20]. Those addicted to work prioritize their jobs, define their self-worth through work, spend excessive time on work, ignore health issues, and often return to work prematurely after illness. This addiction can cause severe physical and mental health problems, including fatigue, hypertension, insomnia, cardiovascular issues, gastritis, alopecia, anxiety, and depression [21].

Rates of work addiction vary widely among different occupational groups and are due to the use of diverse models. Merchaoui et al. [22] report that rates among physicians range from 8.3% to 30%. A study on 444 French university hospital physicians found that 13% exhibited high work addiction, while 35% showed mild addiction [23]. Rates of work addiction among nurses are similar. Ruiz-Garcia et al. [24] found that 28.3% of a total of 219 Spanish emergency and critical care nurses exhibited scores indicative of work addiction. To our knowledge, no study has been conducted on work addiction among Romanian EM personnel.

Responsive distress is the tendency to experience negative emotions in response to others' distress, focusing on the observer's discomfort rather than concern for the suffering person. Unlike empathic concern, which involves care for others, responsive distress

centers on one's own negative feelings. Additionally, blunted reward responsiveness is a key risk factor for depression, particularly under stress. Studies show that individuals with lower reward responsiveness at baseline are more vulnerable to anxiety and depression during stressful events, highlighting the need to address this vulnerability in stress-related interventions [25,26].

Self-discipline, defined by Harrison Gough and measured by the Self-Discipline Scale of the California Psychological Inventory [27], refers to the ability to exercise self-control and prioritize actions over emotions. This trait is crucial for EM personnel, particularly those in the Emergency Mobile Service for Resuscitation and Extrication (SMURD), which operates as a military-like structure within the Inspectorate for Emergency Situations (ISU). Burnout, a significant issue in emergency nursing, is linked to higher turnover rates, traumatic events, shift work, violence, and stress from the COVID-19 pandemic [28]. Building resilience through self-discipline, optimism, and goal-oriented behaviors is essential to combating burnout in this high-stress environment.

Stress is an undeniable reality of human life and almost everyone will experience a stressful situation during their lifetime [29]. Post-traumatic growth or stress-related growth is defined as the experience of useful changes following exposure to a stressful event [30]. Stress-related growth works in three distinct directions: improved social relations, increased trust in personal resources, and enhanced coping abilities. Park et al. [31] argue that stress-related growth has been observed even in extremely stressful situations such as the death of a loved one, severe illness, divorce, or accidents.

Stress is a universal experience that can lead to stress-related growth, manifesting as improved social relationships, enhanced personal resources, and better coping abilities [29–31]. During the COVID-19 pandemic, healthcare workers (HCWs), particularly physicians, faced significant trauma but also demonstrated post-traumatic growth (PTG). A study of 691 healthcare workers in Kosovo highlighted that despite mental health challenges, including anxiety, depression, and insomnia, positive changes were noted in areas like relating to others and personal strength [32]. Physicians, who exhibited higher resilience than the general population, were identified as an "at-risk" group due to their exposure to infected environments. Resilience in physicians is crucial, as it not only mitigates anxiety and depression but also fosters PTG. Ensuring adequate social and psychological support, along with time for rest and family, is essential in helping HCWs, especially physicians, manage the mental health impacts of the pandemic and promote long-term PTG [33].

Research on burnout and stress-related issues among Romanian emergency medical (EM) personnel is limited [16]. To the best of our knowledge, no studies have yet explored the relationship between burnout, work addiction, and stress management variables (such as stress-related growth) within this population. Additionally, there has been little investigation into the differences between various categories of EM personnel, including physicians, nurses, and paramedics.

Therefore, our objective was to evaluate burnout, work addiction, distress response, self-discipline, and stress-related growth among EM personnel, while also identifying differences among physicians, nurses, and paramedics. We focus on two main hypotheses. First, we hypothesize that EM personnel exhibit moderate to high levels of burnout and work addiction, while simultaneously demonstrating strong self-discipline, low distress responses, and a significant capacity for stress-related growth. Second, we anticipate identifying significant differences among EM physicians, EM nurses, and paramedics across all relevant variables, given their distinct roles, training backgrounds, and experiences within EM teams.

2. Materials and Methods

2.1. Participants and Procedure

This cross-sectional descriptive and comparative study of 266 EM personnel included physicians, nurses, and paramedics. A total of 728 individuals were addressed for this study (170 EM physicians, 400 EM nurses, 158 paramedics), from 5 out of the

41 counties from Romania. A total of 266 individuals completed the online form, indicating a 36.5% response rate.

Informed consent was obtained through the online form, which was distributed to prehospital EM personnel from 5 Romanian counties, between December 2023 and February 2024. A total of 266 individuals completed the online form, comprising 41 physicians, 74 nurses, and 151 paramedics.

In Romania, the paramedics are part of ISU, a military structure under the Ministry of Internal Affairs that also operates the firefighting services. Physicians and nurses are employed by the local or regional public hospitals, and team up with paramedics within the prehospital EM mobile teams, part of the SMURD, operating within the ISU regional structures. Paramedics work by rotation within two types of EM teams: the mobile intensive therapy units (1 physician, 1 nurse, and 2 paramedics) and the specialized first-aid units (3 paramedics).

2.2. Measures

Participants completed an online form comprised two sections. First section focused on demographic and professional characteristics, including age, gender, field experience, type of EM personnel (physician, nurse, paramedic), health characteristics: smoking status, self-evaluated health status on a five-point scale, acute and chronic medical conditions, sleep duration outside working shifts, stress-related post-work activities, work satisfaction, and subjective well-being. The last two variables were both assessed on a ten-point scale, ranging from 1 (extremely low) to 10 (extremely high).

Section two comprised five scales: The Oldenburg Burnout Inventory (OLBI), The Dutch Work Addiction Scale (DUWAS), Responsive Distress Scale (RD), Self-discipline Scale (SFD) and The Stress-Related Growth Scale (SRGS). First four scales were retrieved from Romanian version of International Personality Item Pool (IPIP) at https://researchcentral.ro (accessed on 25 March 2024). SRGS is part of the Clinical Assessment System (SEC), a licensed set of psychometric instruments developed, adapted, and distributed by RTS Romanian Psychological Testing Services.

OLBI is a 16-item self-reported scale assessing two core dimensions of burnout: disengagement and exhaustion [34]. It is based on the job demands–resources model [35], which states that working conditions can be delimited in two broad categories: demands and resources, the lack of which leads to disengagement. Exhaustion (8 items) refers to feelings of emotional drain and emptiness, fatigue (physical and mental), and the need for rest. Disengagement from work (8 items) refers to the tendency of distancing oneself from work, while manifesting a negative perception about work and even cynical attitudes and behaviors towards it [34]. Items are scored on a 4-point Likert scale, where 1—strongly disagree to 4—strongly agree. Total score can range between 16 and 64, with higher scores indicating higher levels of exhaustion, disengagement, or burnout (cumulative score of the two dimensions). For our sample, reliability for the entire scale was adequate, Cronbach's Alpha = 0.826, disengagement (0.444), and exhaustion (0.817).

DUWAS-10 is used to assess work addiction and its two dimensions: working excessively (WE) and working compulsively (WC). Each subscale has 5 items with scoring on a four-point Likert scale, 1—never to 4—always. Raw scores are divided per number of items and can range from 1 to 4. Scores above 75th percentile are considered relevant for the construct [36,37]. Reliability for DUWAS-10 was good for our sample, Cronbach's Alpha = 0.881, WE (0.805), and WC (0.693).

RD scale is part of the IPIP, Emotional Intelligence 7 components, proposed by Barchard [25]. It has 10 items with dichotomous responses yes versus no. Total scores can range between 0 and 10, higher scores showing greater responsive distress. In our study, Cronbach's Alpha for RD was 0.955.

SFD scale was retrieved from IPIP, Romanian version, and was originally developed by Gough [26]. It has 10 items with yes or no response. Higher scores mean better self-discipline. For our study, reliability of scale was moderate, Cronbach's Alpha = 0.469.

SRGS was developed by Park et al. [31]; it has 15 items with scoring on a three-point scale, 0—disagree, 1—somewhat agree, 2—strongly agree. Scores can range from 0 to 30. The cut-off score is 28, with scores above showing individuals with relevant stress-related growth. Reliability in our sample was high, Cronbach's Alpha = 0.855.

2.3. Statistical Procedures

Data collected from the online form were systematized and analyzed using IBM SPSS Statistic 20 software. Statistical procedures used for parametric data were Pearson correlation, independent sample t test, One-Way ANOVA, and for nonparametric data and not normal distributions, rho Spearman, Chi-square test, Fisher's exact test, U Mann–Whitney test, Kruskal–Wallis test, and Generalized Linear Models [38]. Distribution normality was tested with One-Sample Kolmogorov–Smirnov test. Significance level for p-value was set at 0.05.

3. Results

3.1. Demographic and Health Characteristics of Participants

Our study includes 266 prehospital EM health workers. Among the participants, 67.3% are men. The age of participants ranges between 20 and 57 years, with a mean age of 38.71 years. Age distribution is normal ($p = 0.216$). Additionally, 29.7% of participants are smokers, 25.6% have an acute medical condition at the time of assessment, and 30.5% have a chronic medical condition (Table 1).

Table 1. Demographic and health characteristics of participants.

Variable	M	SD	Median	Minimum	Maximum
Age (yrs)	38.71	9.18	40	20	57
Field experience (yrs)	12.32	8.04	13	1	38
Sleep duration outside working shifts (h)	7.83	1.12	8	4	10
Variable			frequency		percent
Gender	men		179		67.3
	women		87		32.7
Smoking status	non-smokers		187		70.3
	smokers		79		29.7
Self-evaluation of health status	very poor		1		0.4
	poor		4		1.5
	average		33		12.4
	good		71		26.7
	very good		157		59.0
Acute medical condition	no		198		74.4
	yes		68		25.6
Chronic medical condition	no		185		69.5
	yes		81		30.5

M—mean value of variable, SD—standard deviation.

Field experience ranges from 1 to 38 years, with an average at 12.32 years, with a distribution significantly different from a standard normal distribution ($p = 0.001$). Sleep duration outside working shifts ranges from 4 to 10 h, with a mean of 7.83 h. The distribution of sleep duration differs significantly from a standard normal distribution ($p < 0.001$). Three participants report sleep durations of less than 6 h.

Women in our study tend to be older than men, with a mean age (M) of 42.25 for women, standard deviation, SD = 8.61 and M = 36.99 years for men, SD = 8.987; difference

is significant at $p < 0.001$. Men and women have similar percentages of smokers, with 29.1% and 31.0%, respectively. Women (M = 14.20 years, SD = 8.59) are more experienced than men (M = 11.41 years), and this difference is significant at $p = 0.008$. However, when controlling for age, the difference in experience is significant at $p = 0.076$, indicating that the initial difference in experience can be explained by the age difference between men and women in our study.

Women exhibit a significantly higher percentage of acute medical conditions (34.5%) compared to men (21.2%), with the difference being statistically significant ($p = 0.025$.) This trend is also observed in chronic medical conditions, where women have a much higher percentage (50.6%) compared to men (20.7%), with the difference being highly significant ($p < 0.001$). Additionally, men rate their own health more positively than women, which is also statistically significant ($p < 0.001$).

3.2. Descriptive Statistics for Entire Sample

Participants were asked to evaluate their health status on a five-point scale, ranging from 1 (very poor) to 5 (very good). The scores ranged from 1 to 5, with an average of 4.42, and a median of 5 (SD = 0.79). The results show that most of the participants (85.7%) consider themselves to be in good and very good health (scores of 4 and 5).

Participants evaluated their work satisfaction (WS) on a ten-point scale, ranging from 1 (extremely low) to 10 (extremely high). The average score was 7.47, median = 8, SD = 1.40, scores ranging from 3 to 10. The majority of subjects (54.1%) have high levels of work satisfaction (scores of 8, 9, and 10), while only 7.1% consider their work satisfaction to be below average. No participants consider themselves as extremely and very low satisfied at work.

We requested participants to rate their subjective well-being (SWB), on a scale from 1 (extremely low) to 10 (extremely high). The average score for SWB was 9.01, median = 9.50, SD = 1.25, showing very high levels in general, with 86.5% reporting scores of 8, 9, and 10 (high SWB), and only 1.5% had scores below average (scores ≤ 5).

Participants were asked to provide information about their stress-relieving activities after working hours, which were grouped in eight broad categories. Preferences for each category are as follows: physical/sports (50.0%), intellectual (9.0%), social (3.0%), cultural/artistic (7.1%), online/PC (7.9%), house activities (7.9%), passive relaxation (9.8%), and others (5.3%).

General scores for OLBI range between 17 and 64 (Table 2), with an average of 53.41 and a median of 58, indicating that scores tend to be quite high. Although there is no established cut-off score for the Romanian population, we categorized scores into low burnout (scores $\leq$ 25th percentile), moderate burnout (scores between 25th and 75th percentiles) and high burnout (scores $\geq$ 75th percentile) [36]. The score for 25th percentile was 49.75 and for 75th percentile was 61, showing that 24.8% of the EM personnel have low burnout, 49.3% report moderate burnout, and 25.9% report high burnout. OLBI scores do not follow a normal distribution ($p < 0.001$).

Threshold scores for disengagement were 25 (25th percentile) and 29 (75th percentile). This indicates that 25.2% of the EM medical staff exhibited low disengagement, 23.3% moderate disengagement, and 51.5% high disengagement. For exhaustion, threshold scores were 25 and 32, meaning that 24.8% had low exhaustion, 46.3% had moderate exhaustion, while 28.9% showed high exhaustion.

The average scores for working excessively and working addictively (WA) are 1.96 and 2.39, respectively, both below the cut-off score of 75th percentile. In contrast, the average score for working compulsively is 2.82, which is above the cut-off score. Additionally, 39.1% of the participants show relevant scores for working excessively, 36.1% for working compulsively, and 35% for work addiction. The DUWAS scores do not follow a normal distribution ($p < 0.001$).

Table 2. Descriptive statistics for OLBI, DUWAS, RD, SFD, SRGS (N = 266).

Variable	M	SD	Median	Minimum	Maximum
Disengagement	26.78	3.97	29	9	32
Exhaustion	26.63	6.36	29	8	32
Burnout	53.41	9.68	58.00	17	64
Working excessively (WE)	1.96	1.03	1.60	1.00	3.40
Working compulsively (WC)	2.82	0.83	2.20	1.00	4.00
Work addiction (WA)	2.39	0.90	1.90	2.00	7.40
Responsive distress	4.40	1.31	5.00	1	8
Self-discipline	9.63	0.77	10	5	10
Stress-related growth	26.48	4.80	30	13	30

OLBI—The Oldenburg Burnout Inventory, DUWAS—The Dutch Work Addiction Scale-short version, RD—Responsive Distress Scale, SFD—Self-discipline Scale, SRGS—The Stress-Related Growth Scale, N—number of participants.

The average score for RD is relatively low (4.40), with scores ranging between 1 and 8. No member of the EM personnel showed high responsive distress. The average score for self-discipline is relatively high (9.63), 74.1% showing the maximum possible score, and no participant scoring below 5. The average score for stress-related growth (SRGS) is also relatively high (26.48), with 61.2% scoring above the cut-off score of 28. The distributions for RD, SFD, and SRGS scores are significantly different from a standard normal distribution, all p-values being below 0.001.

Men show higher levels of subjective well-being, disengagement, exhaustion, burnout, responsive distress, and self-discipline, while women show higher levels of work satisfaction, excessive and compulsive work, work addiction, and stress-related growth. All differences are significant at $p < 0.05$ (Table 3).

Table 3. Differences on burnout, work addiction, responsive distress, self-discipline, and stress-related growth, regarding gender, smoking status, acute and chronic medical conditions (U Mann–Whitney test).

	Disengagement	Exhaustion	Burnout	Working Excessively	Working Compulsively	Work Addiction	Responsive Distress	Self-Discipline	Stress-Related Growth
Gender									
men (M/SD)	27.74/2.83	28.99/4.41	56.73/6.49	1.79/1.05	2.76/0.83	2.27/0.93	4.77/1.07	9.76/0.55	26.04/4.80
women (M/SD)	24.79/5.11	21.77/6.98	46.56/11.4	2.32/0.87	2.95/0.82	2.63/0.80	3.63/1.44	9.36/1.03	27.37/4.70
p-value	<0.001	<0.001	<0.001	<0.001	0.019	<0.001	<0.001	<0.001	0.003
Smoking status									
no (M/SD)	26.52/4.28	26.58/6.67	53.1/10.36	1.94/1.04	2.81/0.83	2.38/0.92	4.50/1.34	9.61/0.79	26.36/4.86
yes (M/SD)	27.38/3.05	26.75/5.57	54.13/7.85	2.00/1.00	2.82/0.81	2.41/0.88	4.16/1.22	9.67/0.71	26.76/4.65
p-value	0.490	0.600	0.927	0.349	0.914	0.415	0.057	0.468	0.725
Acute medical condition									
no (M/SD)	27.02/3.67	27.24/5.95	54.26/9.04	1.95/1.04	2.83/0.82	2.38/0.91	4.43/1.26	9.64/0.76	25.95/5.09
yes (M/SD)	26.07/4.71	24.84/7.17	50.91/11.0	2.00/0.99	2.79/0.84	2.39/0.89	4.31/1.47	9.60/0.77	28.01/3.43
p-value	0.145	0.003	0.020	0.423	0.673	0.673	0.330	0.666	0.019
Chronic medical condition									
no (M/SD)	27.50/2.88	28.04/5.29	55.54/7.56	1.89/1.06	2.81/0.82	2.35/0.93	4.51/1.20	9.73/0.68	25.91/5.01
yes (M/SD)	25.12/5.39	23.41/7.36	48.53/12.0	2.11/0.93	2.83/0.84	2.47/0.85	4.14/1.52	9.40/0.90	27.78/4.01
p-value	0.001	<0.001	<0.001	0.037	0.794	0.129	0.005	<0.001	0.001

M—mean value of variable, SD—standard deviation.

As shown in Table 3, acute medical conditions are relevant for exhaustion, burnout, and stress-related growth, and not relevant for disengagement, working excessively, working compulsively, work addiction, responsive distress, and self-discipline, while chronic medical conditions are relevant for disengagement, exhaustion, burnout, working excessively, responsive distress, self-discipline, and stress-related growth, and not relevant for working compulsively and work addiction.

Smoking status is not associated with any of the variables presented in Table 3.

A significant association is found between stress-recovery activities and subjective well-being ($p = 0.022$), exhaustion ($p = 0.004$), burnout ($p = 0.021$), and responsive distress

($p = 0.033$). Participants who prefer physical/sport activities and house activities tend to show higher well-being, while the lowest well-being is observed in participants who prefer social and intellectual activities. Those who prefer intellectual and social activities show lower levels of exhaustion, while the highest level of exhaustion is observed in those engaged in physical/sport and house activities. Participants engaged in cultural and other activities show lower levels of responsive distress, while the highest levels of responsive distress are observed in those who prefer intellectual and sport/physical activities.

3.3. *Differences among Physicians, Nurses, and Paramedics (EM Personnel)*

The emergency team includes a physician, a nurse, and paramedics. Therefore, we have divided the participants in three groups: physicians, nurses, and paramedics. Group distribution is 41 physicians (15.4%), 74 nurses (27.8%), and 151 paramedics (56.8%).

There is a significant difference in gender distribution, with paramedics being overwhelmingly men (98%), while nurses and physicians are mainly women, 74.3% and 70.7%, respectively. Age differs among the groups with paramedics being significantly younger than physicians and nurses. When controlling for gender, age difference between groups is significant at $p < 0.001$ (ANCOVA), with an effect size of 0.107, showing that difference in age is not only due to differences in gender distribution, with paramedics being mainly men, and nurses and physicians predominantly women, but also that women amongst paramedics are younger than women amongst nurses and physicians, and men paramedics tend to be younger than men amongst nurses and physicians (Table 4).

Table 4. Structure and differences of the 3 groups investigated: physicians, nurses, and paramedics.

Variable		EM Nurses N = 74	EM Physicians N = 41	Paramedics N = 151	Difference (p Value)
Gender	M (%) W (%)	19 (25.7) 55 (74.3)	12 (29.3) 29 (70.7)	148 (98) 3 (2)	<0.001 *
Age (yrs)	mean SD	42.01 9.05	44.61 8.10	35.50 8.14	<0.001 **
Field experience (yrs)	mean SD	13.65 9.11	17.59 7.86	10.24 6.66	<0.001 ***
Smoking	yes (%) no (%)	29 (39.2) 45 (60.8)	11 (26.8) 30 (73.2)	39 (25.8) 112 (74.2)	<0.109 *
Acute medical condition	yes (%) no (%)	27 (36.5) 47 (63.5)	12 (29.3) 29 (70.7)	29 (19.2) 122 (80.8)	0.017 *
Chronic medical condition	yes (%) no (%)	34 (45.9) 40 (54.1)	24 (58.5) 17 (41.5)	23 (15.2) 128 (84.8)	<0.001 *
Sleep (h)	mean SD	7.78 1.11	7.95 1.22	7.81 1.10	0.621 ***
Self-evaluated health status	mean SD	4.16 0.84	4.15 0.88	4.63 0.68	<0.001 ***

* Chi-square test; ** One-Way ANOVA; *** Kruskal–Wallis test, EM—emergency medicine, N—number, M—men, W—women, SD—standard deviation.

Physicians show the highest levels of field experience (M = 17.59 years), while paramedics have the lowest (10.24 years). When controlling for age, there is still a significant difference in field experience between groups ($p = 0.038$), although the effect size is small (eta squared = 0.025), with physicians remaining the most experienced.

Paramedics comprise a significantly lower percentage of individuals with acute and chronic medical conditions compared to nurses and physicians. The highest percentage of individuals with acute medical conditions is found in nurses, while the chronic condition rate is highest among physicians. Paramedics tend to evaluate their health status higher than nurses and physicians, although all participants report good health.

Groups differ significantly regarding their post-work stress-recovery activities ($p = 0.001$; Chi-square test) (Table 5). Paramedics show a greater percentage towards physical/sport activities (61.6%) in comparison with physicians (36.6%) and nurses (33.8%). Physicians show a greater percentage towards intellectual activities (19.5%) than nurses (12.2%) and paramedics (4.6%). Nurses have higher percentages of social, cultural, and passive relaxation activities, while physicians have higher percentages of online/PC and house activities. Results should be extrapolated with caution as 41.7% of the distribution is underrepresented (cell frequency less than 5).

Table 5. Group distribution and differences on post-work stress-relieving activities.

Variable		EM Nurses N = 74	EM Physicians N = 41	Paramedics N = 151
Physical/Sport activities	%	33.7	36.6	61.6
Intellectual activities	%	12.2	19.5	4.6
Social activities	%	6.8	2.4	1.3
Cultural activities	%	12.2	7.3	4.6
Online activities	%	6.8	12.2	7.3
House activities	%	4.1	12.2	8.6
Passive relaxation	%	14.8	4.9	8.6
Others	%	9.4	4.9	3.4

EM—emergency medicine, N—number.

To assess differences among groups for work satisfaction, subjective well-being, burnout, work addiction, responsive distress, self-discipline, and stress-related growth, we used Generalized Linear Models. This approach was chosen because the dependent variables do not show normal distributions. The models also controlled for covariates (age, sleep, experience, health status) and other factors (gender, acute and chronic medical condition, post-work stress-recovery activities) that were associated with the dependent variables.

OLBI scores per groups show that 64.9% of nurses, 36.7% of physicians, and 2% of paramedics show low burnout (scores ≤ 49), 33.7% of nurses, 43.8% of physicians, and 58.3% of paramedics show moderate levels of burnout (scores between 50 and 60), and 1.4% of nurses, 19.5% of physicians, and 39.7% of paramedics show high levels of burnout (scores ≥ 61).

Low levels of disengagement were observed in 58.1% of nurses, 26.8% of physicians, and 8.6% of paramedics. Moderate disengagement was reported by 28.4% of nurses, 22% of physicians, and 21.2% of paramedics. High disengagement was observed for 13.5% of nurses, 51.2% of physicians, and 70.2% of paramedics.

Exhaustion was reported as low by 66.2% of nurses, 36.6% of physicians, and 1.3% of paramedics, as moderate by 32.4% of nurses, 61% of physicians, and 49% of paramedics, and as high by 1.4% of nurses, 2.4% of physicians, and 49.7% of paramedics.

Work addiction was present for 33.8% of nurses, 63.4% of physicians, and 27.8% of paramedics. Rates for working excessively were 17.6%—nurses, 26.8%—physicians and 27.8%—paramedics, while for working compulsively the rates were 36.5%—nurses, 58.5%—physicians, and 29.8%—paramedics.

Most of the nurses (93.2%) and physicians (70.7%) show below moderate levels of responsive distress, but not very low, while the majority of paramedics (92.7%) show moderate levels of responsive distress. Self-discipline is quite high in all groups (scores of 9 or 10): 83.8%—nurses, 90.2%—physicians, and 98.7%—paramedics.

A total of 71.6% of nurses show SRGS scores equal to or above the cut-off score, as well as 80.5% of physicians and 50.3% of paramedics, accounting for moderate and high levels of stress-related growth among EM personnel.

There are significant differences for work satisfaction, disengagement, exhaustion, burnout, working excessively and compulsively, work addiction, responsive distress, and self-discipline (Table 6).

Table 6. Group differences for stress-related variables.

Variable		EM Nurses N = 74	EM Physicians N = 41	Paramedics N = 151	Generalized Linear Model (p Value)
Work satisfaction *	mean	8.14	8.59	6.83	<0.001
	SD	0.89	0.92	1.37	
Subjective well-being *	mean	8.43	8.37	9.46	0.847
	SD	1.42	1.36	0.90	
Disengagement *	mean	23.72	26.95	28.23	<0.001
	SD	4.36	4.96	2.29	
Exhaustion *	mean	20.07	24.66	30.38	<0.001
	SD	6.53	6.07	2.08	
Burnout *	mean	43.78	51.61	58.61	<0.001
	SD	10.12	10.41	3.60	
Working excessively *	mean	2.11	2.64	1.70	0.003
	SD	0.86	0.70	1.08	
Working compulsively *	mean	2.86	3.21	2.69	0.027
	SD	0.83	0.80	0.80	
Work addiction *	mean	2.49	2.93	2.17	0.008
	SD	0.82	0.69	0.93	
Responsive distress *	mean	3.27	4.17	5.01	<0.001
	SD	1.02	1.84	0.78	
Self-discipline *	mean	9.23	9.41	9.88	<0.001
	SD	1.00	0.95	0.40	
Stress-related growth *	mean	26.92	28.76	25.64	0.263
	SD	5.21	2.65	4.83	

* Distribution differs significantly from a standard normal distribution (p-value for One-Sample Kolmogorov–Smirnov test below 0.05), EM—emergency medicine, N—number, SD—standard deviation.

4. Discussion

Given the stressful nature of emergency medical care, we aimed to understand how EM professionals are affected by burnout, work overload, health issues, and distress they face on a daily basis, but also to identify potential emotional and behavioral coping factors like responsive distress and self-discipline. Lastly, we aimed to identify specific characteristics of each group of professionals to better understand their strengths and weaknesses.

We observed that 25.9% of the EM professionals show high burnout levels, 51.5% exhibit high levels of disengagement, and 28.9% show high levels of exhaustion. A previous study on 184 Romanian EM personnel found that 30.2% are at risk of burnout [16]. Colville et al. [10] argue for high levels of burnout amongst EM health workers, ranging from 30% to 60%, with a greater risk for physicians than nurses. Similar burnout levels, ranging between 30 and 40%, have been identified in various studies [2,8]. However, Ma et al. [39] reported significantly lower burnout levels, as low as 2.3%. Other studies, however, show higher burnout rates, ranging from 40 to 60% [9,11–15,40].

Work addiction among EM care professionals appears to be quite high, with 39.1% showing relevant scores for working excessively, 36.1% for working compulsively, and 35% for work addiction. Yet 54.1% of the participants report high levels of work satisfaction, which could mean that work addiction might not be all that dysfunctional for everyone with DUWAS high scores. Some might be classified as work enthusiasts, who are high in involvement but also enjoyment regarding work, but low on drive [18,41]. Assessing work addiction, Merchaoui et al. [22], report that 24% of physicians showed relevant scores and no less than 56% were at high risk for work addiction.

We observe that EM personnel exhibit moderate levels of responsive distress, indicating that while they are not overly affected by daily distress, they maintain a balance

between cognitive coping and emotional sensitivity. They remain empathetic and attuned to the distress of others, including patients and their families.

Self-discipline is notably high among EM personnel, reflecting their strong adherence to procedures, which is crucial given the time-sensitive and high-responsibility nature of their work. This high level of self-discipline is likely reinforced by the military-like organizational structure of the SMURD teams in Romania, which operate under the national and regional ISU structures.

Additionally, EM personnel demonstrate a high rate of stress-related growth (61.2%), showing their capacity for professional and psychological development by finding positive outcomes from their daily stressful experiences. Identifying individual traits and organizational factors that support this growth could be key in designing effective interventions and support resources for EM medical staff.

Age correlates positively with work satisfaction, work addiction, and stress-related growth, but negatively with well-being, burnout, and responsive distress. Older EM professionals are more satisfied and experience higher work addiction but less burnout and distress. Experience is linked to lower well-being, while more sleep outside shifts correlates with burnout and self-discipline. Better self-reported health improves well-being but also associates with burnout.

Men report higher well-being and burnout, while women show more work satisfaction and stress-related growth. Acute medical conditions lower burnout, while chronic conditions increase work addiction and stress-related growth [2].

Participants who prefer physical or house activities tend to have higher well-being, while those who favor social and intellectual activities report the lowest well-being. Intellectual and social activities are linked to lower exhaustion, whereas physical and house activities correlate with higher exhaustion. Cultural activities are associated with lower responsive distress, while intellectual and sport/physical activities are linked to higher responsive distress.

Paramedics tend to be younger and mostly male, while physicians and nurses are older and predominantly female, with physicians being more experienced. Paramedics report fewer medical conditions and consider themselves healthier, with a preference for physical activities. Physicians favor intellectual and online activities, while nurses prefer social, cultural, and relaxation activities.

Burnout is highest among paramedics (39.7%) and lowest among nurses (1.4%). Paramedics also have the highest rates of disengagement (70.2%) and exhaustion (49.7%). Nurses report lower levels of disengagement, exhaustion, burnout, responsive distress, and self-discipline. Physicians exhibit higher work satisfaction, work addiction, and stress-related growth, while paramedics show higher burnout, distress, and self-discipline but lower satisfaction and work-related growth.

The higher levels of work addiction and satisfaction among physicians warrant further investigation, as they may indicate a greater enthusiasm for their work. The elevated self-discipline in paramedics could be attributed to their involvement in the ISU's military structure. The increased burnout, disengagement, and exhaustion observed in paramedics may reflect the demands of prehospital emergency work, which involves frequent transportation, greater uncertainty, and longer shifts.

These findings suggest that while burnout and work addiction are prevalent among prehospital emergency personnel, protective factors like low to moderate responsive distress, high self-discipline, and stress-related growth are also present.

We must acknowledge certain limitations of our study: the inclusion of only 20% of Romanian counties, an imbalance in the categories of emergency medical (EM) health workers, and the varied types of activities associated with prehospital assessment for the participants (physicians and nurses: in both Emergency Departments and prehospital settings; paramedics: in prehospital settings and as firefighters).

Additionally, a cross-sectional approach does not allow us to capture the longitudinal changes over time that have been observed in other survey-based studies of EM physicians.

Moving forward, a longitudinal study with a larger participant pool and additional comparative studies between prehospital emergency services, both with and without a military component, would be valuable. These studies could further compare levels of burnout, work addiction, self-discipline, responsive distress, and stress-related growth.

5. Conclusions

Our study shows that burnout and work addiction are present at moderate and high levels in prehospital EM medical staff. Yet EM professionals also show good levels of stress-related growth and low responsive distress, meaning that they do have good coping mechanisms even if working in a highly stressful environment.

We have found relevant differences among three categories of EM personnel: physicians, nurses, and paramedics. Physicians show higher levels of work satisfaction and work addiction. Paramedics show higher levels of burnout, responsive distress, and self-discipline, while nurses show lower levels of burnout, responsive distress, and self-discipline. The differences among physicians, nurses, and paramedics highlight the need for tailored approaches to address burnout and work addiction issues within these groups.

Author Contributions: Formal analysis, M.P.; Methodology, G.V. and M.P.; Validation, D.C., A.G. and L.T.R.; Writing—original draft, M.P., L.T.R., M.-B.G., M.A.B., G.V., M.C.-A., D.C., R.M.T. and A.G.; Writing—review and editing, M.P., M.C.-A., D.C. and A.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study involved healthy volunteers who participated by filling out a questionnaire. There were no invasive procedures, no use of biological samples, and no interventions that could potentially harm the participants. The nature of this study posed minimal risk to the participants. According to the European Union's General Data Protection Regulation (GDPR) (Regulation (EU) 2016/679), research involving the processing of personal data may not require ethical approval if the data are anonymized and the research poses minimal risk to the participants. The European Code of Conduct for Research Integrity (revised edition, 2017) states that research involving surveys or questionnaires with non-sensitive and anonymized data does not require ethical approval, provided informed consent is obtained. This study complies with the European regulatory framework for ethical research, which exempts certain types of low-risk research from requiring formal ethical review. This includes surveys and questionnaires where the data collected are non-sensitive, anonymous, and the participants are not vulnerable populations.

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors acknowledge Bistrian Marius Sorin for the support given for statistical analysis.

Conflicts of Interest: The authors declare no conflicts of interest.

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Emergency department point-of-care biomarkers and day 90 functional outcome in spontaneous intracerebral hemorrhage: A single-center pilot study

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Received November 2, 2021; Accepted December 1, 2021

DOI: 10.3892/etm.2022.11123

Abstract. Spontaneous intracerebral hemorrhage (sICH) results in high morbidity and mortality rates, thus identifying strategies for timely prognosis and treatment is important. The present study aimed to analyze the relationship between emergency department point-of-care (POC) blood biomarkers and day 90 functional outcome (FO) in patients with acute (<8 h) sICH. On-site POC determinations, including complete blood count, glucose, cardiac troponin I, D-dimer and C-reactive protein, and derived inflammatory indexes were performed for a cohort of 35 patients. The primary endpoint was a favorable day 90 FO (modified Rankin Score ≤3). Secondary endpoints included early neurological worsening (ENW), day 7/discharge neurological impairment, day 90 independence assessment (Barthel Index <60), hematoma enlargement and perihematomal edema (PHE) growth. A favorable three-month FO was reported in 16 (46%) participants. Older age, previous history of ischemic stroke and initial imagistic parameters, including intraventricular hemorrhage, enlarged contralateral ventricle and cerebral atrophy, significantly predicted an unfavorable FO. The admission D-dimer similarly predicted day 90 FO and the independence status, along with ENW and a more severe day 7/discharge neurological status. The D-dimer also correlated with the initial neurological status and PHE. PHE growth correlated with granulocytes, systemic immune-inflammation index and glycemia. The results suggested that a lower admission D-dimer could indicate an improved day 90 FO of patients with sICH, while also anticipating the development of PHE growth and ENW.

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Key words: spontaneous intracerebral hemorrhage, point-of-care testing, biomarkers, D-dimer, functional outcome, emergency department

Introduction

Spontaneous intracerebral hemorrhage (sICH) has a disproportionate socioeconomic impact considering its low incidence rate (26% of all incident strokes in 2017, with higher prevalence rates in East European countries) (1). An aging population and repeated unsuccessful research endeavors for curative treatment contribute to its high mortality and morbidity, resulting in a 1-month case fatality of 40% with only 12% of patients regaining long-term functional independence (2). At present, the focus is on improving early outcome prediction to individualize patient management, and to also identify individuals at risk before sICH occurs, as to date, no reliable premonitory onset markers have been determined. Therefore, biomarker testing is an area of interest, as it is minimally invasive, low cost and could potentially enable accurate risk stratification and outcome estimation. Routinely, the standard hematologic evaluation consists of complete blood count (CBC), including platelet (PLT) count, coagulation profile and serum glucose (3,4). As sICH is a time-sensitive condition, readily available point-of-care (POC) devices reduce delays and facilitate prompt management.

Over the past decade, the role of inflammation in sICH progression and neurological impairment has been further clarified (5,6), and inflammatory biomarkers are currently regarded as potential prognostication tools. CBC upon admission, which includes hemoglobin (Hb), red blood cells and their distribution width (RDW), and derived inflammatory indexes, such as neutrophils-to-lymphocytes ratio (NLR), lymphocytes-to-monocytes ratio (LMR), platelets-to-lymphocytes ratio (PLR) and C-reactive protein (CRP), have been

associated with mortality (5,7-11). Moreover, early neurological worsening (ENW) (12), hematoma volume expansion and the expansion of the surrounding edema (5,13,14), and day 90 functional outcome (FO) (6,13,15-19) have also been reported to be associated with mortality. Furthermore, systemic immune-inflammation index (SII) has been recently reported as a relevant predictor of poor hospital discharge outcome (20).

Leukocyte count has been consistently associated with larger ICH volumes (5), but no consensus has been reached as to ICH progression, infection risk and mortality (11). Higher admission neutrophils have been associated with larger baseline volumes (21), mortality (10,22,23) and morbidity (10,23). With regard to monocyte (MON) count, increased admission levels are associated with poor outcome and mortality (5), but not with ICH volume (21), as MON are thought to contribute to secondary injury (13). Admission lymphopenia has been correlated with higher stroke severity, larger baseline hematoma volume and intraventricular extension, along with infection risk and 3-month mortality rate (5). NLR and LMR mirror post-ICH proinflammation and immunosuppression (12), as higher NLR has reflected larger baseline volumes, stroke severity, severe perihematomal edema (PHE) growth and poor 3-month outcome (5), and lower LMR has indicated neurologic deterioration and day 90 mortality (12).

CRP has been significantly linked with hematoma growth (HG), ENW, mortality and 3-month outcome (5,7,9,24). Its early presence at the hemorrhagic site could be due to local synthesis or transformation of the circulating liver-synthetized pentameric form (24).

Regarding Hb, anemia is associated with larger ICH volumes (25), increased HG (26) and worse outcomes (26-28). RDW is another inexpensive, automatically generated hematology parameter that is impacted by inflammation and is currently associated with day 30 FO (29).

Moreover, stroke is considered a systemic condition that induces cardiac, lung and immune dysfunctions (5,30); therefore, cardiac biomarkers, such as troponin I (cTnI), have been linked to stroke severity (31), in-hospital mortality (30,32) and unfavorable outcomes (33,34). On the other hand, D-dimer levels have been associated with an increased risk (35,36) and severity (37) of hemorrhagic stroke, and an increased hematoma volume (37), although it has not been proved sufficiently accurate for molecular stroke diagnosis (38). Furthermore, admission hyperglycemia has also been related to mortality (6,8) and day 90 FO (6).

The emergency department (ED) provides a unique opportunity for POC testing, both for standard and additional biomarkers (e.g., cTnI, D-dimer and CRP). When addressing time-sensitive conditions such as sICH, targeted escalation of the standard protocol could benefit these hyperacute patients. The contribution of additional POC testing could enable early risk stratification strategies to be identified and facilitate improvements in outcomes for patients with sICH. Nevertheless, information about the applicability of POC testing on cerebral hemorrhage is scarce.

The present study aimed to assess the predictive role of ED-based POC biomarkers (standard and additional) and derived inflammatory indexes on day 90 FO in patients with acute sICH.

Materials and methods

Patient recruitment. The design and enrolment processes of this prospective, single-center, ED-based pilot study have been previously published (39). To summarize, adult patients presenting with acute sICH (<8 h from onset) to the ED of the County Emergency Hospital (Cluj-Napoca, Romania) were recruited over 18 months (December 2017 to June 2018) provided that Glasgow Coma Scale (GCS) was ≥8 and no exclusion criteria were met. The exclusion criteria were as follows: Identifiable secondary ICH causes, thromboembolic/ischemic disease, seizures, severe pre-ICH disability [modified Rankin Scale (mRS)≥4], coagulopathy, treatment with heparin, low-molecular-weight heparin, glycoprotein IIb/IIIa antagonists or oral anticoagulants, pregnancy/breastfeeding, scheduled neurosurgical/hemostatic treatment, enrolment in other studies within the last 30 days or terminal disease. The study protocol was approved by the Institutional Review Board of the 'Iuliu Haţieganu' University of Medicine and Pharmacy Cluj-Napoca (approval no. 441/24.11.2016). The procedures and interventions in the present study were in accordance with the principles stated by the Declaration of Helsinki. All participants or legal representatives provided written informed consent.

Data sources/measurements. Demographic, clinical and laboratory data were documented upon ED admission. The routine management of patients with acute sICH in our department and the study of specific interventions are present in Fig. 1. ED-based POC whole-blood analyzers included the Fujifilm Dry-Chem NX500 biochemistry analyzer and the Swelab Alfa Plus hematology analyzer. CBC included granulocytes [GRA; composed of neutrophiles (NEU) and the largest proportion of eosinophils (EOS)] and mid-size (MID) population of cells (composed of mid-size population of MON, basophils, EOS, blasts and other immature cells). Calculated hematology indexes included the following ratios: NLR (incorporating GRA values), LMR (incorporating MID values) and PLR, alongside SII [calculated as NEU x PLT/(lymphocytes (LYM) x1,000)] and incorporating GRA results. An ED-based PathFast™ fully automatic chemiluminescence enzyme immunoassay was used to study additional biomarkers, including cTnI, D-dimer and high sensitive CRP (hs-CRP).

Patients were clinically assessed on days 2 and 7 (or on discharge). Follow-up telephone interviews on day 90 included FO (mRS) and independence on daily living activities [Barthel Index (BI)].

Diagnosis and imagistic controls were performed on a General Electric Optima 64 scanner (Cytiva). The hemorrhage volume was measured using manual segmentation with the inclusion of the entire visible lesion area. The post-processing analysis was performed on a General Electric AW Server 2.0 workstation by two independent radiologists, blinded for patient outcome.

Statistical analysis. In this analysis, the primary endpoint was day 90 FO. A favorable outcome was considered as an mRS of 0-3, whereas an mRS of 4-6 was considered as an unfavorable outcome. Secondary clinical endpoints included ENW [defined as a GCS decrease of ≤2 points or a National Institute

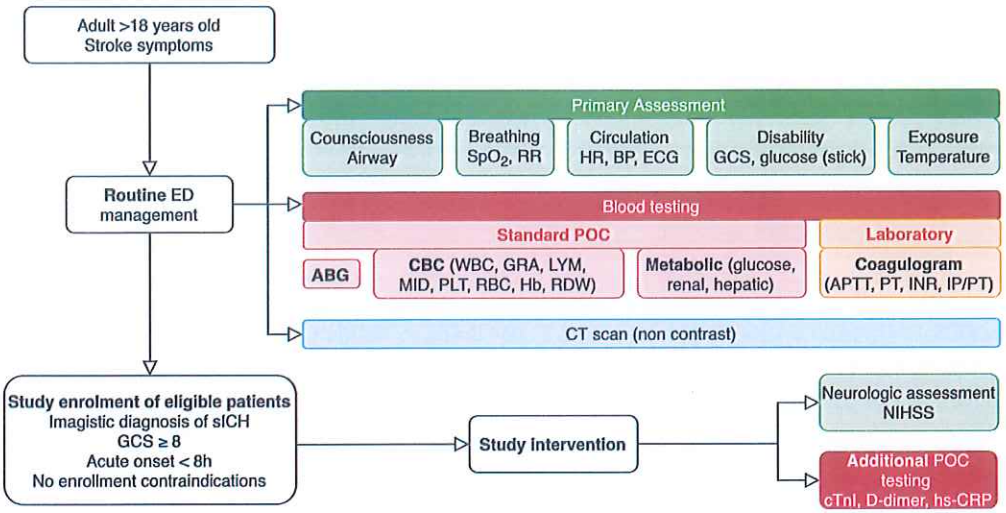

Figure 1. Routine ED baseline assessment of patients with sICH and study specific interventions. ED, emergency department; SpO_2, peripheral oxygen saturation; RR, respiratory rate; HR, heart rate; BP, blood pressure; ECG, electrocardiogram; GCS, Glasgow Coma Scale score; POC, point-of-care; ABG, arterial blood gases; CBC, complete blood count; WBC, white blood cells; GRA, granulocytes; LYM, lymphocytes; MID, mid-cell population; PLT, platelets; RBC, red blood cells; Hb, hemoglobin; RDW, red cell distribution width; APTT, activated partial prothrombin time; PT, prothrombin time; INR, international normalized ratio; IP/PT, prothrombin index/prothrombin time; CT, computer tomography; sICH, spontaneous intracerebral hemorrhage; NIHSS, National Institute of Health Stroke Scale; cTnI, cardiac troponin I; hs-CRP, high-sensitive C reactive protein.

of Health Stroke Scale (NIHSS) increase ≥4], day 7/discharge neurological impairment (NIHSS ≤15), and day 90 assessment of quality of life and independence. Radiological endpoints included HG (change in baseline hematoma volume of >33% or >6 ml by day 2) and PHE growth [difference in the largest PHE linear dimension between the diagnostic and control computed tomography (CT) scans].

Statistical analyses was performed using MedCalc® Statistical software (version 19.6; MedCalc Software byba). Quantitative data was assessed for normality of distribution using the Shapiro-Wilk test, and presented as the median and 25-75th percentiles. Qualitative data are presented as the frequency and percentage. Comparisons between groups were analyzed using the Mann-Whitney U test or χ^2 test. Spearman's rho was used to assess correlations between variables. P<0.05 was considered to indicate a statistically significant difference.

Results

A cohort of 39 patients was recruited, with 35 completing the in-hospital follow-up and 23 (66%) alive on day 90. All deaths were registered within the first month and in-hospital mortality was 23%.

Baseline characteristics of the cohort according to day 90 FO are presented in Table I. Older age, previous history of ischemic stroke, and the presence of intraventricular hemorrhage (IVH), enlarged contralateral ventricle (ECV) and cerebral atrophy on the initial CT scan significantly predicted an unfortunate FO scoring on day 90 follow-up. Median baseline hematoma volume and PHE did not differ significantly between surviving [hematoma volume, 9.15 cm^3 (6.69-22.18); PHE, 8.35 mm (6.81-11.58)] and deceased [hematoma volume, 16.83 cm^3 (9.2-31.89); PHE, 9.35 mm (6.50-13.25)] day 90 outcome groups (P=0.234 and P=0.470, respectively).

Baseline POC biomarker values and calculated indexes according to day 90 mRS are presented in Table II. Higher values in the unfavorable outcome group were documented for RDWa, GRA, NLR, PLR, SII, hs-CRP and D-dimer, but the differences were only significant for D-dimer (P<0.001). When further considering day 90 independence on daily living activities, D-dimer values were significantly higher (P=0.032) in the dependent patients [BI <60; 3.610 μg/ml FEU (0.900-5.010) vs. 0.758 μg/ml FEU (0.383-0.890)] compared with those in the day 90 independent group. Negative correlations were documented between admission D-dimer and admission GCS (rho=-0.342; P=0.044) and day 90 independence status (rho=-0.670; P=0.001).

ENW was documented in 9/35 patients, with only two alive by day 90, equally divided between outcome groups. Baseline median hematoma volume and PHE did not differ significantly (P=0.051 and P=0.094, respectively) between those with [hematoma volume, 24.60 cm^3 (14.16-70.00); PHE, 10.50 mm (9.23-20.63)] and without ENW [hematoma volume, 9.20 cm^3 (5.99-21.25); PHE, 7.5 mm (6.00-10.40)]. All ENW patients received antibiotic treatment by day 7 (ATB 7) and a modest correlation between ENW and ATB 7 was observed (rho=0.367, P=0.033). In patients who developed ENW, white blood cell [WBC; 10.60x10^9/l (8.00-15.25)], GRA [7.30x10^9/l (5.95-11.60)] and D-dimer [3.73 μg/ml FEU (1.22-5.01)] values were significantly higher compared with those in patients without ENW [WBC, 8.10x10^9/l (6.60-10.60); GRA, 5.30x10^9/l (3.90-7.55); D-dimer, 0.86 μg/ml FEU (0.63-1.63)] (P=0.042, P=0.025 and P=0.024, respectively). D-dimer was also significantly higher in the 8/35 patients with a worse day 7 neurological status, defined as NIHSS score ≥16 [2.01 (1.20-5.01) vs. 0.84 (0.59-1.93); P=0.017). By contrast, SII values were not significantly higher in either ENW group [0.45 (0.33-0.86) vs. 0.83 (0.37-1.36); P=0.265], nor in those patients with a worse day 7 neurological status [0.45 (0.32-1.01) vs. 0.49 (0.37-0.81); P=0.315].

The median length of hospital stay was 15 days. Two participants required neurosurgery and another one required

Table I. Baseline clinical and imagistic characteristics.

Characteristic	Day 90 FO		P-value
	Favorable (n=16)	Unfavorable (n=19)	
Median age (range), years	62 (57-68.5)	75 (73-81)	<0.001
>70	3 (18.8)	17 (89.5)	<0.001
Male, n (%)	11 (68.8)	8 (42.1)	0.217
Hypertension, n (%)	12 (75.0)	15 (78.9)	1.000
>2 antihypertensive drugs	7 (43.8)	10 (52.6)	0.854
Diabetes mellitus, n (%)	6 (37.5)	4 (21.1)	0.454
Dyslipidemia, n (%)	7 (43.8)	7 (36.8)	0.945
Statin use prior to admission, n (%)	4 (25)	6 (31.6)	0.723
Antiplatelet agent, n (%)	4 (25.0)	5 (26.3)	1.000
Smoking (former/active), n (%)	11 (68.8)	8 (42.1)	0.217
GCS, median (range)	15 (14; 15)	13 (12; 15)	0.080
NIHSS score, median (range)	8.5 (6.2; 14.5)	15 (6; 21)	0.288
Median SBP (range), mmHg	164.5 (154.5-192.5)	163 (147.2-174.2)	0.174
>170 mmHg, n (%)	7 (43.8)	8 (42.1)	1.000
Median HR (range), beats/min	81.5 (71.5-97.5)	75 (65-86)	0.267
Atrial fibrillation, n (%)	1 (6.2)	0 (0)	0.457
Hematoma location, n (%)			0.581
Supra-tentorial lobar	2 (12.5)	4 (21.1)	
Supra-tentorial deep	14 (87.5)	13 (68.4)	
Supra-tentorial mixte	0 (0)	1 (5.3)	
Infratentorial	0 (0)	1 (5.3)	
Hematoma volume, cm^3, n (%)			0.316
<30	14 (87.5)	14 (73.7)	
30-60	2 (12.5)	2 (10.5)	
>60	0 (0)	3 (15.8)	
Median perihematomal edema (range), mm	9.07 (7.16-28.21)	21.47 (6.97-32.86)	0.371
IVH, n (%)	1 (6.2)	8 (42.1)	0.022
MLS >10 mm, n (%)	3 (18.8)	10 (52.6)	0.086
Mass effect, n (%)	12 (75.0)	17 (89.5)	0.379
CVC, n (%)	12 (75.0)	16 (84.2)	0.677
ECV, n (%)	1 (6.2)	8 (42.1)	0.022
Periventricular leucoaraiosis, n (%)	5 (31.2)	13 (68.4)	0.064
Lacunarism, n (%)	8 (50.0)	16 (84.2)	0.071
Cerebral atrophy, n (%)	4 (25.0)	16 (84.2)	0.001
Median length of hospital stay (range), days	15 (11.5-16.75)	14 (8-19)	0.715
Discharge disposition			0.741
Family care	9 (56.2)	12 (63.2)	
Rehabilitation/lower rank hospital	1 (6.2)	2 (10.5)	

FO, functional outcome; mRS, modified Rankin Scale; TIA, transient ischemic attack; SBP, systolic blood pressure; HR, heart rate; GCS, Glasgow coma scale; IVH, intraventricular hemorrhage; MLS, midline shift; CVC, contralateral ventricle compression; ECV, enlarged contralateral ventricle; NIHSS, National Institute of Health Stroke Scale.

advanced intensive care unit (ICU) airway management. By day 7, medical complications occurred in 20 (57%) participants, all of whom were undergoing antibiotic treatments. A day 90 favorable outcome was reached in only 25% patients. No significant POC biomarker differences were documented between patients with and without day 7 antibiotic treatment.

Control CT scans were performed for 29/35 participants (83%). Only 7 controls were performed within the first 48 h (median time of 6 days and 13 h since the onset of symptoms). Any hematoma expansion occurred in 15/29 participants [median 2.46 cm^3 (1.78-11.18 cm^3)], yet the criteria for HG was fulfilled in only 6 (40%) patients, with 4 dying before the

Table II. Comparison of admission POC biomarkers according to day 90 outcome.

	Day 90 FO		
POC biomarker	Favorable (n=16)	Unfavorable (n=19)	P-value
Hb, g/dl	13.85 (12.75-15.07)	13.40 (12.70-14.80)	0.417
RBC, x10^{12}/l	4.55 (4.33-4.91)	4.55 (4.08-4.94)	0.729
RDWa, fl	60.30 (58.13-65.38)	63.20 (58.20-66.40)	0.943
WBC, x10^9/l	9.35 (6.45-11.58)	9.30 (6.70-11.30)	0.895
GRA, x10^9/l	5.40 (3.80-8.60)	6.20 (4.70-8.30)	0.667
LYM, x10^9/l	2.15 (1.33-2.68)	1.80 (1.40-2.20)	0.127
MID, x10^9/l	0.85 (0.60-1.10)	0.80 (0.60-1.30)	0.934
PLT, x10^9/l	168.50 (140.00-221.50)	162.00 (150.00-192.00)	0.740
NLR	2.26 (1.82-3.70)	3.14 (2.79-5.14)	0.145
LMR	2.11 (1.71-4.33)	2.11 (1.20-3.25)	0.486
PLR	92.94 (55.2-125.00)	95.21 (72.5-116.48)	0.655
SII	0.39 (0.25-1.15)	0.53 (0.36-0.88)	0.868
hs-CRP, mg/l	2.49 (0.89-4.01)	3.27 (0.68-5.20)	0.446
cTnI, ng/ml	0.003 (0.002-0.070)	0.003 (0.001-0.006)	0.181
D-dimer, μg/ml FEU	0.75 (0.38-0.89)	2.31 (0.92-5.01)	<0.001
Glucose, mmol/l	146 (143-168)	140 (119-183)	0.585

Data are presented as the median (range). FO, functional outcome; POC, point-of-care; WBC, white blood cells; GRA, granulocytes; LYM, lymphocytes; MID, mid-cell fractions; PLT, platelets; NLR, neutrophils-to-lymphocytes ratio; LMR, lymphocytes-to-monocytes ratio; PLR, platelets-to-lymphocytes ratio; SII, systemic immune-inflammation index; Hb, hemoglobin; RBC, red blood cells; RDW, red cells distribution width; hs-CRP, high sensitive C reactive protein; cTnI, cardiac troponin I.

follow-up. A median PHE growth of 3.65 mm (1.38-8.38 mm) was documented in 25 participants (12/19 unfavorable outcome group). Moderate or weak negative correlations were detected between POC inflammatory markers and indexes and PHE growth (rho=-0.511, P=0.005 for WBC; rho=-0.548, P=0.002 for GRA; rho=-0.373, P=0.047 for SII; rho=-0.378, P=0.043 for glucose). D-dimer was correlated with admission PHE (rho=0.398, P=0.018).

Cut-off values of the variables mostly associated with the primary outcome were calculated, namely age, admission GCS and D-dimer. As such, an unfavorable day 90 FO was indicated by age ≥72 years [area under the curve (AUC) 0.908 (95% confidence interval (CI), 0.761-0.979), Se 84.2 (60.4-96.6), Sp 93.7 (69.8-99.8), P<0.001)], GCS ≤13 [AUC 0.661 (95% CI 0.482-0.812), Se 52.63 (28.9-75.6), Sp 81.25 (54.4-96.0), P=0.0598)] and D-dimer >0.905 μg/ml fibrinogen equivalent unit [FEU; AUC 0.845 (95% CI 0.683-0.945), Se 84.21 (60.4-96.6), Sp 87.50 (61.7-98.4), P<0.001]. The receiver operating characteristic curve of admission D-dimer predicting day 90 FO is presented in Fig. 2.

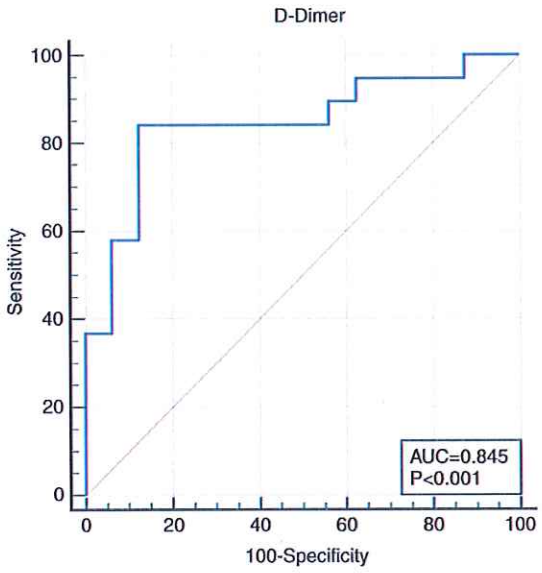

Figure 2. Receiver operation characteristic curve of admission D-dimer predicting day 90 functional outcome. Cut-off, 0.905 μg/ml fibrinogen equivalent units. AUC, area under curve.

Discussion

In this observational cohort of patients with spontaneous ICH, lower admission D-dimer indicated an improved day 90 FO and independence status. Increased age, previous stroke and certain initial imagistic parameters, including IVH, ECV and cerebral atrophy, implied an unfavorable outcome. The results indicated that D-dimer may also anticipate the development of ENW, and modestly reflect admission PHE, whereas certain inflammatory markers may correlate with PHE growth.

Our POC results of baseline D-dimer supported previously published data on the use of D-dimer in prognosing day 90 unfavorable FO (40,41), with an admission level of 0.905 μg/ml FEU predicting a poor 3-month outcome with a

sensitivity and specificity of ~85%. Previous reports identified values ~0.5 mg/l FEU as estimating a poor outcome (41,42). Nevertheless, the correlation between D-dimer and 3-month dependence status should be investigated further to verify the results of the present study. The mechanism through which D-dimer impacts the sICH prognostic value is yet to be established, as it is traditionally a hypercoagulability marker and more recently has been considered for potential application in ischemic stroke diagnosis (43). Evidence of elevated D-dimer as expression of increased fibrinolysis, hence contributing to a hypocoagulable status and extensive hemorrhages, is rather scarce (35,36,44). The present study consistently associated D-dimer with clinical endpoints, including admission neurological status expressed as GCS, throughout the entire follow-up period, alongside baseline PHE. Nevertheless, a correlation with ICH volume was not identified in the present study, despite being frequently reported in previous studies (37,40,41). With ICH volume as a known determinant of admission neurological status and ENW (40,44,45), a larger cohort might associate D-dimer with baseline volume and the evolution of neurological status, thus supporting the hypocoagulability theory.

ICH induces a state of systemic and peripheral inflammation, thus increasing circulating WBC and recruiting certain molecules within the affected area, which amplifies local damage (13,22). Previous studies have reported results for several inflammatory biomarkers and calculated indexes (Hb, absolute RDW, GRA, LYM, PLT, NLR, PLR) that contribute to the existing data on FO prognostication (5,6,17,19,28,29,46,47), but the cohort assessed in the present study did not display statistically significant results, only in-line tendencies with previously published evidence. Furthermore, moderate negative correlations were determined between WBC, GRA, SII and PHE growth. However, these were not consistent with existing theories on the contribution of acute inflammation to PHE enlargement (13), and subsequently to an unfavorable FO (6,13,15,16). This contradiction might reside in the modest sample size of the current analysis and of the fact that the GRA population was reported as a substitute for NEU, the former also including EOS alongside NEU. Recent evidence has shown a significant increase in peripheral WBC, GRA and MON population in patients with acute ICH, whereas the LYM population has decreased (48). The contribution of EOS to sICH prognostication is not completely understood; however, Chen *et al* (14) correlated EOS count with increased risk of HE. Moreover, the short interval from symptom onset to CBC sampling in the present cohort (39) might have prevented the documentation of the activation of local and systemic inflammation (13,22,48). In the present study, MID was documented as a surrogate for MON, but its values incorporate multiple cell populations. SII is a parameter that is documented in regard to hospital discharge outcome of patients with sICH (20); NEU and LYM reflect inflammation, whilst PLT reflect vascular permeability. As such, SII components could depict local PHE metabolism. The results of the present study were in line with previous data on larger SII values in the unfavorable FO subgroup (20), without reaching statistical significance. Furthermore, there was no association with day 7 neurological status (as a proxy for the reported discharge FO) (20) or day 90 FO, which indicated that the timing of the most effective inflammatory panel requires further investigation.

As all reported individual inflammatory markers (WBC, GRA, LYM, MID and CRP) failed to predict day 90 FO, the analysis on calculated indexes (NLR, LMR, PLR and SII) produced similar results. Subsequently, further extensive research is required to validate whether such POC derived indexes can impact outcome prognostication in a similar manner as previous evidence has indicated (5,12,17,46).

At present, the results regarding CRP are inconclusive, despite existing evidence of its association with HG, ENW, mortality and 3-month outcome (5,7,9,24), including in-hospital mortality data reported on a consistent ED-based cohort (9). Currently, hs-CRP assays can only measure plasma pentameric CRP, thus failing to incorporate the extent of the neuroinflammatory response to ICH (24). Furthermore, a pre-existing subliminal inflammatory status, though not detected as an infection or chronic inflammation, might affect the coagulation function and the vessel wall pathophysiology, contributing to persistent vessel leakage.

PLT and PLR are well-known indicators of mortality (8,49) and increase the chances of a negative day 90 FO (6,17-19). Although a similar 25% of each study group was under antiplatelet medication when sICH occurred, the present analysis only documented a moderate negative correlation of PLT with admission ICH volume and day 7 neurological status, in spite of previous discussions on its implications within the existing inflammatory process accompanying sICH (19). Nevertheless, Zhang and Shen (19) demonstrated that ICU rather than ED admission PLR values are relevant for outcome estimation.

In regard to Hb levels, lower mean admission values were recorded in the unfavorable outcome group, without statistical significance or without meeting the criteria of the definition of anemia (39). RDW is another inexpensive automatically generated hematology parameter that is impacted by inflammation (29), and our results indicated a modest correlation with admission PHE, but this was not associated with day 90 FO.

If sICH is considered as having a systemic impact, then metabolic and cardiac biomarkers could indicate its amplitude. However, random admission hyperglycemia did not reflect the severity of sICH (50), nor was it associated with day 90 FO estimation (6), in spite of the modest negative correlation with PHE growth. Mean admission cTnI levels did not differ among outcome groups and the analysis conducted in the present study did not correlate this parameter with any of the study endpoints. Previous studies on troponin levels have concluded that peak serum cTnI values rather than admission values reflect a poor outcome, including mortality (33,34). Therefore, we speculate that the results of the present study suggest further investigating POC testing.

To the best of our knowledge, the present study was one of the few studies on POC testing in acute sICH within an ED, yet its value is limited due to its one-center location and modest sample size, meaning that the current results require further investigation in order to be validated in sICH management. As the sICH population is of an increasing age, D-dimer interpretation should be considered cautiously. The heterogeneity of control CT scan acquisition is another restraint, as the timing of the second scan varied greatly

and, as such, ICH and PHE progression were not reflected uniformly. Therefore, serial sampling of inflammatory biomarkers and a more restrictive protocol for control scans might identify the most relevant time point for a predictive inflammatory panel.

In conclusion, several biomarkers showed modest correlations with the progression of sICH and the day 90 FO, advocating for extensive research on the contribution of ED POC routine biomarkers as outcome assessment tools in hemorrhagic stroke. D-dimer could be a promising maker, as lower admission values could indicate an improved day 90 FO and anticipate the development of PHE growth and ENW. The predictive utility of D-dimer on independence status is a novelty at the present moment and further research is needed to validate the current observations in the acute care setting.

Acknowledgements

Not applicable.

Funding

The present study was supported by the 'Iuliu Haţieganu' University of Medicine and Pharmacy Cluj-Napoca via an internal PhD research program (grant no. 7690/74/15.04.2016 and PCD no. 5200/64/01.03.2017).

Availability of data and materials

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

Authors' contributions

EMM, AG and LPD conceptualized the study. EMM, AG, ML, CC and LPD performed the experiments. Formal analysis was conducted by EMM and SCV. Investigations were performed by EMM, AG, ML, CC and LPD. Resources were accrued by EMM, AG and LPD. The original draft preparation was carried out by EMM. Reviewing and editing of the manuscript were performed by EMM, AG, SCV, ML, CC and LPD. AG and LPD provided supervision. EMM and AG confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of the 'Iuliu Haţieganu' University of Medicine and Pharmacy (approval no. 441/24.11.2016). All patients or legal representatives provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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in vivo *36*: 1534-1543 (2022)
doi:10.21873/invivo.12864

Admission Emergency Department Point-of-care Biomarkers for Prediction of Early Mortality in Spontaneous Intracerebral Hemorrhage

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Abstract. *Background/Aim: Spontaneous intracerebral hemorrhage (sICH) has a significant morbidity and mortality, despite representing a non-dominant hemorrhagic stroke. The aim of the study was to assess the impact of the emergency department (ED) point-of-care (POC) biomarkers on early mortality in sICH patients. Patients and Methods: Demographic data, medical history and admission clinical parameters from adult patients with imaging-based sICH diagnosis were collected retrospectively, upon their ED presentation over a period of 18 months. ED-based POC analyzers were used for blood biomarkers [complete blood count, C reactive protein (CRP), glycemia, hepatic and renal function, D-dimer and cardiac troponin I]. Derived inflammatory indexes were calculated. Mortality endpoints were collected (on day 7 and at discharge). Results: Of the 219 included patients, mortality rates reached 30.14% on day 7 and 46.12% at discharge. In the univariate analysis, day 7 mortality was significantly associated with history of diabetes, atrial fibrillation, ongoing anticoagulant treatment, the need of endotracheal intubation and ED cardiopulmonary resuscitation, and the presence of intraventricular hemorrhage and mass effect on the initial CT scan. White blood cells and granulocytes (but not the neutrophil-to-lymphocytes ratio, nor the CRP) were significantly higher in the deceased groups, alongside serum glucose. Derived inflammatory indexes were not significantly correlated with mortality endpoints. Cut-off values of 9.6×10^9/l for granulocytes and 132 mg/dl for glucose were identified as day 7 mortality predictors. Conclusion: sICH is a potentially severe condition causing high early mortality. Emergency department point-of-care biomarkers could represent a readily available and simple to use prognostic tool.*

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Key Words: Spontaneous intracerebral hemorrhage, point-of-care testing, biomarkers, early mortality, emergency department.

Spontaneous intracerebral hemorrhage (sICH) represents less than 30% of all strokes (1, 2), yet its mortality and disability rates are the highest among survivors (2). The absolute incidence and mortality rates have steadily increased over the last decade, with 2/5 of all stroke-related deaths being caused by hemorrhagic strokes (3).

Early mortality, calculated on day 7, has been rated at 31% (4), with an in-hospital mortality of 24.1% (5). Furthermore, 3-month mortality has been reported to be approximately 35% (4, 6), whereas 1 year mortality reached nearly 60% (4, 6). Such burden, both social and economic, requires better prognostic tools and effective treatments.

Prediction ICH scores usually use demographics, clinical and paraclinical parameters, including imaging data. The

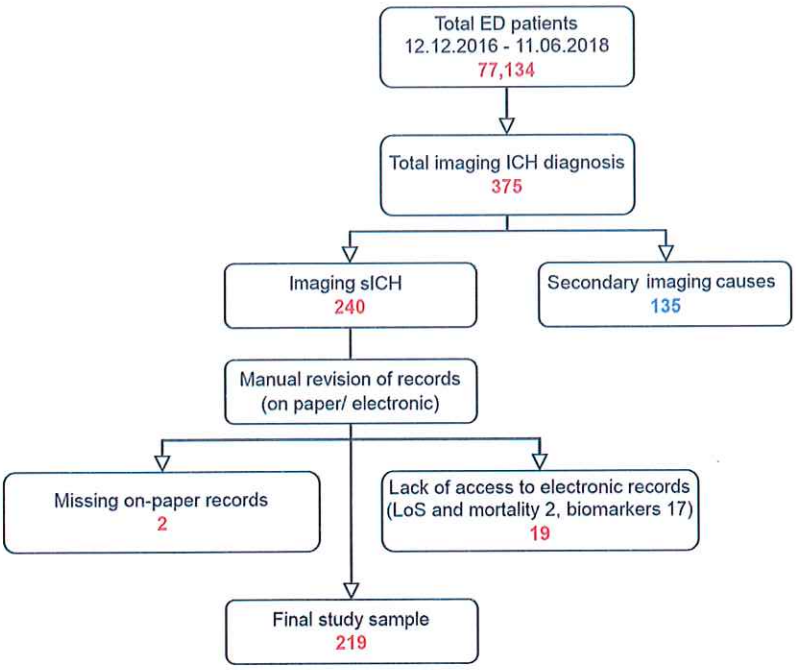

Figure 1. *STROBE diagram of the inclusion process.*

most robust clinical predictors of early mortality in sICH are age (6, 7), stroke severity (6-8) and antithrombotic (including anticoagulant) treatment at the moment of ictus (6, 7), with the first two being indicators of both in-hospital and long-term mortality (6). Additionally, imaging parameters can also be employed in mortality estimation. Large hemorrhagic volumes (8, 9), as well as early hemathoma enlargement (9) are predictors of early mortality. The presence of intraventricular hemorrhage (IVH) and its growth, the hydrocephalus and the need of an external ventricular drainage (8) are strong mortality indicators (6, 8, 10, 11). The absolute growth of perihematomal edema (PHE) seems to be associated with the in-hospital mortality (12), its acute and rapid growth independently predicting death (13).

Recently, blood biomarkers have become of interest for outcome prognosis, as they are easily accessible parameters that could enhance risk stratification. Point-of-care testing (POCT) is the analysis of samples at patient's bedside and is usually performed by personnel without laboratory training. However, it has the advantage of prompt results even in the setting of acute ED care and it can contribute to reducing ED overcrowding when making decisions depending on laboratory data (such as in cardiovascular diseases) (14).

In sICH, short term mortality has been linked with higher values of C reactive protein (CRP), D-dimer, glucose levels and neutrophil-to-lymphocytes ratio (NLR) (6), whilst NLR is also correlated with an unfavorable (including death) 3-month outcome (15, 16). Leucocytosis and neutrophilia are continuously linked to larger ICH volumes, yet their contribution to mortality estimation is conflicting (17); though reports confirm their relevance in mortality estimation (18, 19). Systemic immune-inflammation index (SII) is a predictor of unfavorable hospital discharge (7), while the systemic inflammation response index (SIRI) is a marker of one month mortality in ICH (20). Admission glucose levels anticipate mortality, although the threshold varies from 140 mg/dl to 180 mg/dl (8, 18). Admission cardiac troponin I (cTnI) values are indicative of in-hospital mortality (21) and an unfavorable outcome (22, 23). Recent studies on troponin levels (22, 23) concluded that it is not the admission level, but the peak serum values of cTnI that reflect a poor outcome, including mortality.

The aim of this study was to determine whether emergency department point-of-care biomarkers can contribute to the assessment of early mortality (defined as day 7 and in-hospital mortality) in spontaneous intracerebral hemorrhage patients.

Patients and Methods

This was a retrospective analysis of adult (>18 years old) patients with sICH that have been diagnosed and treated in the Emergency Department of the County Emergency Hospital Cluj-Napoca, Romania over an 18-month period (December 2016 – June 2018). An electronic database search was performed for the terms "hemorrhagic stroke" and "intracerebral hemorrhage", and all the paper records were consulted for identifying non-traumatic intracerebral

hemorrhages. Patients in which a secondary ICH cause was identified were excluded (traumatic, arterio-venous malformation, aneurysm, tumor, infections, hemorrhagic transformation of an ischemic stroke). During the study period, more than 77,000 patients were treated in the ED of the County Emergency Hospital Cluj-Napoca. Figure 1 presents the inclusion process, with 238 patients being diagnosed with sICH and 219 included in the final analysis.

Medical history, clinical, and laboratory data were collected from the earliest emergency presentation that was documented (including lower rank ED or outpatient clinics, from where the patients were referred to our ED for imaging and neurology consultations). Neurology, neurosurgery, ICU, and internal medicine wards from five county hospitals were contacted in order to collect information regarding the length of stay and patients' status upon discharge (dead or alive).

ED blood biomarkers retrospectively collected for the purpose of this study included complete blood count and biochemistry (glucose levels, renal and hepatic function). When available, data were collected on cardiac and inflammatory biomarkers (cTnI, D-dimer, and CRP). For patients presenting directly to the ED of the County Emergency Hospital Cluj-Napoca, complete blood count (CBC) and biochemistry determinations were performed on point-of-care analyzers, namely hematology Swelab Alfa Plus (Boule, Spånga, Sweden), biochemistry Dry-Chem NX 500 (Fujifilm, Tokyo, Japan) and PathFast™ immunoassay analyzer (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) for cTnI, D-dimer and hs-CRP.

Data on blood biomarkers of inpatients referred from lower rank EDs, were generally available upon arrival in our department, expressed as standard CBC and biochemistry. Certain particularities of these standard determinations must be mentioned: CBC included neutrophils (NEU) instead of granulocytes (GRA), whilst monocytes and eosinophils were expressed individually; renal function included urea, as a substitute of blood urea nitrogen (BUN). As the non-POCT results represented a maximum of 13% for CBC and 16% for biochemistry, it was decided to mathematically transform standard clinical laboratory parameters into POCT equivalents, according to the following formulas: 1) GRA=NEU+EOS, 2) MID=MON+BAS and 3) BUN=urea divided by 2.14. cTnI, CRP and D-dimer were available to a lesser extent. cTnI testing was available in 60 patients, with 52 (86.6%) POCT results. CRP and D-dimer were available in 44 patients, of which 43 (97.7%) CRP and all D-dimer results were through POCT.

Furthermore, certain inflammatory indexes were calculated, such as: neutrophil-to-lymphocytes ratio (NLR) incorporating GRA, lymphocytes-to-monocytes ratio (LMR) incorporating MID, platelets-to-lymphocytes ratio (PLR), systemic immune-inflammatory index (SII), NEUxPLT/LYM - incorporating GRA, and systemic inflammation response index (SIRI), NEUxMON/LYM - incorporating GRA and MID.

Diagnostic scans were performed on a General Electric Optima 64 scanner (GE Healthcare, Boston, MA, USA) and interpreted by the on-call radiologist. Data collection included hematoma location and whether IVH, PHE and mass effect were present on the initial scan.

The present study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of County Emergency Hospital of Cluj-Napoca (approval no. 20.992/09.08.2018).

Statistical analysis was performed using the MedCalc® Statistical Software version 20.014 (MedCalc Software Ltd, Ostend, Belgium). Quantitative variables were tested for normality of distribution by Shapiro Wilk test and expressed as median and 25-75 percentiles. Qualitative data were characterized by frequency and percentage. Comparisons between groups regarding quantitative variables were performed using the Mann-Whitney test. Qualitative variables were compared using the chi-square test. AUROC were used to calculate the cut-off values for variables associated with mortality. A p-value <0.05 was considered statistically significant.

Results

Baseline characteristics of the cohort are presented in Table I and Table II, according to mortality endpoints (day 7 and overall, in-hospital mortality). By day seven, 66 patients had died, leading to a mortality rate of 30.14%. Regarding overall in-hospital mortality, 101 patients had died (46.12% of the cohort).

The most frequent risk factors were hypertension, IHD, dyslipidemia, diabetes, and atrial fibrillation. In the univariate analysis, a history of diabetes mellitus, atrial fibrillation and ongoing anticoagulant treatment, the need of endotracheal intubation and ED cardiopulmonary resuscitation, the presence of IVH and a mass effect on the initial CT scan were significantly associated with day 7 mortality. A supratentorial deep location of the hematoma was associated with a favorable day 7 outcome, whilst a prolonged length of stay was associated with a favorable day 7 and discharge outcome.

The need of advanced airway management and CPR were also significantly associated with the overall in-hospital mortality, together with initial IVH and mass effect. Additionally, a history of dyslipidemia, unknown time of onset and the presence of PHE on the diagnostic CT scan were also relevant indicators of in-hospital deaths.

Baseline biomarkers and calculated indexes are presented in Table III and Table IV, according to day 7 and overall, in-hospital mortality. WBC and GRA are significantly higher in the deceased groups, alongside serum glucose. In the mortality subgroups, higher values were recorded for PLT, NLR, SII, SIRI, hs-CRP and D-dimer, alongside MID for day 7 mortality. Anemia and lower LMR were also present in the deceased patients, alongside lymphopenia for day 7 mortality.

Cut-off values for the biomarkers associated with mortality endpoints were calculated. An unfavourable early mortality (day 7 and overall, in-hospital mortality) was predicted by WBC >11.18×10^9/l [day 7 mortality - area under the curve (AUC)=0.599 (95% confidence interval (CI)=0.53-0.664, sensitivity (Se)=60.13 (51.9-67.9), specificity (Sp)=60.61 (47.8-72.4), p<0.0185); overall, in-hospital mortality – AUC=0.591, 95%CI=0.523 to 0.657, Se=62.71 (53.3-71.4), Sp=56.44 (46.2-66.3), p=0.0187] and by GRA >9.6×10^9/l [day 7 mortality–AUC=0.587 (95%CI=0.518-0.653, Se=73.86 (66.1-80.6), Sp=44.62 (32.3-57.5), p=0.0466); overall, in-hospital mortality – AUC=0.590, 95%CI=0.521-0.656, Se=77.12 (68.5-84.3), Sp=42.00 (32.2-52.3),

Table I. *Baseline clinical and imaging characteristics of study cohort, according to day 7 mortality.*

	Day 7 mortality		*p*-Value
	Alive (n=153)	Deceased (n=66)	
Age, years, median (range)	71.59 (60.79-78.71)	72.01 (65.88-86.63)	0.124
>70, n (%)	87 (56.9)	41 (62.1)	0.565
Sex: male, n (%)	77 (50.3)	34 (51.5)	0.989
Previous medical history			
Stroke			
Ischemic stroke or TIA, n (%)	24 (15.7)	6 (9.1)	0.276
Hemorrhagic stroke, n (%)	5 (3.3)	0 (0)	0.326
Hypertension			
Yes, n (%)	96 (62.7)	43 (65.2)	0.852
>2 antihypertensive drugs, n (%)	26 (28.6)	15 (35.7)	0.575
No treatment compliance	15 (16.5)	8 (19)	
Ischemic heart disease, n (%)	37 (24.2)	20 (30.3)	0.436
Venous thrombembolism, n (%)	3 (2.0)	3 (4.5)	0.369
Peripheral arterial disease, n (%)	6 (3.9)	2 (3.0)	1.000
Diabetes mellitus			
Yes, n (%)	20 (13.1)	19 (28.8)	0.042
Insulin, n (%)	3 (2.0)	4 (6.1)	
OAD and insulin, n (%)	2 (1.3)	2 (3.0)	
Dyslipidemia, n (%)	24 (15.7)	18 (27.3)	0.070
Statin use prior to admission, n (%)	23 (15.0)	13 (19.7)	0.512
Atrial fibrillation, n (%)	21 (13.7)	18 (27.2)	0.01
Anticoagulant agent, n (%)	25 (16.34)	20 (30.3)	0.030
Antiplatelet agent, n (%)	32 (20.9)	10 (15.2)	0.420
Current medical condition/baseline status			
Onset to ED >3 h			
Yes, n (%)	41 (26.8)	10 (15.2)	0.083
When waking up in the morning, n (%)	7 (4.6)	5 (7.6)	
Cannot be determined, n (%)	60 (39.2)	36 (54.5)	
GCS*, median (range)	13 (9-15)	14 (11-15)	0.216
Endotracheal intubation			
Prehospital, n (%)	6 (3.9)	13 (19.7)	≤0.001
ED, n (%)	7 (4.6)	13 (19.7)	
SBP*, mmHg			
Median (range)	175 (145,5-209)	168 (152-196)	0.451
>170 mmHg, n (%)	90 (58.8)	32 (48.5)	0.206
HR*, beats/min, median (range)	84 (70.5-100)	73.5 (67-95.5)	0.969
Atrial fibrillation, n (%)	32 (20.9)	20 930.3)	0.185
Cardiopulmonary resuscitation			
Prehospital, n (%)	0 (0.0)	4 (6.1)	≤0.001
ED, n (%)	0 (0.0)	3 (94.5)	
Hematoma location, n (%)			
Supra-tentorial Deep	74 (48.4)	15 (22.7)	0.035
Supra-tentorial Lobar	51 (33.3)	29 (43.9)	
Infratentorial	14 (9.2)	11 (16.7)	
IVH, n (%)	52 (34.0)	45 (68.2)	≤0.001
Perihematomal edema, n (%)	111 (72.5)	56 (84.8)	0.074
Mass effect, n (%)	92 (60.1)	59 (89.4)	≤0.001
Length of hospital stay, days, median (range)	13 (9-17)	2 (1-4)	≤0.001

*First available determination (ambulatory/prehospital/emergency department). mRS: Modified Rankin Scale; TIA: transient ischemic attack; SBP: systolic blood pressure; HR: heart rate; min: minute; GCS: Glasgow coma scale; IVH: intraventricular hemorrhage.

p=0.0209]. A glucose level >132 mg/dl is indicative of day 7 mortality [AUC=0.642 (95%CI=0.574-0.706, Se=42.00 (34.0-50.3), Sp=84.62 (73.5-92.4), p<0.001)], whilst a value >130 mg/dl could anticipate the in-hospital mortality [AUC=0.638 (95%CI=0.570 to 0.702, Se=44.35 (35.1-53.9), Sp=79.00 (69.7-86.5), p<0.001)].

Table II. *Baseline clinical and imaging characteristics of study cohort, according to overall, in-hospital mortality.*

	In-hospital mortality		
	Alive (n=118)	Deceased (n=101)	*p*-Value
Age, years, median (range)	70.36 (60.37-78.63)	73.41 (65.88-80.89)	0.104
>70, n (%)	63 (53.4)	65 (64.4)	0.133
Sex: male, n (%)	59 (50)	52 (51.5)	0.933
Previous medical history			
Stroke			
Ischemic stroke or TIA, n (%)	19 (16.1)	11 (10.9)	0.357
Hemorrhagic stroke, n (%)	4 (3.4)	1 (1)	0.377
Hypertension			
Yes, n (%)	78 (66.1)	61 (60.4)	0.463
>2 antihypertensive drugs, n (%)	22 (29.3)	19 (32.8)	0.760
No treatment compliance	12 (16)	11 (19)	
Ischemic heart disease, n (%)	29 (24.6)	28 (27.7)	0.708
Venous thrombembolism, n (%)	2 (1.7)	4 (4.0)	0.418
Peripheral arterial disease, n (%)	4 (3.4)	4 (4)	1.000
Diabetes mellitus			
Yes, n (%)	16 (13.6)	23 (22.8)	0.268
Insulin, n (%)	2 (1.7)	5 (5)	
OAD and insulin, n (%)	1 (0.8)	3 (3)	
Dyslipidemia, n (%)	16 (13.6)	26 (25.7)	0.035
Statin use prior to admission, n (%)	16 (13.6)	20 (19.8)	0.289
Atrial fibrillation, n (%)	15 (12.8)	24 (23.8)	0.076
Anticoagulant agent, n (%)	19 (16.10)	26 (25.74)	0.111
Antiplatelet agent, n (%)	25 (21.2)	17 (16.8)	0.520
Current medical condition/baseline status			
Onset to ED >3 h			
Yes, n (%)	36 (30.5)	15 (14.9)	≤0.001
When waking up in the morning, n (%)	3 (2.5)	9 (8.9)	
Cannot be determined, n (%)	39 (33.1)	57 (56.4)	
GCS*, median (range)	13.5 (9-15)	14 (9.75-15)	0.710
Endotracheal intubation			
Prehospital, n (%)	4 (3.4)	15 (14.9)	≤0.001
ED, n (%)	2 (1.7)	18 (17.8)	
SBP*, mmHg			
Median (range)	175.5 (148.25-209)	175.5 (148.25-209)	0.206
>170 mmHg, n (%)	71 (60.2)	51 (50.5)	0.193
HR*, beats/min, median (range)	86 (73.25-100)	81.5 (71.75-92.5)	0.145
Atrial fibrillation, n (%)	22 (18.6)	30 (29.7)	0.079
Cardiopulmonary resuscitation			
Prehospital, n (%)	0 (0)	4 (4)	0.015
ED, n (%)	0 (0)	3 (3)	
Hematoma location, n (%)			
Supra-tentorial Deep	59 (50)	30 (29.7)	0.056
Supra-tentorial Lobar	37 (31.4)	43 (42.6)	
Infratentorial	13 (11)	12 (11.9)	
IVH, n (%)	28 (23.7)	69 (68.3)	≤0.001
Perihematomal edema, n (%)	81 (68.6)	86 (85.1)	0.007
Mass effect, n (%)	63 (53.4)	88 (87.1)	≤0.001
Length of hospital stay, days, median (range)	13 (9-17)	4 (2-10)	≤0.001

*First available determination (ambulatory/prehospital/emergency department). mRS: Modified Rankin Scale; TIA: transient ischemic attack; SBP: systolic blood pressure; HR: heart rate; min: minute; GCS: Glasgow coma scale; IVH: intraventricular hemorrhage.

Discussion

Spontaneous intracerebral hemorrhage has a rather high early mortality rate, with only 54% of the patients being discharged alive from hospital. Point-of-care biomarker testing aims to aid risk stratification efforts and alleviate care burden. In our study, higher admission POCT levels of GRA and serum glucose predicted day 7 and overall, in-

Table III. *Baseline biomarkers and calculated indexes, according to day 7 mortality.*

Biomarkers	Day 7 mortality		*p*-Value
	Alive (n=153)	Deceased (n=66)	
Complete blood count and derived indexes (point-of-care)			
Hb, g/dl, median (range)	13.9 (12.53-15.08)	13.7 (12.1-15.05)	0.450
RBC, $\times 10^{12}$/l, median (range)	4.57 (4.15-4.99)	4.57 (4.01-4.96)	0.621
WBC, $\times 10^{9}$/l, median (range)	10.35 (7.63-13.48)	12.33 (8.15-15.00)	0.020
GRA, $\times 10^{9}$/l, median (range)	7.185 (4.7-9.7)	9.1 (5.3-12.05)	0.043
LYM, $\times 10^{9}$/l, median (range)	1.945 (1.4-2.49)	2.0 (1.49-2.66)	0.534
MID, $\times 10^{9}$/l, median (range)	0.8 (0.5-1.2)	0.9 (0.5-1.3)	0.239
PLT, $\times 10^{9}$/l, median (range)	179 (146.25-237.5)	183 (135-224)	0.612
NLR, median (range)	3.58 (2.22-5.92)	4.53 (2.53-5.91)	0.195
LMR, median (range)	2.14 (1.53-3.38)	1.75 (1.08-3.6)	0.318
PLR, median (range)	88.75 (68.75-125)	83.79 (67.28-109.06)	0.619
SII, median (range) GRA×PLT/LYM	634.32 (355.22-1,141)	670.59 (451.17-1,210.48)	0.361
SIRI, median (range) (GRA×MID)/LYM	3.27 (1.46-6.23)	4.52 (1.58-9.61)	0.122
Biochemistry (point-of-care)			
Glucose, mg/dl, median (range)	145 (119-184)	163.5 (144.25-221.5)	0.001
ASAT, U/l, median (range)	26 (22-37)	28 (22-38.25)	0.170
ALAT, U/l, median (range)	22 (15-35)	20 (15.75-30.25)	0.507
BUN, mg/dl, median (range)	18.7 (13.2-23.3)	18.25 (14-27.48)	0.839
CREA, mg/dl, median (range)	0.8 (0.6-1.0)	0.6 (0.85-1.3)	0.071
Others (point-of-care)			
hs-CRP, mg/l, median (range)	2.89 (0.89-6.57)	3.29 (0.37-9.90)	0.959
cTnI, ng/ml, median (range)	0.005 (0.002-0.013)	0.005 (0.001-0.179)	0.916
D-dimer, µg/ml FEU, median (range)	0.898 (0.589-3.17)	1.71 (0.736-5)	0.247

Hb: Hemoglobin; RBC: red blood cells; WBC: white blood cells; GRA: granulocytes; LYM: lymphocytes; MID:mid-cell fractions; PLT: platelets; NLR: neutrophils-to-lymphocytes ratio; LMR: lymphocytes-to-monocytes ratio; PLR: platelets-to-lymphocytes ratio; SII: systemic immune-inflammation index; SIRI: system inflammation response index; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; BUN: blood urea nitrogen; CREA: creatinine; hs-CRP: high sensitive C reactive protein; cTnI: cardiac troponin I.

hospital mortality. Cut-off values of 11.18×10^{9}/l, 9.6×10^{9}/l and 132 mg/dl were identified as predictive for day 7 mortality for WBC, GRA and serum glucose. To our knowledge, this is one of the first studies assessing the impact of emergency department point-of-care biomarkers on early mortality of sICH patients. Additionally, the presence of IVH and a mass effect on the initial CT scan is also indicative of early mortality.

sICH poses a great risk of death, even in the early period after onset (5). Day 7 mortality is known to vary between 16% (5, 24, 25) and 31% (4), reaching 30% in our cohort. The mean length of stay of our cohort was 13 days in the surviving groups, whereas deceased patients spent a significantly shorter time in the hospital (2 and 4 days, in day 7 and overall, in-hospital mortality groups, $p\leq0.001$), emphasizing the impact of sICH on early mortality rates.

The in-hospital mortality rate of our cohort was 46% and similar to previous reports on other national cohorts (18). Nevertheless, its value is considerably higher than other reports ranging from 17.7% (26) to 24.1% (5) and even 32.6% in patients on vitamin K oral anticoagulants (27). A downwards trend has been identified in a 10 years study, with a relative reduction of 24% (24).

Surprisingly, an older age and a history of hypertension did not correlate with any mortality endpoints in our study, although are known as predictors of death and unfavorable outcome (5) and having frequency similar to previous reports (5). Male sex was slightly more predominant in our cohort, both generally and in the deceased subgroups, in opposition with literature reports (5, 18).

Diabetes, atrial fibrillation and prior use of anticoagulant were significantly more frequent in patients deceased by day 7, yet they do not maintain their relevance when dichotomizing patients according to in-hospital mortality. Nevertheless, dyslipidemia was more frequent in the overall, in-hospital mortality group, though being more scarcely reported in the literature (18, 28).

Supratentorial lobar hemathomas were the most frequent in both of the deceased groups, though statistical significance was reached only for day 7 mortality endpoint. This

Table IV. *Baseline biomarkers and calculated indexes, based on overall in-hospital mortality.*

Biomarkers	In-hospital mortality		*p*-Value
	Alive (n=118)	Deceased (n=101)	
Complete blood count and derived indexes			
Hb, g/dl, median (range)	13.85 (12.1-15.075)	13.8 (12.6-15.05)	0.968
RBC, $\times 10^{12}$/l, median (range)	4.57 (4.09-4.99)	4.57 (4.11-4.96)	0.985
WBC, $\times 10^{9}$/l, median (range)	10.23 (7.43-13.38)	11.7 (8.09-14.6)	0.020
GRA, $\times 10^{9}$/l, median (range)	7.00 (4.44-9.38)	8.9 (5.39-11.3)	0.022
LYM, $\times 10^{9}$/l, median (range)	1.98 (1.35-2.5)	2.0 (1.5-2.55)	0.625
MID, $\times 10^{9}$/l, median (range)	0.85 (0.52-1.3)	0.85 (0.52-1.3)	0.526
PLT, $\times 10^{9}$/l, median (range)	176 (146.25-235.25)	185 (135-229)	0.963
NLR, median (range)	3.58 (2.1176-6.1)	4.42 (2.49-5.56)	0.219
LMR, median (range)	2.11 (1.53-3.38)	2.089 (1.23-3.6)	0.704
PLR, median (range)	90.91 (63.64-136)	83.06 (69.69-110.19)	0.456
SII, median (range)			
GRA×PLT/LYM	572.73 (328.17-1,232)	722.05 (415.62-1,075.42)	0.281
SIRI, median (range)			
(GRA×MID)/LYM	3.04 (1.35-6.21)	3.98 (1.78-8.57)	0.136
Biochemistry			
Glucose, mg/dl, median (range)	140 (118-182)	163.5 (136.75-211.5)	≤0.001
ASAT, U/l, median (range)	26 (22-35)	28 (22-42)	0.173
ALAT, U/l, median (range)	22 (16-33)	20 (15-33.25)	0.724
BUN, mg/dl, median (range)	18.8 (13.2-24.3)	18.35 (14.08-23.65)	0.958
CREA, mg/dl, median (range)	0.8 (0.6-1.00)	0.8 (0.6-1.1)	0.265
Others			
hs-CRP, mg/l, median (range)	3.47 (1.16-6.04)	2.57 (0.43-8.25)	0.443
cTnI, ng/ml, median (range)	0.055 (0.002-0.02)	0.003 (0.001-0.017)	0.635
D-dimer, µg/ml FEU, median (range)	0.89 (0.441-3.36)	1.03 (0.774-4.683)	0.339

Hb: Hemoglobin; RBC: red blood cells; WBC: white blood cells; GRA: granulocytes; LYM: lymphocytes; MID:mid-cell fractions; PLT: platelets; NLR: neutrophils-to-lymphocytes ratio; LMR: lymphocytes-to-monocytes ratio; PLR: platelets-to-lymphocytes ratio; SII: systemic immune-inflammation index; SIRI: system inflammation response index; ASAT: aspartate aminotransferase; ALAT: alanine aminotransferase; BUN: blood urea nitrogen; CREA: creatinine; hs-CRP: high sensitive C reactive protein; cTnI: cardiac troponin I.

observation is in line with existing evidence (5, 25), especially when considering that anticoagulant treatment was present in nearly one fourth of the cohort.

Timely estimation of ICH outcome is of clinical relevance, as it enables individualized management and facilitates interdisciplinary communication. Day 30 mortality has been successfully estimated using ED based POCT (29), yet the debate remains open whether or not POCT leads to shorter length of stay (LoS) in a financially sustainable manner (14). Effective strict glucose control seems to benefit from POCT, yet in critical ICU patients it might be inaccurate due to hypoperfussion, anemia, hypoxia or tissue edema (14).

With ICH being more frequently regarded as a systemic condition that leads to immune system activation (17), inflammatory mechanisms are thought to play defensive roles in opposition to the cerebral injury and are part in ICH progression. Tapia-Perez *et al.* (19) reported a 16% 30-day mortality rate and based on a small cohort, concluded that admission WBC >12.5 was related to this outcome. A recent study on a similar population (18) reported a 44% in-hospital mortality rate, that was associated with higher WBC, NEU and NLR values.

The present study identifies novel early mortality POCT inflammatory biomarkers, that warrant further exploration. Our research determined cut-off values of 11.18×10^{9}/l and 9.6×10^{9}/l for WBC and GRA as predictors of day 7 and in-hospital mortality and we consider these results of interest as at this moment the evidence on GRA contribution to sICH prognostication is scarce. Jiang *et al.* (28) reported that the GRA population was significantly increased in peripheral blood, alongside WBC and MON populations, concluding that dramatic cellular immune system changes occur immediately after ICH occurrence. The hereby determined GRA subpopulation also included the largest part of EOS, which has been mentioned to be linked to hematoma growth (30). Yet the present research has not assessed the evolution of hemorrhage volume or PHE, therefore we cannot hypothesize on the mechanism through which increased GRA levels are a relevant mortality marker. This

particularity may also justify the inability of the present research to confirm the NLR substitute (GRA-to-LYM ratio) as a mortality predictor.

The hemorrhagic volume induces a direct injury to neuronal tissues, which in return releases chemokines (10), the attractants of inflammatory molecules, with NEU being the first attracted cells (2), which afterwards contribute to the expansion of inflammation and perihematomal formation. By day 2, NEU are present at the injury site in most cases; by day 5, in all cases and have been observed even by day 12 (31). However, a time-dependent increase of the NEU population has been reported (18, 19). However, day 3 NLR and not admission NLR have been proposed as independent predictors of in-hospital mortality (18).

CPR, D-dimer and cTnI were not routinely determined in our cohort and therefore our analysis could not determine the contribution of these parameters to early mortality prognostication. However, existing evidence supports their utility. CRP is a known acute inflammation marker that has been correlated with HG, ENW, mortality and three-month functional outcome of sICH (17, 32-34), including a greater in-hospital mortality of patients presenting an ED admission hsCRP ≥5.5 mg/l. At hemorrhage site, CRP can be locally produced or transformed from the circulating liver produced pentameric form (34). hsCRP assays are currently able to determine only the plasma pentameric form, hence failing to quantify the degree of neuroinflammation induced by ICH.

D-dimer POCT has been associated with shorter ED LoS and lower hospital admission rates (14). A pilot prospective study we have previously conducted identified that admission POCT D-dimer values higher than 0.905 μg/ml FEU may predict three-month poor outcome (35). cTn POCT proved advantageous when a clinical laboratory was not permanently available on site (14).

Admission hyperglycemia has been recorded in half of the acute ICH patients (36), yet its contribution to sICH progression is still to be determined, as it could represent a pre-existing condition, an alteration due to inflammatory cell activation (25, 37) or a component of the mechanisms leading to PHE development and increased mortality (36). Once the blood-brain barrier is destructed, the neuronal tissue performs anaerobic glycolysis using the excess glucose, leading to alterations of cerebral blood flow and tissue oxygenation, increasing the cerebral edema area and subsequently impacting mortality (36). Nevertheless, recent evidence advocates for hyperglycemia being a consequence of the stress imposed by the hemorrhagic event, and not a self-standing contributor to the augmentation of PHE (37). Lastly, glucose metabolism disorders might be induced by the activation of the inflammatory cells (25), and the presence of intraventricular hemorrhage and the subsequent adrenergic activation due to the exposure of hypothalamic structures to blood breakdown products (36).

Admission glucose values over 140 mg/dl (8) have proved to be significant mortality risk factors, and values >180 mg/dl are indicative of in-hospital mortality (18). The present study identified admission POCT cut-off values of 132 mg/dl and 130 mg/dl as significant for day 7 and in-hospital mortality endpoints, respectively.

Diabetes appears to be a condition consistently associated with sICH patients, with 30% of the patients deceased by day 7 having this diagnosis, in comparison to less than 20% in previous reports (5, 28). Additionally, a connection with longer hospital stay and increased in-hospital mortality has been documented (38).

The association of endotracheal intubation and mortality end-points in non traumatic ICH patients has been previously reported (26) and it was estimated that this intervention was rather uncapable of preventing death, and not necessarily causing it. This procedure is indicated for airway protection in patients with severely altered mental status or those in cardiac arrest. Our study did not specifically investigate the impact of induction medication and subsequent intravenous anaesthesia on mortality, yet caution is advised (26) when taking such risk harboring therapeutic decisions.

As our research used retrospective data and a uniform reinterpretation of diagnostic CT acquisitions was not performed, we were only able to document the presence or absence of IVH, mass effect and PHE, with no quantification of these parameters. As such, their significant association with mortality end-points can only be hypothesized. The presence of IVH and its progression have been associated with a mortality increase (6, 11) and its contribution to inflammatory processes (including hyperglycemia) has been previously debated. PHE is a known imaging surrogate of inflammation and secondary lesions, being present from the first day after stroke (31) and contributing to early neurological worsening and death (13). Not only does its presence alter local metabolism by deteriorating blood-brain barrier (13), but the compression of the local vital structures is further decreasing cerebral perfusion and hence enlarging secondary neurological lesions (31, 39). In our cohort, the presence of PHE was significantly more frequent in the patients who deceased during hospital stay, possibly advocating for the time-dependent progression of PHE (13). As our research identified inflammatory POCT biomarkers and imaging parameters as predictors of early mortality after sICH, we consider these findings relevant for further studies exploring the underlying physiopathological mechanisms and confirming these ED POCT biomarkers as mortality indicators in sICH patients.

Beyond the main aim of this research, we must report a significant trend of late presentations in our cohort, with only 27% of the patients arriving at the hospital within the thrombolysis window of 3 hours and their percentage being significantly higher in the in-hospital deceased group of

patients ($p<0.001$). Such delayed presentations and therefore prolonged lack of blood pressure lowering medication can be reasonably suspected to contribute to larger bleeding volumes and hence its adverse consequences.

The limitations of our study are noteworthy. Its retrospective character impacted the consistency of non-routine POCT (D-dimer, cTnI and hs-CRP) and possibly the lack of significant results. Furthermore, not all biomarker determinations were performed on POCT devices, as for clinical purposes there was no need to repeat the non-POCT parameters that had already been determined in the initial ED. As such, mathematical conversion was needed in approximately 12% of results, converting NEU and EOS into GRA. However, a thorough database search was possible, and the inclusion rate was significantly higher than previous prospective studies implemented in our ED (35). Though being a single center study, our hospital was functioning as the only county ED with a 24/7 functioning CT scan and therefore, all stroke suspicions would have been referred here for imaging. Regarding imaging, our study protocol did not include a uniform diagnostic CT scan reinterpretation nor a control CT scan and subsequently a great number of imaging parameters were lost (hemathoma and PHE initial size and subsequent growth, IVH volume).

The current study confirms that spontaneous ICH is a potentially severe condition causing high early mortality. Emergency department point-of-care biomarkers could represent a readily available and simple to use prognostic tool, with GRA and serum glucose predicting mortality endpoints.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization: E.M.M., A.G. and L.P.D.; methodology: E.M.M., A.G., S.C.V., I.G. and L.P.D.; formal analysis: E.M.M. and S.C.V.; investigation: E.M.M. and I.G.; writing—original draft preparation: E.M.M.; writing—review and editing: E.M.M., A.G., S.C.V., I.G. and L.P.D.; supervision: A.G. and L.P.D. All Authors have read and agreed to the published version of the manuscript.

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Received March 2, 2022
Revised March 22, 2022
Accepted March 23, 2022

Journal of
Clinical Medicine

MDPI

Article

Resistin and Cardiac Arrest—A Prospective Study

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Received: 6 November 2019; Accepted: 24 December 2019; Published: 25 December 2019

Abstract: The systemic response to ischemia-reperfusion that occurs after a cardiac arrest (CA) followed by the return of spontaneous circulation leads to endothelial toxicity and cytokine production, both responsible for the subsequent occurrence of severe cardiocirculatory dysfunction and early death. Resistin is emerging as a biomarker of proinflammatory status and myocardial ischemic injury and as a mediator of endothelial dysfunction. The study aimed to analyze the possible associations between several clinical and biological variables and the serum levels of resistin in CA survivors. Forty patients with out-of-hospital resuscitated CA, were enrolled in the study. Demographic, clinical and laboratory data (including serum resistin measurements at admission and at 6, 12, 24, 48 and 72 h) were recorded. For resistin, we calculated the area under the curve (AUC) using the trapezoidal method with measurements from 0 to 12 h, 0 to 24 h, 0 to 48 h and 0 to 72 h. Fifteen (37.5%) patients died in the first 72 h after CA. Cardiovascular comorbidities were present in 65% of patients. The majority of patients had post-CA shock (29 (72.5%)). Resistin serum levels rose in the first 12–24 h and decreased in the next 48–72 h. In univariate analysis, advanced age, longer duration of resuscitation, high sequential organ failure assessment score, high lactate levels, presence of cardiovascular comorbidities and the post-CA shock were associated with higher resistin levels. In multivariate analysis, post-CA shock or cardiovascular comorbidities were independently associated with higher AUCs for resistin for 0–12 h and 0–24 h. The only identified variable to independently predict higher AUCs for resistin for 0–48 h and 0–72 h was the presence of post-CA shock. Our data demonstrate strong independent correlation between high serum resistin levels, cardiac comorbidities and post-CA shock. The impact of the post-CA shock on serum concentration of resistin was greater than that of cardiac comorbidities.

Keywords: cardiac arrest; resistin; post-cardiac-arrest shock

1. Introduction

One of the relatively common presentations in the emergency department (ED) is that of a patient who suffered an out-of-hospital cardiac arrest (OHCA). Regardless of the etiology of cardiac arrest (CA), the physicians' efforts are centered on the early control of all consequences secondary to the interruption of blood flow to organs and return of spontaneous circulation (ROSC). They are known under the term of post-cardiac-arrest syndrome (PCAS)—which is responsible for the high mortality of

post-resuscitation patients [1,2]. In PCAS, four components have been described: post-cardiac-arrest brain injury, post-cardiac-arrest myocardial dysfunction, systemic response to ischemia/reperfusion and persistent precipitating pathology [2]. Although post-cardiac-arrest brain injury remains an important cause of mortality and morbidity among CA patients, the other elements of PCAS (like systemic response to ischemia/reperfusion) also lead to multiple organ failure and early death [2,3].

The pathophysiology of PCAS is very complex and involves ischemia-reperfusion injury and activation of nonspecific mechanisms of systemic inflammatory response. Summarizing the process, the oxygen supply during ischemia is reduced and the cellular metabolism is affected, ultimately resulting in an increase in the intracytoplasmic calcium concentration responsible for the first cellular and tissue lesions. During the reperfusion phase, following restoration of the blood flow, reactive oxygen species formed during the ischemic phase induce cell death through their cytotoxic effect (inactivation of cytochromes, alteration of membrane transport proteins, inducing lipid peroxidation of the membrane). The pro-oxidant state that occurs inside the cells marks the transition to the next stage, characterized by aggressive endothelial toxicity. The onset of vascular endothelial lesions paves the way to systemic inflammation via the ischemia-reperfusion mechanisms: cytokine production, complement activation, arachidonic acid synthesis, leukocyte adhesion to endothelial cells and triggering of activation and chemotaxis of polymorphonuclear neutrophils at the origin of the inflammatory response. All of these are responsible for the subsequent development of multiple organ failure. Of note, the activation of the systemic inflammatory response is also associated with changes in coagulation (intravascular coagulation dissemination), which generate additional endothelial lesions. This creates a vicious circle where inflammatory lesions and coagulation abnormalities induce further organ damage by accentuating pre-existing lesions and enhancing the persistence of the precipitating pathology of CA, more likely in close dependence with the duration of resuscitation and the rhythm of CA [4–7].

In the past few years, the research community has been focused on identifying biomarkers able to adequately predict the severity of the lesions that underline the pathophysiological processes in CA.

Resistin is a cysteine-rich, adipose-derived peptide hormone encoded by the *RETN* gene that is highly expressed in circulating monocytes, macrophages and vascular endothelium [8–10]. It is involved in numerous pathological processes (obesity, disorders of glucose and insulin metabolism, atherosclerosis, malignancies, rheumatic diseases, chronic kidney disease, etc.) [11–14]. Resistin has been suggested as a marker of the severity of myocardial ischemic lesion [8,12] and proposed as a mediator of endothelial dysfunction [8,12,15–17]. Moreover, resistin has been potentially introduced as a marker of proinflammatory status (cytokine-like) in relation to sepsis and in other nonseptic critical pathologies [8,12–14]. In a previous study, we investigated the role of resistin as a biomarker for predicting mortality after CA. The results showed that elevated serum resistin levels were highly predictive of mortality in critically ill patients who survived a CA [14,18].

Taking into account the proposed mechanisms of action of resistin and the pathophysiology of CA, the aim of our study was to investigate the clinical and biological variables that correlate with serum resistin levels in CA survivors.

2. Materials and Methods

A prospective, analytical, longitudinal, observational cohort study included consecutive patients resuscitated after the OHCA and admitted to the ED of County Emergency Hospital Cluj-Napoca between May 2016 and October 2017. Informed consent for inclusion in the study was obtained from patients' proxies in all cases. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, with registration number 59/14.03.2016.

The inclusion criteria were as follows: age between 18–85 years and resuscitated OHCA. The exclusion criteria were as follows: ages under 18 or over 85 years, pregnancy, re-arrest with unsuccessful

resuscitation within 6 h from hospital arrival, inmates, absence of informed consent and CA due to trauma, acute bleeding from nontraumatic condition, hypothermia or terminal neoplastic disease.

2.1. Study Protocol and Laboratory Assays

The management protocol of the patients admitted to the study, the post-CA shock definition and the lab protocols were previously described [18].

Each patient with out-of-hospital CA admitted to the study was resuscitated by emergency medical team members according to the recommendations of the European Resuscitation Council 2015 [19,20]. Fluids infusion and vasoactive drugs (adrenaline, noradrenaline, dopamine, dobutamine), alone or in combination, were administered in order to maintain mean arterial pressure ≥65 mmHg and urine output ≥0.5 mL/kg/h. For patients remaining comatose after successful resuscitation, able to maintain a systolic blood pressure above 90 mmHg (mean arterial pressure—MAP ≥65 mmHg) and without sepsis, controlled therapeutic hypothermia was administered in the first 24 h, in order to maintain a central temperature with a target between 34–35°C, using ice bags and cooling blanket. Reheating was slow, at a rate of 0.25–0.5 °C/h. According to local protocols which follow current guidelines, hyperthermia, seizures and hyperglycemia were avoided and immediately treated [21,22].

Blood samples were drawn from a peripheral vein where no medication was administered, at 0-time interval (emergency admission), 6, 12, 24, 48 and 72 h following resuscitation. Five-milliliter biochemistry vacutainers with serum separator clot activator were used for blood sample collection. To collect blood samples, we used 5 mL biochemistry vacutainers with serum separator clot activator. The identified hemolyzed samples were excluded and blood samples were immediately repeated. Samples were centrifuged at 3000 rotation/minutes during the first 60 min after collection and were stored at −70 °C. Subsequently, serum concentrations of biomarkers (resistin, S-100B and NSE) were analyzed using a quantitative sandwich immunoassay technique (ELISA; BioVendor, LM, Czech Republic) according to the manufacturer's instructions. After processing, defrosted blood samples were no longer used and underwent destruction.

For every patient, the following data were recorded: demographic (age, gender), clinical (presence of cardiovascular diseases and/or strong risk factors for cardiovascular disease (arterial hypertension, coronary artery disease, valvular heart disease, congestive heart failure, history of stroke, diabetes mellitus and obesity), the rhythm of OHCA, duration of resuscitation, body mass index (BMI), presence of post-CA shock), sequential organ failure assessment (SOFA) score at admission and laboratory data (lactate and glycemia at admission; resistin at 6, 12, 24, 48 and 72 h). Obesity was defined by a body mass index (BMI) ≥30 kg/m^2. The overweight was classified at a BMI between 25 and 29.9 kg/m^2. Post-CA shock was defined as the need to administer vasoactive/inotropic therapy to maintain a MAP >65 mmHg for at least 6 h immediate after return of spontaneous circulation, although fluid therapy was adequate.

2.2. Statistical Analysis

Statistical analysis was performed using the MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2019). Quantitative data normality was assessed using the Shapiro–Wilk test, measures of skewness and kurtosis and histograms. Quantitative data were expressed as median and interquartile range (IQR). Qualitative data were characterized by frequency and percentage. For resistin, we calculated the area under the curve (AUC) using the trapezoidal method with measurements from 0 to 12 h, 0 to 24 h, 0 to 48 h and 0 to 72 h. The sample size was calculated from a pilot study (13 patients with post-CA shock and 4 patients without post-CA shock). Calculated AUC for resistin, for 0–12 h measurements, showed a 24 ng × h/mL mean difference between the two groups. For a type 1 (a) error of 0.01 and a type 2 (b) error of 0.05, we calculated a sample size of 34 patients. The power of the study was calculated as 95%. Correlations between quantitative variables were assessed using the Spearman's rank correlation coefficient. The differences between groups were verified with Mann–Whitney test. In order to find

out which variables can be independently linked to resistin, we constructed several models using multiple linear regressions. Due to the fact that the resistin values followed a non-normal distribution, we performed a logarithmic transformation. We introduced the variables that were significantly associated with the AUCs for resistin during the univariate analysis. A p-value of less than 0.05 was considered statistically significant.

3. Results

Forty patients admitted to ED who met the inclusion criteria were included in the study. Patient characteristics are described in Table 1. On the first and second day, 12 (30%) patients died, and by the third day there were another three (7.5%) deaths. The 25 survivors after 72 h were followed for 30 days, and we recorded the deaths of 13 of them in this interval. Most of the recorded CA rhythm was asystole. Of the total number of patients admitted in our study, only 11 (28.5%) patients did not develop immediate post-resuscitation shock. Of the total admitted patients, 14 (35%) were obese and 20 (50%) were overweight.

Table 1. Baseline characteristics of the study group.

Characteristics		Eligible Patients with CA (n = 40)
Age, years, median (IQR)		67 (59.2 to 76.0)
Gender, n (%)	Female	12 (30.0)
	Male	28 (70.0)
Presenting rhythm, n (%)	Asystole	23 (57.5)
	PEA	5 (12.5)
	VF	11 (27.5)
	VT without pulse	1 (2.5)
Duration of CPR, minutes, median (IQR)		15 (7.7 to 28.7)
Current smoking, n (%)		4 (10)
Chronic alcohol consumer, n (%)		5 (12.5)
Medical history, n (%)	Non-cardiovascular comorbidities	18 (45.0)
	Cardiovascular comorbidities	26 (65.0)
	Arterial hypertension	23 (57.5)
	Coronary artery disease	17 (42.5)
	Valvular heart disease	8 (20%)
	Congestive heart failure	15 (37.5)
	Stroke	3 (7.5)
	Diabetes mellitus	7 (17.5)
BMI, median (IQR)		28 (26.0 to 31.0)
Obesity, n (%)		14 (35)
SOFA score, median (IQR)		15 (12.0 to 16.0)
Patients with post-CA shock, n (%)		29 (72.5)
Lactate (mmol/L), median (IQR)		10.42 (7.6 to 12.9)
Blood glucose (mg/dL), median (IQR)		249.0 (156.0 to 330.0)

IQR = interquartile range; PEA = pulseless electrical activity; VF = ventricular fibrillation; VT = ventricular tachycardia; CPR = cardiopulmonary resuscitation; BMI = body mass index; SOFA = sequential organ failure assessment score; CA = cardiac arrest.

For serum resistin levels we calculated the AUCs using the trapezoidal method with measurements from 0 to 24 h, 0 to 48 h and 0 to 72 h. Resistin levels and AUCs showed an increase in the first 12 h after admission, followed by a gradual decrease in the next 60 h (Table 2).

Table 2. Median serum levels of resistin and the AUC for resistin during the first 72 h.

Variable		Median (IQR)
Resistin, (ng/mL)	at 0 h	7.1 (4.6 to 11.8)
	at 6 h	9.8 (4.4 to 17.7)
	at 12 h	13.5 (5.5 to 21.0)
	at 24 h	12.3 (6.7 to 21.0)
	at 48 h	7.2 (3.5 to 14.6)
	at 72 h	7.4 (3.6 to 11.9)
AUC resistin, (ng × h/mL)	in the first 12 h	26.0 (11.5 to 43.2)
	in the first 24 h	25.8 (15.2 to 44.7)
	in the first 48 h	16.6 (10.4 to 35.1)
	in the first 72 h	34.6 (17.9 to 46.5)

AUC = area under the curve; IQR = interquartile range.

We found that SOFA score and serum lactate values at admission were the most important clinical and laboratory parameters associated with serum resistin levels (strong positive correlation to all repeated measurements) (Table 3). The serum resistin levels were not influenced by BMI.

Table 3. Correlations between the AUCs for resistin and the study quantitative variables.

Variable	AUC for 0–12 h		AUC for 0–24 h		AUC for 0–48 h		AUC for 0–72 h	
	r	*p*	r	*p*	r	*p*	r	*p*
Age, years	0.316	0.04	0.360	0.03	0.467	0.01	0.356	0.08
Duration of CPR, minutes	0.364	0.02	0.386	0.02	0.414	0.02	0.357	0.08
BMI	0.039	0.8	–0.148	0.4	–0.183	0.3	–0.141	0.5
SOFA score	0.586	<0.001	0.579	<0.001	0.510	0.006	0.529	0.007
Lactate (mmol/L)	0.499	<0.001	0.592	<0.001	0.501	0.007	0.509	0.009
Blood glucose (mg/dL)	0.185	0.2	0.417	0.01	0.176	0.3	–0.023	0.9

AUC = area under the curve; CPR = cardiopulmonary resuscitation; BMI = body mass index; SOFA = sequential organ failure assessment score; r = correlation coefficient.

The AUCs for resistin were higher in patients who presented asystole or PEA rhythm of CA (especially), cardiovascular comorbidities, history of congestive heart failure, arterial hypertension or post-CA shock (Table 4). We found no associations between AUCs for resistin and history of coronary artery disease, stroke, diabetes mellitus, obesity, smoking or alcoholic beverages.

Several models based on multiple linear regression were used in order to determine the independent association between clinical/laboratory data and the AUCs for resistin. The variables that were significantly linked to the AUCs in the univariate analysis were introduced in the models. Due to the fact that the resistin values followed a non-normal distribution, we performed a logarithmic transformation. When we introduced the history of congestive heart failure or arterial hypertension as separate variables, we found no statistically significant association with the log AUCs for resistin. Post-CA shock or cardiovascular comorbidities were independently associated with the log AUC for resistin for 0–12 h and 0–24 h. The only identified variable independently linked to the log AUC for resistin for 0–48 h and 0–72 h was the presence of post-CA shock (Table 5).

Table 4. Associations between the AUC for resistin and the qualitative variables studied.

Variable		AUC for 0–12 h		AUC for 0–24 h		AUC for 0–48 h		AUC for 0–72 h	
		Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*	Median (IQR)	*p*
Gender	Female	26.5 (23.0 to 42.5)	0.4	30.0 (17.4 to 44.1)	0.8	20.6 (13.3 to 49.2)	0.3	25.0 (17.1 to 74.0)	0.7
	Male	23.0 (10.2 to 43.2)		25.2 (13.8 to 44.7)		15.9 (9.3 to 33.8)		35.3 (18.7 to 46.1)	
Presenting rhythm of CA	Asystole/ PEA	30.5 (22.0 to 47.7)	0.002	30.3 (19.9 to 51.4)	0.002	23.5 (14.5 to 38.7)	0.009	37.8 (25.0 to 69.8)	0.01
	VF/VT without pulse	10.5 (4.2 to 22.5)		14.3 (8.0 to 24.1)		12.4 (4.8 to 16.0)		23.4 (13.8 to 31.9)	
Cardiovascular comorbidities	present	29.0 (22.0 to 45.5)	0.03	37.2 (18.3 to 50.2)	0.01	22.7 (14.2 to 38.1)	0.06	37.4 (23.6 to 64.0)	0.08
	absent	16.5 (4.7 to 31.5)		18.8 (11.1 to 25.8)		13.7 (5.3 to 28.4)		27.6 (15.0 to 36.7)	
History of arterial hypertension	present	28 (22 to 45)	0.1	37.2 (18.2 to 49.1)	0.05	22 (14.1 to 39)	0.1	37.8 (22.2 to 67.3)	0.1
	absent	18 (7 to 37)		19.9 (14.3 to 26.4)		14.5 (5.8 to 27.3)		29.3 (15.4 to 36.9)	
History of congestive heart failure	present	30.5 (23 to 46.5)	0.04	38 (29.9 to 49.7)	0.02	34.8 (19.6 to 46.4)	0.02	52.8 (23.5 to 92)	0.04
	absent	18.5 (7.5 to 32.7)		19.3 (9.8 to 31.6)		14.5 (9.2 to 23.9)		27.6 (16.9 to 36.8)	
Post-CA shock	present	31.0 (24.0 to 47.5)	<0.001	30.3 (24.1 to 51.4)	<0.001	30.8 (15.9 to 38.7)	0.002	41.8 (23.4 to 70.8)	0.01
	absent	10.0 (4.0 to 15.0)		13.4 (8.0 to 18.8)		12.4 (4.8 to 15.8)		27.5 (12.1 to 34.6)	

AUC = area under the curve; IQR = interquartile range; PEA = pulseless electrical activity; VF = ventricular fibrillation; VT = ventricular tachycardia; CA = cardiac arrest.

Table 5. Multiple linear regression for the AUCs for resistin.

Variables for the log of AUC for 0–12 h	B	p	95.0% CI for B	
			Min	Max
(Constant)	0.784	<0.001	0.581	0.988
Post-CA shock	0.528	<0.001	0.309	0.747
Cardiovascular comorbidities	0.214	0.04	0.009	0.419
Variables for the log of AUC for 0–24 h	**B**	*p*	**95.0% CI for B**	
			Min	**Max**
(Constant)	0.954	<0.001	0.768	1.140
Post-CA shock	0.415	<0.001	0.211	0.619
Cardiovascular comorbidities	0.201	0.04	0.004	0.397
Variables for the log of AUC for 0–48 h	**B**	*p*	**95.0% CI for B**	
			Min	**Max**
(Constant)	0.939	<0.001	0.739	1.139
Post-CA shock	0.470	0.001	0.212	0.727
Variables for the log of AUC for 0–72 h	**B**	*p*	**95.0% CI for B**	
			Min	**Max**
(Constant)	1.321	<0.001	1.154	1.488
Post-CA shock	0.303	0.01	0.079	0.526

AUC = area under the curve; B = standardized beta coefficient; CA = cardiac arrest.

4. Discussions

CA involves the most severe form of circulatory failure. The complex changes produced by disruption of cell morpho-functional integrity during the general ischemia phase do not stop with the return of spontaneous circulation and are subsequently supplemented by those appearing during the reperfusion phase. The release of proinflammatory cytokines with the onset of systemic inflammatory response syndrome and endothelial damage (with coagulation/anticoagulation and fibrinogenesis/fibrinolysis imbalance) are intricate mechanisms that ultimately contribute to organ failures with negative impact prognosis of resuscitated patients [6,8,13,14,23].

In our previous study, we investigated for the first time the serum levels of resistin as a possible predictor of mortality after CA. Our results were promising, showing that high serum values of resistin accurately predicted death at 30 days, making resistin a marker with a high predictive value of survival [14,18].

However, resistin levels can be influenced by a variety of factors, such as the presence of atherosclerosis, obesity or sepsis. In light of this, we investigated the possible correlations of several clinical and biochemical factors with the serum concentration of resistin in patients with CA, for a better understanding of its role in CA [18].

Initially described in 1994 as a way of quantifying organ dysfunction by evaluating respiratory, cardiovascular, hepatic, renal, neurological and coagulation systems [24], the SOFA score remained useful over the years and is now being used with accuracy in quantifying the prognosis of critically ill patients [25]. The ischemia-reperfusion lesion is one of the most important mechanisms that link CA to multiple organ failures, including circulatory and cardiac dysfunction. Our results showed that there is a strong correlation between the severity of the disease (quantified by the SOFA score) and serum levels of resistin, a potential marker that may correctly reflect organ failures.

Over time, elevated levels of resistin have been associated with increased risk of coronary heart disease, especially with myocardial infarction (but not with stroke) [26] and with the degree of heart failure, both responsible for increasing the rate of cardiac events, including the risk of death [26,27]. At the same time, obesity, diabetes, high carbohydrate and unsaturated fat diet and chronic alcohol

consumption, but not smoking, were described as cardiovascular risk factors correlated with elevated human serum resistin levels [28–31]. In our study, we found no associations between resistin levels in patients with CA and history of coronary artery disease, stroke, diabetes mellitus, obesity, smoking or alcoholic beverages. This reinforces the idea that in an acute critical illness high levels of resistin (or other adipokines) are mostly due to inflammatory status and not to adipose tissue mass or pre-existing unhealthy lifestyle [32].

At multivariate analysis, we found that the presence of post-CA shock and cardiovascular comorbidities were independently associated with serum resistin levels in the first 24 h after CA.

In fact, the presence of post-CA shock was the only independent variable associated with serum resistin levels at 48 and 72 h following CA. These results show that the elevated serum concentrations of resistin might be influenced by the post-CA shock, rather than by pre-CA cardiovascular comorbidities. The shock that occurs after CA is the result of myocardial dysfunction, vasoplegic shock and systemic inflammatory response [25]. Part of a complex vicious circle, as this shock becomes more refractory to treatment, cardiocirculatory dysfunction evolves in turn into a more severe form, resulting in multiple organ failures responsible for early death. The strong association of resistin with post-CA shock and with the presence of cardiovascular comorbidities may support the theory that serum resistin levels correlate equally with both the amplitude of the inflammatory process and cardiac dysfunction after resuscitation.

In previous studies, increased serum levels of lactate upon admission to the emergency department and intensive care units were associated with the negative prognosis of patients with acute critical illness [18]. Our data revealed that high serum levels of lactate at admission correlate strongly with serum resistin levels. This may support the idea that resistin is directly involved in the process of systemic inflammation in CA pathogenesis, seeing as elevated lactate levels are in fact associated with severe cardiocirculatory dysfunction [33]. However, at multivariate analysis, the aforementioned correlation did not remain statistically significant, suggesting that there were other important factors that interfere in the CA physiopathological sequence.

To our knowledge, this is the first study that evaluated the factors that influence the kinetics of resistin after CA. These results were obtained on a small number of patients, although statistically significant. The high number of measurements present an accurate kinetics of resistin after a CA, with a peak at 12–24 h and a rapid decrease to admission values after 48 h. This is important for future studies on acute events, as it shows that the focus on resistin should be especially in the first 24 h.

In order to strengthen our hypothesis, it is essential that we develop further/future studies on larger groups of patients. Also, they must include other markers of acute inflammation, with a special interest for those reportedly correlated with resistin during acute cardiovascular events: tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), high-sensitivity C-reactive protein (hs-CRP) and other proinflammatory cytokines [34]. Resistin promotes the production of TNF-α, IL-1β, IL-6 and other cytokines [12,35]. There are several drugs that have been shown to reduce the levels of resistin in chronic administration: statins, anti-TNF-α monoclonal antibodies and folic acid [36–38]. Experimental animal or in vitro studies in acute situations with drugs that lower resistin concentration are worth considering.

Other markers that could provide insights into the functions and pathophysiological implications of resistin are the microvesicles (large extracellular vesicles that appear from different cells after apoptosis) [39]. Platelet-derived microvesicles are a source of TNF-α and IL-6, while endothelial-derived microvesicles are stimulated by TNF-α [40]. Elevated levels of endothelial-derived microvesicles were found in acute coronary syndrome patients, but they were not evaluated in patients that survived a CA; one can speculate that investigating this class of micro-vesicles will offer valuable data [41,42].

Markers that evaluate post-cardiac-arrest myocardial dysfunction should be studied in any future research on patients after a successfully resuscitated CA. Left ventricular systolic dysfunction is present in almost 60% of patients resuscitated after CA [33]. The assessment of left ventricle ejection fraction, biomarkers of ventricular dysfunction and the correlation with proinflammatory markers will generate a better understanding of the complexity of PCAS. The N-terminal pro-B-type natriuretic peptide

(NT-proBNP) and marinobufagenin are reliable indicators of ventricular dysfunction and, as such, can serve as excellent candidates for future studies on OHCA [43,44].

Even though the multivariate analysis showed that the post-CA shock was independently associated with higher levels of resistin, an important bias could be the presence of cardiovascular comorbidities. The myocardial systolic and diastolic dysfunctions appear in post-CA shock, even if the patient does not have a prior coronary disease [45]. Future studies should include patients with noncardiac causes of CA, because the presence of cardiac diseases aggravates the left ventricular dysfunction. Other diseases that were proven to have an influence on the resistin concentrations should be excluded (nonalcoholic fatty liver disease, asthma, autoimmune disease, chronic kidney disease) [46]. That could provide a clearer picture about the association between post-CA shock and resistin kinetics.

5. Conclusions

Our findings demonstrate strong independent correlation between high serum resistin levels, cardiac comorbidities and post-CA shock. The impact of the post-CA shock on serum concentration of resistin was greater than that of cardiac comorbidities.

Author Contributions: Conceptualization, R.M.T., Ş.C.V. and D.I.; Data curation, R.M.T., A.G. and R.R.; Formal analysis, R.R., Ş.C.V. and D.I.; Funding acquisition, R.M.T.; Investigation, R.M.T. and A.G.; Methodology, R.M.T. and Ş.C.V.; Supervision, D.I.; Writing—original draft, R.M.T., A.G., Ş.C.V. and D.I. All authors have read and agreed to the published version of the manuscript.

Funding: "Iuliu Haţieganu" University of Medicine and Pharmacy: 7690/42/15.04.2016.

Acknowledgments: The study was partially funded by "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, through the Doctoral Research Project-2015[No. 7690/42/15.04.2016]. The financial support allocated from the grant was used for the acquisition of biomarkers and laboratory supplies.

Conflicts of Interest: The authors declare no conflict of interest.

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PLOS ONE

RESEARCH ARTICLE

Resistin—Can it be a new early marker for prognosis in patients who survive after a cardiac arrest? A pilot study

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OPEN ACCESS

Citation: Tat RM, Golea A, Vesa ŞC, Ionescu D (2019) Resistin—Can it be a new early marker for prognosis in patients who survive after a cardiac arrest? A pilot study. PLoS ONE 14(1): e0210666. https://doi.org/10.1371/journal.pone.0210666

Editor: Tatsuo Shimosawa, International University of Health and Welfare, School of Medicine, JAPAN

Received: September 30, 2018

Accepted: December 28, 2018

Published: January 16, 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information file.

Funding: This study was partially funded by the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, through the Doctoral Research Project-2015 (No. 7690 / 42 / 15.04.2016). The financial support allocated from the grant was used for the acquisition of biomarkers and laboratory supplies. The sponsor had no involvement in study design, collection, analysis and interpretation of data, writing of the

Abstract

Aim

The aim of our study was to evaluate the potential role of resistin in estimating the 30 days prognosis in patients with hypoxic-ischemic organ injury who survived after a cardiac arrest (CA).

Materials and methods

The study included 40 patients resuscitated after a non-traumatic out-of-hospital CA admitted in Emergency Department (ED). All patients were followed for 30 days after CA or until death. Clinical data on admission were recorded. Blood samples were collected on admission in ED (0-time interval), and at 6, 12, 24, 48- and 72-hours following resuscitation. Serum concentrations of resistin, S100B and neuron specific enolase (NSE) were measured. Several predictive scores for the mortality at 30 days were created with logistic regressions.

Results

At each time interval, median serum levels of resistin and S100 B were significantly higher in non-survivors compared to survivors. For NSE, plasma levels were significantly lower in survivors as compared to non-survivors at 48 and 72 hours, respectively. Accurate predictive scores for 30-days mortality were the ones which included the values of resistin and S100B measured at 12 hours after admittance [AUC 0.938 (0.813–0.989), sensitivity 85.71% (67.3%– 96%), specificity 91.67% (61.5%'99.8%), p<0.001], which included the values of all three markers measured at 12 hours after admittance [AUC 0.955 (0.839–0.995), sensitivity 82.14% (63.1%'93.9%), specificity 100.00% (73.5%'100.0%), p<0.001] and the that included the values of resistin and S-100B at 6 hours together with serum lactate on

manuscript or decision to submit the manuscript for publication. There was no additional external funding received for this study (the authors supported part of the study).

Competing interests: The authors have declared that no competing interests exist.

admission [AUC = 0.994 (0.901–1.0), sensitivity 96.4% (81.7%'99.9%), specificity 100.00% (73.5%'100.0%), p<0.001].

Conclusion

In our study, serum levels of resistin or a combination of resistin with S-100B or resistin with S-100B and lactate, were highly predictive for 30 days mortality in resuscitated patients after CA. Further studies on large number of patients are needed to confirm our data.

Introduction

At present, hypoxic-ischemic organ injury after cardiac arrest (CA) remains a major health problem due to increased mortality, morbidity and length of hospital stay. Most of the hospital stay is pent in intensive care units, which leads to increased costs and implications for health system, patient and family. Majority of these patients have poor outcomes. Comatose patients with brain injury after out-of-hospital cardiac arrest (OHCA) have survival rate of approximately 30% [1–3].

Therefore, numerous attempts have been made to identify those patients with high chances of favorable outcome after return of spontaneous circulation (ROSC). In these cases efforts should be made to obtain full recovery. Thus, in the past years a number of studies focused on the potential role of biomarkers, along with clinical and imagistic criteria in estimating outcomes in patients who suffer an out-of-/in-hospital CA [4–8].

Neuron specific enolase (NSE), isomeric form of glycolytic enolase, is found almost exclusively in neurons and neuroendocrine cells. It is now accepted that increased levels of NSE in patients with hypoxic encephalopathy after CA are especially correlated with the severity of neurological injuries and less with the risk of death (the current recommendations of the guidelines are to make it a prognostic marker after the first 3 days of CA) [1–3, 9–11].

S100 protein family has an important regulatory role in different cellular processes. It has been shown that S-100B has a high sensitivity in detecting brain damages, but it can also be released from other injured sources such are adipocytes or myocardial cells [1, 9]. Increased plasma levels of S-100B protein were well correlated with the severity of post-anoxic neurological damages and with unfavorable outcome in patients with cerebral hypoxic injuries [2, 6, 9, 12, 13].

At the moment, it is not possible to correctly predict patients' outcome after ROSC strictly based on these biomarkers due to their increased heterogeneity and variability in time [3, 8].

Resistin (ADSF- adipose tissue-specific secretory factor) is a protein belonging to resistin-like molecules family and is expressed in humans by a number of cells including adipocytes, peripheral blood mononuclear cells, macrophages and bone marrow cells [14, 15]. Recent studies have drawn attention on the potential role of resistin as a biomarker than can predict mortality in patients with cardio-vascular disease (in close connection with the ability of resistin to influence glucose and insulin metabolism, thrombosis, angiogenesis and smooth muscle cell dysfunction, but as a factor regulating expression of Vascular Cell Adhesion Molecule-1 on endothelial cells) and as a marker of inflammation that can predict survival in critically ill patients. At this point, this correlation was frequently associated with organ dysfunction in patients with sepsis [16–19], but its role in patients who have a successfully resuscitated CA (although in this case, inflammatory processes underlie hypoxic organs injury) is unknown.

The aim of our study was to evaluate the potential role of resistin in estimating 30 days prognosis in patients with hypoxic-ischemic organ injury who survived after a CA.

Materials and methods

The study was prospective, analytical, longitudinal, observational and cohort type. It included all consecutive patients resuscitated after non-traumatic OHCA admitted to the Emergency Department (ED) of County Emergency University Hospital Cluj-Napoca, who were subsequently transferred in intensive care units, between May 2016 and October 2017. All patients were followed for 30 days after CA or until death. This study was approved by the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy, with registration number 59/14.03.2016. Informed consent for inclusion in the study was obtained from patients' proxies in all cases. Inclusion criteria were age between 18–85 years and CA. Exclusion criteria were age under 18 and over 85 years, pregnancy, CA due to trauma, acute bleeding from non-traumatic condition, re-arrest with unsuccessful resuscitation within 6 hours from hospital arrival, arrest secondary to hypothermia, terminal neoplastic disease, inmates and absence of informed consent.

Study protocol

CA was managed in all patients according to European Resuscitation Council Guidelines 2015. Resuscitation was initiated on-site by emergency medical team members [20, 21]. All the study patients were treated with conventional cardiopulmonary resuscitation (CPR). Extracorporeal cardiopulmonary resuscitation (ECPR) has not been applied to any patient in our study. Fluid infusion and vasopressors/inotropes, alone or in combination (based on individual clinician judgment) were administered to maintain mean arterial pressure $\geq$ 65 mmHg and urine output of $\geq$ 0.5 ml/kg/hour. After successful resuscitation, for comatose patients that were able to maintain a systolic blood pressure above 90 mmHg (mean arterial pressure—MAP $\geq$ 65 mmHg, with or without vasopressors) and did not present with sepsis, controlled therapeutic hypothermia was administered in the first 24 hours. A core temperature between 34–35˚C was achieved using ice bags and cooling blankets. Reheating was slow at a rate of 0.25–0.5C˚/hour. Hyperthermia, convulsions and hyperglycemia were avoided and treated according to current resuscitation protocols [3, 22].

Post-CA shock was defined as need for continuous vasopressors/inotropes infusion to maintain MAP > 65 mmHg despite adequate fluid loading, for more than 6 hours after obtaining ROSC. The cause of death was defined as related to post-CA shock (death occurred as a direct consequence of shock, including subsequent multiorgan failure), to hypoxic-ischemic brain injury or to sepsis.

Clinical data such as initial Sequential Organ Failure Assessment (SOFA) score on admission to ED [23], highest SOFA (highest score registered every 24 hours after admission for the first 3 days or until death), age, gender, primary rhythm of CA, duration of resuscitation, the presence or absence of cardiovascular and non-cardiovascular comorbidities were registered.

Blood samples and laboratory assays

Blood samples were drawn from a separate peripheral vein at admission in ED (0-time interval), and at 6, 12, 24, 48- and 72-hours following resuscitation. To collect blood samples, 5 ml serum separator clot activator biochemistry vacutainers were used. The hemolyzed samples were excluded and blood samples were immediately repeated. Samples were centrifuged at 3000 rotation/minutes during the first 60 minutes after prelevation and were stored at -70˚C. Subsequently, serum concentrations of biomarkers resistin, S-100B and NSE were determined

using a quantitative sandwich immunoassay technique (ELISA; BioVendor, LM, Czech Republic) according to the manufacturer's instructions.

Statistical analysis

Statistical analyzes were performed using the MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org/; 2018). Quantitative data were expressed as median and interquartile range (non-normal distribution), and qualitative data were characterized by frequency and percentage. Comparisons between groups were performed using the Chi-Square test, Fisher's exact test, or Mann-Whitney test, whenever appropriate. To identify predictors of death, baseline characteristics were presented stratified by 30-day survival. For each marker we calculated the area under the curve using the trapezoidal method. This included all determinations of serum concentrations of biomarkers studied over time from the first measurement up to 12, 24, 48 or 72 hours. In order to find out how accurate a marker differentiates the deceased form the survivors, we used the area under the receiver operating characteristics (AUROC) curves. The cut-off value for each biomarker was calculated where specificity and sensibility were maximal. A predictive score for the mortality at 30 days was created with a logistic regression and it included the best performing markers. The score was calculated using the following formula: score = $\exp (c + b_i x_i) / 1 + \exp(c + b_i x_i)$; where exp is the base of natural logarithms; c is the constant of the logistic regression; b is the coefficient of the predictor variable; x is value of the marker. A p value < 0.05 was considered statistically significant.

Results

Of the 152 consecutive patients resuscitated after CA and admitted to the ED, 40 were eligible and have been included in the study with a median age of 67 years (IQR: 59.2 to 76). In this study group 28 (70.0%) patients were men. Most frequent rhythms of CA were asystole and pulseless electrical activity, found in 28 cases (70.0%), with a median duration of resuscitation of 15 minutes (IQR: 7.75 to 28.75). Most patients developed post CA-shock after initial resuscitation [n = 29 (72.5%)] with a high mortality rate [n = 25 (86.2%)], especially due to post-CA shock [n = 18 (72.0%)] (Fig 1).

Cardiovascular disease was the main cause of CA with 29 (72.5%) cases and acute myocardial infarction (40.0%) was the most common presentation (all patients receiving coronary revascularization immediately after resuscitation) (Table 1).

Of the 40 enrolled patients, 12 (30.0%) survived and 28 (70.0%) died before 30 days: 6 patients (15.0%) died in the first 24 hours after admission but not within the first 6 hours, while other 6 patients (15.0%) died before reaching 48 hours of admission; 3 patients (7.5%) died between 48–72 hours and 13 patients (32.5%) died after 72 hours of admission but before completing 30 days of follow-up.

Cardiovascular comorbidities and higher disease severity scores were registered in non-survivors as otherwise expected. In survivors, the initial rhythm at presentation was ventricular fibrillation/pulseless ventricular tachycardia, with a shorter duration of resuscitation compared to those who died most often presented with asystole/pulseless electrical activity (Table 2).

Biomarkers assay

At each time interval, median serum levels of resistin and S-100B were significantly higher in non-survivors compared to survivors ($p < 0.05$). For NSE, plasma levels were significantly lower in survivors compared to non-survivors at 48 and 72 hours, respectively ($p < 0.05$) (Table 3).

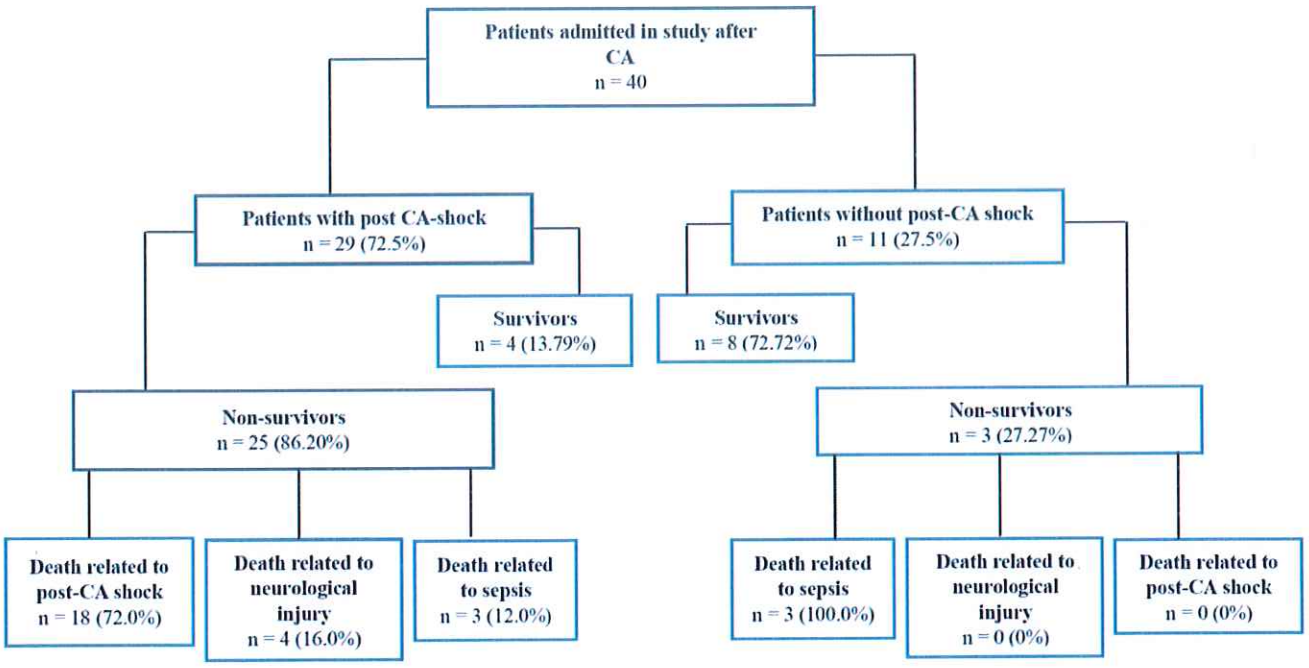

Fig 1. Flowchart.

https://doi.org/10.1371/journal.pone.0210666.g001

Comparing serum concentrations of biomarkers at different time intervals, in non-survivors and survivors, shown that levels were significantly higher in non-survivors for resistin and S-100B ($p < 0.05$), with a relatively constant AUCs for resistin determinations (Table 4).

For predicting the mortality at 30 days, we created several predictive models using logistic regressions (Table 5). In each model we included 2 or 3 variables that achieved the highest AUC alone (Tables 3 and 4) in order to established which score could better predict the

Table 1. Causes of CA registered in study patients and the causes of death at 30 days in relation to post-CA shock, neurological injury and sepsis.

Causes of cardiac arrest n = 40 (100%)	Non-survivors n = 28 (70.0%)		
	Post-CA shock	Neurological	injury Sepsis
Acute myocardial infarction 16 (40.0%)	4 (14.80%)	3 (10.71%)	0 (0%)
Pulmonary Embolism 2 (5.0%)	2 (7.14%)	0 (0%)	0 (0%)
Acute pulmonary edema 4 (10.0%)	4 (14.28%)	0 (0%)	0 (0%)
Rhythm disorders related to heart disease 7 (17.5%)	1 (3.57%)	0 (0%)	3 (10.71%)
Rhythm disorders related to electrolyte imbalances 2 (5.0%)	2 (7.14%)	0 (0%)	0 (0%)
Acute respiratory failure 3 (7.5%)	1 (3.57%)	0 (0%)	2 (7.14%)
Stroke 2 (5.0%)	0 (0%)	1 (3.57%)	1 (3.57%)
Septic shock 4 (10.0%)	4 (14.8%)	0 (0%)	0 (0%)

https://doi.org/10.1371/journal.pone.0210666.t001

Table 2. Baseline characteristics and SOFA scores stratified by 30 days survival.

Characteristics		AUC	Non-survivors (n = 28)	Survivors (n = 12)	p
Age, years, median (IQR)		0.735	69.0 (IQR:66.0 to76.7)	60.50 (IQR:45.0 to 66.7)	0.02
Sex, n (%)	Female	–	9 (32.1)	3 (25.0)	0.72
	Male	–	19 (67.9)	9 (75.0)	
Primary rhythm (%)	VF/VT	–	2 (7.1)	10 (83.3)	<0.001
	Asystole/PEA	–	26 (92.9)	2 (16.7)	
Duration of CPR, minutes, median (IQR)		0.713	15.0 (IQR:10.0 to 30.0)	6.0 (IQR:4.2 to 20.0)	0.03
Cardiovascular comorbidities, n (%)		–	22 (78.6)	4 (33.3)	0.01
Non -cardiovascular comorbidities, n (%)		–	14 (50.0)	4 (33.3)	0.49
SOFA score at admission, median (IQR)		0.815	15.0 (IQR:13.0 to 17.0)	11.0 (IQR:6.2 to 13.0)	0.002
TH, n (%)		–	22 (81)	5 (18.5)	0.03
Lactate at admission, median (IQR)		0.890	11.3 (IQR: 10.1 to 13.3)	5.7 (IQR: 2.8 to 9.1)	<0.001
Creatinine at admission, median (IQR)		0.734	1.4 (IQR: 1.1 to 1.9)	1 (IQR: 0.9 to 1.3)	0.008

PEA = pulseless electrical activity; VF = ventricular fibrillation, VT = ventricular tachycardia

CPR = cardiopulmonary resuscitation; IQR = interquartile range

SOFA = Sequential Organ Failure Assessment Score; Highest SOFA = the highest SOFA score recorded in the first 72 hours

TH = therapeutic hypothermia

https://doi.org/10.1371/journal.pone.0210666.t002

mortality. The score was calculated using the equation described in materials and methods and the coefficients were provided by the regression.

After calculating the scores, we used the AUROC to determine which biomarker could differentiate more accurately the non-survivors from the survivors. The most accurate scores are

Table 3. Median serum levels and AUC of resistin, S-100B and NSE at admission and during the first 72 hours.

Variable	Time interval	AUC	Non-survivors (n = 28)	Survivors (n = 12)	p
Resistin (ng/ml)	0 hours	0.751	9.35 (IQR: 5.6 to 13.7)	5.80 (IQR: 1.9 to 6.9)	0.01
	6 hours	0.807	11.80 (IQR: 7.1 to 19.8)	4.30 (IQR: 3.2 to 9.3)	0.002
	12 hours	0.875	15.85 (IQR: 10.8 to 24.2)	4.50 (IQR: 1.6 to 11.2)	< 0.001
	24 hours	0.795	16.50 (IQR: 10.2 to 26.5)	6.75 (IQR: 3.6 to 9.6)	0.001
	48 hours	0.779	13.45 (IQR: 5.2 to 21.0)	3.65 (IQR: 1.6 to 7.4)	0.004
	72 hours	0.763	11.00 (IQR: 6.2 to 17.8)	5.35 (IQR: 2.5 to 8.0)	0.02
S-100B (pg/ml)	0 hours	0.798	52.80 (IQR: 15.7 to 122.8)	5.95 (IQR: 3.7 to 20.9)	0.003
	6 hours	0.918	40.90 (IQR: 4.8 to 190.3)	4.80 (IQR: 3.3 to 8.9)	< 0.001
	12 hours	0.839	79.90 (IQR: 9.5 to 165.2)	4.60 (IQR: 3.3 to 12.8)	0.001
	24 hours	0.782	56.50 (IQR: 10.9 to 199.5)	4.60 (IQR: 3.3 to 12.8)	0.001
	48 hours	0.776	31.50 (IQR: 11.6 to 201.5)	6.60 (IQR: 3.6 to 15.7)	0.009
	72 hours	0.798	25.10 (IQR: 7.8 to 80.7)	4.80 (IQR: 3.3 to 12.8)	0.01
NSE (ng/ml)	0 hours	0.509	7.30 (IQR: 3.27 to 15.87)	8.05 (IQR: 2.10 to 18.15)	0.92
	6 hours	0.618	15.40 (IQR: 4.77 to 47.77)	10.85 (IQR: 3.92 to 19.62)	0.24
	12 hours	0.652	20.05 (IQR: 6.52 to 80.77)	12.10 (IQR: 1.77 to 20.12)	0.13
	24 hours	0.506	19.85 (IQR: 6.65 to 97.65)	10.60 (IQR: 4.67 to 20.17)	0.17
	48 hours	0.779	72.20 (IQR: 9.70 to 211.87)	5.40 (IQR: 1.35 to 11.70)	0.006
	72 hours	0.750	67.00 (IQR: 4.75 to 194.60)	4.00 (IQR: 1.90 to 23.95)	0.03

AUC = area under the curve; IQR = interquartile range

https://doi.org/10.1371/journal.pone.0210666.t003

Table 4. Comparison of area for markers measurements between non-survivors and survivors.

Variable		AUC	Non-survivors (n = 28)	Survivors (n = 12)	P
Area for resistin ng x h/ml	0–12 hours (3 measurements)	0.792	26.0 (IQR: 14.0–37.5)	9.5 (IQR: 4.2–22.5)	<0.001
	0–24 hours (4 measurements)	0.788	27.2 (IQR: 18.3–37.5)	14.9 (IQR: 8.3–21.8)	0.001
	0–48 hours (5 measurements)	0.795	22.0 (IQR: 14.5–38.7)	12.7 (IQR: 5.3–15.9)	0.003
	0–72 hours (6 measurements)	0.814	44.9 (IQR: 30.5–71.9)	23.4 (IQR: 15.4–33.7)	0.008
Area for S-100B pg x h/ml	0–12 hours (3 measurements)	0.833	75.0 (IQR: 24.5–233.0)	12.0 (IQR: 7.2–30.5)	<0.001
	0–24 hours (4 measurements)	0.647	64.8 (IQR: 15.5–129.3)	23.2 (IQR: 12.9–30.2)	0.011
	0–48 hours (5 measurements)	0.692	55.1 (IQR: 22.6–251.8)	26.4 (IQR: 15.1–31.1)	0.041
	0–72 hours (6 measurements)	0.750	122.5 (IQR: 38.0–372.0)	37.8 (IQR: 22.5–73.8)	0.034
Area for NSE ng x h/ml	0–12 hours (3 measurements)	0.455	19.0 (IQR: 9.0–61.5)	27.0 (IQR: 6.0–38.0)	0.215
	0–24 hours (4 measurements)	0.551	39.8 (IQR: 11.2–47.5)	23.5 (IQR: 10.7–52.2)	0.097
	0–48 hours (5 measurements)	0.763	123.0 (IQR: 28.5–313.5)	19.0 (IQR: 6.25–37.5)	0.008
	0–72 hours (6 measurements)	0.737	108.0 (IQR: 25.0–318.0)	16.5 (IQR: 8.5–48.2)	0.044

https://doi.org/10.1371/journal.pone.0210666.t004

presented in Table 6. We did not find statistically significant differences between any of the AUCs of the scores.

When we compared the predictive value of resistin alone at 12 hours with the combination of S-100B and resistin at 12 hours we found a p = 0.07. The sensitivity was almost the same (85.7%) but the specificity was lower for resistin (75% vs. 91.6%). When we compared the predictive value of resistin alone at 12 hours with the area for resistin and S-100B combined score between 0–12 hours we found a p = 0.08. When we compared the predictive value of resistin alone at 12 hours with the resistin, NSE and S-100B combined score at 12 hours we found a p = 0.1. When we compared the predictive value of resistin alone at 12 hours with area for

Table 5. Logistic regression for 30-days mortality.

Variables		B	P	OR	95% C.I. for OR	
					Lower	Upper
Model for resistin and S-100B combined score at 12 hours	S-100B pg/ml at 12 hours	0.058	0.1	1.060	.975	1.153
	Resistin ng/ml at 12 hours	0.262	0.020	1.300	1.042	1.621
	Constant	-3.057	0.01	0.047	–	–
Model for resistin, NSE and S-100B combined score at 12 hours	S-100B pg/ml at 12 hours	0.136	0.2	1.146	0.899	1.461
	Resistin ng/ml at 12 hours	0.374	0.02	1.453	1.050	2.010
	NSE ng/ml at 12 hours	-0.024	0.1	0.977	0.949	1.006
	Constant	-4.078	0.04	0.017	–	–
Model for area for resistin and S-100B combined score between 0–12 hours	Area for resistin 0–12 hours	0.135	0.02	1.145	1.019	1.285
	Area for S-100B 0–12 hours	0.033	0.1	1.034	0.993	1.076
	Constant	-3.423	0.01	0.033	–	–
Model for areas for resistin, NSE and S-100B combined score between 0–12 hours	Area for resistin 0–12 hours	0.204	0.02	1.226	1.026	1.465
	Area for S-100B 0–12 hours	0.055	0.1	1.056	0.983	1.134
	Area for NSE 0–12 hours	-0.014	0.1	0.986	0.969	1.003
	Constant	-4.530	0.03	0.011	–	–
Model for resistin and S-100B at 6 hours and lactate at 0 hours combined score	Lactate mmol/L at 0 hours	1.880	0.2	6.553	0.206	208.9
	S-100B pg/ml at 6 hours	1.415	0.3	4.116	0.261	64.824
	Resistin ng/ml at 6 hours	1.269	0.2	3.559	0.493	25.701
	Constant	-37.919	0.2	0	–	–

https://doi.org/10.1371/journal.pone.0210666.t005

Table 6. AUCs for the predictive scores.

Predictive score	AUC (CI95%)	Cut-off	Sensitivity (CI 95%)	Specificity (CI 95%)	p
Resistin and S100B combined score at 12 hours	0.938 (0.813–0.989)	> 0.65	85.71 (67.3–96.0)	91.67 (61.5–99.8)	<0.001
Resistin, NSE and S100B combined score at 12 hours	0.955 (0.839–0.995)	> 0.73	82.14 (63.1–93.9)	100.00 (73.5–100.0)	<0.001
Area for resistin and S100B combined score between 0–12 hours	0.937 (0.813–0.989)	> 0.58	82.14 (63.1–93.9)	91.67 (61.5–99.8)	<0.001
Area for resistin, NSE and S100B combined score between 0–12 hours	0.958 (0.843–0.996)	> 0.69	85.71 (67.3–96.0)	100.00 (73.5–100.0)	<0.001
Resistin and S100B at 6 hours and lactate at 0 hours combined score	0.994 (0.901–1)	> 0.57	96.4 (81.7–99.9)	100 (73.5–100.0)	<0.001

https://doi.org/10.1371/journal.pone.0210666.t006

resistin, NSE and S-100B combined score between 0–12 hours we found a p = 0.1. When we compared the predictive value of resistin alone at 12 hours with resistin and S-100B at 6 hours and lactate at 0 hours combined score we found a p = 0.03.

We examined the usefulness of the markers for predicting the mortality at 24 hours, since guidelines usually cannot indicate the predictors for such an early event. Only high resistin values had a statistically significant association with the 24-hours morality: at 0 hours (p = 0.008), at 6 hours (p = 0.02) and at 12 hours (p = 0.03).

Considering that at this point the NSE is useful for predicting mortality, if is measured in dynamics and after 48–72 hours, we compared it with the score that combined resistin and S100 at 12 hours (Fig 2). We obtained an AUC of 0.911 (0.742 to 0.985) for the score and an AUC of 0.810 (0.618 to 0.932) for NSE alone. The difference between AUCs was not statistically significant (p = 0.2).

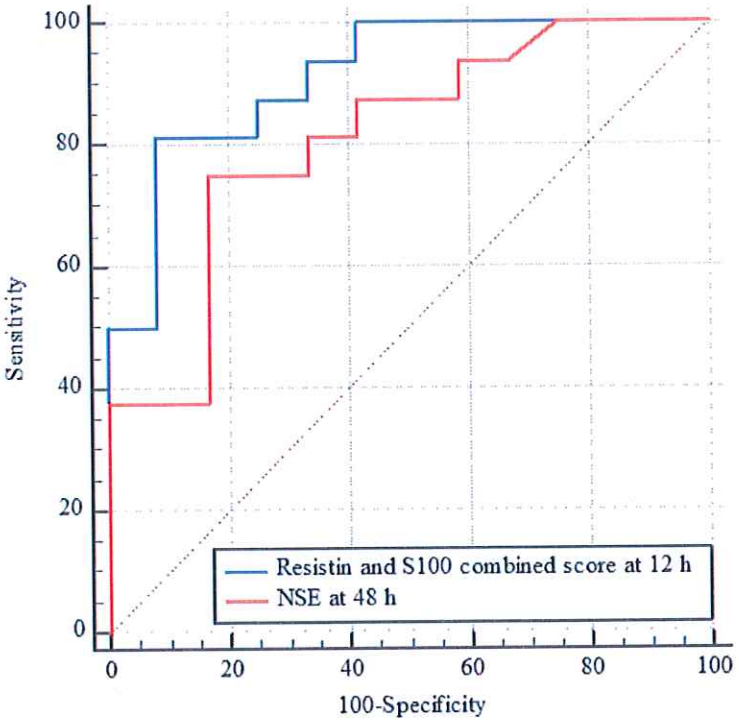

Fig 2. Comparison between AUCs for differentiating the 30-days mortality.

https://doi.org/10.1371/journal.pone.0210666.g002

Discussions

In the last few years it has been shown that resistin levels are well correlated with prognosis of critically ill patients [18]. This correlation may be attributed to the fact that resistin is involved in the release of increased levels of pro-inflammatory biomarkers—IL-6, IL-12 and TNF-α- that are involved in organ failure caused by sepsis [14, 16].

Taking into consideration that an inflammatory process is involved in hypoxic-ischemic organ injury after CA, we investigated a possible correlation between resistin serum levels and the outcome in resuscitated patients and possible predictive value of resistin combined with S-100B and NSE. To our knowledge this is the first study to evaluate resistin as a biomarker for the outcome of resuscitated patients.

Previous studies have focused on the role of NSE and S-100B protein [3, 22] in predicting outcome in patients resuscitated after CA, in order to direct maximal resources for the treatment of these patients [4–6, 9–12], although the time interval for markers assay seems to be crucial in defining their role for predicting patients' outcome [3, 5]. Today these markers are included in resuscitation guidelines.

Stammet P et al., found that NSE levels were well correlated with the outcome in resuscitated patients after CA, as an indicator of increased risk of death and poor neurologic outcome [5]. Similarly, Wiberg S et al. showed that NSE is a strong marker for predicting mortality at 48 hours after resuscitation, in patients with initially successfully resuscitated CA although a cut-off value with 100% specificity (false positive rate of 0) and high sensitivity could not yet be determined [4, 6].

Our study found similar results, showing that NSE levels were significantly elevated in non-survivors, with the highest sensitivity and specificity for predicting mortality if measured at 48 hours from the event.

Wiberg S. et al showed that S-100B is an important marker in predicting mortality after successfully resuscitated CA [6], although it's role in resuscitation is not fully attributable. Similar results are reported by us in this study. We showed that S100B levels were significantly higher in patients who died, at all-time intervals, making S100B a highly predictive and more accurate biomarker for 30-day mortality than NSE.

For resistin, our results showed that levels (including median serum concentrations levels) were significantly increased at each time interval of assay in non-survivors compared to survivors with relatively constant AUCs and high predictive value for 30-day mortality compared with NSE.

Starting from these results, for predicting the mortality at 30 days, we created several models following the pattern that could better differentiate the non-survivors from the survivors. Taking into account the cost-effective ratio (the number of determinations over time, the current cost of biomarker kits), we could demonstrate that six biomarkers determinations that combined resistin with S100 in the first 12 hours is sufficient for early prediction of outcome in patients who survived after a CA (deceased versus survivor).

We must agree and point out, that both biomarkers had a lower sensitivity than specificity for all determinations (although they are very close). This reinforces the suggestion that interpreting the values of these biomarkers in relation to post-cardiac arrest survival should be part of a multifactorial approach of the clinician.

One of the biological variables of interest in determining the prognosis of critically ill patients is serum lactate. Recent studies demonstrate that elevated serum lactate levels determined upon patient arrival in the emergency department is associated with unfavorable prognosis of patients who presented an episode of CA [24, 25]. The results were promising and from the many combinations of biomarkers lactate value at arrival and serum resistin and

S100B values at 6 hours best predict mortality (AUC = 0.994, Sp = 100%, Se = 96.4%, $p < 0.001$). Although we are tempted to believe that we have found the ideal predictive combination, the fairly wide confidence interval of the regression variables on which we built the score impose the need for a larger group of patients to increase the accuracy of the model.

Regarding the usefulness of biomarkers in the prediction of 24-hour early mortality in a patient who initially survived a successfully resuscitated CA, resistin was the only one that was statistically significantly associated with morality.

All of these findings support the idea that resistin may be an important future biomarker in predicting early prognosis after resuscitation, due to its role in the inflammatory processes underlying the hypoxic-ischemic organ injuries after a CA. By combining resistin with S-100B and with S-100B and lactate, predictive value of these biomarkers increased.

Our study is limited by the inclusion of a small number of resuscitated patients, comparable however with other similar studies. At the same time, we could not validate the predictive score due to the difficulty in recruiting patients. We mention that all the study patients were treated with conventional CPR and the results of the study could not extrapolate to patients treated with ECPR.

Conclusion

In our study, serum levels of resistin alone or a combination of resistin with S-100B or resistin with S-100B and lactate were highly predictive of 30 days mortality in resuscitated patients after CA. Further studies on larger number of patients are needed to confirm our hypothesis.

Supporting information

S1 Database.
(XLSX)

Acknowledgments

This study was partially funded by the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, through the Doctoral Research Project-2015 (No. 7690 / 42 / 15.04.2016). The financial support allocated from the grant was used for the acquisition of biomarkers and laboratory supplies.

The sponsor had no involvement in study design, collection, analysis and interpretation of data, writing of the manuscript or decision to submit the manuscript for publication.

There was no additional external funding received for this study (the authors supported part of the study).

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Original papers

Med Ultrason 2017, Vol. 19, no. 3, 265-271
DOI: 10.11152/mu-1015

Non-alcoholic fatty liver disease, bulb carotid intima-media thickness and obesity phenotypes: results of a prospective observational study

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Abstract

Aims: The objective of this prospective study was to assess the correlation between carotid intima-media thickness at the common carotid (CIMTc) and carotid bifurcation (CIMTb) level, hepatic fat accumulation, and obesity phenotypes. **Material and methods:** Two hundred obese adults, in which CIMTc and CIMTb thickness was determined, were included. According to body mass index (BMI) and presence of metabolic syndrome (MetS), patients were classified as metabolically healthy obese (MHO, obesity without MetS) and metabolically unhealthy obese (MUHO, obesity with MetS). MHO patients were further classified as MHO1 (obese with increased waist circumference) and MHO2 (obese with increased waist circumference plus one of the 4 criteria for MetS). Non-alcoholic fatty liver disease (NAFLD) presence was assessed by fatty liver index (FLI). **Results:** CIMTc and CIMTb increased with obesity phenotypes from 0.74 mm and 1.04 mm in MHO1 to 0.84 mm and 1.23 mm in MHO2 and 0.88 mm and 1.74 mm in MUHO. Obesity phenotypes were significantly correlated with CIMTb. NAFLD frequency increased from 66.0% in the MHO1 to 73.0% in the MHO2 and 84.2% in the MUHO ($p<0.05$). Independent of age, BMI, total cholesterol, HbA1c, and HOMA-IR, the CIMTc was significantly associated with FLI in all obesity phenotypes and CIMTb only in MHO2 and MUHO. **Conclusions:** Our results suggest that subclinical atherosclerosis varies according to obesity phenotypes and is correlated with the hepatic fat accumulation.

Keywords: carotid intima-media thickness; non-alcoholic fatty liver disease; obesity phenotypes

Introduction

Obesity represents a public health problem due to its increasing prevalence despite public awareness programs [1]. Based on cardiovascular (CV) risk factors, clinical studies have identified 2 types of obesity – metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUHO). MHO are characterized by the presence of obesity as defined by a body mass index (BMI) equal or over 30 kg/m^2 without metabolic CV risk factors. MUHO associates obesity with the presence of metabolic CV risk factors and an increased risk of diabetes and CV diseases [2-4]. Studies assessing the health risks associated with MHO have shown conflicting results, some showing similar or lower risk of CV disease and diabetes when compared to MUHO. Despite numerous clinical cross-sectional and prospective epidemiological studies evaluating the CV risk associated with this obesity phenotype and its clinical implications, controversies surrounding the health risks associated with MHO remain [5-7]. Therefore, it still under debate whether MHO represents a distinct phenotype compared with MUHO (lower health associated risks during lifetime or just a MUHO precursor) [8].

In parallel with the increasing prevalence of obesity an increased prevalence of nonalcoholic fatty liver disease (NAFLD) has been reported [9]. Obesity and ab-

Received 19.02.2017 Accepted 18.05.2017
Med Ultrason
2017, Vol. 19, No 3, 265-271
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dominal obesity, metabolic syndrome, and type 2 diabetes have been identified among risk factors for NAFLD [10], which in turn is associated with an increased risk of fatal and non-fatal CV events and increased risk of total and CV diseases mortality [11]. The gold standard for NAFLD diagnosis is the liver biopsy but its invasive nature limits its use. Fatty liver index (FLI) score [12] is a noninvasive method widely used in epidemiological studies for NAFLD screening, showing good sensitivity compared with magnetic resonance spectroscopy for detecting fatty liver [13].

Carotid intima-media thickness (CIMT) is a simple and non-invasive method of the assessment of subclinical atherosclerosis and has been shown to be an independent predictor of CV disease risk [14-16]. Evaluation of CIMT includes evaluation of common carotid artery (CIMTc), bifurcation (bulb; CIMTb), and internal carotid artery and it has been shown that the association of the CIMT with CV risk factors varies according to the segment assessed [17]. The association between CIMT and NAFLD has been reported in the past years and this association was independent of other CV risk factors [18,19].

Currently, limited data are available on the relationship between hepatic fat content and atherosclerosis according to obesity phenotypes. A recent study showed that MHO participants had significantly lower levels of CIMT and intrahepatic triglycerides content compared with the MUHO participants and intrahepatic triglycerides content was independently associated with metabolic syndrome (MetS) components and increased CIMT [20].

In this context, we aimed to investigate the correlation between subclinical carotid atherosclerosis assessed by CIMT at common carotid and carotid bifurcation level, hepatic fat, and obesity phenotypes.

Material and methods

This was a prospective study performed in the Emergency County Clinical Hospital Cluj-Napoca, Romania. We included 200 obese patients as defined by a BMI ≥30 kg/m^2, who presented, between February 2014 and November 2015 for nutritional and metabolic status evaluation in the Diabetes, Nutrition and Metabolic Diseases Clinic. We excluded from the study patients under 18 years of age, with prior diagnosis of autoimmune, viral (hepatitis virus B, C, D), toxic or uncertain etiology hepatitis, with high alcohol consumption (>140 g/week), diabetes mellitus, pregnancy, with hypolipemic and/or weight loss medication. The patients signed an informed consent prior to enrollment and Institutional Ethics Committee approval was obtained.

Clinical assessments

We recorded for all patients demographic and clinical variables such age, weight, height, waist circumference, BMI [calculated as weight (kg)/height (m^2)], associated disease (hypertension, dyslipidemia, etc), and their medication.

Waist circumference (WC) was measured with the patients standing, with a measuring tape halfway between the ribcage and the iliac crest, horizontally, at the end of a complete expiration.

Blood pressure (BP) was measured according to the guidelines [21] after a 5 minute resting, in sitting position. Two measurements were performed for each arm at 2 minutes interval. The arm with the highest BP was chosen and the average value of the measurements was computed.

Metabolic syndrome definition

Classifying patients as MHO or MUHO was performed after a series of explorations to identify the MetS components according to the International Diabetes Federation criteria [22]: 1) abdominal obesity: WC >94 cm (men) and >80 cm (women); 2) hypertriglyceridemia: ≥150 mg/dl; 3) low levels of HDL-C: <40 mg/dl (men) and <50 mg/ dl (women) or specific treatment; 4) hypertension: ≥130/85 mmHg or specific treatment; 5) high fasting glucose: ≥100 mg/dl.

Assay and indices assessment

Blood samples were drawn from the cubital vein after a 12-h fasting. Fasting plasma glucose (FPG), triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, gamma-glutamyl transferase (GGT), transaminases, uric acid, fasting insulinemia (for insulinoresistance), glycated hemoglobin (HbA1c), apolipoprotein A1 and B were determined using a Beckman Coulter UniCel DxI 600. Insulin resistance was estimated using homeostasis model assessment (HOMA-IR) as [fasting glucose (mg/dL) × fasting insulin (μUI/mL)]/405 [23].

Based on FPG values, patients were classified as having dysglicemia (prediabetes) if they had an FPG level from 110 to less than 126 mg/dl [24]. NAFLD was diagnosed using the fatty liver index (FLI) score. FLI requires for calculation BMI, WC, triglycerides, and GGT [13]. A FLI score > 60 is considered to be suggestive for the presence of NAFLD [13].

Carotid Ultrasound Measurements

Ultrasound evaluations were performed by a single examiner (with a 15-year expertise, certified for carotid ultrasonography) using a 3-10 MHz VF8-3 linear Transducer (ACUSON X300 Ultrasound System). The examination was performed with the patient lying in supine position, with a lateral probe position using a standardized protocol [25]. Wall thickness was measured in lon-

gitudinal view, at the far wall level, with the transducer positioned strictly perpendicular to this wall (the lumen-intima and media-adventitia interfaces were clearly defined), including the carotid bifurcation in the image plane. CIMT was measured at 10, 15, and 20 mm below the end of the common carotid artery (CCA), at a plaque-free point on the far wall and the average was considered the CIMTc on that side. Any atherosclerotic thickening ≥1.5 mm was considered a plaque. CIMTc was calculated as the average of the CIMT for left and right CCA. CIMTb was calculated as CIMT average in the thickest point (including plaque) of the left and right internal carotid bulb. Variation coefficients of the measurements for the examiner were <5%.

Establishing the groups of patients according to obesity phenotype

According to BMI and presence of MetS [22], participants were classified as MHO (obese without MetS) or MUHO (obese with MetS). MHO patients were further classified as MHO1 (obese with increased WC) and MHO2 (obese with increased WC plus one of the criterions for MetS).

Statistical analysis

Statistical analysis was performed with SPSS version 20. Kolmogorov-Smirnov tests were used to evaluate the distribution of investigated variables. Data was presented as proportions for qualitative variables, mean and standard deviation (SD) or median for continuous variables. The chi-square-test was used to compare categorical variables and t-test for continuous variables. Mann-Whitney test or Kruskal-Wallis test was used for non-normal distribution variables. Correlations were assessed with Spearman or Pearson coefficients, according to the variables distribution.

Univariate linear regression was performed for the relation between CIMT and FLI score in the whole sample and by obesity phenotype, multiple regression analysis was applied for the relation between CIMT and FLI adjusted for age, BMI, total cholesterol, HbA1c, and HOMA-IR. A two-sided p value ≤0.05 was considered statistically significant.

Results

Baseline characteristics for the 200 patients who met the inclusion criteria and were included in the study are shown in Table I. MHO patients were younger, had significantly lower BMI, WC, systolic and diastolic BP comparing with MUHO. Comparing with MHO, the MUHO patients had significantly lower HDL-cholesterol levels, higher triglycerides, uric acid, and CRP levels (p <0.05 for all). The frequency of dysglycemia was 11.5% in the MHO2 group and 74.0% in the MUHO group. In the MHO2 group 21 patients (40.4%) had increased WC plus hypertension, 18 patients (34.6%) plus low HDL-cholesterol, 7 patients (13.5%) plus high triglycerides, and 6 patients (11.5%) plus dysglycemia. CIMTc, CIMTb and FLI score were significantly higher in MUHO patients. .

Comparing MHO1 and MHO2 patients, those with MHO2 had significantly higher WC, glycemia, HbA1c, insulinemia, C-peptide, HOMA-IR, total and LDL-cholesterol, triglycerides, CRP and uric acid. CIMTc and CIMTb were significantly higher in MHO2 patients than in MHO1 patients. NAFLD frequency was 66.0% in the MHO1 and 73.0% in the MHO2 (p<0.05). FLI score increased from 63.44 in the MHO1 to 68.01 in the MHO2.

In all groups both CIMTc and CIMTb were directly and significantly correlated with FLI score. Correlation coefficients between CIMTc and FLI score were 0.343, 0.425, and 0.343 (MHO1, MHO2, and MUHO, respectively); 0.763, 0.443, and 0.754 for CIMTb and FLI score in the three groups (Table II).

Obesity phenotypes were statistically significant correlated only with CIMTb (p <0.05 for all; Table III).

In the univariate regression model, CIMTc was associated with the FLI score in the whole sample and CIMTb was associated with the FLI score only in the MHO1 group. After adjusting for age, BMI, total cholesterol, HbA1c, and HOMA-IR, a statistically significant association was observed between the CIMTb and FLI score in the MHO2 and MUHO, and CIMTc with the FLI score in all obesity phenotype groups (Table IV).

Discussions

MHO prevalence varies largely, up to 40%, according to the clinical study and the definition criteria, being more common in younger persons [8,26].

Our observations are similar to previous reports – MHO has a lower CV risk profile compared to MUHO [26,27]. Elevated CIMTc and CIMTb, found in several MHO1 patients were correlated with ApoB/ApoA1 ratio, but not with LDL levels. Therefore, our results support the recommendations of the guidelines, to assess apoB even when LDL levels are normal because apoB correlates with atherosclerosis and predicts CV events [28].

Marini et al showed that CIMT increased from 0.68 mm in non-obese to 0.79 in MHO and 0.89 in obese with insulin resistance [27]. From our findings, increasing values were observed from MHO1 to MHO2, reaching the highest value in MUHO. Supposedly both CIMTc and CIMTb could increase with the number of MetS components. Previous studies have shown that CIMT and carotid plaque prevalence are associated with the

Table I. Clinical, anthropometric, and metabolic characteristics of patients grouped according to the presence MetS.

Assessed parameters	MHO				MUHO	p#
	All N=100	MHO1 N=48	MHO2 M=52	p*	N=100	
Age (years)	41.02±11.33	39.23±10.13	42.33±12.12	0.001	46.31±14.82	0.001
Weight (kg)	88.06±11.81	88.06±11.81	103.87±26.89	0.001	103.87±26.89	0.001
Waist (cm)	106.90±16.77	100.00±10.27	111.50±18.79	0.01	118.47±12.51	0.010
BMI (kg/m^2)	31.72±5.42	29.98±4.45	32.8±15.77	0.23	36.40±5.75	0.006
SBP (mmHg)	127.13±22.64	113.24± 8.75	134.21±10.13	0.021	148.21±19.34	0.002
DBP (mmHg)	78.56±16.12	62.44 ±6.21	89.24±12.13	0.024	95.21±19.43	0.008
Total cholesterol (mg/dL)	186.39±43.21	179.60±43.37	196.80±42.28	0.023	206.63±50.23	0.120
LDL-cholesterol (mg/dL)	123.61±39.09	105.14±24.81	125.26±51.68	0.015	130.26±51.68	0.600
HDL-cholesterol (mg/dL)	49.18±10.70	52.13±8.02	45.26±11.91	0.01	41.20±11.84	0.019
Triglycerides (mg/dL)	106 (83.23; 120.4)	88 (75.5; 111.5)	112 (91; 122.5)	0.12	135 (77;191.5)	0.002
ApoB/ApoA1	1.43±0.550	0.85±0.33	0.71±0.14	0.23	1.44±.230	0.929
Glycemia (mg/dL)	76.83±37.30	72.75±26.94	79.56±38.08	0.01	97.28±36.13	0.052
HbA1c (%)	5.55 (5.3;5.9)	5.3 (5.20; 5.45)	5.6 (5.45; 5.95)	0.01	6.1 (5.85; 6.25)	0.001
Insulinemia (µUI/mL)	9 (7.5;11.3)	8.7 (7.60; 9.30)	10 (8.15; 16.65)	0.001	15.45 (13.3;23.85)	0.027
C-peptide (ng/mL)	2.25 (2.1;4.0)	2.1 (1.8; 2.2)	2.7 (2.2; 4.4)	0.009	3.25 (2.45; 5.65)	0.038
HOMA-IR	1.91 (1.02; 5.45)	2.43 (1.02; 3.25)	4.16 (2.25; 6.22)	0.001	6.12 (5.44; 7.51)	0.001
ASAT (IU/mL)	23.02±11.45	20.13±10.34	26.47±14.03	0.13	43.37±13.02	0.035
ALAT (IU/mL)	25.12±13.42	21.03±11.02	27.01±13.49	0.19	45.23±15.27	0.031
GGT (IU/mL)	34.25±10.89	24.38±11.02	35.67±14.89	0.21	48.38±16.72	0.037
Uric acid (mg/dL)	5.63±1.53	4.96±1.20	6.11±1.59	0.001	6.70±1.05	0.013
CRP (mg/dL)	1.90 (1; 3.3)	2.56 (0.95; 3.10)	1.40 (1.05; 3.75)	0.000	4.8 (2.3; 7.5)	0.035
CIMTc (mm)	0.81±0.19	0.74±0.17	0.84 ± 0.19	0.001	0.88±0.17	0.036
CIMTb (mm)	1.2 (1; 1.3)	1.2 (0.95; 1.3)	1.2 (1.1; 1.3)	0.01	1.5 (1.3; 2)	0.008
FLI score	66.65±26.52	63.44±25.32	68.01±26.82	0.009	79.89±26.97	0.002
NAFLD	70.0	66.7	73	0.005	84.2	0.013

The results are expressed as number (%), mean±SD or median (Q1;Q3). *p values are provided for the comparison between MHO1 and MHO2.

#p values are provided for the comparison between MHO and MUHO.N = number of participants; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1c – A1c glycated hemoglobin; HOMA-IR = homeostasis model assessment; ASAT = aspartate transaminase; ALAT = alanine transaminase; GGT = gamma-glutamyl transferase; CRP = C-reactive protein; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; FLI = fat liver index; NAFLD = non-alcoholic fatty liver disease; MHO = metabolically healthy obese; MHO1 = obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

presence of MetS [23, 29] and the number of its components [29-33]. Each additional component of the MetS is associated with a 0.02 mm increase in the CIMTc, independent of age, gender, family history of CVD, and smoking [34].

We found that only CIMTb was correlated with the obesity phenotype and systolic and diastolic BP in all groups, suggesting that BP-induced shear stress could explain the higher yearly growth rate of CIMTb compared to CIMTc [35]. Polak et al [17] showed that FPG and diastolic BP had a stronger association with CIMTc while hypertension, diabetes, and smoking with CIMTb.

We showed that FLI score increased in parallel with obesity phenotype groups. The prevalence of NAFLD increased from MHO1 to MUHO. Similarly, Zhang et al showed that the intrahepatic triglyceride content was significantly lower in MHO compared to MUHO and this content is a better predictor for MUHO than BMI, WC or percentage of body fat [20].

Intrahepatic fat accumulation and NAFLD are associated with a more adverse CV risk profile [36-40], while NAFLD is associated with insulin resistance, MetS and an atherogenic lipid profile [37, 41]. Furthermore, NAFLD patients have a higher prevalence of coronary artery lesions [36], higher CIMT, and atherosclerotic plaques [38], as well as a higher incidence of CVD, and increased CV mortality [39, 40]. We found significant correlations between the FLI score, presence of NAFLD, CIMTc, and CIMTb in all obesity phenotypes. Independent of age, BMI, total cholesterol, HbA1c, and HOMA-IR, the CIMTc was significantly associated with FLI in all obesity phenotypes, unlike CIMTb (only in MHO2 and MUHO), suggesting that hepatic fat accumulation plays a role in the determination of the obesity phenotype asso-

Table II. Correlations of carotid-intima media thickness with measured parameters according to the obesity phenotype.

	MHO1		MHO2		MUHO	
Assessed parameters	**CIMTc**	**CIMTb**	**CIMTc**	**CIMTb**	**CIMTc**	**CIMTb**
Age (years)	**0.569***	**0.523***	0.289	0.083	**0.678***	0.550
Waist (cm)	0.523	0.110	**0.503***	**0.336***	-0.165	0.218
BMI (kg/m2)	0.368	0.667	0.137	-0.065	-0.337	-0.600
SBP (mmHg)	0.505	**0.514***	0.523	**0.544***	0.505	**0.603***
DBP (mmHg)	0.489	**0.563***	0.512	**0.570***	0.489	**0.563***
LDL cholesterol (mg/dL)	-0.413	-0.306	0.001	0.355	0.297	0.218
HDL cholesterol (mg/dL)	0.042	0.518	0.212	-0.315	0.338	0.327
Triglycerides (mg/dL)	0.200	-0.409	-0.056	0.222	0.463	0.491
ApoB/ApoA1	**-0.629***	-0.691	-0.015	0.462	-0.036	-0.522
Glycemia (mg/dL)	**0.750***	**0.710***	0.083	-0.442	-0.493	0.327
HbA1c (%)	-0.209	-0.662	-0.110	-0.050	-0.021	0.806
HOMA-IR	-0.267	-0.564	0.332	-0.500	0.327	0.866
Uric acid (mg/dL)	0.407	0.218	0.231	0.077	0.528	0.794
CRP (mg/dL)	-0.360	0.051	0.389	**0.780***	-0.118	0.500
FLI score#	**0.343***	**0.763***	**0.425***	**0.443***	**0.343***	**0.754***
NAFLD	**0.368***	**0.792***	**0.502***	**0.434***	**0.368***	**0.783***

*p values <0.05 showing a statistically significant association; #FLI score included in the correlation analysis as a continuous variable; N = number of participants; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1c – A1c glycated hemoglobin; HOMA-IR = homeostasis model assessment; CRP = C-reactive protein; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; FLI = fat liver index; NAFLD = non-alcoholic fatty liver disease; MHO = metabolically healthy obese; MHO1 =obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

Table III. Correlations of the carotid-intima media thickness with the obesity phenotypes.

Assessed parameters	**MHO/MUHO**	**MHO1/MHO2/MUHO**
CIMTc	0.286 (p=0.060)	0.204 (p=0.184)
CIMTb	**0.483 (p=0.015)**	**0.518 (p=0.008)**

CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; MHO = metabolically healthy obese; MUHO = metabolically unhealthy obese.

Table IV. Association of carotid-intima media thickness and with fatty liver index score in the whole sample and by obesity phenotypes

Assessed parameters	**CIMTc**		**CIMTb**	
	β	**p**	**β**	**p**
All sample	**0.283**	**0.005**	0.072	0.416
MHO1	0.160	0.351	**0.763**	**<0.001**
MHO2	0.030	0.891	0.126	0.449
MUHO	-0.151	0.378	0.052	0.671
All sample#	0.116	0.226	0.058	0.763
MHO1#	**-0.577**	**0.001**	-*	-*
MHO2#	**-1.199**	**<0.001**	**1.586**	**<0.001**
MUHO#	**-7.713**	**<0.001**	**-0.980**	**0.004**

#Models adjusted for age, BMI, total cholesterol, CRP, HbA1c, HOMA-IR. *Could not be calculated due to high number of covariates compared to sample size; β – coefficient of correlation; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; MHO = metabolically healthy obese; MHO1 = obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

ciated with subclinical atherosclerosis, probably through increased cytokines production [42]. Currently, there is scarce data on the association of CIMT and hepatic fat accumulation in obesity phenotypes. The only available study we could identify showed that irrespective of obesity phenotype and independent of percentage of body fat, an increase in the intrahepatic triglyceride content was associated with a higher risk of increased CIMT [20].

Our study has some limitations that we must acknowledge. The sample size was relatively small and therefore, future prospective evaluation in a larger scale study is required. We could not collect accurate information on the smoking status for all patients (years of smoking, passive smoking, etc) and therefore we could not assess its impact on CIMT. NAFLD was assessed by the FLI score and not by liver biopsy nor by an ultrasound exam. However, FLI score was shown to have a good performance in NAFLD identification [43,44]. The study subjects were selected from ambulatory patients and not from the general population. Also we did not take into account the different effects of associated therapy on MetS components (anti-inflammatory drugs, contraceptives, alternative therapies etc). Because of the small number of patients, dividing them into groups according to the association of MetS component would not have allowed an accurate statistical analysis. Therefore we do not have results on the impact of each MetS component – WC pair on NAFLD and CIMT.

Conclusions

Our results support previous findings suggesting the degree of subclinical atherosclerosis varies according to obesity phenotypes and is associated with hepatic fat accumulation. Hepatic fat accumulation increased according to the obesity phenotype and may represent a predictor of metabolic changes in obesity. Further studies investigating the association between NAFLD and CIMT progression in all obesity phenotypes are required.

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Short Communication

DOI: 10.1515/rrlm-2017-0012

Investigation of biomarker variations post-return of spontaneous circulation following an out-of-hospital cardiac arrest

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Abstract

***Objective**: The objective of this research was to describe evolution of several biomarkers post-return of spontaneous circulation (ROSC) following an out-of-hospital cardiac arrest (OHCA).*

***Methods**: Thirteen adult patients were divided in 2 groups according to their survival status at 30 days, survivors (alive at 30 days or discharged alive) and non-survivors (not alive at 30 days). Glycemia, lactate, C-reactive protein (CRP), neurofilament heavy chain (NfH) and presepsin were assessed at pre-set time-points, during OHCA and the first 72 hours post-ROSC.*

***Results**: In survivors, lactate levels decreased steadily throughout the 72 hours from a maximum observed during OHCA; in non-survivors, it increased during ROSC, then decreased abruptly at 2 hours post-ROSC and remained lower than in survivors for up to 24 hours. Glycemia at all-time points within the first 24 hours and CRP levels at 2 hours post-ROSC were higher in non-survivors, but this observed difference was not statistically significant. The variation of NfH was bi-modal, with peaks at 12 and 48 hours. The interpretation of NfH was limited by the large number of samples outside the limit of detection.*

***Conclusion**: Glycemia, lactate and CRP showed different patterns of evolution in survivors and non-survivors and should be further investigated as potential predictors of survival after ROSC.*

***Keywords**: out of hospital cardiac arrest, return of spontaneous circulation, biomarkers*

Received: 11th December 2016; Accepted: 6th March 2017; Published: 16th May 2017

Introduction

Out-of-hospital cardiac arrest (OHCA) is one of the major causes of death in Europe and USA. Survival after cardiac arrest and survival with good neurological function remains a major public healthcare problem and prediction of survival and especially survival with good neurological function remains a huge unmet need of the healthcare systems. Post cardiac arrest syndrome, consisting of anoxic brain injury,

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myocardial dysfunction, and systemic ischemia/ reperfusion response following the return of spontaneous circulation (ROSC) after complete whole-body ischemia, remains a major cause of mortality with little changes in survival during the past 50 years despite major advances in the treatment of cardiac arrest [7].

Several biomarkers, such as serum lactate, C-reactive protein (CRP) and neurofilament heavy chain (NfH) have been proposed as predictors of survival following ROSC. However, research results have been mixed and with questionable applicability in the real-life settings in terms of risk stratification after ROSC. Lactate level has been shown to be associated with mortality in trauma, burns, sepsis and ROSC after cardiac arrest [8-11]. NfH belongs to the intermediate filaments class, are abundant in axons and are present in both central and peripheral nervous system, playing a central role in axon growing [12]. Increased serum levels of NfH have been reported in states associated with neuronal injury [13-14], and it has been proposed as surrogate markers of brain injury after cardiac arrest [15]. Post-cardiac arrest immune-inflammatory response, especially the level of CRP, has been correlated with patients' survival following cardiac arrest [16]. The levels of N-terminal fragment of the soluble CD14 form, known as presepsin, produced by hepatocytes and involved in the immune responses of endothelial and epithelial cells [17] increases in the serum during the inflammatory stress, and has been proposed as a serum biomarker for early diagnosis of sepsis [17, 18] but its value as an inflammatory biomarker following ROSC has yet to be established.

We hypothesised that in patients with ROSC following cardiac arrest, the levels of several biomarkers such as lactate, glycemia, CRP, NfH, and presepsin may display rapid changes within the first hours and days that are not yet fully described and that these fluctuations can have an impact on their ability to predict outcomes.

Objective

The main objective of the research presented here was to describe the variation in the first 72 hours post-ROSC of lactate, glycemia, CRP, NfH, and presepsin according to patients' survival status at 30 days or discharge.

Materials and method

Study design and patients

This was a prospective observational research conducted in the Prehospital Intensive Care Units and Emergency Department of the Emergency Clinical County Hospital Cluj between July 2014 and April 2015. Adult patients with resuscitated non-traumatic OHCA, with ventricular fibrillation as initial arrest rhythm at the ambulance presentation or during the resuscitation, and for whom the family members consented for data collection and blood sampling were included in the study. Patients with oncological medical history, and patients who did not achieve a return of spontaneous circulation in pre-hospital setting were excluded from the study.

Eligible patients were followed until discharge or death, whichever came first. Patients were divided in 2 groups for the analysis: survivors (patients who were alive at 30 days and patients discharged alive) and non-survivors (patients who were not alive at 30 days after ROSC). Pre-hospital treatment and treatment in the Emergency Department and subsequently in the Intensive Care Unit (ICU) was performed according to the Emergency Clinical County Hospital Cluj procedures.

The research was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Ro-

mania (No.107/28.02.2014) and conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki, as revised in 2000. One of the family members signed the informed consent for the participation to the study.

Study assessments

Data on age, sex, medical history, initial arrest rhythm, time from dispatch call to start of advanced life support maneuvres (time to ALS), the presence or absence of bystander CPR, number of cardiac resuscitation attempts, adrenaline dose (mg), systolic and diastolic blood pressure at ROSC and number of days of admittance were collected from the Prehospital Intensive Care Units and Emergency Department records.

Glucose, lactate, CRP, presepsin, and NfH were collected at pre-set time-points, as per protocol, before and after the ROSC. Samples for glucose and lactate were collected during the cardiac arrest, at ROSC, at admittance in the Emergency Department, and at 2, 6, 12, 24, 36, 48 and 72 hours following the ROSC. Samples for CRP and presepsin were obtained before admittance in the Emergency Department, and at 2, 6 and 12 hours following the ROSC and those for NfH were collected at 2, 6, 12, 24, 36, 48 and 72 hours following ROSC.

Routine enzymatic methods (Konelab 30, Thermo Fisher Scientific Inc, Finland) were used for the assessments of plasma glucose levels. Presepsin and CRP were measured from whole blood samples on PATHFAST™ analyzer (Mitsubishi Chemical Holdings Corporation, Tokyo, Japan) by PATHFAST™ Presepsin chemiluminescent enzyme immunoassay (Mitsubishi Chemical Holdings Corporation, Tokyo, Japan), assay range 20 – 20000 pg/mL and by PATHFAST® hsCRP enzyme immunoassay (Mitsubishi Chemical Holdings Corporation, Tokyo, Japan), assay range 0.05-30 mg/dL. Lactate and arterial pH were determined by routine methods using RAPIDPoint® 500 Blood Gas Systems (Siemens Healthcare GmbH, Erlangen, Germany).

The samples for NfH assessment were immediately centrifuged to separate the blood cells, frozen and stored at - 70°C until the analysis. The assessments were performed using commercially available kits (MyBioSource.com, San Diego, CA, United States) using a standard ELISA assay. Assay ranges: 15.6-1000pg/mL.

Statistical analysis

Descriptive statistic (mean, standard deviation, median, quartile 1 and quartile 3) were computed for continuous variables. Categorical variables were summarized with frequency tables (number and percentage). Student t-test, independent samples median test and chi-square test were used to compare variables between the survivors and non-survivors groups. A p value below 0.05 was considered statistically significant. For the statistical analysis, the CRP and NfH values below or above the limits of quantification of the tests were not included in the analyses of medians and are presented separately.

Statistical analysis was carried out using IBM® SPSS® Statistics (IBM, Armonk, NY, USA).

Results

Of the 46 cases which fulfilled the inclusion criteria and without any exclusion criteria, blood samples were obtained from 13 patients. Overall, 7 of the 13 patients (53.8%) survived up to 30 days or to discharge (survivors) and 6 patients (46.2%) died before discharge (non-survivors). The demographic and clinical characteristics of the survivors and non-survivors group are presented in Table I.

Overall, serum lactate had the highest levels pre-hospital during the cardiac arrest (median value 11.5 mmol/L), displaying a decreasing trend up to 6 hours post cardiac arrest (median value 2.5 mmol/L) and plateaued thereafter up

Table 1. Baseline characteristics of the patients

Parameter	Total N=13	Survivors N=7	Non-survivors N=6	p-value
Age, years	59.9 (18.6)	52.83 (20.23)	68.40 (13.85)	0.180
Men, n (%)	9 (69.2%)	6 (85.7%)	3 (50.0%)	0.164
CVD history, n (%)	7 (53.8%)	4 (57.1%)	3 (50.0%)	0.967
Initial arrest rhythm, n (%)				
• PEA	1 (7.7)	0 (0%)	1 (16.7%)	0.131
• Asystole	3 (23.1)	3 (42.9%)	0 (0%)	
• VF	9 (69.2)	4 (57.1%)	5 (83.3%)	
Presence of bystander CPR, n (%)	6 (46.2)	3 (42.9%)	3 (50.0%)	0.797
Time to ALS (min), n (Q1; Q3)	5.0 (4.0; 6.5)	5.0 (4.0; 5.0)	5.0 (4.0; 8.0)	1.000
Number of resuscitation attempts, n (Q1; Q3)	3.00 (2.00; 4.00)	2.00 (1.50; 4.00)	3.50 (3.00; 4.00)	0.592
Adrenalin dose, mg	6.4 (3.7)	5.71 (3.68)	7.40 (3.78)	0.457
SBP, mmHg	138.2 (48.2)	121.29 (50.71)	158.00 (40.89)	0.184
DBP, mmHg	90.9 (27.4)	84.71 (26.66)	98.17 (28.90)	0.401

All continuous variables results are displayed as mean (standard deviation) if not specified otherwise.
n (%), number (percentage); PEA, pulseless electrical activity; VF, ventricular fibrillation; CVD, cardiovascular disease; SBP, systolic blood pressure; DBP, diastolic blood pressure

to 24 hours (median value 2.3 mmol/L; Figure 1A). After 24 hours the serum lactate started to decrease reaching its minimum values at 48 and 72 hours post-ROSC (1.6 mmol/L). The pattern of serum lactate levels observed during the first 36 hours was different in survivors than in non-survivors. In survivors, the maximum lactate value was recorded in the first pre-hospital sample (median value 12.1 mmol/L) and the lactate decreased steadily up to 2.0 mmol/L at 36 hours. In non-survivors the median lactate levels increased from 8.0 mmol/L in the first pre-hospital sample to 9.4 mmol/L at admittance, started to decrease following the admittance reaching 1.6 mmol/L at 24 hours. The difference observed between the serum lactate levels in the 2 study groups was not statistically significant at any timepoint ($p > 0.05$ for all).

Glucose median values increased from 91.0 mg/dL during the cardiac arrest to 225.5 mg/dL after RCOS and 281.0 mg/dL at admittance and then steadily decreased up to 36 hours (median value 127.5 mg/dL) and plateaued thereafter up to 72 hours. The trend and the levels was similar in both survivals and non-survivals ($p > 0.05$ for all; Figure 1B).

In the whole population analysed CRP median values decreased from 6.7 mg/L pre-hospital admittance to 2.5 mg/L at 2 hours after ROSC and then started to increase. The increasing trend was maintained at 12 hours post-ROSC. A similar trend was observed in the survivors. In non-survivors, the CRP values increased steadily from 3 mg/dL pre-hospital and reached the maximum level at 12 hours (14.7 mg/L; Figure 1C).

Median presepsin levels increased from pre-hospital (500.0 pg/mL) reaching its peak at 2 hours post-ROSC (889.0 pg/mL) and then decreased to 483.0 pg/mL at 6 hours post ROSC. In survivors presepsin levels remained relatively stable and high from 2 to 12 hours post-ROSC; in non-survivors presepsin levels started to decrease after 2 hours post-ROSC and maintained the decreasing trend at 12 hours post-ROSC. At all-time points presepsin levels were higher in

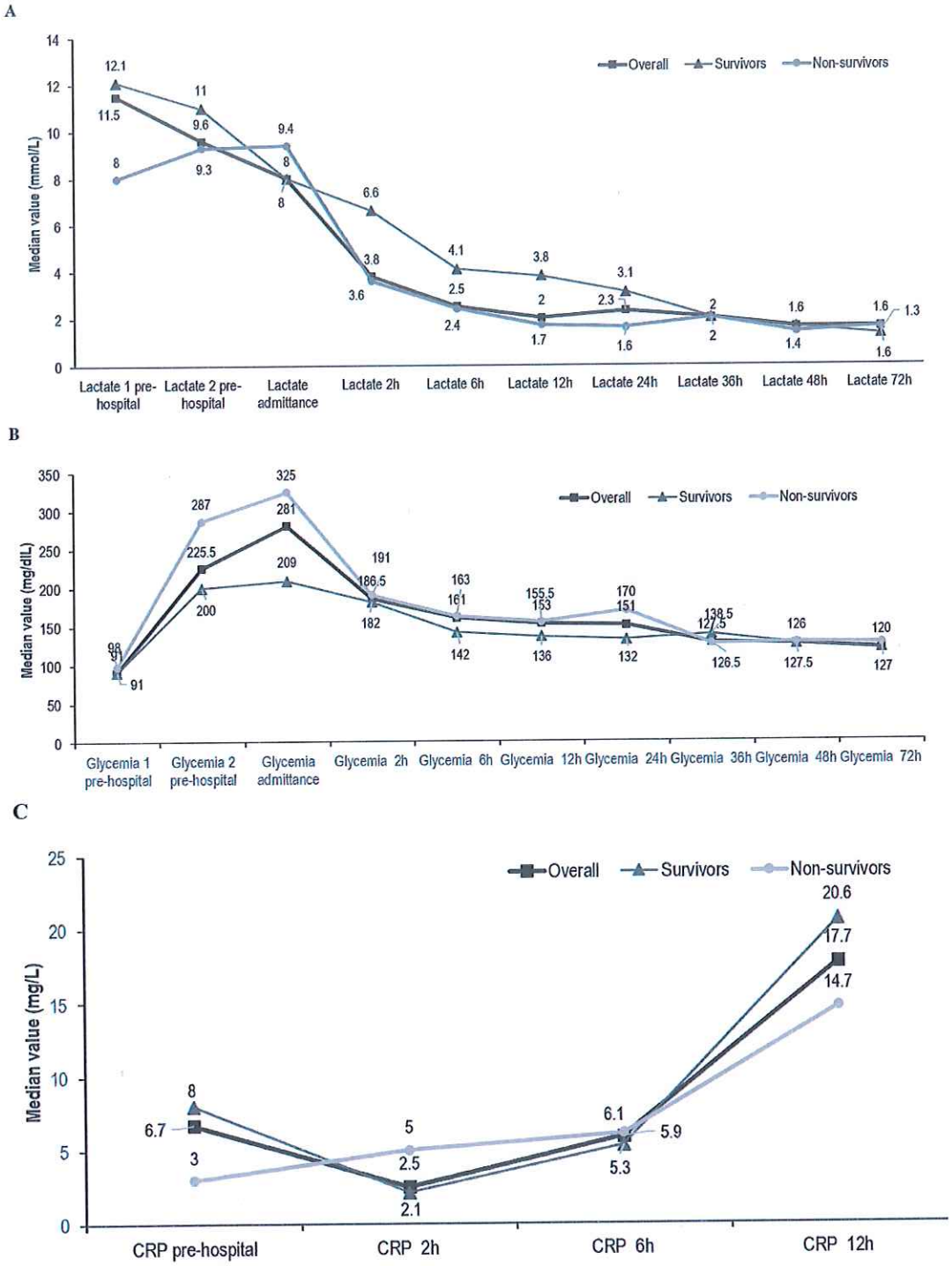

Figure 1. Evolution of median lactate (panel A), glycemia (panel B) and CRP (panel C) levels in the whole group included in the analysis and separate in survivors and non-survivors

lactate 1 pre-hospital, lactate collected during cardiac arrest; lactate 2 pre-hospital, lactate collected at ROSC; glycemia 1 pre-hospital, glycemia collected during cardiac arrest; glycemia 2 pre-hospital, glycemia collected at ROSC; ROSC, return of spontaneous circulation; CRP, C-reactive protein

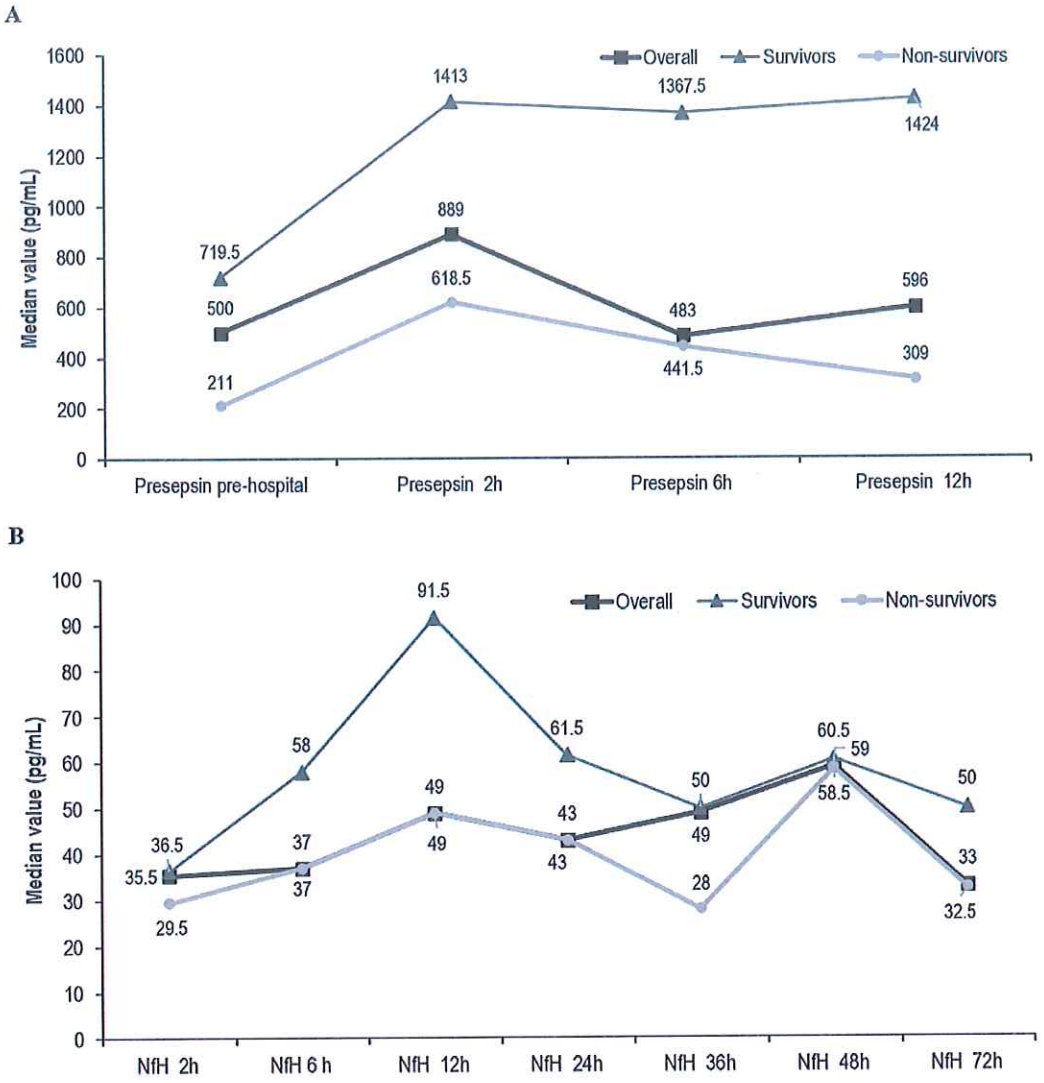

Figure 2. Evolution of median presepsin (panel A) and neurofilament heavy chain (panel B) in the whole group included in the analysis and separate in survivors and non-survivors
NfH, neurofilament heavy chain

survivors than in non-survivors, but the differences did not reach statistical significance ($p > 0.05$; Figure 2A).

Overall, median NfH values ranged between 33.0 pg/mL and 59.0 pg/mL. During the time interval 2-72 hours post-ROSC NfH had a bimodal evolution with the minimum values observed at 2 and 72 hours post-ROSC and 2 peaks – one at 12 hours post-ROSC and one at 48 hours post ROSC. This bimodal evolution of the median NfH levels was observed in both survivors and non-survivors, who had similar NfH levels at all timepoints ($p > 0.05$ for all; Figure 2B).

CRP levels exceed the limit of quantification of the test of 30 mg/L at all time points in 2 patients from the survivors group and at 12 hours in 3 patients from the non-survivors group. Of the 80 available NfH samples for all patients, 13 were below and 3 above the limit of quantification for the test.

Discussion

We performed a study which aimed to describe the short-term fluctuations of a number of biomarkers within the first 72 hours post-ROSC in non-traumatic OHCA patients according to their survival status at 30 days or discharged alive from hospital.

In post-cardiac arrest patients, lactic acidosis may occur due to increased lactate production as a consequence of microcirculatory and mitochondrial dysfunction leading to inadequate tissue perfusion and oxygen supply or decreased lactate clearance [11, 19]. In a retrospective study including 128 patients with ROSC following OHCA, it was shown that mortality increased in parallel with serum lactate levels collected within one hour from ROSC, from 39% in those with a lactate level <5 mmol/L to 92% in those with lactate level ≥10 mmol/L [11]. We did not observe any statistically significant difference between the lactate levels in the 2 study groups. These results may be explained by various etiologies of cardiac arrest or comorbidities known to be associated with high lactate levels, such as hepatic failure or thiamine deficiency, and which were not evaluated. Also lactate levels are influenced by the duration of the cardiac arrest [20]. We were not able to accurately collect data on the duration of cardiac arrest and the only variable available was the time to ALS, which was similar in survivors and non-survivors. Our data showed that in the survivors' group lactate levels decreased steadily throughout the 72 hours following cardiac arrest. In non-survivors, lactate increased during ROSC and then decreased abruptly at 2 hours following ROSC. The pattern observed in the non-survivors' group may suggest that rapid fluctuations in lactate levels within the first 2 hours following ROSC are critical in establishing a relationship between lactate levels and survival at 30 days.

Numerous studies have shown that hyperglycemia is an important outcome predictor in critically ill patients with or without diabetes. In a prospective study on 1,000 consecutive patients admitted in an intensive care unit, each 1 mmol/l increase in peak blood glucose during the first 48 hours was associated with a 20% increase in risk of death in patients with critical illness-associated hyperglycemia and in those with established diabetes and HbA1c levels <7 % [21]. In a group of 134 patients who survived to hospital admission after an OHCA, in those who were alive at discharge blood glucose levels remained stable between pre-hospital and admission, while in non-survivors there was a significant increase between these 2 time points [22]. We also observed that survivors had lower levels of blood glucose than non-survivors, although the difference did not reach statistical significance.

In patients with successfully resuscitated cardiac arrest, a "systemic inflammatory response syndrome" was described in the first hours following resuscitation [23]. Higher levels of inflammatory cytokines on admission were seen in non-survivors as compared to survivors [23]. We observed higher CRP levels at 6 and 12 hours in survivors than in non-survivors, although this difference was not statistically different. No studies reporting levels of presepsin in patients with ROSC following cardiac arrest were found in the literature. In our study, pre-hospital presepsin values in the whole group were 500 pg/mL, similar to levels seen in a group of patients with cardiogenic shock with or without infectious complications [18] and a rapid increase of presepsin was observed at 2 hours for both survivors and non-survivors. We did not collect patient diagnosis and therefore we cannot exclude the presence of sepsis and its association with higher presepsin levels in the whole sample and higher CRP levels in survivors.

We found a bi-modal variation of NfH, with peaks at 12 and 48 hours for NfH. A similar pattern with latter peaks of NfH values (>24 hours) was reported in a study on 90 patients treated

with hypothermia after cardiac arrest [15]. The interpretation of NfH levels in our study is limited by the large number of samples outside the limit of detection (16 out of 80 samples). If for those above the limit of quantification the dilution should be used, we had several samples below the limit of detection of the assay thus limiting its utility in patients post-ROSC. The same limitation in the assay ranges for commercially available kits was reported in the study of Rundgren et al [15], where for a large proportion of patients were reported NfH levels of 0 pg/mL, with the value of 0 attributed to non-measurable levels.

Several limitations of our study should be acknowledged. The main limitation was the small number of patients evaluated due to objective difficulties in evaluating cardiac arrest patients during CPR and immediately after ROSC. Thus, the statistical comparison of between group differences may have not been accurate and of limited interpretability. Although no statistically significant difference between study groups was observed in terms of age, the survivors were 16 years younger than non-survivors and this may had influenced the survival and thus also the pattern of studied markers.

Conclusions

Although no statistically significant difference was observed in our patients, glycemia, lactate and CRP levels seem to display different patterns of evolution in survivors and non-survivors following ROSC after cardiac arrest. Studies including larger samples, allowing for adjustment for other potential cofounders known to influence the survival and including clinical parameters are needed to further investigate the evolution of these markers as potential predictors of survival after ROSC.

Abbreviations

ALS - advanced life support; CRP – C-reactive protein; NfH - neurofilament heavy chain; ICU – Intensive Care Unit; OHCA - out-of-hospital cardiac arrest; ROSC - return of spontaneous circulation

Acknowledgement

This study was supported by a grant financed by "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania (contract no.1493/19/28.01.2014); equipment used from Hospital and County Ambulance Service according to the university ethical approval of the study (No.107/28.02.2014).

Conflict of interest

Authors have no conflicts of interest to declare.

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Original papers

Med Ultrason 2016, Vol. 18, no. 4, 419-424
DOI: 10.11152/mu-866

Ultrasonography as an integrated tool in clinical decision-making in the Emergency Department

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Abstract

The **aim** of this retrospective study was to identify the role of ultrasonography as a decision-making and screening tool in emergency patients with pathological changes. **Material and method:** The study was carried out for 28 months in the Emergency Department of the County Emergency University Hospital, Cluj-Napoca. An ultrasound examination was performed as part of the clinical algorithm within the first hour of treating non-critical patients after they had been triaged. The diagnostic decision based on the results of the ultrasound examination was compared with the final diagnosis on discharge from the Emergency Department. **Results:** In study were included 1565 patients with a mean age of 50.61±19.21 years. Ultrasound changes were detected in a statistically significant number of patients from all the examined subgroups ($p \leq 0.002$). The concordance between clinical and ultrasound findings was of 54.06%. Of all the examined patients, 20.63% were referred to surgery department based on the results of the ultrasound examination. Surgery was the final therapeutic decision in 5.06% of all the patients with normal ultrasound findings. **Conclusions:** Ultrasonography as an integrated tool in the clinical examination algorithm allowed the identification of non-critical patients who required emergency surgery (20.63%). The integration of point-of-care ultrasound into the clinical examination allows the management of emergency patients through the ranking of decisions: hospital admission for surgery and medical treatment, other diagnostic investigations, referral to outpatient care and family physician.

Keywords: ultrasonography, emergency medicine, syndrome, clinical decision-making.

Introduction

Emergency medicine is a high-risk field when it comes to diagnostic errors or delayed treatment initiation. The large number of patients resulting in reduced examination times, often coupled with lack of access to the patient's history, hinders the early detection of acute conditions with high morbidity and mortality, especially when the clinical picture is non-specific. Bedside ultrasound (US) allows rapid point-of-care evaluation guided by previously identified clinical signs and symptoms for the early diagnosis of conditions that require surgery or immediate care [1]. In 2001, the American College of Emergency Physicians introduced emergency US guidelines in order to increase the quality of diagnosis and follow-up, as well as to avoid diagnostic or treatment errors [1]. Thus, the following were defined as clinical contexts in which US examinations are indicated: during resuscitation, for diagnostic purposes, within clinical algorithms guided by sign/symptom, for guiding invasive procedures or monitoring emergency treatment and hemodynamic/ respiratory status. Emergency US is a direct examination focusing on the detection of a sign/ symptom/syndrome for the diagnosis of life-threatening conditions that require immediate treatment [1].

The portable and non-radiating character of US machines used for clinical bedside examinations transforms them into a visual stethoscope that is able to determine

Received 20.07.2016 Accepted 29.08.2016
Med Ultrason
2016, Vol. 18, No 4, 419-424
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whether the patient has fluid accumulation/ inflammatory process/complicated stones/venous thrombosis. Appropriate imaging training is required in order to reduce operator errors. Clinical evidence gathered for the past 15 years has allowed the inclusion of US examinations into the diagnostic and treatment algorithms for acute appendicitis, aortic aneurysms, acute heart failure, closed abdominal trauma, etc [1-5]. All these led to the introduction of basic training in US for the management of clinical problems in diagnostic and treatment algorithms [6]. Emergency US examinations provide morphological and functional information for patients' monitoring. Equipping Romanian emergency services with US machines has allowed their introduction in current emergency practice, provided that adequate training can also be supplied.

The **aim** of this study was to identify the role of ultrasonography as a decision-making and screening tool in emergency cases with pathological changes with the purpose of reducing the incidence of diagnostic and treatment errors and improving adequate patient flow.

Material and method

A retrospective study was carried out between October 2011 and January 2014 in the Emergency Department (ED) of the County Emergency University Hospital of Cluj-Napoca. This regional medical unit with out-of-hospital CT examination facilities serves patients over 14 years of age with all acute pathologies. US examinations were performed by a specialist with 12 years' experience in general US, as part of the clinical examination algorithms, 6 shifts of 12 hours per month. The examinations were conducted within the first hour of treating non-critical patients after the patients had been triaged and were guided by a previously-identified clinical syndrome. The diagnostic decision based on the US examination was compared with the final diagnosis at discharge from the emergency unit.

The inclusion criteria were as follows: non-critical patients over the age of 14, who presented for triage with a clinical picture of: biliary and pancreatic emergencies (biliary dyskinesia, biliary colic, jaundice syndrome, acute pancreatitis, pain in the right hypochondrium), digestive emergencies (epigastric pain, pain in the right iliac fossa, abdominal pain, bowel sub-obstruction, hyper-emesis syndrome, diarrhea syndrome, acute gastroduodenitis), acute surgical abdomen, renal and urologic emergencies (renal colic, macroscopic hematuria, oliguria and anuria), vascular emergencies such as venous thrombosis, gynecologic emergencies, closed thoracic and abdominal trauma, other non-specific emergencies (anemia, fever, ascites).

The following exclusion criteria were applied: patients under 14 years of age, patients with hemodynamic and respiratory instability, polytrauma patients, or patients with other clinical pictures then specified.

A Fukuda Denshi Ultrasound Color Doppler UF-850XTD was used. The machine is fully digital; it has multi-function B/W ultrasound systems and three transducers: convex (2.5-5 MHz), linear (6-9 MHz) and sector (2.5-5 MHz). The protocol included serial examinations in order to detect the cause of a previously identified sign/ symptom, to evaluate fluid accumulation in the peritoneal/ pleural/pericardial space and to carry out serial examinations of abdominal organs and tissues in clinical syndromes that are difficult to diagnose in emergency practice (table I). During the 168 selected shifts for the study, we received in ED 8904 patients and we selected in our study 1565 adult patients according with the inclusion criteria.

Table I. Clinical syndrome identified, ultrasound examination performed, and its aim within the integrated clinical algorithm.

Clinical syndrome	Ultrasound examination	Aim of examination
Closed thoracic and abdominal trauma	FAST, EFAST, general abdominal ultrasound	Fluid accumulation, free air, parenchyal and visceral lesions
Biliary and pancreatic emergencies	Serial ultrasound with visualization of the biliary tree, coupled with general abdominal ultrasound	To identify changes in the gallbladder, biliary ducts and pancreas
Digestive emergencies	Serial ultrasound of the digestive tube and general abdominal ultrasound	Parietal changes in the digestive tube, inflammatory processes and free intra-peritoneal air, stenosis or dilation of the digestive tube and fluid accumulation
Acute surgical abdomen	Serial ultrasound of the digestive tube and general abdominal ultrasound	Distended bowel without movements, parietal changes and the presence of free intra-peritoneal air
Renal and urologic emergencies	Serial ultrasound of the kidneys, urethra and urinary bladder	Renal stones and obstructive complications
Vascular emergencies such as venous thrombosis	Ultrasound of the venous region of the affected limb	Changes such as intravascular thrombosis and incompressibility
Gynecologic emergencies	Serial ultrasound of the uterus and ovary and general abdominal ultrasound	Complicated ovarian cysts, inflammatory processes or torsions
Other emergencies	General abdominal ultrasound	Abscesses, tumors and peritoneal fluid accumulation

The data was recorded in emergency patient records and then transferred into a database. Pathological images were stored as *.jpg and cine loop files. The patients signed an informed consent form before the investigation. In order to maintain patient confidentiality, the information was transferred into the study database by recording the electronic registration number assigned on admission to the emergency care unit. The study was approved by the Ethics Committee of the County Emergency University Hospital of Cluj-Napoca.

Statistical analysis

Statistical analysis was conducted according to the type of investigated data. Quantitative variables were summarized as median and interquartile ranges whenever data proved not to follow the normal distribution. Categorical data was reported as percentages and associated 95% confidence intervals calculated using an exact method [7]. The Kruskal-Wallis ANOVA test was applied in order to assess differences between groups whenever quantitative data did not follow the normal distribution.

The Z-test for proportions was used to compare groups on categorical data. Statistical analysis was conducted with the Statistica program (v. 8, StatSoft) and p-values lower than 0.05 were considered statistically significant.

Results

One thousand five hundred sixty five patients aged between 14 and 92 years with a mean of 50.61±19.21 years were included in the study, with significant differences within the group (Kruskal-Wallis ANOVA, p<0.0001). The demographic data and the frequency of clinical syndromes are detailed in table II. The highest percentage was represented by the biliary and pancreatic emergencies (36.10%), closely followed by the digestive emergencies (31.57%). Age proved to be significantly higher in subjects with acute abdomen. Patients from the urban area predominated in the studied group (66.58%). The analysis according to gender revealed the predominance of female patients for all types of emergencies, except for thoracic and abdominal emergencies, where male patients predominated, and for vascular emergencies, where no statistically significant differences were recorded.

Ultrasound changes were observed in a statistically significant number of patients in all the studied subgroups (p=0.002 for other syndromes; p-values for all other plots <0.0001. Significant concordance between clinical and ultrasound findings was observed (55-83%) in the clinical syndromes examined (54.05% of all patients, 72.3% of those with pathologic ultrasound findings, respectively), except for digestive emergencies, abdominal and thoracic trauma and other non-critical syndromes (0-33%). Surgery was recommended based on the ultrasound examination in 77.5% of the patients with acute surgical abdomen and in 34.5% of the patients with biliary and pancreatic emergencies, respectively, which accounts for 20.64% of all the patients examined (table III).

Although the need for other investigations was higher in patients with acute abdomen (65%) and thoracic and abdominal trauma (81%), it only represented a low percentage from the total number of patients who underwent ultrasound examinations (19.48%).

Significant co-morbidities were found in patients with positive ultrasound findings who presented with vascular emergencies (50%) and biliary and pancreatic emergencies, respectively (21%) (fig 1).

Normal US findings were observed in a reduced percentage of patients with acute abdomen (1.26%, 95% CI [0-4]), gynecologic emergencies (0.91%, 95% CI [0-5]), digestive emergencies (2.27%, 95% CI [8-11]) and other clinical syndromes (2.43%, 95% CI [1-4]) who required surgery (1.27% of all patients, 5.06% of all normal ultrasound findings, respectively).

The percentage of FAST positive was of 6.36% while the percentage of parenchymal lesions was of 8.18%, without significant differences (p=0.6334). Among those with parenchymal lesions, a significantly lower percentage also had other lesions (p<0.0001). Interventional therapy was required in 21.06% of subjects (95% CI [17.88-24.78]) with biliary and pancreatic emergencies. 5.56% (95% CI

Table II. Demographic data on the investigated sample.

Clinical syndrome	Number of patients	Age (years)	Environmental origin (u/r)	Sex (f/m)
Digestive emergencies	494 (31.56)	47.79±19.20	68/32	63/37*
Gynecologic emergencies	38 (2.43)	30.68±12.18	76/24	100/0*
Vascular emergencies	36 (2.30)	57.36±15.88	67/33	53/47
Acute abdomen	40(2.56)	61.38±19.10	45/55	68/33*
Renal and urologic emergencies	238 (15.20)	43.81±18.94	70/30	55/45*
Biliary and pancreatic emergencies	565 (36.10)	56.27±17.10	65/35	64/36*
Abdominal/thoracic trauma	110 (7.03)	47.10±19.50	67/33	38/62*
Other emergencies	44 (2.81)	59.77±19.46	66/34	61/39*

*p<0.003, u=urban, r=rural, f=female, m=male. Data are expressed in number (percent) or mean±standard deviation.

Table III. Pathological ultrasound findings

Clinical syndrome	US-path	US–verify CDG	US-Surg	US-Med	US-Other
Digestive emergencies	65.59 [61-70]	33.00 [29-37]	13.56 [11-17]	38.87 [35-43]	33.20 [29-38]
Gynecologic emergencies	73.68 [58-87]	55.26 [40-71]	13.16 [5-29]	23.68 [11-39]	23.68[11-39]
Vascular emergencies	80.56 [64-92]	55.56 [39-72]	2.78 [0-14]	55.56[39-72]	61.11 [45-78]
Acute abdomen	90.00 [78-97]	57.50 [40-72]	77.50 [63-90]	82.50 [68-92]	25.00 [13-40]
Renal and urologic emergencies	89.50 [85-93]	83.19 [78-88]	0.84 [0-3]	82.35 [77-87]	33.61 [28-40]
Biliary and pancreatic emergencies	87.08 [84-90]	72.04 [68-76]	34.51 [31-39]	80.53 [77-84]	24.42 [21-28]
Abdominal/thoracic trauma	19.09 [12-27]	0.00 [0-4]	10.00 [5-17]	10.91 [5-18]	10.00[5-17]
Other emergencies	61.36 [47-75]	31.82 [18-48]	25.00 [14-41]	22.73 [11-39]	20.45 [9-36]
All plots	74.75 [73-77]	54.06 [52-57]	20.64 [19-23]	59.23 [57-62]	28.31 [26-31]

US-path=pathological changes detected; US-verify CDG=US verified clinical diagnosis, US-Surg=emergency surgical treatment required, US-Med=emergency medical treatment required, US-Other=US detected other pathological changes, but not emergencies. Data are expressed as percent with 95% confidence interval (% [95% CI])

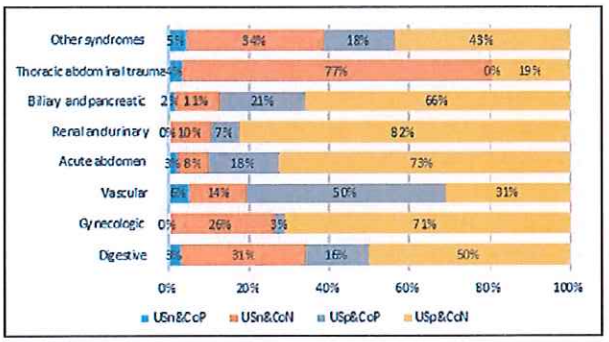

Fig 1. Incidence of comorbidities in the examined patients according to ultrasound results (USn&CoP=normal ultrasound associated with comorbidities; USn&CoN=normal ultrasound without comorbidities; USp&CoP=ultrasound changes associated with comorbidities; USp&CoN= ultrasound changes without comorbidities).

[38.97-72.15]) of subjects with vascular emergencies had confirmed thrombosis. 15.79% (95% CI [5.33-31.51]) of patients with gynecologic emergencies had confirmed gynecologic pathology requiring treatment.

Discussions

Although decisions in emergency medicine are based on anamnesis/the patient's history, clinical and paraclinically examinations. US investigations can influence subsequent investigations or immediate treatment choices. Lichtenstein DA described "ten reasons" for the use of US in critical care: the differential diagnosis of acute respiratory failure, acute circulatory failure, cardiac arrest, assistance during venous access, assessing ARDS and ventilated lungs, finding the cause of fever, decreased radiation, practicing a holistic approach to the heart, practicing visual diagnosis in modern medicine [8]. In 2009, Donald Medd addressed a letter to the editor of the American Journal of Ultrasound in Medicine in which he stated that US, as an integral part of the physical examination, can increase the safety of medical practice and reduce healthcare costs through the early detection of potentially critical situations [9]. Three years later, Gillman and Kirkpatrick introduced the concept of portable US as the visual stethoscope of the 21st century and defined its usefulness as a clinical examination tool rather than as a diagnostic test [10].

As far as the age decades with the highest frequency of presentation to the ED are concerned, the literature describes an increased number of presentations in patients over 45 years of age, with a further increase over 85 years [11]. In our study, the average age was above 45 years in six subgroups, except for gynecologic, renal and urologic emergencies. We recorded a statistically significant higher number of female, similar with the over 20% difference registered by "Healthcare Agencies" in ED from the Unites States of America [10]. The high percentage of urban patients in our studied groups was also in accordance with the literature data [10].

An increased occurrence in non-differentiated digestive emergencies was also recorded in our patients, which, correlated with the available literature data (18% increase between 2006 and 2011 in the USA [12]) underlines the role of imaging investigations in the clinical examination. This is also supported by the increased percentage of pathological changes detected by US and the low clinical concordance between clinical and imaging findings, the US examination having modified the clinical diagnosis in 32% of patients. Montanari et al highlighted that the use of a "*pocket ultrasound device in biliary disease*" confirmed a suspected diagnosis in 77% of patients [13]. Similar results were obtained in our study, given that the US identified pathological changes in 87.08% of biliary and pancreatic emergencies and confirmed the clinical diagnosis in 72.04% of patients. Studies demonstrated the good specificity of US examinations for the detection of biliary lithiasis and its complications [14,15]. The impor-

tance of the ultrasound examination was validated in our study, where it identified the need for interventional therapy (21.06%), and emergency surgery (34.5%) respectively, thus improving healthcare services and reducing costs.

The strategy for diagnosing acute abdominal pain on presentation in the ED aims to diminish the number of missed diagnoses, to reduce examination times and to avoid patient radiation. Literature data shows that the use of the US as a first-line examination, followed by a CT scan in case of a negative or inconclusive finding represents the best solution, with only a 6% chance of losing emergency patients [16]. The results obtained in our study were in agreement with literature data as only 5.06% of the patients with normal ultrasound findings (2.27% of digestive emergencies; 2.43% of other syndromes) required surgery (1.27% of all patients) after complementary investigations were performed. Although the need for further investigations was higher in the subgroup of patients with acute abdomen (65%) and trauma (81%), overall it did not exceed 20%, which confirms the usefulness of the US as first-line examination for reducing times and radiation.

The novelty of our study lies in the analysis of US examinations as a rapid diagnostic and treatment tool in emergency cases compared to X-ray/CT/MRI examinations, which require time and specialized physicians. Thus, we found that in biliary emergencies/ other syndromes, 34.51% versus 25% of patients were referred to surgery after US, which is in agreement with the literature data on emergency exploratory surgery for abdominal pain (24.1%) [17].

We evaluated the role of US in the early administration of medical treatment (antibiotics, anticoagulants, anti-inflammatory drugs, IV therapy) and observed that imaging findings led to medical treatment initiation in over 80% of patients with acute abdomen, biliary, renal and urologic emergencies. This result, which has to be confirmed by other published studies, proves the usefulness of US in early management of emergency patients.

Studies showed that the prevalence of pathological changes increases with age and co-morbidities, which are frequent causes of complications, thus further strengthening the need for emergency ultrasound examinations [18]. In our study, patients with co-morbidities presented a higher percentage of US changes compared with normal US (3-50% versus 0-6%, the extreme being represented by vascular emergencies with 50% vs. 6%). Hasani et al also showed that 41.3% of the patients with acute abdominal pain, who underwent an emergency ultrasound examination, had co-morbidities [19]. The percentage of normal US was 34% (39.3% in case of radiologist with experience) for acute nonspecific abdominal pain, concordant with the study's Hasani and the errors were at a reduced rate (2.27%) in our study compared with theirs (7%) [19]. Literature data shows that experience and training in US examination (normal US – 34% for highly experienced emergency physician in our study, 51.3% for emergency physicians, 39.3% for radiologists) can improve the accuracy and usefulness of the method [19].

As far as the role of US as part of the clinical algorithm in patients with trauma and vascular emergencies was concerned, we identified a lower percentage of confirmed diagnoses compared with literature data: 6.36% FAST positive (15.10% [20] and 78% [21] when hypotension was present) and 5.56% (18% [22] - 24% [23]) for diagnosed deep vein thrombosis.

The accidental detection of other changes that can influence the patient's evolution is another argument in favor of emergency ultrasonography. Soultati et al registered accidental detections in 28.2% of patients (up to 20% requiring further treatment), more frequent on abdominal level and in elderly patients [24]. In our study, accidentally detected ultrasound changes were of 10% (trauma subgroup) up to 61.11% (vascular emergencies subgroup), with an overall incidence of 28.31%, which highlights the importance of emergency US screening for the prevention of unfavorable outcomes.

Thus, the results of our study demonstrate the importance of US as a part of the clinical algorithm for confirming a suspected diagnosis, for guiding the diagnosis in the absence of specific clinical findings, for identifying patients who require immediate medical and surgical treatment or for detecting morphological and pathological changes that could influence the patient's evolution. Our data was in agreement with the study carried out by Mjolstad, who advocated the introduction of this method as a tool for improving the diagnosis and treatment of abdominal and cardiac conditions [25].

The limitation of our study included its retrospective, nonrandomized design. Another limitation of this study was the fact that the US examination integrated in clinical examination was performed by an experienced emergency physician. From this point of view we do not know how our findings correspond to non-experts users. The stay in ED is mostly limited and a more realistic setting will probably be the US examination performed by residents. In spite of this, US examination may still be of significant value in case of critical patients which were excluded from our study. Another limitation of the study was the lack of verification of the negative US findings by other imaging techniques.

Conclusions

In conclusion the US as part of the clinical examination algorithm allowed the identification of non-critical pa-

tients who required emergency surgery, thus the immediate initiation of a different course of treatment was possible in 20.64% of patients. Regardless of the physician training, clinical diagnosis alone is insufficient, whereas non-specific clinical-symptoms lead to diagnostic errors, which are frequently detected late or during autopsy. The integration of point-of-care ultrasonography into the clinical examination enables better patient flow management in the emergency department by allowing the prioritization of decisions: patients requiring admission for medical and surgical treatment; patients who will undergo further diagnostic investigations; patients that can be referred to a family physician or outpatient care. The accidental identification of other morphological and pathological changes (28.31%) is crucial for the prevention, the monitoring and the evolution of patients, thus avoiding complications and further costs.

Conflict of interest: none

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