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Contact system activation in human sepsis – 47kD HK, a marker of sepsis severity?

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Summary

Aim: This pilot study seeks to determine whether contact system activation (CSA) occurs in human sepsis patients and to characterise blood levels of the 47kD light chain of high-molecular weight kininogen (47kD HK).

Methods: Six consecutive patients with clinical suspicion of sepsis were evaluated on days 1, 2, 3 and 6–8 for 47kD HK blood levels expressed in U/ml of whole blood and as percent of total HK. 47kD HK was measured in whole blood by quantitative immunoblot analysis.

Results: On study day 1 or 2, analysis of 47kD HK in U/ml of whole blood identified CSA in 3/6 patients. When 47kD HK levels were expressed as percent of total HK, 4/6 patients were identified with CSA before day 3. The degree of CSA as assayed by the presence of 47kD HK correlated with the severity of the systemic inflammatory syndrome (SIRS), i.e. mean CSA increased progressively from basal levels in healthy controls (0.08 U/ml or 10.4%) to patients without SIRS (0.10 U/ml or 15.1%), to patients with sepsis (0.12 U/ml or 15.0%), and finally to patients in a combined category of severe sepsis and septic shock (0.13 U/ml or 17.4%).

Conclusion: CSA, defined by increased 47kD HK, occurred early on in the course of sepsis in a subset of sepsis patients. 47kD HK levels, an indicator of bradykinin release, correlated with sepsis severity. Future larger studies will need to evaluate the role of 47kD HK as a biomarker for both prognosis and treatment response in human sepsis.

Key words: systemic inflammatory response syndrome; sepsis; high-molecular weight kininogen; contact system activation; bradykinin

Introduction

Sepsis is a clinico-pathological syndrome arising from infection and is defined by the presence of a systemic inflammatory process called systemic inflammatory response syndrome or SIRS. SIRS may result from infectious (i.e. sepsis) or non-infectious causes. SIRS and sepsis can therefore be seen as overlapping entities [1]. Prospective analysis of 3708 patients with SIRS and sepsis has shown that there is a clinical progression from SIRS to sepsis, to severe sepsis, and septic shock. 28-day mortality increases from 7% to 16%, to 20% and 46% respectively [2].

The systems of coagulation and inflammation interact in many ways [3]. On the hypothesis that one can alter the outcome of sepsis, i.e. reduce sepsis-related mortality by influencing coagulation parameters, the effects of drugs that alter the coagulation system have been studied. To date activated protein C is the only coagulation inhibitor known to improve survival in sepsis patients. Other studies involving factors such as tissue factor pathway inhibitor (TFPI), antithrombin III (ATIII) or C1 inhibitor (C1Inh) have so far failed

Conflict of interest: none of the authors has a conflict of interest to declare.

Abbreviations

activated partial thromboplastin time	aPTT
antithrombin III	AT III
bradykinin	BK
C1 inhibitor	C1Inh
contact system activation	CSA
control	Con
factor XI	FXI
factor XII	FXII
high molecular weight kininogen	HK
intensive care unit	ICU
kallikrein	KK
kilo Dalton	kD
molecular weight	MW
normal human plasma	NHP
patient	Pt
prekallikrein	PK
standard deviation	SD
systemic inflammatory response syndrome	SIRS
tissue factor pathway inhibitor	TFPI

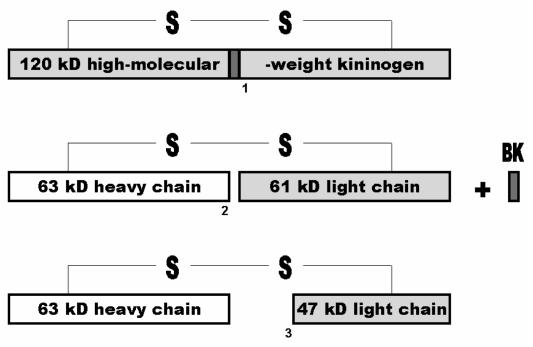
to reduce mortality in human sepsis or were not sufficiently powered to address the issue [4–9]. The search for disease markers allowing identification of high-risk subpopulations of patients likely to benefit most from targeted therapeutic strategies is ongoing.

Bradykinin, a vasoactive peptide which mediates inflammatory signs and sepsis-induced hypotension, is generated in vivo by a three-step sequential cleavage from a 120kD precursor molecule, high molecular weight kininogen (HK; see figure 1) [10–15]. Plasma kallikrein is the serine protease responsible for endovascular HK cleavage [16]. Direct measurement of bradykinin is laborious and prone to preanalytical artifacts due to the very short half-life of 30–50 seconds and basal plasma concentration in the pmol/l range [17–21]. We therefore designed an assay measuring the cleaved 47kD band of HK with a long half-life as a direct indicator of contact system activation (CSA) and an indirect indicator of bradykinin release (see Figure 1).

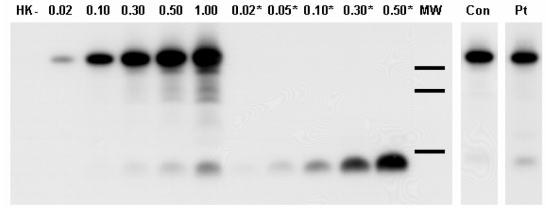
We hypothesise that contact system activation occurs early on in the course of sepsis and hence may be a candidate marker of clinical relevance. A quantitative assay for high molecular weight kininogen cleavage was designed to study contact system activation. We characterised this test by studying the evolution of test results over time in six patients with suspected sepsis and compared these results with those of six healthy controls. Finally, we investigated whether there is a correlation between the severity of the inflammatory syndrome and the severity of contact system activation.

Figure 1

High-molecular weight kininogen cleavage. Circulating HK is cleaved by plasma kallikrein in three sequential steps (see 1, 2 and 3 in panel A). The first cleavage generates 63kD heavy chain and 61kD light chain. The next cleavage liberates the polypeptide bradykinin from the heavy chain. Finally, the third cleavage generates 47kD light chain. Using a polyclonal rabbit antiserum directed against 47kD light chain our assay detects three bands (depicted in gray): 120kD, 61kD and 47kD. 61kD band is almost undetectable on the immunoblots due to increased electrophoretic mobility and short plasma half-life (see Material and Methods).
Panel B shows an immunoblot used to generate a standard curve, and immunoblots of a control sample (Con) as well as a sepsis sample (Pt). For the standard curves HK deficient plasma (HK-) was spiked with normal human plasma to contain the amounts of HK as indicated (lane 1: only HK- plasma = 0%, lane 2: HK-:NHP = 1:50 or 2%, lane 3 HK-: NHP = 1:10 or 10%, etc). Whereas 1 µl of plasma was applied per lane for standard curves, only 0.4 µl of whole blood was added for test samples. Lane 10 shows three molecular weight (MW) markers at 112, 84 and 52kD.



A



B

Patients and methods

Patients

Patients were recruited from the internal medicine ward, the medical intensive care units (ICU) or the surgical ICU on the basis of clinical suspicion of sepsis of recent onset on day 0 of the study. The inclusion criterion was the presence of at least two SIRS defining criteria (see below) on day 1. Seven consecutive patients were enrolled. One patient who died on day 2 was excluded from the study. Patient characteristics were as follows (patient number: sex, age, (colour used in figures): reason for transfer to ICU and comorbidities): **1**: female, 71, (purple): fever – valvular heart disease (mechanical mitral valve and severe aortic stenosis); **2**: male: 77, (blue) pseudomonas sepsis 8 days after aortic valve replacement – valvular heart disease (mechanical aortic valve), ulcerative proctitis, peripheral artery disease; **3**: female, 24, (green): fever – haemophagocytic syndrome; **4**: female, 54, (yellow): aspiration pneumonia acquired during generalised epileptic attack – pituitary insufficiency after resection of a prolactinoma; **5**: male, 57, orange: sigma perforation – alcohol abuse, chronic renal failure and recurrent gout; **6** male, 44, red: resection of small intestine – comorbidities not specified. Six healthy volunteers, 3 females and 3 males, with ages ranging from 27–47 years (mean: 37 years) served as controls.

Study protocol

Patients had blood drawn on 4 occasions on days 1, 2, 3 and 6–8. Contact system activation testing was performed on all occasions. Patients' clinical status pertaining to sepsis was ascertained for each of the 4 study days on the basis of information documented in the patient charts.

The American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference definitions of sepsis and related disorders were used [22]. In brief, 4 SIRS defining criteria were applied: (i) body temperature <36 or >38 °C, (ii) heart rate >90 beats/minute,

(iii) tachypnoea with respiratory rate >20/minute or hyperventilation with P_{aCo2}<32 Torr (4.3 kPa), and (iv) alteration of white blood count <4 or >12 G/l or the presence of >10% immature neutrophils (bands). SIRS was defined by the presence of two or more of the above criteria. Sepsis was defined as SIRS due to infection. Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion or hypotension. Septic shock was defined as a subcategory of severe sepsis characterised by persistent hypotension unresponsive to adequate fluid resuscitation.

Internal review and patient consent

This study protocol was reviewed and approved by the Ethical Committee of the University of Berne and the patients' administration department of the University Hospital in Berne. Doctors involved in the direct care of the patients obtained informed consent from patients or their families.

Contact system activation assay

A quantitative immunoblotting method for high molecular weight kininogen was developed on the basis of previously established methods [23]. Subjects' whole blood (WB) was diluted 1:50 in 0.01 M Tris, 0.14 M sodium chloride, pH 7.4 containing 1 mg/ml bovine serum albumin and 100 µg/ml soybean trypsin inhibitor (SBTI). 20 µl of diluted WB were added to 30 µl sample buffer containing 10% sodium dodecyl sulfate (SDS) and immediately denatured by heating to 100 °C for 5 minutes in a waterbath. Whole blood was used instead of plasma to minimise handling time and prevent preanalytical sample degradation. All samples were denatured within 20 minutes of the blood draw.

Pretest variables were tested in preliminary experiments. Citrate proved to be the optimal anticoagulant as heparinised samples showed less reproducibility of results after storage. Storage of denatured samples at room temperature was superior to freezing/defrosting, which induced excessive sample precipitation. Heating the samples to 100 °C for 5 min. in a waterbath resolubilised small precipitates which formed in some samples and permitted sample loading without losses due to non-entry into the stacking gel. Results of immunoblots produced using fresh samples and samples stored for 8 days under these conditions were highly reproducible (data not shown).

SDS PAGE was performed using a standard discontinuous buffer system [24]. Prior to electrophoresis samples were reduced with dithio-D-L-threitol (DTT) and alkylated with iodoacetamide (IAA). Gels contained 10% of acrylamide (wt/vol). Bisacrylamide was added to make up 2.6% of total monomer. Four gels with 12 lanes each were run in parallel overnight, permitting evaluation of 2 subjects in one session

Two separate standard curves were established for uncleaved and cleaved (47kD band) HK (see figure 1B). For uncleaved HK (120kD band) a standard curve was produced using the dilutions of non-activated normal human plasma (NHP) supplemented in HK-deficient plasma to assure equal total plasma content. A similar dilution scheme was applied to generate a standard curve for the 47kD cleavage product of HK. For this purpose NHP was activated by exposure to dextran sulphate (final concentration: 1.25 µg/ml) for 180 min. before being diluted.

Negative controls (1 µl plasma deficient in HK, HK) were run on each gel in lane 1; lane 12 was used for prestained molecular weight standards (BioRad, Hercules, Ca; 161–0305). For all patient/control samples the equivalent of 0.4 µl of whole blood was applied per lane. Internal controls (0.5 or 1.0 µl NHP) were included on each gel to assure comparability of gels that were run in parallel.

The immunoblotting technique has been described previously [23]. HK cleavage was analysed using a rabbit polyclonal antibody directed against reduced and alkylated 47kD light chain of HK (see figure 1).

Autoradiographs of the immunoblots were scanned using a personal laser densitometer (Molecular Dynamics, Sunnyvale, CA) and commercial software (MD Image Quant, version 3.3) [25]. Calibration curves were generated from the data using Table Curve 2D for Windows (Jandel Scientific Software, San Rafael, CA, version 3) and a transition function [logistic dose response curve: $y = a/(1+(x/b)^c]$.

Normal values were established in six healthy volunteers based on 4 blood draws each. These 24 values were distributed normally. Figure 1 schematically illustrates HK cleavage (1A) and a normal *vs.* a sepsis sample (1B). The antibody detects 120kD, 61kD and 47kD forms of HK. The 61kD fragment has an increased electrophoretic mobility resulting in partial migration through the nitrocellulose membrane (data not shown). This issue and the probable short half-life of the fragment led to the very low levels that we detected for this fragment. We determined absolute concentrations (U/ml of whole blood) of each of these bands using two different standard curves and a band scanning procedure. Based on the absolute concentrations of each fragment, we then calculated the relative concentrations in % of total HK. For this analysis total HK (100%) was determined as the sum of uncleaved HK + 61kD HK + 47kD HK. We defined contact system activation as any result, for either absolute concentration (U/ml) or for relative concentration (%), that falls outside the normal range defined by the mean +/- t x SD (t for n = 6 or five degrees of freedom = 2.57).

To assure reproducibility, runs with 4 gels were electrophoresed in parallel, blotted in parallel and their autoradiographs were exposed in parallel. On the basis of the internal controls on each gel the intra-run coefficient of variability (CV) was calculated: mean CV 8.0%, median CV 7.3%, range 1.6–15.4%. The inter-run CV could not be calculated as no calibrated source of purified HK (uncleaved and cleaved) was available to us.

Coagulation and blood chemistry studies

Coagulation studies such as prothrombin time, aPTT, thrombin time, reptilase time, ethanol gelation assay, fibrinogen concentration, clotting activities for vitamin K-dependent coagulation factors (FII, FVII, FX), for FV and for contact factors (FXII, FXI, HK and PK) as well as C-reactive protein and creatinine levels were determined according to standardised procedures [25, 26].

Statistical analyses

Sigma Plot for Windows 9.0 (Jandel Scientific) was used to perform the statistical calculations. Mann Whitney rank sums test was used to evaluate the data in table 2. For Figures 2 and 3 standard deviations of the control population were calculated using a two-stage sampling approach [27].

Results

Clinical course of SIRS/sepsis

Four of the six patients had blood culture-positive sepsis. In retrospect, in three patients these cultures turned positive on day 0 of the study (patients 1 (purple), 2 (blue) and 3 (green) with *Streptococcus bovis*, *Pseudomonas aeroginosa* and *Streptococcus mitis* respectively. One patient's culture dating from one day before study inclusion (day –1: patient 6 (red) with *Hafnia alvei*) returned a positive result. Cultures remained negative in two of the patients (4 (yellow) and 5 (orange)), who in view of their clinical presentation were classified as culture-negative sepsis. Sepsis was complicated by features characterising septic shock in three patients (patients 3–5). One of these patients still presented severe sepsis on the last study day (patient 5), one presented signs and symptoms indicative of sepsis (patient 3), and one

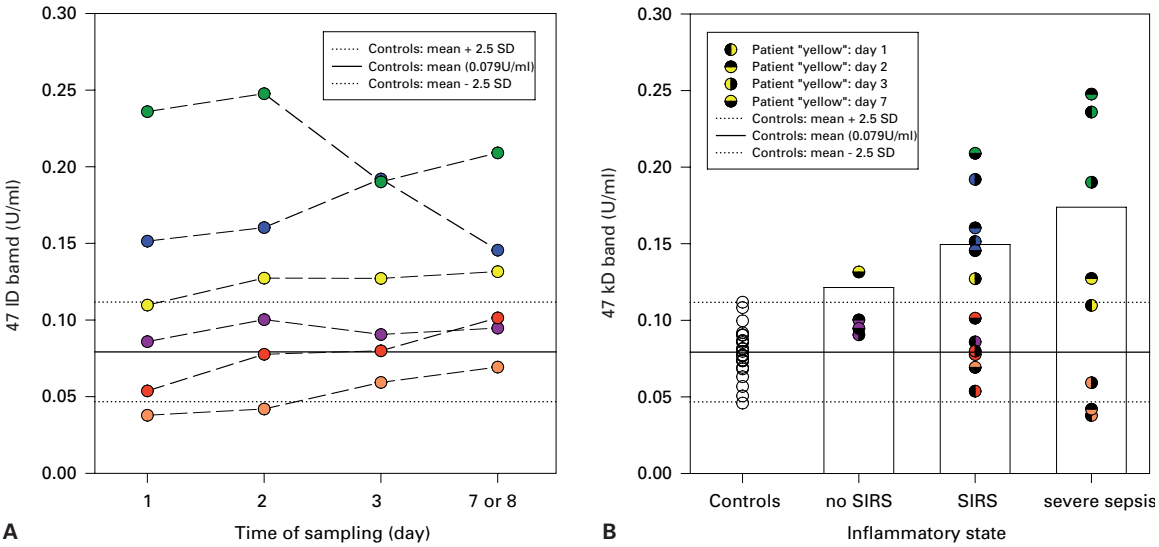


Figure 2

Contact system activation in sepsis *vs.* controls (analysis in U/ml).
Panel A shows the six individual patients' results over time. Panel B shows the analysis of 47kD light chain in U/ml as a function of SIRS severity. The solid line depicts the mean of all normal controls; whereas the dotted line delimits the mean ± two standard deviations. The categories on the x-axis are normal controls (empty circles), patients without SIRS (all patients upon inclusion in the study had at least two SIRS criteria; on subsequent days of testing some patients only presented 1 or 0 SIRS defining criteria and therefore were categorised in the "no SIRS" group), patients with sepsis and patients with severe sepsis (including septic shock). The insert correlates the symbols with the respective patients and time of blood draw. The empty bars indicate the mean CSA for each category.

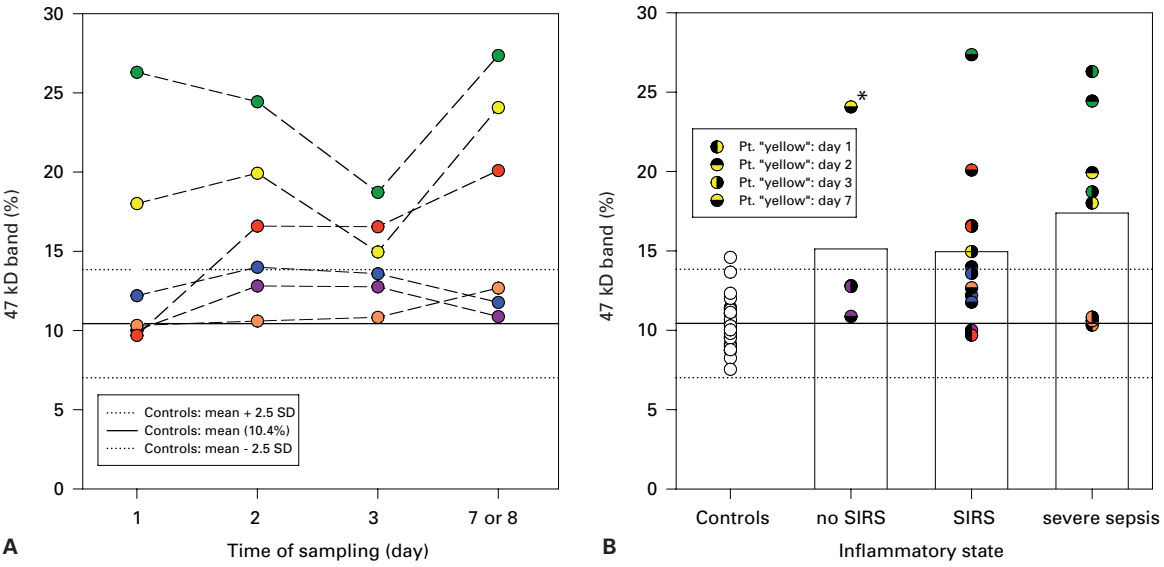


Figure 3

Contact system activation in sepsis *vs.* controls (analysis in %).
Panels A and B are organised similarly to Figure 2. Panel A shows the analysis for 47kD light chain in % of total HK. For this analysis the sum of all three detected bands (120kD + 61kD + 47kD) was considered to equal 100%. As well as illustrating increased CSA as a function of SIRS severity in sepsis patients, this figure shows that in healthy controls approximately 10% of HK circulates in its cleaved form under basal conditions. This result suggests a basal turnover of HK with a persistence of the 47kD fragment in the circulation. See Results section for comments regarding *.

Table 1
HK split products

		Sepsis patients	Controls
U/ml	120kD band	0.63 (0.11)	0.67 (0.08)
	61kD band	0.02 (0.01)	0.02 (0.01)
	47kD band	0.12 (0.03)*	0.08 (0.01)*
	Total HK	0.77 (0.12)	0.78 (0.08)
%	120kD band	81.8 (3.2)	86.3 (1.0)
	61kD band	2.4 (1.0)	3.3 (0.5)
	47kD band	15.8 (2.3)*	10.4 (0.7)*
	Total HK	100	100

Results are given as arithmetic mean
(± the value to obtain the 95% confidence interval).

shock patient no longer showed signs of SIRS by the end of the study period (patient 4). Of the three patients who did not have severe sepsis or septic shock, two still classified for sepsis by day 8, whereas patient 1 no longer presented the requisite criteria for that diagnosis.

Contact system activation in sepsis

The time course of contact system activation for the six patients as defined by the absolute amount of 47kD band of HK in U/ml is depicted in figure 2A. CSA correlates with the severity of the underlying inflammatory state (figure 2B). Using this analysis, three patients (2, 3 and 4) had pathologically high levels of 47kD HK. One patient (5) had results below the normal range. Controls are indicated for comparison. The solid line represents the mean control value; dotted lines represent the range of normal values.

Analysis by relative abundance of 47kD HK, expressed as % of total HK, is depicted in figures 3A and 3B. This analysis identified the same three patients from the above-mentioned group (patients 2, 3, 4) and a fourth patient, patient 6, who had pathological results on days 2, 3 and 8.

The empty bars in figures 2B and 3B depict the mean contact system activation of the SIRS/sepsis patients. The values ranged from the lowest values in controls (0.08 U/ml or 10.4%; as depicted by the solid line), to patients without SIRS (0.10 U/ml or 15.1%), to patients with SIRS (0.12 U/ml or 15.0%), and finally to patients with severe sepsis or septic shock (0.13 U/ml or 17.4%). There was one outlier, day 8 of patient 4 in Figure 3B: see asterix. If this outlier is excluded from the analysis of means, the resultant mean CSA for the "no SIRS" group in figure 3B would be 12.1% (in contrast to 15.1%, see above).

Table 1 shows that, while HK expressed in absolute terms (U/ml) did not differ in the sepsis and control groups (0.77 *vs.* 0.78 U/ml of whole blood), there was significantly more 47kD HK present in sepsis patients (see asterix). This holds true both in U/ml and in % of total HK.

Other Coagulation Studies

Clotting activity for FXII, HK, PK and FXI was assayed in all six patients on the four study dates. Table 2 shows the standard markers of CSA used in previous studies. The implications of these studies are commented on in the discussion. In our patient cohort at least one of the 4 assays showed decreased values in each of the six patients. However, the majority of measurements were normal or increased (60 out of 96). In contrast to 47kD HK, no correlation between reduced contact system factors (FXII, HK, PK or FXI) and severity of SIRS was detectable.

	Patient 1 (purple)				Patient 2 (blue)				Patient 3 (green)				Patient 4 (yellow)				Patient 5 (orange)				Patient 6 (red)			
Day	1	2	3	8	1	2	3	8	1	2	3	8	1	2	3	8	1	2	3	6	1	2	3	8
SIRS sc.	**2**	1	0	1	**3**	**3**	**3**	**2**	**3**	**3**	**3**	**2**	**3**	**3**	**2**	1	**3**	**3**	**3**	**2**	**2**	**2**	**2**	**2**
SIRS cat.	**S**	o	o	o	**SS**	**SS**	**SS**	**S**	**SS**	**SS**	**SS**	**S**	**SS**	**SS**	**S**	o	**SS**	**SS**	**SS**	**S**	**S**	**S**	**S**	**S**
FXII	**60**	71	65	76	86	64	66	68	81	93	100	82	61	74	80	78	**48**	**27**	**27**	**24**	**20**	**38**	**45**	**47**
PK	**64**	65	78	**52**	**61**	**59**	**60**	73	**59**	97	96	86	72	**56**	**51**	92	**44**	**25**	**29**	**24**	**38**	**38**	**41**	87
HK	129	87	94	91	130	118	102	114	90	101	192	129	89	103	126	154	**56**	**54**	75	**50**	94	102	108	103
FXI	79	**56**	87	79	95	**62**	81	67	**63**	87	99	95	**46**	**64**	82	107	**39**	**28**	**36**	**44**	**49**	81	73	76

Table 2

Standard parameters of CSA. Mann Whitney rank sum test.
Abbreviations and comments: score (sc., SIRS score according to [22]), category (cat.): no SIRS (o, white) SIRS (S, light grey)), severe sepsis and/or septic shock (SS, dark grey), clotting factor activity for FXII (normal range: 61–145%), PK (65–120%); HK (65–140%) and FXI (65–140%); bold characters indicate pathological results.

Discussion

We have shown that contact activation can be detected early in a subset of patients with sepsis. Defining CSA as an increase in the absolute concentration of 47kD HK (U/ml), we identified three patients with contact system activation. The test turned positive early on in the course of sepsis, namely on day 1 or 2, staying positive thereafter. If one uses the relative concentration of 47kD band (% of total HK), the same three patients were identified on day 1 or 2 as well as one

additional patient (patient six). Three out of these four patients remained positive for the rest of the study period, whereas one patient's results normalised on day 3 and 8. Out of the four patients who had a positive test in either analysis, three had severe forms of sepsis (severe sepsis or septic shock). Two patients did not test positive. Other CSA parameters were negative or inconclusive in our study patients. This suggests that our assay identified sepsis associated with adverse outcome in a subpopulation of patients.

Several studies have reported CSA in sepsis in the past, with first reports dating back to the 1970s [28–38]. The main confounding factor in these studies was the lack of consensus on how to define CSA. Decreased coagulation factor levels for factor XII, prekallikrein and HK have been proposed as measures of CSA. However, different reports have shown these values to be increased, normal or reduced in different sepsis patients, making analysis difficult. Tables 1 and 2 illustrate how contact system activation may be overlooked if one analyses only the total concentration of a contact system protein. Previous reports [39] and our own results support the notion that absolute concentrations of contact system proteins at the time of study enrolment into the relevant study do not reliably include or rule out CSA or predict patient outcome. Assays for activated contact factors such as FXIIa have been proposed but also remain debatable. Complexes of coagulation factor inhibitors bound to activated coagulation factors such as C1Inh-FXIIa, C1Inh-KK, or α_1-antitrypsin-FXIa and α_2-macroglobulin-KK have also been studied. Again, no single assay has established itself for definition of CSA in sepsis. We propose 47kD HK as a marker for contact system activation in the context of sepsis. Our assay has two advantages over other assays: i) it defines CSA through the generation of a specific and persistent product, the 47kD light chain of HK, and ii) it indicates that an important mediator in sepsis, the vasoactive peptide bradykinin, has been released (fig. 1).

In our patients the degree of CSA correlated with the severity of SIRS/sepsis. Mean (depicted by empty bars in the figure) and median CSA (not depicted) increased from controls to patients without SIRS, to those with SIRS and finally to those with severe sepsis in the 47 kD HK analysis by U/ml (figure 2A) and in % (figure 2B) with one exception. In the analysis of CSA in % the mean CSA of patients without SIRS was smaller than that of patients with SIRS (15.1 vs 15.0; the higher average in the no SIRS group is attribuable to one outlier: see asterisk). Our data are however preliminary and based on a small patient population. Confirmation of this finding in a larger cohort is necessary. Such confirmation is of significant clinical interest since patients with severe sepsis and septic shock have the highest mortality risk. We predict that patients with high levels of 47kD would benefit most from treatment whih specifically targets the contact system, such as C1Inh. Any treatment aimed at reducing mortality in sepsis should target a pathway that is active in these patients.

Earlier studies on CSA in culture negative *vs.* culture positive sepsis [30, 31] either used varying definitions of sepsis, adopted criteria of severity not predictive of an adverse outcome, did not describe criteria of patient recruitment, used patients from previous studies, used less sensitive methods to detect HK cleavage and non- or less specific markers for CSA, or described CSA only at single time points. In this study we adopted a definition of SIRS and sepsis that is based on a consensus statement [22] for which the criteria of severity have been prospectively validated [2, 40]. Moreover, we followed our patients over 4 time points. However, our study has several limitations. It is a preliminary study based on a small patient population. The normal ranges were defined using 24 values, which *sensu stricto* were not independent measurements. We were unable to assay HK cleavage using an alternative method. Disease severity at study inclusion was not prospectively registered.

Surfaces on which contact system activation occurs in vivo have been identified in recent years. Such surfaces include endothelial cells [41–44], polymorphonuclear neutrophils [45], platelets [46, 47] and various bacteria [48–50]. It is interesting that all of the above *in vivo* targets also play central roles in the pathogenesis of sepsis, and hence are sites of potential therapeutic intervention. Although the site of CSA in our study is undefined, comparative studies in whole blood, platelet rich plasma and platelet poor plasma demonstrated a cell bound fraction of HK (data not shown) suggesting a role of HK bound to circulating cellular elements.

To date the only coagulation-modifying drug that has been shown to reduce sepsis-related mortality in a prospective randomised study with predefined outcomes is activated protein C [5]. Most trials investigating drugs which modulate the contact system in SIRS/sepsis patients have failed to show an improved patient outcome. Trials in humans and animals have investigated monoclonal antibodies directed against FXIIa [51], direct BK antagonists [52], or also inhibitors of contact factors such as ATIII [6] and C1 inhibitor [9]. A meta-analysis of controlled trials of anticoagulant treatments for sepsis concluded that, since the degree of benefit from the various treatment regimens depends on disease severity, "the safety and efficacy of these agents may be enhanced by refinement in techniques of clinical stratification" [53]. Future studies will have to show whether our assay represents such a refinement.

In conclusion, our results show that the contact system became activated early on, that the activation was persistent in a subset of sepsis patients, and that the activation correlated with the severity of the inflammatory syndrome. 47kD HK

warrants further research into its potential role as a sepsis marker for the prognosis of outcome or treatment response.

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