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Elevations of inflammatory markers PTX3 and sST2 after resuscitation from cardiac arrest are associated with multiple organ dysfunction syndrome and early death

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Abstract

Background: A systemic inflammatory response is observed after cardiopulmonary resuscitation. We investigated two novel inflammatory markers, pentraxin 3 (PTX3) and soluble suppression of tumorigenicity 2 (sST2), in comparison with the classic high-sensitivity C-reactive protein (hsCRP), for prediction of early multiple organ dysfunction syndrome (MODS), early death, and long-term outcome after out-of-hospital cardiac arrest.

Methods: PTX3, sST2, and hsCRP were assayed at ICU admission and 48 h later in 278 patients. MODS was defined as the 24 h non-neurological Sequential Organ Failure Assessment (SOFA) score ≥12. Intensive care unit (ICU) death and 12-month Cerebral Performance Category (CPC) were evaluated.

Results: In total, 82% of patients survived to ICU discharge and 48% had favorable neurological outcome at 1 year (CPC 1 or 2). At ICU admission, median plasma levels of hsCRP (2.8 mg/L) were normal, while levels of PTX3 (19.1 ng/mL) and sST2 (117 ng/mL) were markedly elevated. PTX3 and sST2 were higher in patients who developed MODS ($p<0.0001$). Admission levels of PTX3 and sST2 were also higher in patients who died in ICU and in those with an unfavorable 12-month neurological outcome ($p<0.01$). Admission levels of PTX3 and sST2 were independently associated with subsequent MODS [OR: 1.717 (1.221–2.414) and 1.340, (1.001–1.792), respectively] and with ICU death [OR: 1.536 (1.078–2.187) and 1.452 (1.064–1.981), respectively]. At 48 h, only sST2 and hsCRP were independently associated with ICU death.

Conclusions: Higher plasma levels of PTX3 and sST2, but not of hsCRP, at ICU admission were associated with higher risk of MODS and early death.

Keywords: cardiac arrest; pentraxin 3 (PTX3); ST2.

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Introduction

Despite an initially successful return to spontaneous circulation (ROSC), prognosis of cardiac arrest remains poor, with the majority of resuscitated patients dying early due to post-cardiac arrest syndrome [1]. Most prominent, together with myocardial failure and ischemic brain damage, is the systemic inflammation related to whole body ischemia and reperfusion and including plasma cytokine elevation with deregulated cytokine production, presence of endotoxin in plasma, coagulation abnormalities, and adrenal

dysfunction [2, 3]. Accordingly, this systemic inflammatory response observed after cardiopulmonary resuscitation (CPR) brings evident similarities with sepsis and septic shock, generally described as a "sepsis-like" syndrome [2]. Indeed, the role of inflammation in the progression towards worse circulatory shock, multiple organ dysfunction syndrome (MODS), and poor outcome of resuscitated patients is under research [1–3].

Thus, the importance of circulating markers in patients resuscitated from cardiac arrest for early prognostic stratification is expanding towards markers of inflammation [2–6]. Pentraxin 3 (PTX3) and soluble suppression of tumorigenicity 2 (sST2) are two novel markers that are emerging as new candidate indicators of inflammation, infection, and cardiovascular disease [7–13]. PTX3 is a prototypic long pentraxin produced mainly by dendritic cells, macrophages, and endothelial cells in response to primary inflammatory stimuli, while ST2 is a member of the toll-like/interleukin (IL)-1 receptor host defence/inflammation family, participating in inflammatory processes [9, 13].

Since the systemic inflammation following resuscitation from cardiac arrest includes leucocyte activation and endothelial injury, as well as innate and immune responses, important elevations in plasma levels of PTX3 and ST2 might be expected [1, 2]. Thus, in the present study, we examined circulating levels of PTX3 and sST2, in a large population of patients resuscitated from out-of-hospital cardiac arrest. The classic short pentraxin high-sensitivity C-reactive protein (hsCRP) has been used as a benchmark biomarker of systemic inflammation [14]. In response to primary inflammatory stimuli CRP is produced mainly by the liver, while PTX3 and sST2 by diverse cell types, so we hypothesized that PTX3 and sST2 would be acute-phase markers more closely related than hsCRP to the severity of post-cardiac arrest syndrome and therefore would be better prognostic indicators in this context.

Materials and methods

Study design, setting and selection of participants

The study was an observational cohort study, in which plasma levels of inflammatory biomarkers, hsCRP, PTX3, and sST2, were assessed in adult patients resuscitated from out-of-hospital cardiac arrest. Patients included in the present study were part of FINNRESUSCI, a prospective observational cohort study conducted at 21 hospitals in Finland between 1 March, 2010 and 28 February, 2011 [15]. The study was approved by the Ethics Committee of the Helsinki and Uusimaa Hospital District (FINNRESUSCI TUTKIMUS §10, 20.1.201), in addition to local ethics approvals by Päijät-Häme, Etelä-Karala, Satakunta, Kymenlaakso Central Hospitals and Tampere and Turku University Central Hospitals. The study complied with the World Medical Association Declaration of Helsinki. Informed consent from the patient's next of kin was obtained for data collection and blood sampling. All cardiac arrest patients in whom blood samples were obtained at intensive care unit (ICU) admission were included in the study. Details on post-resuscitation care are reported in the original publication of the FINNRESUSCI study [15].

Data collection, processing and outcomes

The participating hospitals were a part of the Finnish Intensive Care Consortium (FICC) and used the same electronic data management system and data validation software (Web Validator, Tieto, Helsinki, Finland). Data on study patients were prospectively collected using an Internet-based case report forms. Pre-hospital data were collected by the paramedics in accordance with the Utstein Guidelines and included: whether the arrest was witnessed or not; the administration of bystander initiated life support; the time from call to the dispatch centre and ROSC; and the use of adrenaline. In-hospital care data were collected electronically and comprised the use of vasopressors and therapeutic hypothermia, the Sequential Organ Failure Assessment (SOFA) score, Acute Physiology and Chronic Health Evaluation (APACHE) II score, and ICU mortality. The condition of MODS was defined as a SOFA score ≥12 [16]. More specifically, the entire SOFA score was used and it was reported as worst value during the first 24 h of ICU care. A specialist in neurology blinded to management in the ICU contacted patients discharged from the hospital by phone 1 year after cardiac arrest and determined neurological outcome according to the Pittsburgh Cerebral Performance Categories (CPC). We defined 12-month favorable outcome as CPC 1-2 (absent, mild or moderate neurological disability), and 12-month unfavorable outcome as CPC 3-5 (severe neurological disability, persistent vegetative state or death) [17].

Methods of measurement

Plasma levels of inflammatory biomarkers, hsCRP, PTX3, and sST2, at ICU admission and 48 h later, were measured blinded to case identity. Blood samples were collected into ethylenediaminetetra-acetic acid tubes, centrifuged and plasma stored at –70 °C. Plasma samples were collected in each participating center and then shipped in dry ice to a central repository where they were stored at –70 °C. Upon analysis, samples were thawed and divided into aliquots. The biomarkers were assayed in two different laboratories, one for hsCRP and PTX3, and the other for sST2. HsCRP levels were assayed using the latex immunoassay CRP Vario-High Sensitivity Method, by instrument Ci16200 Architect (Abbott Diagnostics USA) [3]. PTX3 levels were assayed with a non-commercial ELISA based on the monoclonal antibody MNB10 and rabbit anti-serum [7]. sST2 levels were assayed using a commercial enzyme immunoassay (Presage ST2, Critical Diagnostics) after dilution of samples by 1/150.

Statistical analysis

Categorical variables are presented as proportions and continuous variables as mean with SEM or median with interquartile range (IQR).

Baseline characteristics by outcomes occurrence were investigated with the χ^2-test for categorical variables; continuous variables

were compared by analysis of variance or by the non-parametric Kruskal-Wallis test for continuous non-normally distributed data. Multivariable linear regression was used to identify the independent factors at resuscitation influencing inflammatory biomarkers levels at ICU admission and 48 h later. Results of linear regression are reported in terms of β coefficients with 95% confidence interval (CI) and p-Values. Multivariable logistic regression was used to identify factors that were predictors of MODS, ICU mortality, and 12-month unfavorable outcome. All variables associated with the outcome in the univariate analysis ($p<0.05$) were included in the multivariable model. Odds ratios (OR) with the corresponding 95% CI were calculated and p-Values were considered statistically significant if they were <0.05. Prognostic accuracy of inflammatory biomarkers was evaluated by receiver operating characteristic curve (ROC) analyses and compared with Delong test [18]. All statistical analyses were performed with SAS software, version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

The FINNIRESUSCI study included 548 patients [15]. Among these, informed consent for blood sampling was obtained in 278 patients, and they were all included in the study. However, for some patients only the 48 h blood sample was available due to delayed consent. Eighty-two percent of these 278 patients survived to ICU discharge and 48% had favorable neurological outcome (CPC 1 or 2) at 12 months (Table 1).

Baseline characteristics and factors influencing ICU survival and 12-month outcome are shown in Table 1. Pre-hospital factors univariately associated with ICU survival were a shockable rhythm, no use of adrenaline, a shorter time to ROSC, and the use of therapeutic hypothermia, while those associated with long-term survival and favorable neurological outcome included also a younger age and a witnessed cardiac arrest (Table 1).

Inflammatory markers and factors related to resuscitation

Upper limits of normality for plasma hsCRP, PTX3, and sST2 in the general population are 3 mg/L [19], 4.76 ng/mL [20], and 45.6 ng/mL (manufacturer's data from Presage ST2 assay, Critical Diagnostics), respectively.

In our patients, at ICU admission, median plasma levels of hsCRP were normal, 2.8 (1.2–9.8) mg/L, while levels of PTX3 and sST2 were already markedly elevated, 19.1 (9.2–41.8) ng/mL and 117 (67–218) ng/mL, respectively. Levels of hsCRP and sST2 were significantly higher in patients with an initial non-shockable rhythm compared to those with a shockable rhythm, while levels of PTX3 were significantly higher in the case of longer time to ROSC (Table 2).

Each inflammatory marker significantly increased 48 h later as shown in Figure 1. Forty-eight hour plasma levels of PTX3 were significantly higher in the instance of older age, shockable rhythm, and longer time to ROSC, while sST2 levels were significantly higher in the case of non-shockable rhythm and longer time to ROSC (Table 2). At 48 h after resuscitation, all the inflammatory markers were significantly higher in patients who were treated with therapeutic hypothermia.

By linear regression models, the common independent determinants of elevated levels of the three inflammatory markers assayed were: the presence of a non-shockable rhythm and the use of adrenaline, at ICU admission; and the APACHE II score, at 48 h (Table 3).

Table 1: Baseline characteristics and clinical factors at resuscitation in all patients and between survivors and non-survivors and patients with favorable and unfavorable outcome at 12 months.

	Whole population (n=278)	ICU survival		12-month survival[a]		12-month outcome[a]	
		Yes (n=229)	No (n=49)	Yes (n=143)	No (n=133)	Favorable (n=133)	Unfavorable (n=143)
Age, mean (SD)	63±13	63±12	64±14	61±12[b]	65±13	60±12[c]	65±13
Sex (male), n (%)	229 (82)	189 (83)	40 (82)	117 (82)	111 (83)	109 (82)	119 (83)
Shockable rhythm, n (%)	180 (65)	163 (71)[d]	17 (35)	115 (80)[b]	64 (48)	108 (81)[c]	71 (50)
Witnessed cardiac arrest, n (%)	254 (91)	212 (93)	42 (86)	136 (95)[e]	116 (87)	128 (96)[c]	124 (87)
Bystander initiated BLS, n (%)	158 (57)	133 (58)	25 (51)	87 (61)	71 (53)	82 (62)	76 (53)
Adrenaline used, n (%)	186 (67)	141 (62)[d]	45 (92)	74 (52)[b]	111 (83)	67 (50)[c]	118 (83)
Time to ROSC in min, mean (SD)	21±11	20±11[d]	25±10	18±10[b]	24±11	18±10[c]	24±11
Therapeutic hypothermia, n (%)	202 (73)	173 (76)[f]	29 (59)	112 (78)[e]	89 (67)	104 (78)	97 (68)

BLS, basic life support; SD, standard deviation. [a]Data on 12-month survival/outcome were missing for two patients; [b]$p<0.01$ vs. 12-month death; [c]$p<0.01$ vs. unfavorable outcome at 12 months; [d]$p<0.01$ vs. ICU death and [e]$p<0.05$ vs. 12-month death and [f]$p<0.05$ vs. ICU death.

Table 2: High-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) levels by age, cardiac arrest (CA) presenting rhythm, time to ROSC, and therapeutic hypothermia.

Variable	n	hsCRP, mg/L	PTX3, ng/mL	sST2, ng/mL
Admission levels				
Age, year				
<59	79	2.3 (1.4–5.1)	17.5 (8.7–39.9)	107 (66–202)
59–68	86	2.4 (1.1–8.7)	20.3 (7.6–43.5)	117 (72–210)
>68	81	4.6 (1.5–11.7)	24.0 (12.4–44.7)	144 (69–240)
p-Value		0.251	0.211	0.452
CA presenting rhythm				
Shockable	156	2.3 (1.2–5.4)	17.4 (8.7–36.2)	105 (60–189)
Non-shockable	89	6.2 (1.9–29.5)	24.5 (11.2–60.3)	175 (95–373)
p-Value		<0.0001	0.054	<0.0001
Time to ROSC, min				
1–15	83	3.0 (1.7–11.7)	14.1 (6.6–29.8)	102 (65–210)
16–24	83	4.3 (1.5–14.6)	19.1 (8.2–48.2)	147 (66–277)
25–57	80	1.9 (1.0–4.4)	24.9 (15.0–46.1)	123 (74–203)
p-Value		0.005	0.002	0.287
48 h levels				
Age, year				
<59	75	115 (77–150)	37.1 (18.3–55.1)	299 (136–556)
59–68	84	131 (93–165)	44.3 (21.0–79.3)	277 (176–461)
>68	68	131 (92–172)	50.1 (24.8–81.8)	275 (186–442)
p-Value		0.373	0.038	0.988
CA presenting rhythm				
Shockable	162	124 (87–161)	47.6 (24.3–72.7)	256 (157–189)
Non-shockable	65	124 (73–161)	33.2 (12.6–51.4)	353 (208–808)
p-Value		0.742	0.004	0.001
Time to ROSC, min				
1–15	79	116 (74–147)	33.2 (16.8–62.6)	225 (118–392)
16–24	74	133 (78–163)	42.1 (21.8–69.5)	277 (205–416)
25–57	74	131 (97–169)	47.0 (25.9–93.5)	341 (189–597)
p-Value		0.056	0.033	0.003
Therapeutic hypothermia				
Yes	180	131 (92–165)	47.3 (23.7–77.2)	298 (184–534)
No	47	102 (56–143)	28.6 (16.8–45.7)	219 (97–381)
p-Value		0.005	0.001	0.007

Data are reported as median and (IQR).

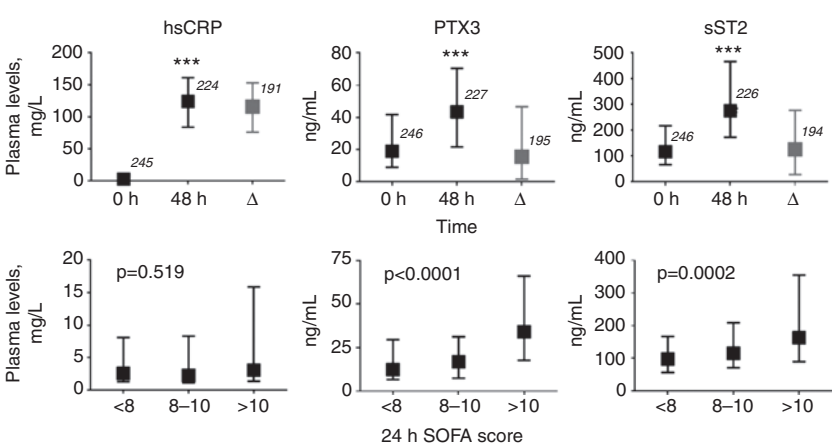

Figure 1: Plasma levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) at ICU admission (0 h) and 48 h later, and absolute Δ of changes during the 48 h (on the top).
The number of patients is in italics. Plasma levels of the same inflammatory biomarkers at ICU admission in relationship to the 24 h entire SOFA score (on the bottom). p<0.0001 vs. plasma levels at ICU admission.

Table 3: Independent determinants of levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2), at ICU admission (0 h) and 48 h later.

Biomarker	Variable	β	p-Value
hsCRP, 0 h	Age	0.007	0.308
	Sex (male)	−0.215	0.373
	Shockable rhythm	−0.738	0.001
	Bystander CPR	−0.279	0.166
	Adrenaline	0.484	0.043
	Time to ROSC	−0.020	0.048
PTX3, 0 h	Age	0.011	0.052
	Sex (male)	−0.004	0.982
	Shockable rhythm	−0.159	0.356
	Bystander CPR	0.044	0.788
	Adrenaline	0.521	0.008
	Time to ROSC	0.011	0.177
SST2, 0 h	Age	0.003	0.490
	Sex (male)	−0.179	0.220
	Shockable rhythm	−0.455	0.000
	Bystander CPR	−0.116	0.339
	Adrenaline	0.428	0.003
	Time to ROSC	−0.001	0.848
hsCRP, 48 h	Shockable rhythm	0.056	0.617
	Bystander CPR	−0.043	0.643
	Adrenaline	0.197	0.061
	Time to ROSC	0.003	0.513
	Therapeutic hypothermia	0.281	0.022
	Apache II score	0.010	0.089
PTX3, 48 h	Shockable rhythm	0.604	0.004
	Bystander CPR	0.194	0.257
	Adrenaline	0.129	0.508
	Time to ROSC	0.003	0.709
	Therapeutic hypothermia	0.238	0.297
	Apache II score	0.024	0.032
sST2, 48 h	Shockable rhythm	−0.408	0.0009
	Bystander CPR	−0.016	0.867
	Adrenaline	0.246	0.032
	Time to ROSC	0.009	0.101
	Therapeutic hypothermia	0.357	0.008
	Apache II score	0.029	<0.0001

Multivariable linear regression model.

Inflammatory markers at ICU admission and MODS

At ICU admission, plasma levels of PTX3 and sST2 were significantly higher in patients with higher 24 h SOFA scores (Figure 1). Moreover, PTX3 and sST2 levels were significantly higher in patients who developed MODS compared to those who did not (Figure 2). The AUCs of the ROC curves for prediction of MODS were 0.78 ($p<0.0001$) and 0.74 ($p<0.0001$) for PTX3 and sST2 ($p=0.204$ PTX3 vs. sST2), respectively (Figure 2). A plasma level of 24 ng/mL of PTX3 had a sensitivity of 0.8 and a specificity of 0.7 to predict development of MODS. Similarly, a plasma level of 141 ng/mL of sST2 had a sensitivity of 0.8 and a specificity of 0.7 to predict development of MODS (Figure 2).

Plasma levels of hsCRP were also significantly higher in patients who developed MODS compared to those who did not, but with a lower accuracy (AUC of 0.6, $p=0.033$) compared to PTX3 ($p<0.003$) and sST2 ($p<0.014$). The univariate odds ratios for prediction of MODS were: 1.922 (95% CI 1.351–2.736, per 1 SD, $p=0.0003$) for PTX3; 1.565 (1.181–2.074, per 1 SD increase, $p=0.0003$) for sST2; and 1.316 (95% CI 1.004–1.724, per 1 SD increase, $p=0.0466$) for hsCRP. Plasma levels of PTX3 and sST2, but not hsCRP, were also independently associated with development of MODS in the multivariable analysis: OR 1.717 (95% CI 1.221–2.414, per 1 SD, $p=0.0019$) for PTX 3; OR 1.340 (95% CI 1.001–1.792, per 1 SD, $p=0.048$), respectively) for sST2; and OR 1.115 (95% CI 0.833–1.491, per 1 SD increase, $p=0.464$) for hsCRP.

Inflammatory markers and ICU survival

Plasma levels of PTX3 and sST2 at ICU admission and levels of hsCRP and sST2 48 h later were significantly higher in patients who died compared to those who survived at ICU discharge (Figure 3). The odds ratios for prediction of ICU mortality of these inflammatory markers are detailed in Table 4.

In a multivariable model, including the initial cardiac arrest rhythm (shockable or non-shockable), time to ROSC, use of adrenaline, and whether therapeutic hypothermia was applied or not (Table 1), plasma levels of PTX3 and sST2 at ICU admission and of sST2 and hsCRP 48 h later were independently associated with ICU death (Table 4).

The AUCs of the ROC curves for prediction of ICU death were 0.71 ($p<0.0001$) for PTX3 at ICU admission ($p=0.078$ vs. sST2 and $p=0.005$ vs. hsCRP), 0.78 ($p<0.0001$) and 0.72 ($p<0.0001$) for sST2 ($p=0.008$ vs. PTX3 and $p=0.417$ vs. hsCRP) and hsCRP ($p=0.175$ vs. PTX3), respectively, at 48 h after ICU admission (Figure 4). The optimal cut-off levels for prediction of ICU death were: 24 ng/mL for PTX3 at ICU admission (sensitivity of 0.7 and a specificity of 0.7); 604 ng/mL for sST2 (sensitivity of 0.6 and a specificity of 0.9) and 145 mg/L for hsCRP at 48 h (sensitivity of 0.7 and a specificity of 0.7) (Figure 4).

Inflammatory markers and 12-month unfavorable outcome

Plasma levels of PTX3 and sST2 at ICU admission and of sST2 48 h later were significantly higher in patients who

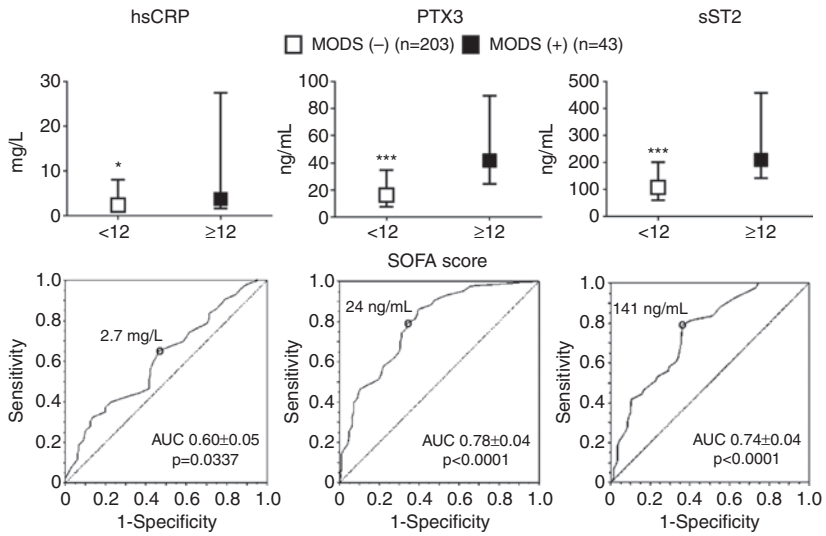

Figure 2: Plasma levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) at ICU admission in patients with or without multiple organ dysfunction syndrome (MODS) (on the top).
Corresponding receiver operating curves (ROC) curves and area under the curves (AUC) shown on the bottom panels. *p<0.05 and ***p<0.0001 vs. MODS (-). p=0.204 AUC PTX3 vs. AUC sST2; p=0.003 AUC PTX3 vs. AUC hsCRP; p=0.014 AUC sST2 vs. AUC hsCRP.

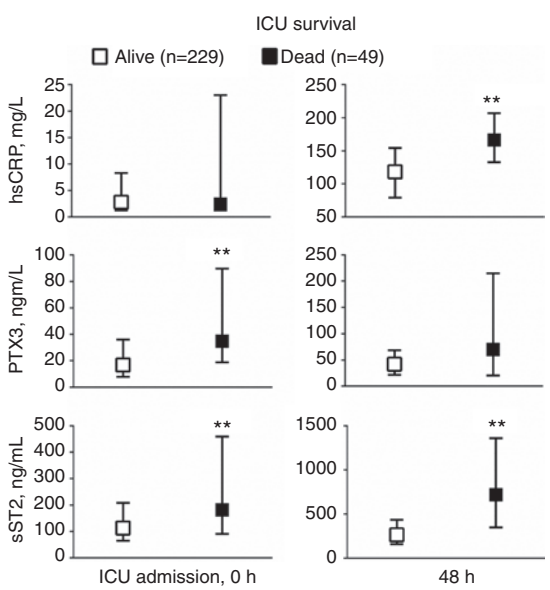

Figure 3: Plasma levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) at ICU admission (0 h) and 48 h in ICU survivors and non-survivors.
**p<0.01 vs. alive.

had a unfavorable outcome at 12 months compared to those who had a good recovery (Figure 5). However, in the multivariable model, none of the studied biomarkers was independently associated with 12-month unfavorable outcome (Table 4). The odds ratios for prediction of 12-month unfavorable outcome of these inflammatory markers at ICU admission and 48 h later are detailed in Table 4.

Discussion

The present study showed that plasma levels of PTX3 and sST2 were markedly elevated early after resuscitation from out-of-hospital cardiac arrest. Higher plasma levels of PTX3 and sST2 at ICU admission were associated with higher 24 h SOFA score and with development of MODS and were independently associated with death in ICU. In contrast, hsCRP increased later compared to PTX3 and sST2, so that only 48 h levels were associated with ICU outcome. Plasma levels of PTX3 and sST2 at ICU admission were also significantly higher in patients with an unfavorable 12-month outcome compared to those with a favorable outcome, although they were not independently associated with it in the multivariable model.

PTX3 is a prototypic long pentraxin, component of the humoral arm of innate immunity that can regulate inflammatory processes. It shares similarities with CRP, a prototypic short pentraxin, but differs in terms of structural domains, gene organization, cellular and tissue sources, inducing stimuli, and ligands [13]. Indeed, PTX3 is produced mainly by myeloid cells and vascular endothelial cells in response to primary inflammatory stimuli [7, 13, 21, 22]. In addition the protein is stored by neutrophils in

Table 4: Univariate and multivariable logistic regression models for prediction of ICU death, and 12-month unfavorable outcome.

	Univariate			Multivariable		
	OR	95% CI	p-Value	OR	95% CI	p-Value
ICU death						
hsCRP, 0 h	1.342	1.027–1.754	0.031	1.056	0.778–1.432	0.728
hsCRP, 48 h	2.091	1.374–3.182	0.0006	1.986	1.263–3.123	0.003
PTX3, 0 h	1.763	1.274–2.439	0.0006	1.536	1.078–2.187	0.0174
PTX3, 48 h	1.247	0.931–1.762	0.139	1.230	0.896–1.688	0.201
sST2, 0 h	1.820	1.331–2.488	0.0002	1.452	1.064–1.981	0.019
sST2, 48 h	2.151	1.539–3.006	<0.0001	1.856	1.272–2.707	0.0013
12-month unfavorable outcome						
hsCRP, 0 h	1.488	1.053–2.101	0.024	1.073	0.741–1.552	0.710
hsCRP, 48 h	1.105	0.849–1.439	0.457	0.883	0.642–1.214	0.444
PTX3, 0 h	1.618	1.073–2.440	0.022	1.183	0.813–1.723	0.380
PTX3, 48 h	1.398	0.899–2.173	0.137	1.229	0.775–1.950	0.381
sST2, 0 h	1.888	1.235–2.884	0.003	1.303	0.873 –1.946	0.196
sST2, 48 h	1.896	1.329–2.704	0.0004	1.318	0.910–1.907	0.144

hsCRP, high-sensitivity C-reactive protein; OR, odds ratio per 1 SD increase; PTX3, pentraxin 3; sST2, soluble suppression of tumorigenicity 2.

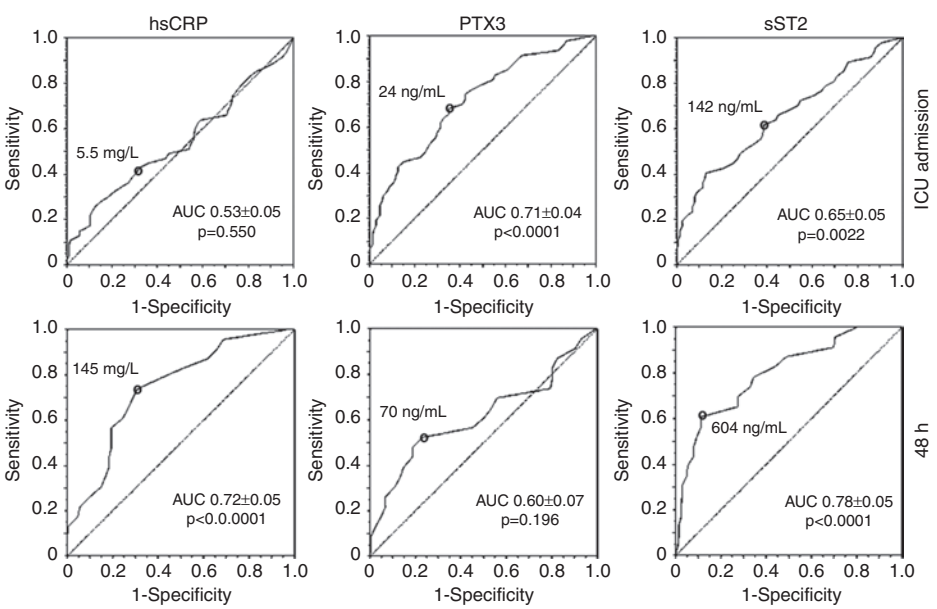

Figure 4: Receiver operator curves (ROC) and area under the curves (AUC) for plasma levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) at ICU admission (0 h) and 48 h for prediction of ICU death. p=0.078 at ICU admission and p=0.008 at 48 h for AUC PTX3 vs. AUC sST2; p=0.005 at ICU admission and p=0.175 at 48 h for AUC PTX3 vs. AUC hsCRP; p=0.035 at ICU admission and p=0.417 at 48 h for AUC sST2 vs. AUC hsCRP.

specific granules and is promptly released in response to tissue damage [23]. Thus, in contrast to CRP that is primary synthesized in the liver and thereby reflects systemic inflammation, PTX3 is, at least in part, synthesized at the site of inflammation [21], behaving as an acute phase response protein that increases rapidly and peaks within 6–8 h after the insult [24]. In the instance of cardiac arrest, as early as 3 h after resuscitation, blood concentrations of soluble intercellular adhesion molecule-1, soluble vascular-cell adhesion molecule-1, and P- and E-selectins increase, suggesting leucocyte and endothelial activation, which together with the protein released by neutrophils, account for rapid PTX3 release [2, 23, 25, 26]. In our study, the median PTX3 levels at ICU admission were, in fact, already 10-fold higher compared to normal values, in contrast to normal levels of hsCRP.

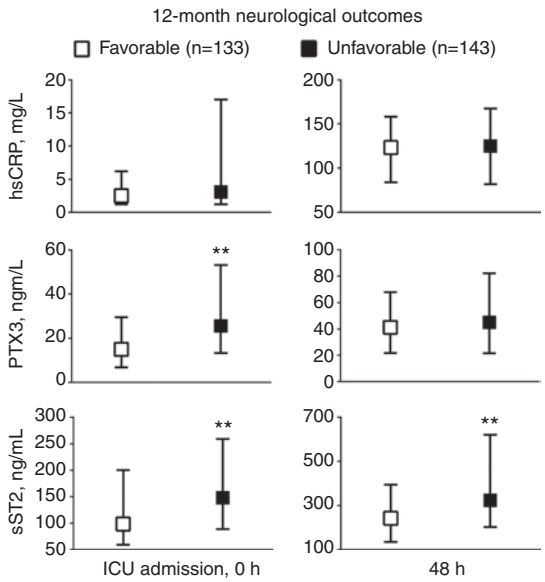

Figure 5: Plasma levels of high-sensitivity C-reactive protein (hsCRP), pentraxin 3 (PTX3), and soluble suppression of tumorigenicity 2 (sST2) at ICU admission (0 h) and 48 h and long-term neurological outcome based on Cerebral Performance Category (CPC 1–2, favorable and CPC 3–5, unfavorable).
**$p<0.01$ vs. favorable outcome.

We have previously reported that PTX3, compared with other biomarkers considered reliable predictors of mortality in acute cardiovascular events, i.e., natriuretic peptides and cardiac troponins, appeared as an earlier and stronger predictor of worsening of heart failure and death in patients with ST-elevation myocardial infarction as well as in patients with chronic heart failure [7, 20, 27]. Moreover, high plasma levels of PTX3 have also emerged as prognostic markers for severe sepsis and septic shock, with a gradient from systemic inflammatory response syndrome to septic shock [8, 28]. In our study, PTX3 has now emerged as a useful biomarker predictive of the severity of post-resuscitation syndrome and early death after cardiac arrest. Patients with a median value of PTX3 >24 ng/mL at ICU admission had a 92% higher risk of developing MODS, and a 54% higher risk of dying in ICU, compared to patients with lower values.

PTX3 is a multifunctional protein with in vivo roles that remain in part controversial [22]. In fact, PTX3 was known to bind C1q with consequent activation of the classic pathway of the complement when bound to immobilized ligands [29]. Therefore, locally produced PTX3 was recognized to cause and amplify tissue damage [7]. Moreover, PTX3 was also known to inhibit angiogenesis and tissue repair, by binding and inactivation of fibroblast growth factor [30]. Recent observations, however, support the possibility that PTX3 may act as a molecule at the crossway between pro- and anti-inflammatory stimuli, regulating leukocyte recruitment and perhaps balancing the over activation of pro-inflammatory cascades [31, 32]. Indeed, it has been shown that fluid-phase PTX3 can sequester C1q and prevent complement activation [33]. Thus, PTX3 may exert a dual role and contrasting effects on complement activation in ischemia/reperfusion [7, 32]. PTX3-deficient mice, in fact, had increased C3 deposition and amplified tissue damage in the ischemic myocardium, while administration of exogenous PTX3 reduced such a complement deposition exacerbated damage after reperfusion [32]. Therefore, the high level of circulating PTX3 in our patients may be considered as both an indicator of the severity of post-ROSC inflammation, as well as one of the pathophysiological players in the post-cardiac arrest syndrome, thus explaining its association with death.

ST2 is a protein of the IL-1 receptor family that binds IL-33. Its homology with the toll-like receptor and other IL-1 family members suggests that it may play a central role in innate and adaptive immune responses [9, 10]. As suggested for PTX3, in cardiac arrest patients, sST2 may be, therefore, not only a marker/indicator of the severity of the post-ROSC inflammatory status, but it may also be directly involved in its pathophysiology. Indeed, earlier investigations have revealed sST2 participation in the regulation of inflammatory processes, particularly regarding mast cells, type 2 CD4+ T-helper (Th) cells and the production of Th2-associated cytokines [9, 10]. Although sST2 gene is markedly induced in the instance of mechanically overloaded cardiac myocytes, i.e., during acute and chronic heart failure, ST2 seems to be produced also in response to inflammatory stimuli [34, 35]. Circulating ST2 levels have never been reported in cardiac arrest. Thus, of interest were the levels of sST2 in our patients, 117 ng/mL at ICU admission and 276 ng/mL 48 h later, that were 2–4-fold greater than those reported in acute heart failure patients [36]. The initial sST2 increase might have been related to the post-cardiac arrest systemic inflammation, while the further increase to the overloaded myocardial cells within the condition of severe post-resuscitation myocardial dysfunction [1].

In over 800 patients with an acute ST-elevation myocardial infarction, levels of sST2 at hospital admission were significantly higher in those who died or developed heart failure [10]. Similarly, in large cohorts of patients with acute heart failure, sST2 was associated with a more decompensated hemodynamic profile with left and right ventricular dysfunction, and with higher risk of hospitalization and death [11, 36–38]. Moreover, levels of circulating sST2 were elevated also in patients

admitted to ICU with a diagnosis of sepsis or after severe trauma [12]. Similarly to PTX3, in our study, sST2 has emerged as another useful biomarker predictive of the severity of post-resuscitation syndrome and early death. Patients with a median value of sST2 >142 ng/mL at ICU admission had a 57% higher risk of developing MODS, and a 45% higher risk of dying in ICU, compared to patients with lower values. Association with early death remained, although with a reduced sensitivity, for sST2 levels at 48 h, with an 86% increased relative risk when values were above 600 ng/mL.

Role of CRP after cardiac arrest is controversial. Indeed, several studies in small cohorts of patients have described important post-resuscitation increases in CRP, peaking in 48–72 h independently from infection [39]. A significant correlation between peak CRP levels, cardiovascular dysfunction, and SOFA score has been reported, together with association with death and poor neurological outcome [39–41]. Other studies, however, have reported no correlation between CRP levels and severity of post-cardiac arrest syndrome or outcome [4, 42]. Our study further confirmed the weak association of admission levels of hsCRP with the severity of post-resuscitation syndrome and no association with ICU survival. Nevertheless, high hsCRP levels at 48 h after resuscitation were independently associated with ICU death.

In agreement with previous reports, inflammatory markers levels in our study were higher in patients with non-shockable rhythm, with longer time to ROSC, and that received adrenaline during CPR [41]. As previously suggested, because non-shockable rhythms are usually associated with other pathological conditions, the high levels of our markers may reflect the presence of a general inflammatory status that preceded and/or led to cardiac arrest [41]. Moreover, a longer duration of cardiac arrest, including both no-flow and low-flow times, and the use of adrenaline, are known to be associated with a greater severity of post-cardiac arrest syndrome, and plausibly accounted for the higher levels of inflammatory markers [1, 43]. Finally, at 48 h post-ROSC, patients treated with hypothermia presented significantly higher levels of hsCRP and sST2, similar to earlier investigations [4]. The continuous rising of inflammatory markers after hypothermia has been explained as a consequence of the endothelial activation that declines during cooling while it increases again following rewarming [4, 5].

We acknowledge several limitations in the interpretation of our findings. First, this was a biomarker substudy of the FINNRESUSCI trial. Nevertheless, all the patients with a blood sample were included in the study and the biomarker analyses, data collection, outcome evaluation and statistics were performed independently. Second, we did not report data on cardiac disease or other comorbidities. Indeed, all the biomarkers are associated with cardiovascular risk so it is possible that their levels on admission might have been influenced by the presence of a pre-existing cardiac disease [44].

In conclusion, after cardiac arrest, the temporal release patterns of the inflammatory markers PTX3, sST2, and hsCRP showed large differences with an early cellular and vascular response, leading to PTX3 and sST2 release, whereas the organ derived inflammatory marker hsCRP was released more slowly. Thus, ICU admission levels of PTX3 and sST2 are independent predictors of MODS and early death after cardiac arrest, while CRP is not. Nevertheless, PTX3, sST2 and hsCRP levels continued to rise at 48 h after cardiac arrest, suggesting that systemic activation of the pro-inflammatory response is prolonged and could be therefore a potential target for intervention.

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