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Research Article

Role of Conjunct Thromboelastometry and Aggregometry for Identification of Clotting Abnormalities and Early Severity Stratification in the Emergency Department in Patients with Sepsis: A Prospective Study

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Abstract

Objective: Sepsis and septic shock are frequently observed in the Emergency Department (ED). Early identification of sepsis is a prime target for optimal treatment. Inflammation and coagulation are closely linked and early alterations of the clotting system and platelets, with major thrombotic and bleeding complications, may be distinctive of sepsis. Thrombelastometry (Rotem®) and Impedance Aggregometry (Multiplate®) are Point-of-Care (POC) technologies with a potential diagnostic value in critically ill patients. The former is a viscoelastic method able to quickly assess the whole plasmatic coagulation process according to different activation pathways; the latter evaluates global platelet function in response to different stimuli. The application of these POC tests in the ED may be helpful in defining peculiar coagulation profiles in patients with sepsis, increasing diagnostic effectiveness and speed and identifying high risk patients.

Methods: In this prospective observational study conducted in the ED we enrolled 40 patients with clinical diagnosis of sepsis, 38 with septic shock and 31 control patients. Rotem® tests (Extem, Intem, Fibtem and Aptem) and Multiplate® tests (ASPI, ADP, TRAP, COL and RISTO test) were performed at first presentation, together with traditional laboratory exams and clinical scores. Controls were compared to Sepsis (S) and Septic Shock (SS) patients.

Results: Significant differences were observed in Clotting Times (CT) among controls, sepsis and septic shock populations ($p<0.05$). Analysis of Maximum Clot Firmness (MCF) displayed significant differences in Extem and Fibtem ($p<0.05$). Rapidity of clot formation (alpha angle) was also significantly increased in patients with sepsis and septic shock. Multiplate® analysis demonstrated a widespread tendency to hypo-aggregability in subjects with sepsis/septic shock, via ADP, COL and TRAP tests ($p<0.05$). Diagnostic accuracy of POC tests in discriminating sepsis was adequate and far superior to some classical laboratory variables and clinical scores. In this study POC tests did not associate with mortality.

Conclusions: Early alterations of the coagulation system and global platelets hypo-aggregability were observed with POC tests in patients with sepsis and septic shock since the first phases of the disease during ED presentation. These modifications draw a peculiar coagulative profile able to identify sepsis and its complications with good accuracy.

Keywords: Coagulopathy; Diagnosis; Impedance Aggregometry; Sepsis; Thromboelastometry

Introduction

Sepsis and septic shock, are frequent in the Emergency Department (ED) and still deserve great attention due to morbidity and mortality rates [1]. Sepsis definition was until recently based on the presence of suspected infection and Systemic Inflammatory Response Syndrome (SIRS) criteria and is more than 20 years old now [2]. This approach, however, has been criticized because of low specificity and unclear sensitivity [3-5], and a recent retrospective study demonstrated that SIRS criteria failed to identify one in eight septic patients [6]. SIRS can moreover have non-infectious causes such as trauma or burns and sometime differential diagnosis can be extremely challenging [7]. In particular among patients in the ED almost 40% of those with SIRS-positive severe sepsis have an infection, but about 20% of those with SIRS-negative have severe sepsis [8]. Recently published new definitions [9], based on epidemiologic studies and clinical trials, suggest to replace the concept of SIRS in order to facilitate earlier recognition and more timely management of patients, but this concept still needs to be fully endorsed by the Scientific Community and presents some limitations too. The Sequential Organ Failure Assessment (SOFA) score is in fact elevated in many patients presenting to the ED and could difficultly be used as a screening tool in this context. Its quickest version, the qSOFA, has been proposed as an alternative screening tool, and in the ED, it seems to predict organ failure and mortality in patients with suspected infection with superior predictive ability than SIRS. However, low sensitivity was described for the qSOFA too, and further confirmatory tests for organ failure are needed [10]. In another large-scale study among patients presenting to the ED with suspected infection the use of qSOFA resulted in greater prognostic accuracy for in-hospital mortality [11]. Sepsis is still a leading cause of mortality, with high related costs. All the scoring systems currently in use have significant limitations in specific conditions and we are still missing valid prognostic instruments in order to modulate our approach.

Biomarkers, such as procalcitonin (PCT), have long been used in the critical care setting to diagnose sepsis and to guide antimicrobial therapy, with some left limitations [12]. We know from previous studies that inflammation and coagulation are closely linked [13]. Systemic infection itself activates the coagulation pathway, from a mild pro-thrombotic state to overt Disseminated Intravascular Coagulation (DIC). The coagulation system may in turn influence the inflammatory response, partly contributing to the pathogenesis and outcome of sepsis. Thrombocytopenia is frequently associated with sepsis and may be one determinant of poor outcome [14]. Platelet aggregation appears to be notable in inflammation, but precise mechanisms still need to be defined: some studies describe enhanced aggregation in presence of infection [15], some others show decreased platelet activity during severe sepsis [16]. Assessment of coagulation in septic patients may be complex: recent evidences suggest that traditional tests like Activating Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) may have limited accuracy in vivo.

Point-of-Care (POC) thromboelastometry (Rotem®) evaluates whole-clot formation and dissolution and could be promising in diagnosing clotting alterations during sepsis [17]. Previous studies conducted on Intensive Care Unit (ICU) patients with severe sepsis and septic shock demonstrated a correlation between thromboelastometry alterations and the presence of sepsis, rate of organ dysfunction, incidence of DIC and 30-day survival [18,19]. Viscoelastic and aggregometric POC tests (e.g. Multiplate®) are able to measure platelets aggregation in response to different stimuli and are potentially useful for bedside diagnosis of sepsis: platelets aggregation was reduced in ICU patients during severe sepsis and correlated with survival rates [20].

Early identification of sepsis is a prime target for optimal treatment [21]. The application of coagulation POC tests may increase diagnostic effectiveness in high risk patients since their first presentation to the ED. In this work we report the results of a prospective study conducted in the ED on adult patients presenting with clinically suspected sepsis or septic shock to investigate diagnostic role, efficacy and prognostic power of Rotem® and Multiplate® in comparison to a control population. We hypothesized that POC tests can define a peculiar coagulation profile helpful to facilitate the diagnosis of sepsis.

Patients and Methods

Patients and Study Design

We conducted a cohort, prospective, observational study on patients with at least 2 SIRS (Systemic Inflammatory Response Syndrome) criteria: study group was composed by patients for whom sepsis was suspected as the primary diagnosis, control group by patients with major trauma (Injury Severity Score - ISS> 15). The study was performed in the main Medical ED and in the Major Trauma Center of Turin University Hospital, Italy. One hundred and nine patients were enrolled. Inclusion criteria were presence of at least two among: Temperature >38°C or < 36°C; Heart Rate > 90'; Respiratory Rate > 20'; Systolic blood pressure <90 or drop ≥ 40 mm Hg of normal; clinically suspected diagnosis of sepsis or septic shock (defined as hypotension with vasopressors requirement to maintain MAP ≥65 mm Hg; serum lactate level >2 mmol/L) [2,21]; major trauma (ISS>15) with positive SIRS criteria and trauma team involvement; patients who could undergo a blood test. Exclusion criteria were: age less than 18 years old, incapability

to give informed legal consent and evidence of a clear different diagnosis. The study was approved by the Ethics Committee of Turin University Hospital according to the Helsinki Declaration (1964). Participants provided their written or verbal (when written was not feasible due to clinical priorities) informed consent to participate in the study. Consent was recorded on ad hoc forms, approved by the Ethics Committee. Forty patients with suspected sepsis, 38 with septic shock and 32 severe trauma patients were consecutively included in the study. Clinical parameters, laboratory values, Rotem® and Multiplate® tests were recorded at first presentation in the ED. Controls (C) were compared to sepsis (S) and septic shock (SS) patients; the former group was furthermore compared to overall septic population (S + SS). Blood samples were collected in the ED before any medical treatment in hospital and sent for analysis to the main Clinical Chemistry laboratory of the Institution. Initial SOFA score and Simplified Acute Physiology Score II (SAPS II) score were calculated. Definitive diagnosis, in-hospital length of stay and survival rates were obtained in the aftermath from clinical records. Thirty-day in hospital-mortality rates were blindly matched to biomarkers values.

Measurement Methods

Thromboelastometry

Thromboelastometry was used to assess whole-blood coagulation [22]. Citrated blood samples were placed into a disposable cuvette using an electronic pipette and analyzed few minutes after collection by using preconstituted single-use Rotem® kits according to the manufacturer's recommendations (TEM International GmBH, München, Germany). For each sample 4 tests were simultaneously performed: Extem (with Tissue Factor and $CaCl_2$), Intem (ellagic acid and $CaCl_2$), Fibtem (Extem plus cythocalasin D) and Aptem (Extem plus aprotinin). The samples were run for about 30'; Clotting Time (CT), Clot-Formation Time (CFT), Maximum Clot Firmness (MCF), alpha angle, and 30-minute lysis index were determined and recorded.

Impedance aggregometry

Multiplate® was used for platelets function tests [23]. Aggregation analysis was performed simultaneously with Rotem®. 300 µl saline and 300 µl of citrated whole blood were added to the test disposable cell according to the manufacturer´s recommendations (Cobas, Roche Diagnostics, Mannheim, Germany). After three minutes of incubation at 37°C, samples were activated with arachidonic acid (ASPI test), Adenosine Diphosphate (ADP test), Collagen (COL test), Thrombin Receptor Activating Peptide 6 (TRAP test) and Ristocetin (RISTO test) preconstituted kits. The tests were run simultaneously for six minutes and platelet aggregation was defined by the area under curve in arbitrary units (U).

Statistical analysis

We designed the study as a proof of concept, therefore sample size calculation was avoided, but a size effect of 2.32 with an alpha of 0.5 and a power of 0.8 were estimated.

We performed non-parametric tests, based on data distribution, in order to evaluate differences among each group at the first evaluation in the ED. Receiver Operating Characteristics (ROC) curves were used to assess diagnostic accuracy of POC parameters. Kaplan-Meyer analysis was applied to evaluate the prognostic efficacy in 30-day mortality prediction. A P-value <0.05 was considered significant. Statistical tests were performed using MedCalc® statistical software version 10.0.1.0 (MedCalc Software, Acacialaan 22, B-8400 Ostend, Belgium), Graph Pad prism® 5.02 software and STATA 11.0

Results

Patient Characteristics

A total of 109 patients were enrolled. Thirty-one of them presented with SIRS related to trauma (Median ISS 23.20) and were included in the control group. Forty patients were diagnosed with suspected sepsis and 38 had septic shock. Demographic characteristics, SOFA and SAPS II score median values at presentation are reported in Table 1. Nine patients in the sepsis group, 8 in the septic shock group and none in the control group were receiving anticoagulants at enrollment; two controls, 9 sepsis and 11 septic shock patients were antiaggregated.

Clinical Variables	Controls (C)	Sepsis (S)	Septic Shock (SS)	P-Value
Number of patients	31	40	38	
M/F	23/08	23/17	25/13	
Age Median (Range)	45 (18-81)	64.5 (18-95)	73.5 (35-98)	<0.05[1]
SOFA Score Median (Range)	3 (0-15)	2 (1-11)	6 (2-16)	>0.05[1]
SAPS II Score Median (Range)	34 (14-69)	33 (12-53)	48 (23-76)	>0.05[1]
Length of Admission Median (Range)	17.5 (1-45)	12 (1-60)	18 (5-84)	<0.05
Abbreviations: M: Males; F: Females; 1 According to Kruskal Wallis test				

Table 1: Clinical variables of patients in the three groups at presentation in the Emergency Department.

Main laboratoristic variables were tested and the majority of them are summarized in Table 2. White blood cells count at presentation was higher in the control group with respect to S and SS patients; hemoglobin and platelets count did not significantly differ in the three groups. Traditional clotting tests aPTT and Antithrombin III (AT III) were within normal ranges in the three groups, with minor differences in the former; INR differences did not result significant when patients with warfarin overdosage (three patients in the SS group) were excluded from analysis; fibrinogen was higher in the S and SS population, while D-dimer resulted increased in controls, as expected in patients presenting with major trauma. Differences in Base Excess (BE) and lactate levels were significant, but could not distinguish between controls and SS group. Mean serum creatinine levels were elevated in the SS group, probably due to older median age, more comorbidities and absence of fluid treatment in the pre-hospital phase. PCT demonstrated a high diagnostic accuracy, as previously reported [12]: levels were higher in the SS groups, with rare false positive in the control group.

Laboratoristic Variables	Controls (C)	Sepsis (S)	Septic Shock (Ss)	P-Value [1]
WBC 10^6/L , median (range)	18200 (5900-33500)	9320 (2200-32190)	11980 (200-68300)	<0.05
Hb g/dL, mean (s.d.)	13.02 (2.5	11.74 (0.33)	11.84 (0.37)	=0.05*
PLTS 10^6/L, median (range)	212000 (35000-471000)	200000 (21000-725000)	175000 (14000-643000)	NS
INR, median (range)	1.07 (0.93-1.43)	1.07 (0.92-2.5)	1.31 (0.97-6.63)	NS[2]
aPTT Ratio, median (range)	0.85 (0.69-1.2)	1.07 (0.92-2.5)	1.04 (0.82-2.06)	<0.05
Fibrinogen mg/dL, median (range)	248 (144-412)	581 (152-1054)	529.5 (90-826)	<0.05
AT III %, median (range)	92 (48-122)	89 (30-121)	81.5 17-124)	NS
D-Dimer µg/ml, median (range)	13.6 (0.24-40)	1.7 (0.8-19.7)	3.55 (0.7—40)	<0.05
BE, median (range)	-3 (-11.5-5.7)	0.1 (-17.2-5.4)	-3 (-21.1-4.7)	<0.05
Lactate mmol/L, median (range)	2.35 (0.8-8.1)	1.35 (0.6-3.8)	3.7 (0.9-12.5)	<0.05
Serum Creatinine mg/dL, median (range)	0.97 (0.65-1.92)	0.89 (0.46-6.89)	1.61 (0.48-7)	<0.05
PCT ng/mL, median (range)	0.05 (0.2-3.26)	0.35 (0.11-88.9)	3.07 (0.14-139)	<0.05

Controls (C) were compared to sepsis (S) and Septic Shock (SS) patients; the former group was furthermore compared to overall septic population (S + SS). Abbreviations: WBC: White Blood Cells; Hb: Hemoglobin; PLTS: Platelets; AT III: Antithrombin III; BE: Base Excess; PCT: Procalcitonin.

*According to ANOVA.

[1]According to Kruskal Wallis test.

[2]Patients with markedly altered INR (>4) due to warfarin over dosage were excluded from thromboelastometric analysis.

Table 2: Main laboratoristic variables of patients in the three groups at presentation in the Emergency Department.

Thromboelastometry Variables

Results in groups C, S and SS were within the normal ranges provided by a previous study conducted on a normal population with no clotting abnormalities [21].

However, in comparison to controls, S and SS showed increased CTs in all tests performed; CFT was longer in controls with respect to patient with sepsis and septic shock. MCF showed a diffuse increase in patients with S and SS, in particular with Extem and Fibtem tests. Alpha angle was also augmented in the septic population with Extem, Intem and Aptem tests. Table 3 shows all the numbers in details. Thirty-minutes lysis indexes (data not shown) did not significantly differ among the three populations.

Thromboelastometric Variables Median (Range)	Test	Controls (C)	Sepsis (S)	Septic Shock (SS)	Total Septic Patients (S+Ss)	P-Value*
CLOTTING TIME (CT) (seconds)	EXTEM	49 (29-106)	58 (34-94)	65 (34-226)	56 (29-226)	<0.05
	INTEM	144.5 (99-211)	160 (99-264)	160.5 (128-206)	155 (99-206)	<0.05
	FIBTEM	54 (41-81)	59 (31-171)	66 (46-406)	58 (31-406)	<0.05
	APTEM	49 (43-83)	57 (37-97)	61 (26-362)	55 (26-362)	<0.05
CLOT FORMATION TIME (CFT) (seconds)	EXTEM	126 (72-185)	60 (30-242)	66 (29-558)	77.5 (29-558)	<0.05
	INTEM	77.5 (44-210)	58 (30-220)	63 (30-518)	65 (30-518)	<0.05
	APTEM	126 (65-203)	53 (24-296)	69.5 (27-462)	77 (24-462)	<0.05
MAXIMUM CLOT FIRMNESS (MCF) (millimiters)	EXTEM	57 (39-71)	70 (42-81)	70 (10-82)	65 (10-82)	<0.05
	INTEM	62 (52-75)	70 (41-80)	65 (5-82)	66 (5-82)	<0.05
	FIBTEM	10 (4-21)	29 (9-68)	25 (4-43)	23 (4-68)	<0.05
	APTEM	58 (45-72)	69 (39-83)	65 86-82)	65 (6-83)	<0.05
ALPHA ANGLE (α) (degrees°)	EXTEM	69 (57-80)	81 (57-86)	80 (28-85)	77 (28-86)	<0.05
	INTEM	74 (52-81)	79 (56-84)	78 (37-84)	77 (37-84)	<0.05
	APTEM	68 (54-78)	81 (47-83)	80 (34-85)	79 (34-87)	<0.05
Controls (C) were compared to sepsis (S) and Septic Shock (SS) patients; the former group was furthermore compared to overall septic population (S + SS). *According to Kruskal Wallis test						

Table 3: Main thromboelastometric variables in the three groups at presentation in the Emergency Department.

Impedance Aggregometry Variables

Reduced platelet aggregation was shown with three of the five activators in patients with S and SS when compared to controls (Figure 1). Arbitrary Units (U) recorded with ADP test were within normal values (according to the producer) in controls and S patients, but severely reduced in patients with SS. TRAP test showed analogous results, with significant differences between SS and controls, and between the former and S patients too. COL test demonstrated markedly reduced aggregability in both patients with S and patients with SS, in comparison with controls. ASPI and RISTO tests showed aggregation curves within normal ranges, with no significant differences between the three populations, Diagnostic accuracy, ROC curves and prognostic role

AGGREGOMETRIC VARIABLES Median (Range)	CONTROLS (C)	SEPSIS (S)	SEPTIC SHOCK (SS)	TOTAL SEPTIC PATIENTS (S+SS)	p-VALUE*
ADP-TEST (U)	75.2 (12.8-221.7)	53.3 (15.9-163.8)	38.2 (3-212.7)	54.5 (3-221.7)	<0,05
ASPI-TEST (U)	95.25 (13.2-216.5)	61.3 (8.1-183.3)	68.1 (8.4-233)	78.6 (8.1-233)	NS
COL-TEST (U)	66.4 (4.9-243)	38.1 (10.4-170.8)	29.3 (2.1-97.3)	40.6 (2.1-243)	<0,05
TRAP-TEST (U)	94.7 (12.1-210.3)	83.7 (6.5-80.4)	52.7 (2.8-162)	79.7 (2.8-210.3)	<0,05
RISTO-TEST (U)	14.9 (0-52.6)	11.7 (4.3-75.6)	13.6 (0-42.5)	12.7 (0-75.6)	NS

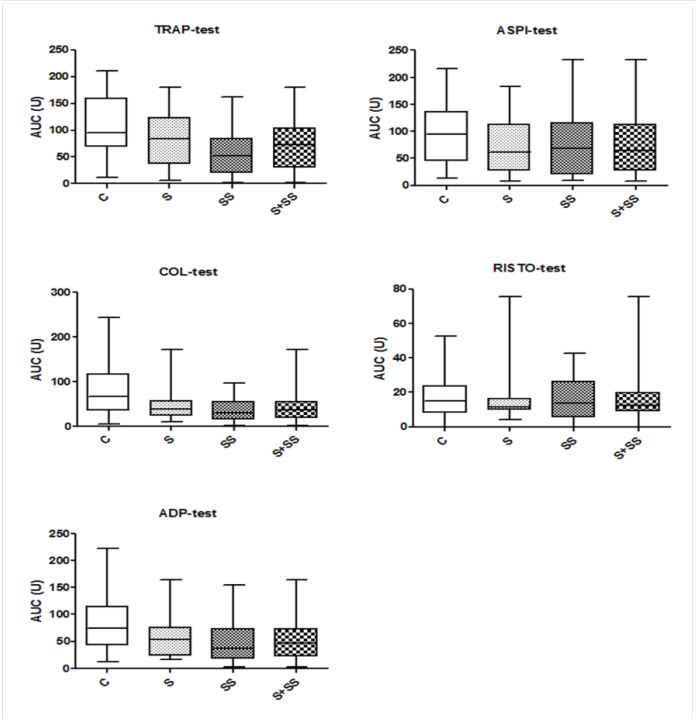

Figure 1: Median (box-plots) values of aggregometric variables in controls, sepsis and septic shock populations at first evaluation in the ED. In the table mean values and Standard Errors (S.E.) of the different tests are reported. Controls (C) were compared to Sepsis (S) and Septic Shock (SS) patients; the former group was furthermore compared to overall septic population (S + SS). Abbreviations: AUC: Area Under the Curve; U: Arbitrary Units, NS: Non-Significant. *According to Kruskal Wallis test.

Figure 2 shows ROC curves, in order to assess discrimination between C, S and SS groups. All Rotem® and Multiplate® parameters show significant discrimination between groups ($p<0.05$). This indicates that thoromboelastometry and whole blood impedance aggregometry are potentially able to differentiate between these conditions. Areas under the curve (AUC) and optimal cut-off points (related to best sensitivity and specificity) are reported in the table. CT, MCF and CFT both in Extem and Intem displayed good diagnostic accuracy; aggregometric tests using ADP, COL, TRAP as activators were also significant. The conventional biomarker PCT demonstrated the most significant discriminatory power (AUC 0.95); SOFA and SAPSII scores (data not shown), together with lactate plasmatic levels were associated with non-significant curves.

ROC	AUC	SE	95% CI	CUT-OFF	p-VALUE
LACTATE	0,50	0,08	0,39-0,64		NS
SOFA	0,55	0,06	0,43 – 0,65		NS
SAPS-II	0,56	0,06	0,46 – 0,66		NS
PCT	0,95	0,02	0,88-0,99	<0,12	<0,05
E-CT	0,72	0,06	0,62-0,8	>53	<0,05
I-CT	0,73	0,05	0,63-0,82	>157	<0,05
E-MCF	0,76	0,05	0,66-0,84	>67	<0,05
I-MCF	0,67	0,06	0,57-0,77	>69	<0,05
E-CFT	0,84	0,05	0,74-0,9	<70	<0,05
I-CFT	0,71	0,06	0,61-0,8	<65	<0,05
ADP-test	0,7	0,07	0,59-0,8	<=38,2	<0,05
COL-test	0,74	0,06	0,64-0,83	<=56	<0,05
TRAP-test	0,67	0,07	0,55-0,76	<=75,2	<0,05

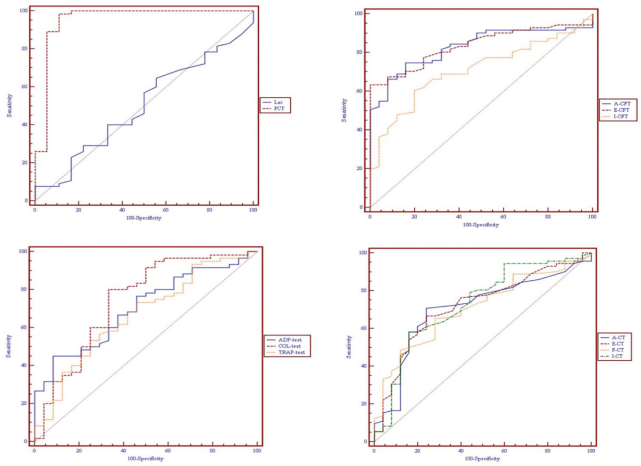

Figure 2: Receiver Operating Characteristic (ROC) curves for thromboelastometric (E-CT, Extem clotting time; I-CT, Intem clotting time; E-MCF/I-MCF, Extem and Intem maximum clot firmness; E-CFT/I-CFT, Extem and Intem clot formation time) and aggregometric (ADP, adenosine diphosphate; COL, collagen; and TRAP, thrombin receptor activating peptide test) variables. Results are reported in the table as area under the curve (AUC), Standard Error (SE), 95% confidence intervals (95%CI) and cut-offs with best sensitivity and specificity for discrimination between septic (sepsis, severe sepsis and septic shock) and non-septic (trauma) populations. Conventional biomarkers reported are procalcitonin (PCT) and lactate.

Thirty-day mortality was significantly higher in the SS group in comparison to C and S (31.6% vs 3.2 and 2.5%, respectively). A diffuse tendency to platelet aggregation reduction was noted in the 30-day mortality group, in particular using COL agonist, but the differences between survivors and non-survivors were not statistically significant; thoromboelastometric variables were indeed more altered in the non-survivors group, but also in this case without significant differences (data not shown).

Discussion

The conjunct role and utility of coagulation and platelets function POC tests have so far not been investigated and established in the ED. In this observational prospective study, we evaluated the association of thromboelastometry and impedance aggregometry in defining a peculiar coagulative picture in patients presenting to

the ED with sepsis or clinical SIRS due to different conditions.

Sepsis may lead to severe alterations of platelets and hemostasis [13] and previous studies reported a potential role for coagulation POC tests in patients with sepsis, severe sepsis or septic shock. Majority of thromboelastometry studies were conducted on ICU patients with an established diagnosis and results are so far controversial: some authors reported peculiar alterations in septic patients [18-19]; while some others revealed an overall normo-coagulative state [25]. Impedance aggregometry showed reduced platelets aggregability in patients with sepsis and related complications, with higher morbidity and mortality in these groups of patients [20,26].

Severe trauma is another potential cause of systemic inflammation and may be associated with peculiar coagulation dysfunctions. Trauma-induced coagulopathy is an imbalance of the dynamic equilibrium between procoagulant factors and systemic anticoagulation, with systemic activation of Protein C pathway and hyperfibrinolysis [27]. Many studies demonstrated that thromboelastometry is feasible in early trauma coagulopathy and can eventually guide specific treatment [28]. Platelet function seems to be impaired too, but mechanisms still need to be elucidated and application of aggregometry in trauma patients is very limited.

In our prospective study the diagnostic role and accuracy of coagulation POC tests Rotem® and Multiplate® were evaluated in critical ED patients. Enrolled subjects presented with SIRS criteria and clinical suspicion of infection (with or without signs of unresponsive hypotension or systemic hypoperfusion) or with severe trauma. The choice to enroll as controls major trauma patients was done on purpose and due to the fact that the condition of systemic inflammation in those patients was definitively not due to disseminated infection in the initial phases, representing therefore an adequate comparison for groups with systemic inflammation secondary to sepsis. Definitive diagnosis was made according to the retrospective analysis of clinical records. Severe sepsis was not mentioned and considered as a unique group with septic shock, due to similar clinical and prognostic features and according to new definitions [9]. Further analysis compared controls to overall population with infections (S and SS). The three groups in the study were homogeneous in terms of size and clinical characteristics. Control trauma patients were younger, as expected, and patients with sepsis and septic shock had more comorbidities and concurrent treatment. Traditional coagulation tests APTT and PT resulted slightly prolonged in patients with severe sepsis, but this was certainly due to concurrent treatment with anticoagulants and some few cases of over-dosage, which were excluded from POC analysis in order to eliminate this bias.

Thromboelastometric variables were within normal values in ED patients with trauma, sepsis and septic shock, but some peculiar differences were observed between the groups. In patients with sepsis and septic shock there was a significant prolongation of Clotting Time in all the assays performed. The possible misleading effect of concurrent anticoagulant treatment was eliminated by the exclusion of patients with markedly altered traditional coagulation tests (INR, aPTT) from further analysis. Clot Formation Time was shortened in patients with sepsis and septic shock in comparison to controls. These values are generally influenced by platelet function, fibrinogen and coagulation factors. In our study Maximum Clot Firmness resulted amplified in patients with sepsis and septic shock in comparison to controls: Fibtem test results suggested that increased circulating levels of fibrinogen were one of the possible reasons. Analysis of Lysis Index (the percentage of remaining clot stability in relation to the MCF) was limited to 30 minutes after CT in order to evaluate a real POC and reduce the time of test performance: no cases of hyperfibrinolysis were observed in the three groups.

Multiplate® showed reduced platelets aggregability in patients with sepsis, severe sepsis and septic shock. Previous studies [20,26] had described this phenomenon, but reduced aggregation was imputed to endothelial alterations and platelets consumption due to an increased activation during the hypercoagulable state. In this study observed platelets hypoaggregability was an extremely precocious phenomenon, present since the very first phases of the disease in the ED: intrinsic platelets dysfunction or circulating inhibitory mediators are therefore a potential alternative explanation, rather than hypercoagulation and consumption phenomena. Thrombocytopenia and concurrent use of anti-platelets drugs like aspirin (as confirmed by ASPI test) had non-determinant roles. These tests demonstrated differences between patients with S with respect to those with SS, suggesting a possible inverse relationship between platelet function and disease severity.

The ROC curves showed an adequate diagnostic accuracy for the thromboelastoemetric and aggregometric variables examined. The widest AUC was anyway calculated for PCT, confirming the role of this biomarker in early identifying sepsis and its complications. Analysis of 30-day mortality showed no differences in Rotem® or Multiplate® values between survivors and non-survivor septic patients. General mortality in the SS group was higher (about thirty percent), but thromboelastometry and impedance aggregometry were probably analyzed too early to provide any correlation, like observed instead in previous studies conducted in different settings [18-20,26].

Limitations

Among the potential problems of this study we must mention heterogeneity of enrolled population, concurrent treatment and comorbidities, but this represents a true “real-life” scenario in the ED. The control population was extremely variegated and

composed by some patients with monocompartimental but very severe injuries (like isolated brain injury) and some others with proper polytrauma. Wider study population and sub-groups analysis would probably be required in future studies to outline differences due to comorbidities, medications and mechanisms of injury. Our sample size was big enough to detect significant differences among groups (as detailed in the statistical analysis section), also if larger prospective studies would be useful. A very large panel of tests was performed with multiple comparisons, and all the available assays for Rotem® and Multiplate® were used in this study, with potential limitations due to cost-effectiveness, necessity of trained staff and potential problems with analysis: nevertheless this was an intentional choice aimed to give the most complete picture possible of the role of available coagulation POC tests: future studies should focus on significant values suggested by this work.

Finally, our analysis was purposely limited to the ED, in order to investigate the early role of these POC technologies in discriminating sepsis from other non-infectious conditions: it is therefore not possible to clarify if any of the patients in the suspected sepsis group progressed to have septic shock and if an association with POC abnormalities was present. This could be the object of future studies.

Conclusions

This study represents the first attempt of a joined application of POC coagulation tests in patients with suspected sepsis and correlated clinical conditions. This is furthermore the first study where thromboelastometry and impedance aggregometry were tested in patients during early presentation to the ED.

Thromboelastometric variables resulted within normal values in all groups, but some significant differences outlined peculiar coagulative panels. Patients with sepsis and septic shock displayed prolonged CT, reduced CFT and increased MCF in comparison to controls. Global platelets aggregability resulted significantly reduced in patients with sepsis and in particular with septic shock since their very first presentation to the ED, with a potential correlation with severity and suggesting a mechanism of early intrinsic platelets dysfunction, which should be better elucidated.

Though further studies are required, this work highlights the potential role of readily available coagulation POC tests in promptly identifying peculiar alterations in patients with sepsis and septic shock in the ED, supporting treatment celerity and appropriateness.

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