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Activated factor XI-antithrombin complex presenting as an independent predictor of 30-days mortality in out-of-hospital cardiac arrest patients

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ABSTRACT

Background: Cardiac arrest and cardiopulmonary resuscitation (CPR) are associated with activated coagulation and microvascular fibrin deposition with subsequent multiorgan failure and adverse outcome.
Objectives: Activated Factor XI-antithrombin (FXIa-AT) complex, activated Factor IX–antithrombin (FIXa-AT) complex and thrombin-antithrombin (TAT) complex were measured as markers of coagulation activation, and evaluated as independent prognostic indicators in out-of-hospital cardiac arrest (OHCA) patients.
Methods: From February 2007 until December 2010 blood samples were collected in close approximation to CPR from patients with OHCA of assumed cardiac origin. Follow-up samples in survivors were drawn 8–12 h and 24–48 h after hospital admission. All measurements were determined by ELISA.
Results: Thirty-seven patients presented with asystole and 77 with ventricular fibrillation as first recorded heart rhythm. At 30-days follow-up, 70 patients (61.4%) had died. All patients had elevated levels of FXIa-AT complex, FIXa-AT complex and TAT. Initial levels were significantly higher in non-survivors compared to 30-days survivors.
A significant increase in risk of 30-days all-cause mortality was observed through increasing quartiles of all three biomarkers in univariate Cox regression analysis. Compared to the lowest quartile (Q1), only FXIa-AT complex levels in Q3 (HR 3.17, $p = 0.011$) and Q2 (HR 3.02, $p = 0.016$) were independently associated with all-cause mortality in the multivariable analysis. FIXa-AT complex and TAT-complex did not behave as independent predictors.
Conclusions: Complexes of FXIa-AT were independently associated with 30-days survival in OHCA-patients.
Clinical trial registration: ClinicalTrials. gov, NCT02886273.

1. Introduction

The prognosis following out-of-hospital cardiac arrest (OHCA) is poor, and even in initially successfully resuscitated patients admitted to the intensive care unit, the mortality rate is high [1]. Cardiac arrest and cardiopulmonary resuscitation (CPR) are associated with derangement in the coagulation- and fibrinolytic systems [2]. Cardiac arrest represents the most severe shock state, and whole-body ischemia-reperfusion in combination with direct tissue trauma during CPR may cause endothelial injury with a subsequent generalized activation of the immune- and coagulation systems [2]. Increased coagulation activation and impairment of fibrinolysis are considered to be closely related to the pathophysiology of post-cardiac arrest syndrome [1,2], which is associated with an adverse outcome following cardiac arrest [1]. Intravascular thrombosis and microvascular fibrin deposition may lead to impaired end-organ perfusion with subsequent multiple organ failure and increased risk of death [3,4].

Blood coagulation results from the activation of a cascade of coagulation factors that interact in a tightly regulated sequential order, eventually leading to the formation of thrombin and subsequently fibrin.

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Activation of blood coagulation may occur either by the extrinsic- or the intrinsic coagulation pathway, which converge in the common pathway at Factor X level. Although considered as two individually activation pathways, they are not directly separated and interact on several steps [5].

Activation of the extrinsic coagulation pathway may be an important mechanism for blood coagulation in cardiac arrest patients, and elevated levels of tissue factor (TF) in association with low levels of tissue-factor pathway inhibitor (TFPI) have been demonstrated during and after CPR in OHCA patients [6]. Up-regulation of the coagulation cascade during cardiac arrest is also supported by increased levels of thrombin-antithrombin (TAT) complexes and fibrin-monomers [7–9]. Less is known about activation of the intrinsic coagulation pathway during cardiac arrest.

Coagulation Factor IX (FIX) may be activated both through the intrinsic- and the extrinsic coagulation pathway [10]. Through the extrinsic pathway, FIX is activated by TF – Factor VIIa complex. The initiating zymogen of the intrinsic pathway is Factor XII (FXII), which is autoactivated after contact with negatively charged surfaces. The activated FXII (FXIIa) enzyme initiates the coagulation cascade by activating FXI to FXIa, which in turn activates FIX to FIXa. With FVIIIa as a cofactor, FIXa activates FX to FXa with subsequent conversion of prothrombin to active thrombin. Through a positive feedback mechanism, thrombin acts as an FXI-activator, thus FXI- and subsequent FIX activation is important for sustained thrombin generation and fibrin formation [10].

In our study, we analysed activated Factor XI-antithrombin (FXIa-AT) complex, activated Factor IX–antithrombin (FIXa-AT) complex and TAT complex as a measure of coagulation activation. The primary aim of our study was to evaluate the independent prognostic utility of these three coagulation markers in a previously described population of OHCA patients with both shockable- and non-shockable heart rhythm [11]. As a second aim, this study was performed to improve our understanding of the pathophysiology related to coagulation activation during cardiac arrest, including both the intrinsic- and the extrinsic pathway.

2. Methods

2.1. Study subjects and design

Patients ≥ 18 years of age with OHCA of assumed cardiac origin, defined according to the Utstein definitions [12], were recruited from the southwestern part of Norway from February 2007 until November 2010. All patients received out-of-hospital advanced cardiac life support by Emergency Medical Services (EMS) paramedics according to the 2005 European Resuscitation Council guidelines with Norwegian modifications [13]. Patients with return of spontaneous circulation (ROSC) were admitted to Stavanger University Hospital and received standardized post-cardiac arrest care at the intensive care unit, including therapeutic hypothermia and coronary artery revascularization procedures. During or immediately after termination of CPR, 20 mL of blood was collected into ethylenediamine tetra acetic acid (EDTA) -tubes, using a venous cannula. Patients admitted without a prehospital blood sample, had blood collected immediately after hospital admission. Follow-up samples were drawn 8–12 h and 24–48 h after hospital admission.

Clinical information obtained from hospital records and additional investigations, including electrocardiograms, echocardiography, and coronary angiography, was applied for verification of an assumed cardiac cause of OHCA and for categorization of patients [14]. Two main groups were defined according to whether the first recorded heart rhythm was asystole or ventricular fibrillation (VF). VF-patients were further categorized into four groups according to the diagnosis of an acute myocardial infarction (AMI) or not, and whether or not they had previously known heart disease.

All survivors gave written, informed consent retrospectively. The next-of-kin were asked for consent on behalf of patients who did not regain consciousness before death. This study was approved by the Regional Board of Research Ethics and the Norwegian Health Authorities, conducted in accordance with the Helsinki Declaration of 1975, as revised in 1989, and registered in ClinicalTrials.gov, identifier: NCT02886273.

2.2. Laboratory methods

After collection, blood samples were centrifuged at 2500 rpm for 10 min within 24 h if stored at room-temperature, or within 48 h if stored at +4 °C. EDTA-plasma was subsequently stored in aliquots at −70 °C.

Complexes of activated coagulation factors XI, IX and thrombin inhibited by antithrombin: FXIa-AT, FIXa-AT and TAT complex, respectively, were quantified in EDTA plasma by in-house assays as previously described [15]. Measurements were performed at the Thrombosis Expertise Center and Cardiovascular Research Institute Maastricht, Maastricht University Medical Center.

2.3. Statistical methods

Descriptive statistics are presented as medians with interquartile range (25th to 75th percentile) for continuous data and as numbers and percentages for categorical data. Differences in baseline characteristics were assessed by the Kruskal-Wallis Test for continuous data and Fisher's exact test for categorical data. The Mann-Whitney *U* test was used to assess between-group differences, comparing median values of FXIa-AT complex, FIXa-AT complex and TAT complex in non-survivors with survivors, between patients who died on scene and survivors until hospital admission, and between OHCA-patients with an AMI and control patients with an AMI without cardiac arrest. Pearson's correlation coefficient was calculated to identify a possible relation between biomarkers levels and duration of resuscitation, and partial correlation was applied to assess a possible association between the levels of the three different biomarkers. Spearman's rank correlation coefficient was calculated to assess a possible relation between admission levels of each of the three coagulation markers and hs-CRP, respectively.

Patients were divided into quartiles (Q1–4) according to their FXIa-AT complex, FIXa-AT complex, and TAT complex concentrations. The Kaplan-Meier product limits were used for plotting the times to event and the log-rank test was used to test for the equality of the survival curves. Cox regression models were fitted for each of the biomarkers with all-cause mortality within 30-days as the dependent variable. For multivariable analysis, we adjusted for age, gender, VF as first recorded heart rhythm, duration of resuscitation, witnessed cardiac arrest, bystander-initiated CPR and the logarithmically transformed value of creatinine ($\log_e$(creatinine)). Hazard ratios (HR) with 95% confidence intervals were calculated for each of the higher quartiles as compared to quartile 1. Subgroup analyses were performed in survivors at hospital admission and for VF-patients.

Statistical analysis was performed using the statistical package SPSS version 25 (IBM Corp. Armonk, NY). All tests were 2 -sided with a significance level of 5% without multiplicity adjustment.

3. Results

3.1. Study population

A total of 114 OHCA-patients were included, 37 patients with asystole and 77 patients with VF as first recorded heart rhythm (Fig. 1). Baseline characteristics for the total population, and for the patients stratified according to 30-days mortality, are listed in Table 1. The median age was 67 (25th to 75th percentile; 56–78) years and 83% were men. Non-survivors, as compared to survivors, were significantly older, had more comorbidities, and worse cardiac arrest conditions, including a higher proportion of asystole as first recorded heart rhythm (Table 1).

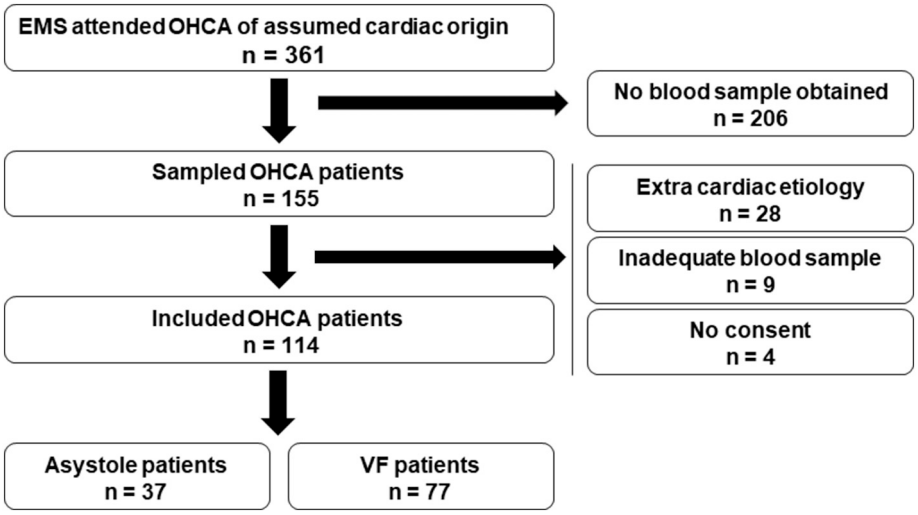

Fig. 1. Flow-chart displaying selection by cause of death of OHCA-patients recruited between February 2007 and November 2010, and classification according to first recorded heart rhythm.

Among survivors, 73% received therapeutic hypothermia. There was no statistically significant difference in the number of patients receiving hypothermia treatment between survivors and non-survivors.

In the total population, 28 patients were on platelet inhibitors and 20 patients were taking anticoagulants prior to the OHCA (Table 1). There were significantly more patients on acetylsalicylic acid among non-survivors as compared to survivors ($p = 0.014$), but there was no significant difference in the use of warfarin between the two patient groups.

Out of 77 patients with VF as primary heart rhythm, 58 (68.8%) were categorized as having an AMI, and 10 out of these had previously been diagnosed with MI. Twenty-one patients (27.3%) suffered sudden cardiac arrest without signs of an AMI, of whom 18 had evidence of prior heart disease, including coronary artery disease and/or chronic heart failure. Three patients could not be classified as having an AMI or not, due to lack of clinical information.

Patient baseline characteristics stratified according to quartiles of FXIa-AT complex, FIXa-AT complex, and TAT-complex are shown in Supplemental Tables 1, 2 and 3, respectively.

3.2. FXIa-AT complex and outcome

At 30-days follow-up, 70 patients (61.4%) had died, 35 (30.7%) died on-scene while 35 patients (30.7%) died in hospital. All patients surviving to hospital discharge (38.6%) were still alive at 30-days follow-up. FXIa-AT complex levels in the first blood sample drawn during or closely after termination of CPR, were significantly higher in patients who died compared to 30-days survivors [median 172, 25th–75th percentile: (112–296) pM vs 117 (54–189) pM, $p < 0.001$] (Fig. 2). All-cause mortality at 30-days follow-up varied across FXIa-AT complex quartiles (Log Rank test $\chi^2(3) = 14.1$, $p = 0.003$), as shown in the Kaplan-Meier plot (Fig. 3a). In univariate analysis, the HR increased with increasing FXIa-AT complex quartiles (Table 2). After adjusting for age, gender, VF as primary heart rhythm, duration of resuscitation, witnessed cardiac arrest, bystander-initiated CPR and $\log_e$(creatinine) in a multivariable Cox regression model, FXIa-AT complex acted as an independent predictor of 30-days mortality in Q3 (HR 3.17, $p = 0.011$) and in Q2 (HR 3.02, $p = 0.016$) (Table 2).

In univariate subgroup analysis, FXIa-AT complex levels in each of the two highest quartiles were significantly associated with 30-days mortality in survivors at hospital admission and in patients with VF as primary heart rhythm, respectively, but these associations were no longer statistically significant in the multivariable model (Table 2).

Patients who died on scene had significantly higher plasma-levels of FXIa-AT complex compared to survivors at hospital admission [194 (103–290) pM vs 133 (73–229) pM, $p = 0.030$]. As compared to 30-days survivors, patients who died during hospitalization had higher plasma-levels of FXIa-AT complex 8–12 h after hospital admission ($p = 0.012$), but there were no significant differences in median FXIa-AT complex levels between the two groups 24–48 h after admission (Fig. 2).

3.3. FIXa-AT complex and outcome

Similar to that of FXIa-AT complex, patients who died had significantly higher first-sample levels of FIXa-AT complex compared to survivors at 30-days follow-up, [403, (280–815) pM vs 241, (163–406) pM, $p < 0.001$]. All-cause mortality at 30-days follow-up varied across FIXa-AT complex quartiles (Log Rank test $\chi^2(3) = 12.8$, $p = 0.005$), as shown in the Kaplan-Meier analysis (Fig. 3b). In univariate analysis, the HR for each of the higher quartiles as compared to Q1 were statistically significant. However, after applying the multivariable model, all-cause mortality at 30-days follow-up did not vary significantly across FIXa-AT complex quartiles, $p = 0.18$ (Table 2). Subgroup analyses of survivors at hospital admission and in VF-patients, respectively, revealed similar, but somewhat weaker results in univariate analyses, and there was no significant association between FIXa-AT complex levels and 30-days all-cause mortality in both subgroups, when applying the multivariable model (Table 2).

There were no significant differences in FIXa-AT complex levels between patients who died on scene and those surviving until hospital admission [400 (284–805) pM vs 306 (199–484) pM, $p = 0.079$]. As compared to survivors, patients who died during hospitalization had significantly higher plasma-levels of FIXa-AT complex in follow-up samples drawn 8–12 h ($p < 0.001$) and 24–48 h ($p = 0.008$), respectively, after hospital admission (Table 1).

3.4. TAT complex and outcome

Median first sample levels of TAT-complex were significantly higher in patients who died compared to survivors at 30-days follow-up [64 (38–102) μg/L vs 39 (14–72) μg/L, $p = 0.001$]. In the univariate analysis, all-cause mortality at 30-days follow-up varied across TAT complex quartiles (Log Rank test $\chi^2(3) = 12.9$, $p = 0.005$) as shown in the Kaplan-Meier plot (Fig. 3c). As compared to Q1, Cox regression analysis resulted in a significant univariate HR for each of the higher quartiles (Table 2). These associations were no longer statistically significant in the

Table 1
Baseline characteristics and laboratory values of patients suffering out-of-hospital cardiac arrest.

	Total population ($n = 114$)	Survivors ($n = 44$)	Non-survivors ($n = 70$)	*P*-value
Age, y	67 (56–78)	61 (51–68)	72 (62–83)	<0.001
Male gender	95 (83)	36 (82)	59 (84)	0.80
Previous history				
Angina pectoris	17 (18)	6 (14)	11 (22)	0.42
Myocardial infarction	33 (31)	10 (23)	23 (36)	0.20
Previous PCI	12 (11)	5 (11)	7 (11)	1.00
Previous CABG	12 (11)	2 (5)	10 (16)	0.12
Heart failure	28 (26)	7 (16)	21 (34)	0.046
Hypertension	53 (52)	19 (43)	34 (58)	0.17
Diabetes mellitus	17 (16)	2 (5)	15 (25)	0.007
Hypercholesterolemia	44 (42)	22 (51)	22 (36)	0.16
Smoking				0.81
Never	18 (21)	7 (18)	11 (24)	
Current smoker	27 (32)	13 (33)	14 (31)	
Ex-smoker	40 (47)	20 (50)	20 (44)	
Cardiac arrest conditions				
Witnessed cardiac arrest	87 (77)	39 (89)	48 (70)	0.022
Bystander-initiated CPR	93 (82)	40 (91)	53 (76)	0.049
Duration of resuscitation, min	23 (10–38)	9 (5–15)	32.5 (23–45)	<0.001
Initial rhythm				<0.001
VF	77 (68)	44 (100)	33 (47)	
Asystole	37 (33)	0 (0)	37 (53)	
Therapeutic hypothermia	57 (50)	32 (73)	25 (36)	0.23
Medication prior to inclusion				
Acetylsalicylic acid	26 (23)	7 (16)	19 (27)	0.014
Clopidogrel	2 (2)	0 (0)	2 (3)	0.18
Warfarin	19 (17)	6 (14)	13 (19)	0.12
LMW Heparin	1 (1)	0 (0)	1 (1)	0.34
Baseline blood samples				
Creatinine (μmol/L)	102 (87–122)	93 (82–116)	110 (92–125)	0.013
Total cholesterol (mmol/L)	4.2 (3.6–5.3)	4.9 (4.1–6.5)	3.9 (3.4–5.0)	0.002
CRP (mg/L)	2.5 (1.1–9.9)	2.1 (1.2–4.6)	3.9 (1.0–18.0)	0.15
Glucose (mmol/L)	12.6 (8.0–16.9)	12.6 (8.8–14.7)	12.6 (6.6–17.8)	0.94
Copeptin (pmol/L)[a]	436 (216–825)	388 (195–825)	445 (244–879)	0.39
hs-cTnT (ng/L)[b]	71 (26–231)	100 (21–289)	66 (26–207)	0.49
NT-proBNP (pmol/L)[c]	61 (25–234)	30 (12–98)	105 (35–495)	<0.001
TAT complex first sample (ug/L)	55 (27–94)	39 (14–72)	64 (38–102)	0.001
TAT complex 8–12 h (ug/L)	8 (5–19)	6 (5–10)	11 (10–35)	<0.001
TAT complex 24–48 h (ug/L)	5 (4–8)	4 (3–7)	6 (5–9)	0.077
FIXa-AT complex first sample (pM)	335 (224–597)	241 (163–406)	403 (280–815)	<0.001
FIXa-AT complex 8–12 h (pM)	188 (140–284)	152 (114–196)	327 (187–741)	<0.001
FIXa-AT complex 24–48 h	150 (111–206)	131 (104–184)	194 (130–357)	0.008
FXIa-AT complex first sample (pM)	143 (92–241)	117 (54–189)	172 (112–296)	<0.001
FXIa-AT complex 8–12 h (pM)	57 (46–80)	51 (39–73)	68 (53–147)	0.012
FXIa-AT complex 24–48 h (pM)	46 (34–64)	45 (32–61)	53 (39–90)	0.26

Data are presented as median (interquartile range) or numbers (%).
Abbreviations: CPR, cardiopulmonary resuscitation; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; CRP, C-reactive protein; hs-cTnT, high-sensitivity cardiac troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TAT, thrombin-antithrombin; FIXa-AT, activated factor IX – antithrombin; FXIa-AT, activated factor XI – antithrombin.

[a] $n = 110$ (96%).

[b] $n = 111$ (97%).

[c] $n = 112$ (98%).

multivariable model adjusting for clinical and demographic variables related to survival of OHCA (Table 2).

Analysis of the subgroups revealed largely similar results. In univariate analysis, higher quartiles of TAT-complex were associated with increased hazard ratio for 30-days all-cause mortality in both admitted survivors and for the VF subgroup of patients (Table 2), but these associations did not remain statistically significant in multivariable analysis.

There was no significant difference in median plasma-levels of TAT-complex in patients who died on scene as compared to survivors at hospital admission [71 (36–106) μg/L vs 50 (25–89) μg/L, $p = 0.090$]. Patients who died during hospitalization, as compared to 30-days survivors, had higher plasma-levels of TAT complex 8–12 h after hospital admission ($p < 0.001$), but there was no difference between the two groups at 24–48 h after admission ($p = 0.077$).

3.5. Associations between FXIa-AT complex, FIXa-AT complex and TAT-complex and baseline characteristics

Plasma-levels of FXIa-AT complex ($r = 0.38$, $p = 0.013$), FIXa-AT complex ($r = 0.43$, $p = 0.004$) and TAT complex ($r = 0.48$, $p = 0.001$) in samples drawn at hospital admission were all separately positively correlated with the duration of resuscitation. In addition, FIXa-AT complex in samples drawn at 8–12 h ($r = 0.32$, $p = 0.028$) and 24–48 h ($r = 0.31$, $p = 0.040$) after hospital admission correlated with duration of resuscitation. There was no correlation between duration of resuscitation and the measured levels of FXIa-AT complex, FIXa-AT complex, and TAT-complex in blood samples drawn on-scene.

We found a positive partial correlation between plasma concentrations of FXIa-AT complex and FIXa-AT complex, controlling for levels of TAT-complex, in the first sample drawn following OHCA ($r = 0.63$, $p < 0.001$) and in samples drawn 8–12 h after hospital admission ($r = 0.30$, $p = 0.035$). There was also a positive partial correlation between first sample FIXa-AT complex levels and levels of TAT-complex ($r = 0.48$, $p < 0.001$), controlling for FXIa-AT complex, with an even higher degree of correlation between these biomarkers in samples drawn 8–12 h after hospital admission ($r = 0.69$, $p < 0.001$).

Median plasma concentrations of FXIa-AT complex, FIXa-AT complex and TAT complex in the first sample drawn following cardiac arrest and in samples drawn 8–12 h after hospital admission were significantly higher in OHCA-patients with an AMI as compared with AMI controls without cardiac arrest (Table 3). Median TAT-complex levels at 24–48 h after admission were also significantly higher in AMI patients with OHCA as compared to AMI controls.

There was no statistically significant correlation between first sample levels of FXIa-AT complex ($r_s = 0.09$, $p = 0.32$), FIXa-AT complex ($r_s = 0.06$, $p = 0.52$) and TAT complex ($r_s = -0.009$, $p = 0.93$) and hs-CRP, respectively.

4. Discussion

Evidence of systemic coagulation activation was consistently present in our cohort of patients suffering OHCA. We demonstrated that TAT-complex, a marker of thrombin generation and general coagulation activation, was markedly increased in the early phase following OHCA. Patients with an AMI complicated with OHCA had almost 18-times higher TAT-values as compared to AMI-patients without cardiac arrest. This is in accordance with previous studies demonstrating a marked coagulation activation with a substantial increase in thrombogenic

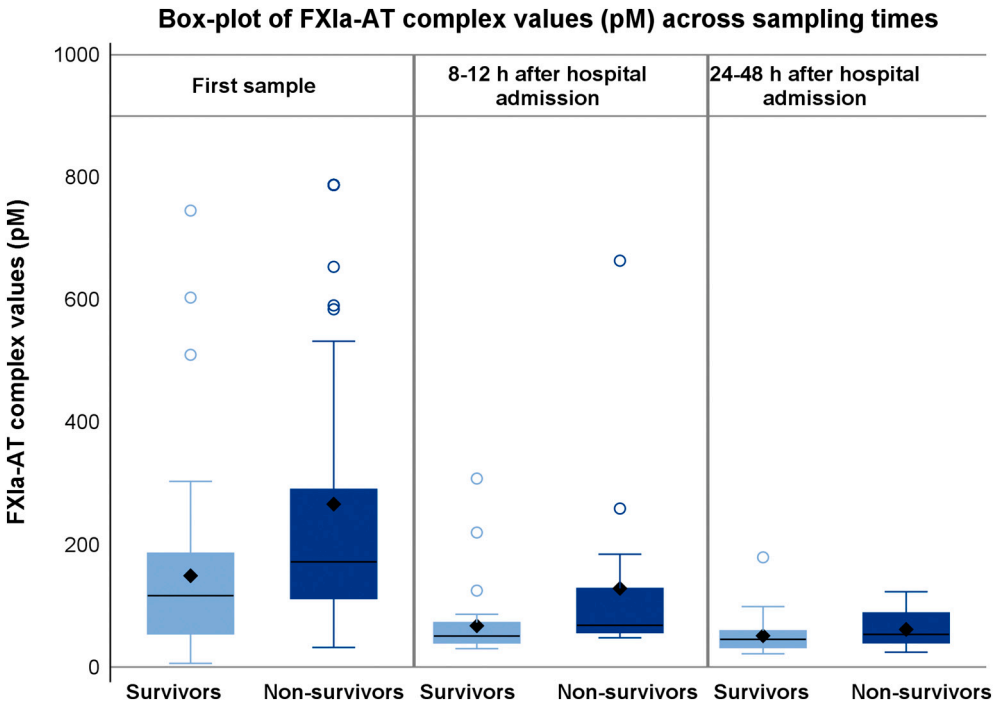

Fig. 2. Boxplot for FXIa-AT complex values stratified according to survival across sampling period. First blood sample (column 1) drawn during or closely after termination of cardiopulmonary resuscitation. Second blood sample (column 2) drawn 8-12 h after hospital admission. Third blood sample (column 3) drawn 24-48 h after hospital admission.

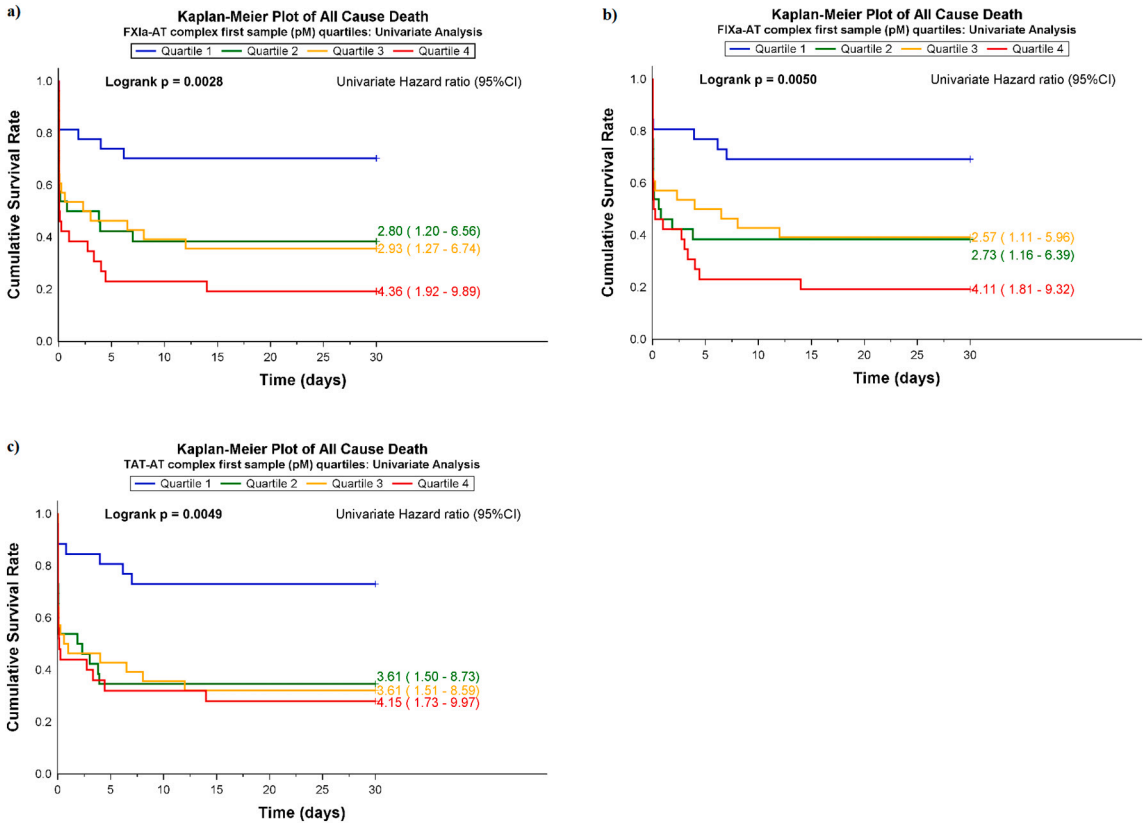

Fig. 3. Survival curves up to 30-days in OHCA-patients stratified by a) first sample FXIa-AT complex quartiles, b) first sample FIXa-AT complex quartiles and c) first sample TAT complex quartiles.

Table 2
Univariate- and multivariable Cox regression models applying quartiles of FXIa-AT complex, FIXa-AT complex and TAT complex.

	Univariate		Multivariable	
	Univariate HR (95% CI)	P-value	Multivariable HR (95% CI)	P-value
Total population				
FXIa-AT complex quartiles		0.006		0.044
Quartile 2	2.80 (1.20–6.56)	0.017	3.02 (1.23–7.41)	0.016
Quartile 3	2.93 (1.27–6.74)	0.012	3.17 (1.30–7.69)	0.011
Quartile 4	4.36 (1.92–9.89)	<0.001	1.95 (0.84–4.52)	0.12
FIXa-AT complex quartiles		0.009		0.18
Quartile 2	2.73 (1.16–6.39)	0.021	2.04 (0.74–5.59)	0.17
Quartile 3	2.57 (1.11–5.96)	0.028	2.42 (0.96–6.11)	0.061
Quartile 4	4.11 (1.81–9.32)	0.001	1.36 (0.54–3.41)	0.51
TAT quartiles		0.011		0.31
Quartile 2	3.61 (1.50–8.73)	0.004	1.99 (0.74–5.35)	0.17
Quartile 3	3.61 (1.51–8.59)	0.004	2.28 (0.87–5.93)	0.093
Quartile 4	4.15 (1.73–9.97)	0.001	1.50 (0.56–4.00)	0.41
Survivors to hospital admission				
FXIa-AT complex quartiles		0.027		0.25
Quartile 2	2.33 (0.58–9.32)	0.23	1.18 (0.26–5.43)	0.83
Quartile 3	5.69 (1.60–20.2)	0.007	2.49 (0.64–9.63)	0.19
Quartile 4	4.70 (1.31–16.9)	0.018	1.18 (0.28–4.94)	0.82
FIXa-AT complex quartiles		0.028		0.17
Quartile 2	6.37 (1.39–29.1)	0.017	3.76 (0.72–19.5)	0.12
Quartile 3	4.07 (0.84–19.6)	0.080	3.76 (0.74–19.0)	0.11
Quartile 4	3.91 (1.24–12.3)	0.020	1.78 (0.37–8.59)	0.47
TAT quartiles		0.091		0.59
Quartile 2	3.47 (0.94–12.8)	0.062	2.69 (0.54–13.3)	0.23
Quartile 3	3.87 (1.03–14.6)	0.046	2.80 (0.54–14.6)	0.22
Quartile 4	5.14 (1.45–18.2)	0.011	2.02 (0.41–9.96)	0.39
VF-patients				
FXIa-AT complex quartiles		0.10		0.34
Quartile 2	2.89 (0.75–11.2)	0.12	3.52 (0.78–15.7)	0.10
Quartile 3	4.48 (1.23–16.3)	0.023	3.29 (0.83–13.1)	0.091
Quartile 4	4.52 (1.24–16.4)	0.022	3.11 (0.73–13.2)	0.12
FIXa-AT complex quartiles		0.079		0.31
Quartile 2	6.64 (1.45–30.4)	0.015	3.15 (0.59–16.8)	0.18
Quartile 3	5.32 (1.13–25.1)	0.035	4.75 (0.91–24.7)	0.064
Quartile 4	7.08 (1.55–32.4)	0.012	2.67 (0.53–13.6)	0.24
TAT quartiles		0.074		0.38
Quartile 2	6.63 (1.45–30.3)	0.015	5.95 (0.73–48.8)	0.097
Quartile 3	5.28 (1.12–24.9)	0.035	6.12 (0.74–50.9)	0.094
Quartile 4	7.30 (1.60–33.4)	0.010	4.83 (0.57–40.5)	0.15

Abbreviations: FIXa-AT, activated factor IX – antithrombin; FXIa-AT, activated factor XI – antithrombin; TAT, thrombin – antithrombin; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 3
Median values of FIXa-AT complex, FXIa-AT complex and TAT complex in OHCA patients with an AMI as compared to control AMI-patients without cardiac arrest.

	OHCA AMI-patients	Control AMI-patients	P-value
FXIa-AT complex (pM)			
First sample	132 (74–209)	52 (41–71)	<0.001
8–12 h	60 (49–78)	50 (43–54)	0.026
24–48 h	48 (41–65)	48 (46–55)	0.99
FIXa-AT complex (pM)			
First sample	306 (221–486)	140 (112–163)	<0.001
8–12 h	191 (146–306)	140 (116–152)	0.001
24–48 h	145 (114–192)	132 (107–143)	0.17
TAT complex (μg/L)			
First sample	54 (22–99)	3 (3–6)	<0.001
8–12 h	9 (5–20)	3 (3–4)	<0.001
24–48 h	5 (4–8)	3 (3–4)	<0.001

Data are presented as medians with interquartile range (25th – 75th percentile). Abbreviations: FIXa-AT, activated factor IX – antithrombin; FXIa-AT; activated factor XI – antithrombin; TAT, thrombin – antithrombin; first sample, sample drawn on scene or at hospital admission; 8–12 h; sample drawn 8–12 h after hospital admission; 24–48 h, sample drawn 24–48 h after hospital admission; OHCA, out-of-hospital cardiac arrest; AMI, acute myocardial infarction.

markers in the presence of cardiac arrest [4,7–9]. In accordance with previous studies [9,16], we found that coagulation activation was more pronounced in non-survivors, with significantly higher TAT-levels in patients who died, as compared to survivors.

Increased thrombin generation is a main pathophysiologic component of disseminated intravascular coagulation (DIC). In patients with cardiac arrest and resuscitation, DIC is associated with multiple organ dysfunction and a poor outcome [4]. Kim et al. [17] reported that an increased DIC-score on admission predicted in-hospital and 6-months mortality in OHCA patients, and Wertz et al. [16] noted that initial TAT-levels were independently associated with survival after resuscitation from cardiac arrest. In our study, we found a significant association between increasing quartiles of TAT-complex and 30-days all-cause mortality in univariate analysis, but the association was attenuated and no longer statistically significant in multivariable analysis. In contrast to the study by Wertz et al. [16], we recruited only OHCA patients, and also included on-scene non-ROSC patients. Moreover, blood sampling was performed closer to the cardiac arrest. Furthermore, our multivariable analysis included duration of resuscitation, witnessed cardiac arrest, bystander-initiated CPR and $\log_e$(creatinine) value, in addition to age, gender and VF as primary heart rhythm. We did not adjust for Charlson Comorbidity index or category of post-arrest illness severity.

Prothrombin is activated by coagulation Factor X which is the common denominator of the extrinsic- and intrinsic coagulation pathways. Its activation results in thrombin formation, which in turn converts fibrinogen to fibrin. Factor X is activated by FIXa in combination with cofactor VIIIa.

As compared to the AMI controls, we found that FIXa-AT complex

levels were significantly elevated in OHCA-patients. FIX may be activated both through the extrinsic- (TF-FVII) and the intrinsic pathway (FXI) [10], and our findings confirm an ongoing general coagulation activation during and in the early phase of cardiac arrest. To our knowledge, this is the first study reporting FIXa-AT complex levels in OHCA-patients. Non-survivors had significantly higher median plasma levels of FIXa-AT complex at all time-points as compared to survivors, and increasing quartiles of FIXa-AT complex were significantly associated with 30-days all-cause mortality in univariate analysis. These findings support previous studies demonstrating increased coagulation activation in non-survivors as compared to survivors following cardiac arrest [4,9]. However, after adjusting for clinical and demographic factors related to cardiac arrest in multivariable analysis, FIXa-AT complex did not reach statistical significance as an independent predictor of all-cause mortality.

OHCA also resulted in early-on initiation of the intrinsic system through FXIa, and we found that FXIa-AT complex levels were independently associated with all-cause mortality at 30-days follow-up. Patients who died on scene had significantly higher levels of FXIa-AT complex compared to survivors at hospital admission, and patients who died during hospitalization had significantly higher plasma-levels of FXIa-AT complex 8–12 h after hospital admission as compared to 30-days survivors.

Coagulation factor XI plays a role in both haemostasis and thrombosis [18,19], and activation of FXI is found to be important for sustained thrombin production and subsequent fibrinolysis inhibition through activation of thrombin-activatable fibrinolysis inhibitor (TAFI) [18,19]. FXI is assumed to play only a modest role in normal haemostasis as severe FXI-deficiency usually is associated with mild and injury-related bleeding [19]. In contrast, FXI may play an important role in thrombosis, as elevated FXI-levels are associated with increased risk of thromboembolic disease, including deep vein thrombosis [20], ischemic stroke [21] and myocardial infarction [19,22]. FXI activation has also been suggested to contribute to the pathogenesis of sepsis with an innate-immune inflammatory response and disseminated intravascular coagulation [4,23], a condition which resembles the post cardiac arrest syndrome [4].

In accordance with previous studies demonstrating an important role of FXI in pathological thrombosis [18,19], we found that FXIa-AT complex levels were markedly increased in OHCA-patients, and that they were independently associated with outcome. This observation may point to a pivotal role of FXIa as a driver of coagulation during cardiac arrest. As noted by others, a substantial fraction of OHCA patients suffer from DIC [4,17], a state of systemic hypercoagulability of which the origin may be obscure. In general, DIC is thought to be triggered by the expression of TF at cell surfaces, induced during the inflammatory state. TF in complex with FVII may directly trigger the extrinsic pathway, and the initial amounts of thrombin formed may back amplify the conversion of FXI to FXIa [24]. Alternatively, or even simultaneously, FXI may be activated by the contact system. Recent work has demonstrated that during a state of systemic inflammation, the contact system was highly engaged [26]. Conceptually, OHCA is a condition in which all systems are maximally active, both the extrinsic and intrinsic system and one of the enzymes that are regulated by both pathways, is FXI. Thus, FXIa could also be an early and sensitive marker for hypercoagulability and a relevant risk indicator in OHCA-patients.

Given the growing evidence suggesting that FXIa plays an important role in thrombosis, a lot of work has been put into the development of inhibitors targeting the factor XI/XIa system [27,28], as this would represent a new generation of anticoagulants which effectively may prevent thromboembolic diseases without life-threatening bleeding risk. In animal models, inhibition of FXI-activation has also been found to be associated with reduced inflammatory and coagulopathic responses and improved outcome in sepsis [23,25]. Our findings of FXI as an early marker of increased coagulation in OHCA patients, may indicate that inhibiting FXIa with one of the currently clinically investigated inhibitor approaches, could be beneficial under these circumstances.

As initially described, we demonstrated generalized coagulation activation through measurements of TAT-complex. We also studied the correlation between the individually measured coagulation factors, and found a positive partial correlation between FXIa-AT complex and FIXa-AT complex and between FIXa-AT complex and TAT complex, reflecting a subsequent activation of coagulation factors during cardiac arrest.

We also demonstrated a statistically significant correlation between admission levels of FXIa-AT complex, FIXa-AT complex and TAT-complex levels and the duration of resuscitation, supporting previous findings of increased thrombin-generation in patients with a longer no-flow- or low-flow time following cardiac arrest [16]. However, there was no statistically significant correlation between the duration of resuscitation and the complex-levels of FXIa-AT, FIXa-AT and TAT in samples drawn on scene, probably due to the fact that samples were harvested during ongoing resuscitation, by which the measured complexes will not reflect the entire resuscitation time.

4.1. Strengths

To our knowledge, this is the first study reporting levels of FXIa-AT complex and FIXa-AT complex in OHCA-patients and relating them to prognosis. Blood samples were collected very early after OHCA and included non-admitted patients without ROSC, a patient category usually missed out in previous studies. Also, the initial cardiac rhythm was recorded in all patients, and we included both patients with asystole and VF as primary heart rhythm. Pre-hospital data were collected in accordance with the Utstein guidelines [12], and advanced cardiac life support was performed by the EMS paramedics according to current guidelines [13]. Levels of on-scene FXIa-AT, FIXa-AT and TAT were largely reproduced in blood samples drawn in survivors at hospital admission, indicating that the conditions related to the on-scene blood sampling may not have influenced the measurements. Furthermore, we performed multivariable Cox regression analysis adjusting for demographic- and clinical parameters closely related to outcome following OHCA.

4.2. Limitations

The small study population is one of the limitations. Inclusion of patients was restricted to the largest ambulance centres in the area located closest to the hospital and to the medical support helicopter, limiting the potential recruitment area. Furthermore, patient recruitment could only be performed when there was enough EMS crew present at the OHCA-scene. There was an unbalanced blood sampling in the ROSC and non-ROSC group of patients. Due to challenging conditions during CPR, blood sampling may result in coagulation activation, which also may occur during the storage conditions prior to processing. However, on-scene results were largely reproduced in admission samples obtained from survivors under strict conditions. A few patients lacked detailed information regarding the OHCA. There may be factors related to coagulation activation and survival following cardiac arrest that we have not been aware of and have not adjusted for in our analysis. Due to the lack of a complete medical history for 25 patients, we were not able to adjust for antiplatelet therapy and the use of anticoagulants in the multivariable analysis. We adjusted for seven variables, known to be associated with outcome following OHCA, in our multivariable Cox regression analysis, both for the total population and the smaller subgroups. As our study is an exploratory study, we applied a significance level of 5% without multiplicity adjustment. The highest quartile contained some non-discriminatory out-of-range measurements, due to analytic limitations, which may have influenced the results pertaining to this quartile.

5. Conclusions

Initial levels of FXIa-AT complex, but not FIXa-AT complex and TAT-complex, were independently associated with 30-days all-cause mortality in OHCA patients.

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CRediT authorship contribution statement

R. Aarsetøy; investigation, formal analysis, writing – original draft. H. ten Cate; resources, writing – review and editing. H. Spronk; resources, writing – review and editing. R. Van Oerle; resources, writing – review and editing. H. Aarsetøy: methodology, investigation, writing – review and editing. H. Staines; formal analysis, writing – review and editing. DWT. Nilsen; conceptualization, methodology, writing – review and editing.

All authors read and approved the final manuscript.

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Availability of data and materials

Local database. The datasets analysed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

Dr. ten Cate reports grants from Bayer, grants from Pfizer, other from Alveron (consultancy), other from Coagulation Profile (shareholder), outside the submitted work.

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