Clinical value of automated fibrin generation markers in patients with septic shock: a SepsiCoag ancillary study

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Summary

An ancillary analysis to the SepsiCoag multicentric prospective observational study on patients entering an intensive care unit with septic shock evaluated the prognostic potential of fibrin generation markers (FGMs) tested at inclusion in the study, on survival at day 30. After centralization of samples, three automated FGMs were compared: D-dimers (DDi), fibrin/fibrinogen degradation products (FDP) and fibrin monomers (FM). FM was the single FGM that was significantly higher in non-surviving patients, area under the receiver-operator characteristic curve (AUC_{ROC}): 0·617, $P < 0·0001$. Significantly higher International Society on Thrombosis and Haemostasis Disseminated Intravascular Coagulation (ISTH DIC) scores were calculated in non-survivors using each of the three FGMs. A dose-effect relationship was observed between ISTH DIC scores and non-survival, with highest significance obtained using FM as the FGM. An overt DIC diagnosis using the ISTH DIC score calculated using FM was a predictor of non-survival at day 30, independently from overt DIC diagnosis based on scores calculated using FDP or DDi. The AUC_{ROC} values testing the ability of the ISTH DIC score to predict non-survival were 0·650, 0·624 and 0·602 using FM, DDi and FDP, respectively, as the FGM. In patients with septic shock, among the commercially-available automated assays, automated FM is the FGM best related with late prognosis.

Keywords: septic shock, coagulation, fibrin monomers, D-dimers, DIC score.

Sepsis is a potentially life-threatening complication of infection, and is almost always associated with some acquired coagulation abnormalities secondary to a systemic activation of the haemostatic system (Levi & van der Poll, 2017). These coagulation abnormalities can range from subtle laboratory signals that can only be detected by specialised assays, classic laboratory features associating fall in platelet counts and increase of global clotting times, to disseminated intravascular coagulation (DIC) syndrome, which is characterised by a widespread microthrombogenesis. DIC could precipitate hypoxia-induced organ failure and fuel DIC consumption of coagulopathy components, with its related haemorrhagic risk.

Septic shock is the most severe form of sepsis, which previously referred to a state of acute circulatory failure associated with infection, but was recently re-defined as a subset of

sepsis in which underlying circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone (Singer *et al*, 2016). Recent results confirm that elevated levels of endothelial cell biomarkers of haemostasis and permeability are associated with increased mortality (Hou *et al*, 2017). Endothelial dysfunction is also central to DIC, which may complicate septic shock. The main pathophysiological mechanisms are inflammatory cytokines-initiated activation of tissue factor-dependent coagulation, insufficient control of anticoagulant pathways, and plasminogen activator inhibitor-1 (PAI-1, now termed serpin family E member 1, SERPINE1) mediated suppression of fibrinolysis (Gando *et al*, 2016).

The diagnosis of DIC is always made in the context of the underlying clinical condition, typically in the setting of an acute clinical deterioration, as is the case for septic shock in septic patients (Levi *et al*, 2009; Gando *et al*, 2016). The diagnosis of DIC is complex, as no single coagulation test can act as a gold standard, but nonetheless, it is well validated that the degree of abnormality in global coagulation tests has pathogenetic relevance in indicating the degree of multiple organ failure and the likelihood of death. The International Society on Thrombosis and Haemostasis (ISTH) thus established a DIC diagnostic scoring system consisting of global haemostatic test parameters, which has now been validated in diverse clinical settings, in which a score of ≥ 5 is indicative of overt DIC (Taylor *et al*, 2001). Among the four test results used to calculate the ISTH scoring criteria for DIC are the plasma levels of fibrin generation markers (FGMs), i.e., circulating fibrin monomers (FM), fibrin/fibrinogen-degradation products (FDP) and D-dimers (DDi). However, these three FGMs have different characteristics: FM generation only depends on active thrombin generation whereas FDP and DDi need thrombin generation followed by active plasmin-mediated proteolysis, in a setting where PAI-1-mediated inhibition of plasmin generation is the rule (Gando *et al*, 2016).

We studied a large prospective multicentre cohort (SepsiCoag study, unpublished observations, NCT01231672) aiming to validate the performance of our previously-derived day 30 mortality prediction score in patients with septic shock entering an intensive care unit (ICU) (Lissalde-Lavigne *et al*, 2008). We used this unique opportunity to analyse the ability of automated FGMs (FM, FDP and DDi), performed in patients as they entered ICU, studied individually and then according to corresponding ISTH DIC scores, to predict death at day 30.

Patients, materials and methods

Patients

The trial from which this ancillary study was derived is a multicentre prospective validation study involving eight central ICUs at French University Hospitals (Clermont-Ferrand, La Conception Marseille, North Hospital Marseille, Montpellier DAR-A, Montpellier DAR-B, Nice, Nimes and Saint-Etienne). It was approved by the South Mediterranean III ethics committee (CPP 2008.11.04 bis), informed consent forms were signed by the patient or a legally acceptable surrogate when the patient was unable to give consent The trial was registered at www.clinicaltrials.gov as NCT01231672.

Eligible patients were aged 18 years or older with a documented or suspected infection characterised by systemic inflammatory response syndrome determined by at least two of the following symptoms: (i) temperature >38·3°C or <36°C; (ii) heart rate >90 bpm; (iii) tachypnea >20 breaths/min, or $PaCO_2$ < 32 mmHg, or mechanical ventilation; (iv) leucocytes >12·0 × 10^9/l, or <4·0 × 10^9/l, or >10% immature forms. Patients with sepsis were included if they had septic shock for less than 24 h, defined as systolic blood pressure (SBP) <90 mmHg or the need for vasopressors to maintain SBP > 90 mmHg despite prior filling (20–30 ml/kg of macromolecules or 40–60 ml/kg of normal saline), or decrease in SBP > 40% in people with hypertension (Bone *et al*, 1992).

The non-inclusion criteria were: age < 18 years, moribund patients and patients with a life expectancy <7 days, patients who opted for care withdrawal or withholding. Patients with septic shock for more than 24 h (from the moment of the initiation of vasopressor infusion) and patients with a current treatment that interfered with coagulation (i.e., efficient preventive and curative anticoagulation) were not included. Moreover, parturient women and patients already included in a recent interventional trial, patients who refused to participate and patients not affiliated to the French national health insurance system were not included.

Patients' data collection

At ICU admission. Age, sex, referring facility, primary and secondary admission diagnoses and associated comorbidities. Treatments during the previous month and admission patterns (i.e., infectious disease, severe haemorrhage) were systematically collected. The Simplified Acute Physiology Score (SAPS-II; Le Gall *et al*, 1993), Sepsis-related Organ Failure Assessment (SOFA; Vincent *et al*, 1996) and McCabe (Delodder *et al*, 2011) scores were calculated during the first 24 h in ICU.

At study inclusion. The infection type (community-acquired or healthcare-associated infection according to the Centers for Disease Control and Prevention (CDC) criteria [www.cdc.gov/hai/infectiontypes.html]) and site were recorded. Organ failure was assessed according to the definition of Bone *et al* (1992): renal (oliguria <0·5 ml/kg/h for at least 2 h), neurological (abrupt – last 24 h – alteration of consciousness), respiratory (partial pressure of arterial oxygen over fraction of inspired oxygen ratio, PaO_2/FiO_2, <300 mmHg or <40 KPa), coagulation (thrombocytopenia 100 × 10^9/l or DIC), cutaneous

(mottled skin and/or capillary refill time >3 s), metabolic (lactataemia >2 mmol/l). Pathogens were specified when available (type of bacteriological sample, when performed, and positive cultures) and the anti-infective drugs were recorded to assess the adequacy of the anti-infective treatment.

Study endpoints

The main objective was to assess the ability of DDi, FDP and FM to predict death at day 30 by using the area under the receiver operating characteristic curve (AUC_{ROC}) and studying the performances of their best cut-off values.

The secondary objective similarly assessed the ability of the ISTH DIC scores calculated using each of the three FGMS to predict death at day 30.

Materials and methods

Fibrin generation markers. Fibrin monomers, FDP and DDi plasma concentrations were tested using commercially available automated immunoagglutination assays from Stago, Asnières, France using the STA-R® automatic coagulation analyser (DDi: STAR-Liatest® D-Di; FDP: STAR-Liatest® FDP; FM: STAR-Liatest® FM) including the corresponding normal and pathological control and standard plasma samples. All these tests were performed centrally at the Haematology Laboratory, University Hospital, Nîmes, using frozen individual platelet-poor plasma samples (PPP) obtained from blood taken at study inclusion (day 0), and during the first 24 h after ICU admission.

Platelet-poor plasma aliquots had been prepared from blood samples taken using a clean venipuncture procedure in which the first 5 ml of blood were used for whole blood cell counting. Blood was collected in tubes containing 1/10 volume of CTAD anticoagulant-antiplatelet mixture (0·109 trisodium citrate, pH 5·4; theophylline; 3·7 mmol/l adenosine; 0·198 mmol/l dipyridamole; Diatube H; Becton Dickinson, Rungis, France). Following double centrifugation at 2500 ***g*** for 15 min, aliquots of PPP were immediately stored at −80°C until sent on dry ice to the central laboratory. The individual frozen PPP samples were thawed just before use, by immersion in a water bath at 37°C for 5 min, followed by a systematic homogenisation using a vortexing device for 30 s.

Other haemostasis-related parameters and blood cell count. Complete blood cell count, including haemoglobin (Hb), was performed at to each centre's facility. All haemostasis-related parameters were determined using a STA-R analyser with commercially available kits and reagents from Diagnostica Stago (Asnières, France), including the corresponding normal and pathological control and standard plasma samples: STA-Neoplastine CI PLUS for prothrombin time (PT) and STA-PTT Automate for activated partial thromboplastin time (aPTT). Results were given as the corresponding patient to standard plasma time ratios (i.e., PT ratio and aPTT ratio). Fibrinogen (Fg) plasma levels were assayed using the STA Fibrinogen kit (according to Clauss' method), factor (F)VII, FX, FV and FII procoagulant activities using the relevant STA-deficient plasma samples. Antithrombin (AT) activity was measured with an amidolytic assay (Stachrom® ATIII, Diagnostica Stago).

ISTH DIC scores. ISTH DIC scores were calculated according to the published recommendations (Taylor *et al*, 2001; Levi *et al*, 2009), using the platelet count, PT prolongation and Fg levels as described. Values of FGMs were used as previously published (Lissalde-Lavigne *et al*, 2008): DDi ≤ 0·5 mg/l = 0; ≤4 mg/l = 2; >4 mg/l = 3 to calculate the "DIC score-DDi". FDP ≤ 5 mg/l = 0; ≤20 mg/l = 2; >20 mg/l = 3 to calculate the "DIC score-FDP". FM ≤ 5 mg/l = 0; ≤10 mg/l = 2; >10 mg/l = 3 to calculate the "DIC score-FM": cut-offs differentiating a FGM score of two from a FGM score of three had systematically been the easy-to-remember values contained in the corresponding 95% confidence interval (CI) of the 90th percentile that had been computed in our originally studied population (Lissalde-Lavigne *et al*, 2008).

Statistical analysis

Statistical analyses were performed using Staview® (Abacus concepts, Berkeley, CA, USA) and XLSTAT® (2015.4.01.20116 release, Addinsoft SARL, Paris France) software. Quantitative variables were described as median values and interquartile range, and qualitative variables as numbers and percentages. Correlations between quantitative variables were tested computing Spearman's rank correlation coefficient ρ. Variables were compared (using a Kruskall–Wallis ANOVA by ranks for the quantitative variables and a χ^2 test for the qualitative variables) between patients who survived at day 30 after admission to the ICUs, and those who did not.

The ability of each studied variable to predict death by the end of the observational period (day 30 following ICU admission) was represented by a receiver operating characteristic (ROC) curve and the corresponding area under the curve (AUC; ROC_{AUC}), all of which were assessed by non-parametric methods (Hanley & McNeil, 1982). ROC_{AUC} values were compared using Wald's test. The maximum value of the computed Youden index, defined as the (sensitivity + specificity −1) value, identified the optimal cut-off values, whose performances were then assessed in terms of sensitivity (Se), specificity (Sp), positive predictive value (PPV), positive likelihood ratio (LR+), negative predictive value (NPV) and negative likelihood ratio (LR−).

As the present study was ancillary to the primary study (NCT01231672), the sample size calculation was determined according the primary study, i.e. 779 patients. The initial derivation cohort which justified the primary study included 158 patients; 66 of whom were dead at day 30. The prognostic performances of day 30 mortality prediction score were:

ROC_{AUC} 0·889, Se: 0·970, Sp: 0·435, NPV: 0·952 and PPV: 0·552. Estimating a ROC_{AUC} value of 0·890 with an absolute precision of 0·025 and a risk value of 0·05, with an event rate of 66 deaths/158 patients required 780 patients in the external validation sample.

The links between the studied variables and the risk of death were also studied by logistic regression analysis, performed on continuous variables (DDi, FDP, FM) then on discrete variables (values of the ISTH DIC scores calculated using the three FGMs).

All tests were two-sided and assessed at a 5% significance level.

Results

Patient characteristics

From April 2009 to September 2013, 2,320 patients with septic shock were admitted to the eight ICUs and 779 patients (34%) were finally enrolled in the study (Fig 1). At admission, the average SAPS-II and SOFA scores were 51·3 ± 17·9 and 9·8 ± 3·8, respectively, and 82% of patients were mechanically ventilated (Table I). Most patients were from another in-hospital unit (previous median hospital stay: 1 day, range 0–129). The most frequent sites of infection were lung (39%) and abdomen (36%). The infection was documented in 587 (75%) patients. The haematological parameters at inclusion are shown in Table II. The mortality rate at day 30 was 30·7%.

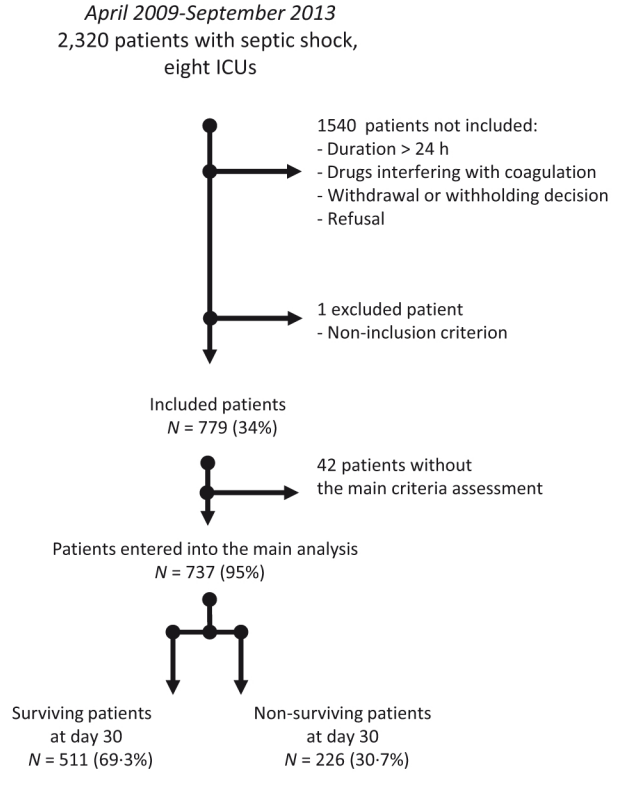

Fig 1. Flowchart of the study. ICU, intensive care unit.

Fibrin generation markers

The FGM levels in patients tested at ICU admission are given in Table II. DDi and FDP values were, overall, very highly correlated ($\rho = 0{\cdot}956$, $P < 0{\cdot}0001$), whereas the correlations between FM and DDi or FDP were statistically significant but lower ($\rho = 0{\cdot}623$, $P < 0{\cdot}0001$ and $\rho = 0{\cdot}608$, $P < 0{\cdot}0001$, respectively).

Values of FGM according to survival at day 30 are given in Table III. FM values were significantly higher in patients who were dead at day 30 ($P < 0{\cdot}0001$; Fig 2). There was no difference in survival rates according to DDi and FDP values.

Analysing FM prognostic performance, the ROC_{AUC} value for predicting death at day 30 (Fig 2) was 0·617, 95% (0·564–0·670), $P < 0{\cdot}0001$. The maximum value of the Youden index was obtained for a FM value = 13·1 mg/l and the computed prognostic performances of this cut-off were: Se: 0·533 (0·466–0·598), Sp: 0·682 (0·638–0723), PPV: 0·440, LR+: 1·675, NPV: 0·757 and LR−: 0·685. The odds ratio (OR) for non-survival at day 30 in patients with a FM value higher than this cut-off was 2·457 (1·767–3·413), $P < 0{\cdot}0001$.

For DDi performance for predicting death at day 30, the ROC_{AUC} was 0·525 (0·469–0·580), $P = 0{\cdot}381$, compared to a ROC_{AUC} of 0·529 (0·473–0·585), $P = 0{\cdot}305$ for FDP.

ISTH DIC scores calculated using each of the FGMs

The frequencies of the ISTH DIC scores calculated according to the three FGMs are depicted in Fig 3A. Whereas strong similarities emerge for scores ranging from 5 to 9, the rates of overt-DIC diagnosis (DIC score values ≥5) being consequently similar regardless of the FGM, the DIC score for FM (DIC score-FM) differs significantly from the other two for values <5.

The comparative analyses of these rates between survivors and non-survivors at day 30 (Fig 3B) overall shows a significant shift to higher rates of high score values in non-survivors, for DIC score values ranging from 4 to 8, most sharply with the DIC score-FM. The value of the ISTH score equal to four is also the one for which the curves of survivors and non-survivors intersect systematically: patients with DIC scores 4–8 were exposed to an increased risk of death at day 30 – DIC score for DDi (DIC score-DDi): OR 1·988 (1·412–2·801), $P < 0{\cdot}0001$; DIC score for FDP (DIC score-FDP): 1·988 (1·412–2·801), $P < 0{\cdot}0001$; DIC score-FM: 3·106 (2·212–4·348), $P < 0{\cdot}0001$.

We subsequently analysed the risk of death associated with individual DIC scores, merging patients with the lowest DIC score values (0, 1 or 2) and the highest DIC core values (7 and 8) in order to have greater numbers of patients per DIC score levels (Table IV). Generally, the higher the DIC score, the higher the risk of non-survival: the ISTH DIC score-FM

Table I. Characteristics of patients with septic shock admitted to eight French intensive care units (N = 779).

Characteristic	Total
Female, *n* (%)	310 (39·1)
Age (years), mean (SD)	66 (± 15)
Body mass index, kg/cm^2 (SD)	26·9 (± 6·99)
Mac-Cabe 0/1/2, *n* (%)	429(55)/295(38)/55(7)
SAPS-II score (SD)	51·3 (± 17·9)
SOFA score (SD)	9·8 (± 3·8)
Origin of patients, *n* (%)	
Home or Emergency Department	289 (37)
In-hospital wards	327 (42)
Other institutions	163 (21)
Type of admission, *n* (%)	
Medical	366 (47)
Surgical emergency	359 (46)
Scheduled surgery	54 (7)
Time between hospital and ICU admissions (median [range])	1 [0–129]
Underlying diseases, *n* (%)	
Cardiovascular	374 (48)
Respiratory	102 (13)
Liver disease	79 (10)
Renal failure requiring renal replacement therapy	18 (2)
Diabetes mellitus	193 (25)
Dyslipidaemia	140 (18)
Tobacco consumption (>20 packets/year)	249 (32)
Alcohol consumption (>3 glasses wine/day)	163 (21)
Solid cancer	169 (22)
Chronic medication, *n* (%)	362 (47)
Statins	165 (21)
Anti-platelet drugs	227 29)
Beta blockers	122 (16)
Angiotensin blockers	97 (13)
Organ failure (specific SOFA score ≥3), *n* (%)	
Cardiovascular SOFA ≥3	724 (91)
Respiratory SOFA ≥3	432 (55)
Neurological SOFA ≥3	145 (12)
Renal SOFA ≥3	168 (22)
Liver SOFA ≥3	47 (1·7)
Haematological SOFA ≥3	72 (9)
Organ support, *n* (%)	
Mechanical ventilation	636 (82)
Renal replacement therapy	134 (17)
Blood components transfusion	190 (24)
Red blood cells	144 (18)
Frozen plasma	90 (12)
Platelets	45 (6)
Sedation	622 (80)
Time between infection initiation and inclusion (median [range])	1 [0-3]
Sources of infection, *n* (%)	
Pneumonia	306 (39)
Peritonitis	281 (36)
Septicaemia	134 (17)
Urinary tract infection	126 (16)
Biliary tract infection	54 (7)
Soft tissues	27 (4)
Miscellaneous	28 (4)
Initial bacteriological test, *n* (%)	775 (100)
Documented infection (at least one isolated micro-organism)	587 (75)
Anti-infective management, *n* (%)	
Surgical or interventional procedure	384 (50)
Anti-infective agents	778 (100)

ICU, intensive care unit; SAPS-II, Simplified Acute Physiology Score; SD, standard deviation; SOFA, Sepsis-related Organ Failure Assessment.

leading to the most harmonious model of this dose-effect relationship, as shown by its higher logistic regression likelihood ratio test.

We also studied the risk of death associated with an overt DIC score (i.e. ≥5), according to the score(s) indicating a positive diagnosis, the reference group being defined by patients with negative overt DIC diagnoses regardless of FGM (Table VA). Interestingly, triple positivity impacted on survival whereas double positivity defined with a negative DIC-FM score did not. Also, double positivity *via* the DIC score-DDi and the DIC-score-FM impacted on prognosis whereas single positivity due to a non-diagnostic DIC score-FM did not. Finally, single positivity altered survival only in the case of positive DIC score-FM. These data indicated the prognostic value of an overt DIC positive diagnosis using FM as the FGM. Logistic regression analysis (Table VB) showed that an overt DIC diagnosis using the DIC score-FM is a predictor of non-survival at day 30, independently from an overt DIC diagnosis using the two other DIC scores.

Analysing the performances of the three FGMs for predicting non-survival at day 30 (Fig 4), the ROC_{AUC} values are 0·650, 0·624 and 0·602 for the DIC score-FM, DIC score-DDi and DIC score-FDP respectively, i.e. very modest values, the maximum value of the Youden index being systematically obtained for a DIC score value = 4, which intrinsic performances are also limited in terms of predictability capacities.

Discussion

In this prospective multicentric study on patients with septic shock admitted into an ICU, the comparative testing at day 0 of three automated FGMs shows that FM is more associated with an increased risk of non-survival at day 30 than DDi and FDP. The FM absolute values are higher in non-survivors with a significant ROC_{AUC} value: this is not the case for DDi or FDP values. Using FM to calculate the ISTH

Table II. Haematological data of the study population at inclusion.

	Minimum	Median	Maximum
Haemoglobin, g/l	56	106	202
White blood cell count, $\times 10^9$/l	0·10	13·6	190
Lymphocytes, $\times 10^9$/l	0	0·74	85·2
Platelet count, $\times 10^9$/l	8	184	1666
Fibrinogen, g/l	0·3	5·1	14·6
D-Dimers, mg/l	0·3	40·1	21
Fibrin/fibrinogen degradation products, mg/l	4	17·9	151
Fibrin monomers, mg/l	5	7·9	151
Antithrombin, %	9	54	140
Factor VII, %	4	49	159
Factor X, %	5	59	169
Factor V, %	6	76·5	215
Factor II, %	8	56	131
aPTT ratio	0·6	1·4	100
PT ratio	0·9	1·4	11·3

aPTT, activated partial thromboplastin time; PT, prothrombin time.

Table III. Values of fibrin generation markers in patients tested at ICU admission, according to survival at day 30.

	Survival at day 30	*P*
DDi (mg/l)		
Negative	4·10, 5·60 (0·27–21)	0·299
Positive	4·10, 4·37 (0·32–21)	
FDP (mg/l)		
Negative	17·99, 29·30 (4–151)	0·189
Positive	17·51, 19·75 (4–151)	
FM (mg/l)		
Negative	14·74, 45·66 (5–151)	<0·0001
Positive	6·65, 17·20 (5–151)	

DDi, D-dimers; FDP, fibrin/fibrinogen degradation products; FM, fibrin monomers; ICU, intensive care unit.

DIC score showed an association between increasing score and the risk of non-survival which is more robust than those obtained with DIC score calculated using the other two FGMs. An overt-DIC diagnosis obtained using the ISTH DIC score-FM indicates a significant risk of non-survival independently from an overt-DIC diagnosis assessed using DDi or FDP to calculate the ISTH DIC score. Thus, FM has some clinical advantages over DDi or FDP in such a setting, making it the best choice in this precise clinical practice.

The prognostic performance of FM appears however only slightly higher than DDi and FDP. Not all hospitals currently offer the measurement of FM. The balance between a modestly higher clinical value with the complexity and costs currently deriving from the routine measurement of FM is thus a concern. The automatic coagulation analyser on which the

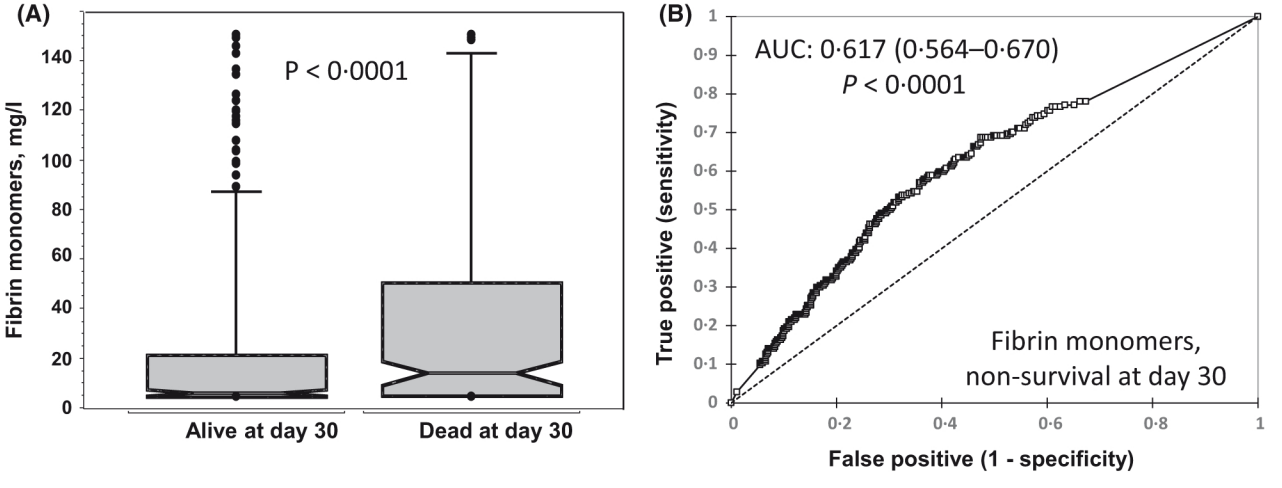

Fig 2. Box-and-whiskers plot describing the fibrin monomers (FM) values tested in septic shock patients at admission to an intensive care unit, according to their survival/non-survival at day 30 (A), and receiver operating characteristic (ROC) curve of FM for predicting non-survival at day 30 (B). AUC, area under the ROC curve.

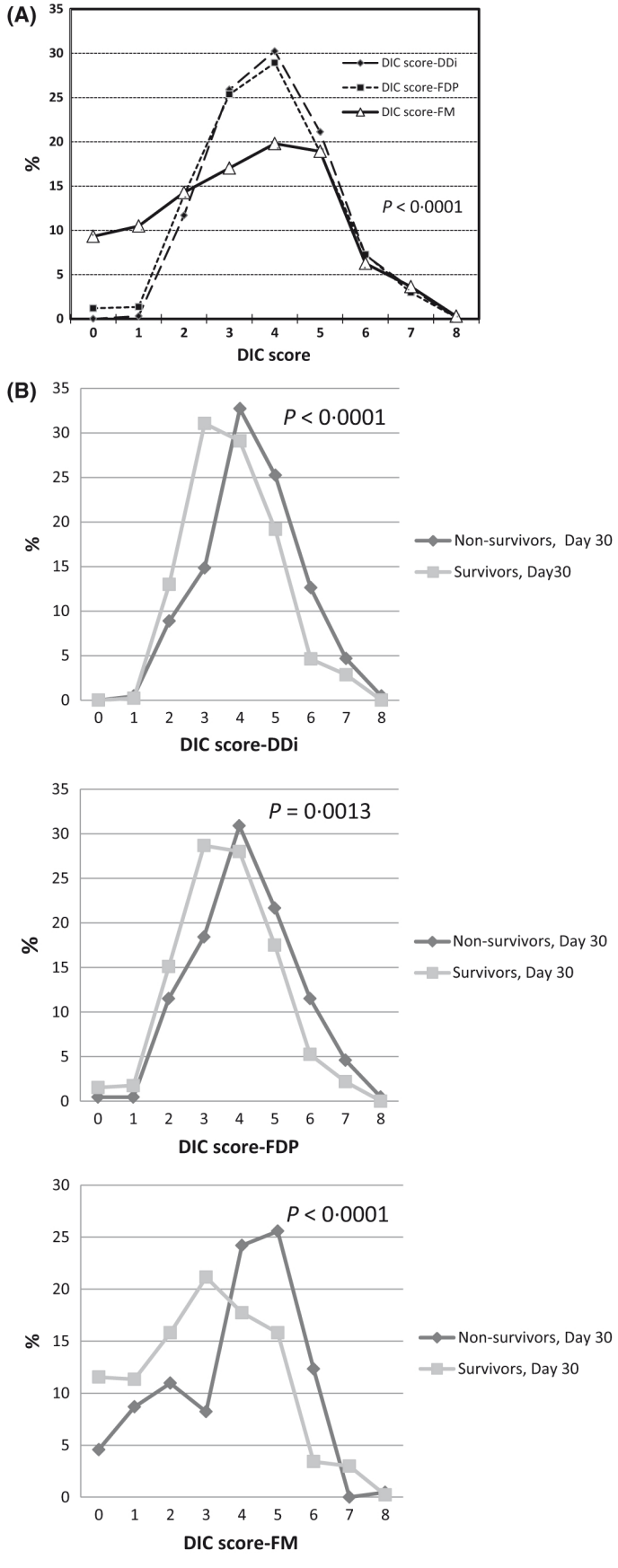

Fig 3. Rates of each of the values of the three International Society on Thrombosis and Haemostasis disseminated intravascular coagulation (ISTH DIC) scores (A) and comparative analyses of these rates between survivors and non-survivors at day 30 (B). DIC score-DDi, ISTH DIC score using D-dimers DDi as the fibrin generation marker; DIC score-FDP, ISTH DIC score using fibrin degradation products as the fibrin generation marker; DIC score-FM, ISTH DIC score using fibrin monomers as the fibrin generation marker.

Table IV. Risk of non-survival at day 30 according to the values of the three ISTH DIC scores calculated at ICU admission.

Score value	Odds ratio	95% CI	*P*
DIC score-DDi			<0·0001 (34·95*)
Reference	1		
3	0·681	0·361–1·299	0·236
4	1·590	0·888–2·849	0·119
5	1·862	1·012–3·425	0·046
6	3·861	1·799–8·264	0·0005
7–8	2·538	0·982–8·264	0·054
DIC score-FDP			0·0004 (22·52*)
Reference	1		
3	0·950	0·543–1·664	0·857
4	1·629	0·963–2·755	0·069
5	1·828	1·041–3·215	0·036
6	3·236	1·595–6·579	0·001
7–8	3·425	1·311–8·929	0·012
DIC score-FM			<0·0001 (55·76*)
Reference	1		
3	0·621	0·345–1·119	0·112
4	2·179	1·376–3·460	0·0009
5	2·584	1·626–4·098	<0·0001
6	5·747	2·890–11·49	<0·0001
7–8	2·732	1·205–6·211	0·016

Patients with the lowest DIC score values (0, 1 or 2) were merged to define the reference, as were patients with the highest DIC core values (7 and 8).

CI, confidence interval; DIC, disseminated intravascular coagulation; DIC score-DDi, DIC score calculated using D-dimers as the FGM; DIC score-FDP, DIC score calculated using fibrin/fibrinogen-degradation products as the FGM; DIC score-FM, DIC score calculated using fibrin monomers as the FGM; FGM, fibrin generation marker; ICU, intensive care unit; ISTH, International Society on Thrombosis and Haemostasis.

*Logistic regression likelihood ratio test, χ^2 value.

tests were derived is, however, widely available and used in hospitals. Those teams that routinely use this equipment employ, in most cases, the DDi test used in this study as their routine FGM test. Focusing on these hospitals and teams, the challenge would be to introduce the FM test for coagulation assessment in septic shock patients. The cost of the reagents required to perform a unit FM test is very slightly cheaper than of a unit DDi test, based on the precise analytical conditions we have used. The introduction of the FM test under these precise laboratory conditions therefore seems acceptable without any increase in costs.

The concentrations of the currently available FGMs do not strictly follow the same mechanisms. This could modulate their clinical properties.

DDi and FDP are the consequences of a complex multistep mechanism. They necessitate thrombin generation; thrombin-mediated fibrinogen attack leading to the generation of fibrin monomers; thrombin-mediated FXIII activation leading to the cross-linking of fibrin monomers; and finally, plasmin-mediated proteolysis of cross-linked fibrin monomers ("fibrinolysis"). FMs only need thrombin-mediated fibrinogen partial proteolysis attack: they do not depend on the activation of FXIII and of the fibrinolytic system. FMs are thus less dependent on acquired abnormalities of FXIII and/or of the components of the fibrinolytic system than DDi and FDP. In view of this, increased levels of PAI-1 leading to inadequate fibrinolysis (Vervloet *et al*, 1998) have been widely reported during septic shock. In addition, the common deletion polymorphism (4G) within the promoter region of the gene encoding PAI-1 (*SERPINE1*), which leads to impaired fibrinolysis, influences the severity and outcomes (Texereau *et al*, 2004). The septic shock-associated hypofibrinolysis may impact the DDi and FDP terminal generation rates, partially uncoupling them from fibrin generation. The generation of FM only depends on unopposed final coagulation activation. This may partly explain the relative superiority of FM in the clinical setting of this study.

The fluctuating volume of distribution of FMs and DDis during septic shock may also modulate the relationship between their circulating concentrations and the intensity of the intravascular fibrin generation process. Septic shock is associated with a strongly altered vascular permeability (Russell *et al*, 2018) related to several pathways (Slit/Robo4, vascular endothelial growth factor, angiopoietin 1 and 2/Tie2 pathway, sphingosine-1-phosphate, and heparin-binding protein). DDis can diffuse out of the vascular bed, their intravascular component can thus be diluted, but circulating DDis concentrations can also be fed by their extravascular generation. FMs, which are bigger molecules, are less susceptible to diffusion. This difference may also impact on the relative capacity of the two markers to closely reflect the intravascular fibrin generation.

Fibrin monomers measurements thus offer advantages in reflecting thrombin action on circulating fibrinogen (Levi *et al*, 2009).

The type of assay to be used as FGM in patients with septic shock and/or in patients investigated with the ISTH overt DIC score has been widely investigated. Elevated levels of DDi and FM are very sensitive for the diagnosis of DIC, and a normal level has a high NPV (Horan & Francis, 2001). The measurement of FM in 1184 patients, half of which had a haematopoietic tumour, with DIC according to Japanese Ministry of Health and Welfare criteria (using FDP as the FGM), suggested that plasma FM might be a useful marker for the diagnosis of not only DIC but also pre-DIC (Wada *et al*, 2003). The work on multiple samples from 359 German patients treated in an ICU, which tested the incorporation of FMs as the FGM instead of DDis in the ISTH overt DIC score, concluded on a small but relevant impact on the prognostic performance of the overt DIC score (Dempfle *et al*, 2004). The study of 165 consecutive Belgium patients evaluating the utility of the ISTH overt DIC scoring system at its first determination in a

Table V. Risk of non-survival at day 30 associated with DIC score values at ICU admission compatible with an overt DIC (i.e. ≥5): (A) according to the type(s) of score indicating a positive diagnosis and (B) global logistic regression models.

(A)

	DDi+	FDP+	FM+	Rate (%)	OR	95% CI	*P*
							<0·0001
Reference	□	□	□	64·81	1		
Overt DIC	■	■	■	23·76	2·381	1·626–3·484	<0·0001
Overt DIC	■	■	□	4·66	0·851	0·357–2·028	0·713
Overt DIC	■	□	■	1·65	7·752	2·028–30·30	0·0028
Overt DIC	■	□	□	1·50	1·946	0·539–7·042	0·309
Overt DIC	□	■	■	0·30	2·915	0·181–47·62	0·451
Overt DIC	□	□	■	3·31	2·915	1·230–6·897	0·0150

(B)

Univariate analysis

	cOR	95% CI	*P*	*
Overt DIC-DDi+	2·075	1·475–2·924	<0·0001	17·472
Overt DIC-FDP+	1·862	1·333–2·632	0·0004	12·291
Overt DIC-FM+	2·646	1·876–3·731	<0·0001	30·686

Multivariate analysis

	aOR	95% CI	*P*
Overt DIC-FM+	3·030	1·681–5·464	0·0002
Overt DIC-FDP+	0·393	0·155–1·001	0·051
Overt DIC-DDi+	2·033	0·820–5·050	0·126

aOR, adjusted odds ratio; CI, confidence interval; cOR, crude odds ratio; DDi+, positive using the DIC score-DDi (D-dimers as the FGM); DIC, disseminated intravascular coagulation; FDP+, positive using the DIC score-FDP (fibrin/fibrinogen-degradationproducts as the FGM); FGM, fibrin generation marker; FM+, positive using the DIC score-FM (fibrin monomers as the FGM); ICU, intensive care unit; OR, odds ratio.

*Logistic regression likelihood ratio test, χ^2 value.

□ overt DIC negative (ISTH DIC score <5).

■ overt DIC positive (ISTH DIC score ≥ 5).

general hospital setting, which used FDP as the FGM, suggested that FMs are useful for describing illness severity (Cauchie *et al*, 2006). Of note, anther Japanese work evaluated the haemostatic abnormalities and the onset of DIC in 613 patients but failed to identify an adequate FM cut-off value to differentiate patients with no DIC from patients with pre-DIC (Okamoto *et al*, 2010). Guidance for diagnosis and treatment of DIC from harmonization of the recommendations of the British Committee for Standards in Haematology, The Japanese Society of Thrombosis and Haemostasis and the Italian Society for Thrombosis and Haemostasis, in its subsection devoted to laboratory tests, states that FMs offer theoretical advantages in DIC, as they give a closer reflection of thrombin action on fibrinogen (Wada *et al*, 2013). However, the downstream markers of FMs, i.e. FDPs and DDis, were more statistically significant than FMs in distinguishing sepsis from systemic inflammatory response syndrome and its known impact on prognosis (Toh *et al*, 2013). A study on 70 Indian patients suspected to develop DIC showed FM to be a better indicator than DDi in distinguishing patients with overt and non-overt DIC from non-DIC patients (Singh *et al*, 2017). A recent Japanese work, focused on 107 septic patients admitted to an emergency and critical care centre of a women's hospital, studied whether coagulation markers may enable earlier diagnosis of DIC: FMs were found to be useful to predict, and also to exclude, a diagnosis of DIC (Masuda *et al*, 2018). To summarise, the available data, mainly obtained from heterogeneous groups of patients, do not show uniform findings. Most of them, however, appear to indicate a clinical benefit, of varying value and intensity, associated with the use of FM as FGM. Our study has some characteristics in that setting. It is a multicentre study, on a significant number of patients. The inclusion criterion is unique: septic shock admitted to an ICE. The three FGMs were tested in a centralised way, using the same analytical technology: immunoagglutination, carried out on the same automated analytical platform. The primary clinical outcome is robust and clinically relevant, i.e. late survival at day 30.

Nevertheless, the ability of FM to predict non-survival at day 30 is limited; its PPV and NPV are modest and FM cannot be viewed as a clinically applicable and relevant marker alone for aiding prognosis and deciding innovative care to

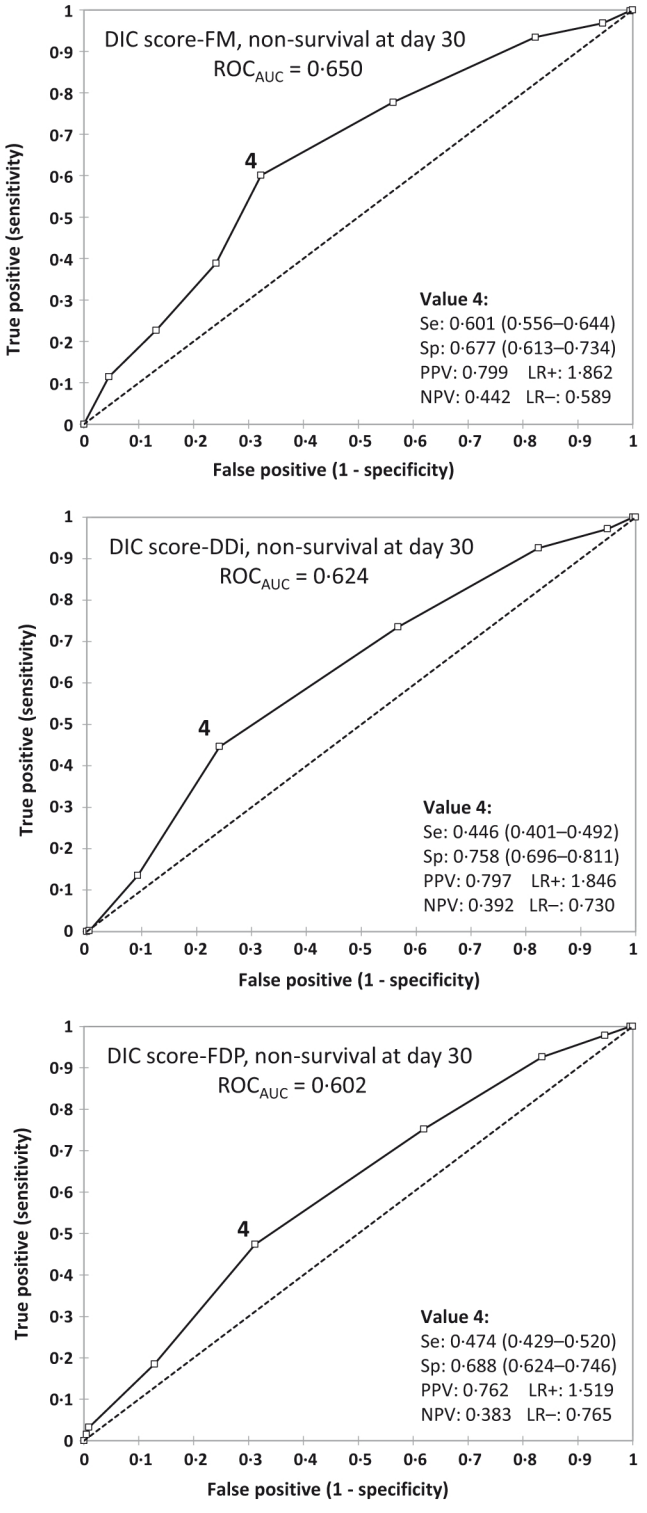

Fig 4. Receiver operating characteristic (ROC) curve of the three ISTH DIC scores for predicting non-survival at day 30. DIC score-DDi, International Society on Thrombosis and Haemostasis disseminated intravascular coagulation (ISTH DIC) score using D-dimers as the fibrin generation marker; DIC score-FDP, ISTH DIC score using fibrin degradation products as the fibrin generation marker; DIC score-FM, ISTH DIC score using fibrin monomers as the fibrin generation marker. ROC$_{AUC}$, area under the ROC curve. The maximum value of the Youden index was systematically obtained for a DIC score value = 4; Se, sensitivity; Sp, specificity; LR+, positive likelihood ratio; LR−, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

improve survival. However, the diagnosis of DIC is a frequent concern in septic patients. This diagnosis is complicated and is usually facilitated by the application of scores, of which the ISTH DIC score is most frequently used in Western ICUs. The ISTH DIC score calculation tests an FGM whose value can be quickly obtained. Among the available commercially available assays, automated FM testing, which is most closely linked to survival prognosis than other automated FGM, can be viewed as a preferential choice. As better supportive treatment of DIC is still questionable in its ability to result in an improvement of clinically relevant outcomes, and as a randomised controlled trial

of heparin in this situation is still urgently warranted (Levi & Scully, 2018), the clinical relevance of biological tools used to diagnose DIC must be determined.

Acknowledgements

We thank the research staff of the "*Direction de la Recherche Clinique et de l'Innovation*" of the University Hospital of Nîmes: S. Clément, C. Meyzonnier, N. Best, A. Megzari, R. Jacquet, E. Dupeyron, S. Granier, B. Lafont, C. Masseguin, H. Obert, H. Léal, O. Albert, C. Suehs, P. Rataboul and M.P. Francheschi. We thank the staff of the "*Département de Biostatistique, Epidémiologie, Santé Publique et Information Médicale BESPIM*" of Nîmes University Hospital: J.P. Daurès and P. Fabbro-Peray. We thank Sarah Kabani for English language editing. We thank Karine Carrière, Arnaud Berthier, Patricia Roger and Dominique Hellio-Morel, from STAGO, Asnières, France, for their technical support and for easy access to their biological reagents.

Competing interests

Financial support was provided by Nîmes University Hospital via an internal funding scheme. The authors have no competing conflict of interest to disclose.

Author contributions

J.-C. G., G. L.-L. and J.-Y. L. contributed to the conception and design of the study. S. J., J.A., J.-M. C, J.-C. O., J. M., M. L., P. D., L. E. and J.-Y. L acquired the samples whereas E C.-N., S.B. and L. E. performed biological analysis. J.-C. G., C. C., G. L.-L. and J.-Y. L. contributed to data analysis and interpretation. J.-C. G. drafted the manuscript and all authors revised it critically and approved the final version.

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