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# Plasma fractalkine is a sustained marker of disease severity and outcome in sepsis patients

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## Abstract

**Introduction:** Fractalkine is a chemokine implicated as a mediator in a variety of inflammatory conditions. Knowledge of fractalkine release in patients presenting with infection to the Intensive Care Unit (ICU) is highly limited. The primary objective of this study was to establish whether plasma fractalkine levels are elevated in sepsis and associate with outcome. The secondary objective was to determine whether fractalkine can assist in the diagnosis of infection upon ICU admission.

**Methods:** Fractalkine was measured in 1103 consecutive sepsis patients (including 271 patients with community-acquired pneumonia (CAP)) upon ICU admission and at days 2 and 4 thereafter; in 73 ICU patients treated for suspected CAP in whom this diagnosis was refuted in retrospect; and in 5 healthy humans intravenously injected with endotoxin.

**Results:** Compared to healthy volunteers, sepsis patients had strongly elevated fractalkine levels. Fractalkine levels increased with the number of organs failing, were higher in patients presenting with shock, but did not vary by site of infection. Non-survivors had sustained elevated fractalkine levels when compared to survivors. Fractalkine was equally elevated in CAP patients and patients treated for CAP but in whom the diagnosis was retrospectively refuted. Fractalkine release induced by intravenous endotoxin followed highly similar kinetics as the endothelial cell marker E-selectin.

**Conclusions:** Plasma fractalkine is an endothelial cell derived biomarker that, while not specific for infection, correlates with disease severity in sepsis patients admitted to the ICU.

## Introduction

Sepsis is a life-threatening condition that represents a great health burden [1] and the most frequent cause of death in non-coronary intensive care units (ICUs) in the developed world [2]. The annual incidence of severe sepsis in the USA is estimated at 300 cases per 100,000 person-years population, which accounts for 10 % of all ICU admissions [3]. The mortality of severe sepsis and septic shock lies between 25 and 50 %, with the extent and number of organs failing as the strongest predictors

of an adverse outcome [2]. The clinical syndrome of sepsis is the consequence of a deregulated immune response to infection that is injurious to the host's tissues and organs. The harmful host response during sepsis involves both disproportionate proinflammatory and immune suppressive anti-inflammatory components [1].

Fractalkine (CX<sub>3</sub>CL1) is a CX<sub>3</sub>C chemokine, which was first described to be produced by endothelial cells [4]. Subsequent research identified several additional cellular sources of fractalkine, including epithelial cells, neurons, microglial cells, osteoblasts, smooth muscle cells, dendritic cells, lymphocytes and macrophages [5, 6]. Fractalkine has a membrane-bound form and a soluble form [7]. The membrane-bound form functions as an adhesion protein, whereas soluble fractalkine acts as a

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chemokine [6]. The receptor for fractalkine, CX<sub>3</sub>CR1, is expressed by T cells, natural killer cell subsets, brain microglia, dendritic cell subsets, blood monocytes, and thrombocytes [5, 8, 9]. Fractalkine has been implicated as a mediator in several inflammatory conditions, including atherosclerosis, atopic dermatitis, airway hyper responsiveness, multiple sclerosis, and Crohn's disease [10–12].

Recent studies have suggested that fractalkine plays a role in the pathogenesis of sepsis. Mice with polymicrobial abdominal sepsis caused by cecal ligation and puncture (CLP) have elevated fractalkine levels in their peritoneal lavage fluid and serum [13–15]. CX<sub>3</sub>CR1-deficient mice have enhanced mortality after CLP, suggesting that fractalkine contributes to protective immunity during sepsis [13]. Knowledge of fractalkine levels in a setting of clinical sepsis is limited to a single study that described elevated serum concentrations in 43 patients with septic shock [16]. In the present study, we analyzed sequential plasma fractalkine levels in 1,103 sepsis patients during the first 4 days of ICU admission and determined their association with the source of infection, organ failure and survival. In addition, we evaluated the possible cellular source of fractalkine by studying healthy humans injected with endotoxin. Finally, we determined whether fractalkine levels can be discriminative of infection upon ICU admission by performing analyses in 344 patients presenting with suspected community-acquired pneumonia (CAP).

## Methods

### Study design, patients and definitions

From January 2011 through July 2013 consecutive patients presenting to the mixed ICUs of two tertiary teaching hospitals (Academic Medical Center in Amsterdam and University Medical Center Utrecht) were included. Data and plasma samples were prospectively collected as part of the molecular diagnosis and risk stratification of sepsis (MARS) project, a large prospective observational study (ClinicalTrials.gov identifier NCT01905033) [17–23]. Organ failure was defined as a score of 3 or greater on the Sequential Organ Failure Assessment (SOFA) score, except for cardiovascular failure for which a score of 1 or more was used [24]. Shock was defined as the use of vasopressors (noradrenaline) for hypotension in a dose of 0.1 mcg/kg/min during at least 50 % of the ICU day. The plausibility of infection was scored post hoc based on all available evidence and classified on a 4-point scale (none, possible, probable or definite) according to Center for Disease Control and Prevention [25] and International Sepsis Forum consensus definitions [26], as described in detail previously [17]. Readmissions and patients transferred from another ICU were excluded, except for patients referred to one of the study centers on the day of admission. Daily (on admission and at 6 a.m. thereafter) left-over

EDTA plasma (obtained from blood drawn for patient care) was stored within 4 hours at –80 °C. The Medical Ethical Committees of both study centers gave approval for an opt-out consent method (IRB no. 10-056C). The Municipal Personal Records Database was consulted to determine survival up to one year after ICU admission.

The primary objective of this study was to establish whether plasma fractalkine levels are elevated in sepsis and are associated with outcome. For this we analyzed all patients with sepsis diagnosed within 24 hours of admission, defined as a 'definite' or 'probable' infection [17] combined with at least one general, inflammatory, or hemodynamic, organ dysfunction, or tissue perfusion parameters derived from the 2001 International Sepsis Definitions Conference [27]. The secondary objective was to determine whether fractalkine can assist in the diagnosis of infection upon ICU admission. For this, we selected all patients with CAP from this sepsis cohort and compared these with all patients presenting with suspected CAP, for which the clinical team started therapeutic antibiotics, but in retrospect were classified as having an infection likelihood of 'none', as described previously [19].

### Experimental human endotoxemia

Five healthy non-smoking male volunteers (mean age 21, range 19–22 years) were intravenously administered 4 ng/kg lipopolysaccharide (LPS) (from *Escherichia coli* O113, CC-RE lot 3), kindly provided by Dr. Anthony Suffredini (National Institutes of Health, Bethesda, MD, USA). Blood was collected in EDTA tubes immediately before injection, and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, and 20.0 hours thereafter. The study was approved by the Medical Ethics Commission of the AMC, Amsterdam, The Netherlands, and written informed consent was obtained from all subjects.

### Assays

Fractalkine, soluble E-Selectin, interleukin (IL)-6, IL-8, IL-10 and TNF- $\alpha$  levels were measured by cytometric bead array (BD Biosciences, San Jose, CA, USA) using a FACS Calibur flow cytometer (BD Biosciences). IL-6 levels in plasma from subjects injected with LPS were determined by Luminex multiplex assay using BioPlex 200 (BioRad, Hercules, CA, USA). Normal biomarker values were acquired from EDTA plasma from 27 age- and gender-matched healthy volunteers, from whom written informed consent was obtained.

### Statistical analysis

Data are represented by box and whisker plots. Two-group comparisons were performed with the Wilcoxon rank-sum test; for multiple groups Kruskal-Wallis analysis followed by Dunn's test was used. The primary endpoint for association with mortality was set at day 30 after ICU

admission. Mixed-effects models were used to compare survivors with non-survivors over time. The log-rank test was performed to compare survival in quartiles of fractalkine levels on ICU admission. Correlation was determined using Spearman's rho test. Multivariable logistic regression was used to establish the independent prognostic value of fractalkine after adjustment for age, Charlson comorbidity index, body mass index, admission type and severity of disease (Acute Physiology and Chronic Health Evaluation (APACHE) IV or Sequential Organ Failure Assessment (SOFA)). Variables in the model were checked for collinearity by calculating the variance inflation factor. The area under the receiver operating characteristics (ROC) curve (AUC, or the *C* statistic) and 95 % confidence intervals (CI), considering 2000 bootstrap replicates, were determined to examine the performance of fractalkine in predicting 30-day mortality. The optimal threshold was determined using the Youden index for selection of the highest sum of sensitivity and specificity. Differences in the predictive power of fractalkine and soluble E-selectin was studied by comparing the ROC AUC models by means of DeLong's test. Prognostic analyses were

complemented with the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) [28]. Calibration of the model was performed with the Hosmer and Lemeshow goodness-of-fit test. For regression analyses, biomarker values were transformed to a log scale. All analyses were performed in R (v3.1.1). *P* <0.05 was considered statistically significant.

## Results

### Patient characteristics

Characteristics of all sepsis patients are shown in Table 1. From a total of 1,103 patients, 786 (71.3 %) survived until day 30 after ICU admission, whereas 305 (27.7 %) patients did not; 12 patients were lost to follow up. The lung was the most common source of infection, followed by abdominal and urinary tract infections; survivors and non-survivors had similar sites of infection. As expected, non-survivors presented with more severe disease, as reflected by higher APACHE IV and SOFA scores, more organs failing and more shock. Thirty-day mortality occurred in 70.2 % of patients in the ICU.

**Table 1** Clinical characteristics and outcome of sepsis patients stratified according to survival status 30 days after admission

	All patients n = 1103	Survivors n = 786	Non-survivors n = 305	<i>P</i>
Demographics				
Age, years, mean (SD)	61.2 (14.7)	59.7 (14.7)	64.9 (13.9)	<0.0001
Gender, male, n (%)	671 (60.8)	481 (61.2)	183 (60)	0.73
Race, white, n (%)	972 (88.8)	689 (87.7)	273 (89.5)	0.45
Body mass index, kg/m <sup>2</sup> , mean (SD)	26 (6.2)	26.2 (6.3)	25.5 (5.8)	0.09
Admission type, medical, n (%)	817 (74.1)	562 (71.5)	247 (81)	0.001
Charlson score, median (IQR)	4 (3–6)	4 (2–6)	5 (3–6)	0.0001
Site of infection				
Pulmonary, n (%)	478 (43.3)	337 (37.1)	135 (44.3)	0.69
Abdominal, n (%)	215 (19.5)	150 (19.1)	61 (20)	0.74
Urinary, n (%)	109 (9.9)	84 (10.7)	24 (7.9)	0.69
Other, n (%) <sup>a</sup>	173 (15.7)	123 (15.6)	49 (16.1)	0.93
Coinfection, n (%)	128 (11.6)	92 (11.7)	36 (11.8)	1
Severity of disease in first 24 hours				
APACHE IV score, median (IQR)	80 (63–101)	74 (59–92)	98 (81–122)	<0.0001
SOFA score, median (IQR) <sup>b</sup>	7 (5–9)	7 (4–9)	9 (6–12)	<0.0001
Shock, n (%)	373 (33.8)	234 (29.8)	135 (44.3)	<0.001
Mechanical ventilation, n (%)	851 (77.2)	590 (75.1)	249 (81.6)	<0.05
Renal replacement therapy, n (%)	115 (10.4)	60 (7.6)	53 (17.4)	<0.001
Outcome				
ICU length of stay, days, median (IQR)	4 (2–10)	5 (2–10)	3 (2–8)	<0.01
ICU mortality, n (%)	227 (20.6)	13 (1.7)	214 (70.2)	<0.001

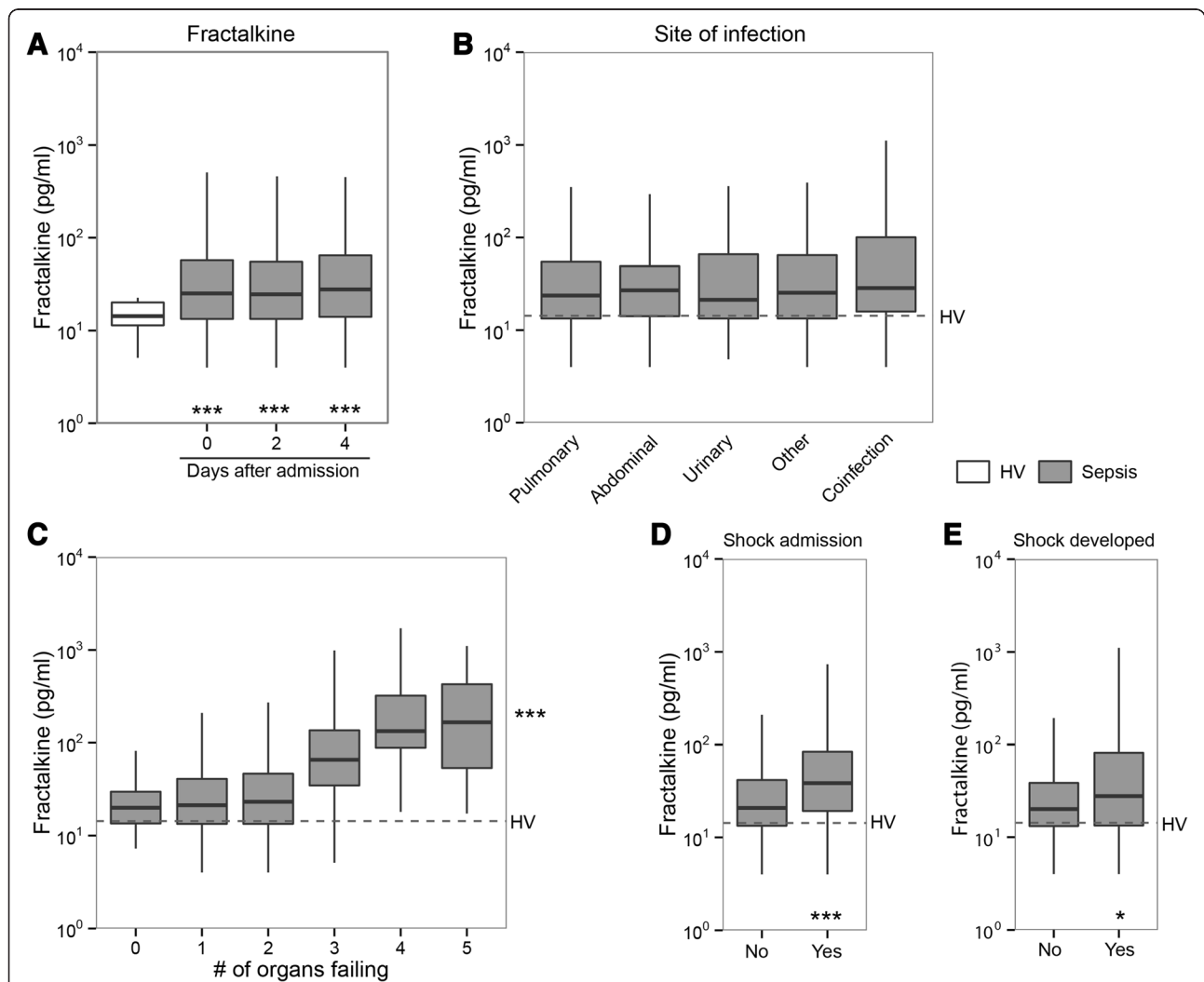
12 patients were lost to follow up.<sup>a</sup>Site of infection: "other" includes cardiovascular infection, mediastinitis and skin infection. <sup>b</sup>Central nervous system not included in score. APACHE Acute Physiology and Chronic Health Evaluation, SOFA Sequential Organ Failure Assessment

**Fractalkine levels are elevated in sepsis patients during the first 4 days after ICU admission and are associated with severity of disease**

Fractalkine levels, measured in plasma obtained within 24 hours after admission (day 0) and at days 2 and 4, were consistently elevated in patients with sepsis relative to healthy controls (Fig. 1a,  $P < 0.0001$  for all time points). On longitudinal analysis there was no effect of time on plasma fractalkine levels ( $P = 0.43$ ). In contrast, in sepsis patients during the first 4 days of the ICU stay there was a time-dependent decline in plasma IL-6, IL-8 and IL-10 (Additional file 1: Figure S1,  $P < 0.0001$  for all analytes), which are well-known cytokine markers in sepsis [29].

Sustained elevated plasma fractalkine levels after admission for sepsis were confirmed upon separate analysis of the two contributing ICUs (data not shown). Fractalkine concentrations did not differ between sepsis patients with different sources of infection (Fig. 1b).

Admission fractalkine levels increased with increasing number of organs failing on admission (Fig. 1c,  $r = 0.42$ ,  $P < 0.001$ ). Patients presenting with shock had significantly higher fractalkine levels than those without shock (Fig. 1d). Of the 730 patients not presenting with shock on admission, 90 (12.3 %) developed shock later during their stay on the ICU; admission fractalkine levels were higher in those septic patients who developed shock



**Fig. 1** Fractalkine levels are elevated in sepsis and correlate with organ failure. Blood was drawn from patients within 24 hours of admission to the ICU (day 0) and on days 2 and 4. **a** Fractalkine levels were elevated in patients compared to healthy volunteers (HV) at all time points, and did not differ between days 0 and 4. **b** Fractalkine levels were not influenced by the primary source of infection. **c** Admission fractalkine levels in patients with increasing numbers of failing organs at admission. Fractalkine levels were higher in patients with shock on admission (**d**) and in patients who developed shock >24 hours after admission (**e**). Box and whisker diagrams depict the median and lower quartile, upper quartile, and their respective 1.5 IQR as whiskers (as specified by Tukey). Gray boxes represent sepsis; open box or dotted lines represent healthy volunteers. \*\*\* $P < 0.001$ , \* $P < 0.05$

while on the ICU when compared with patients who did not (Fig. 1e).

**Fractalkine levels are higher in non-survivors**

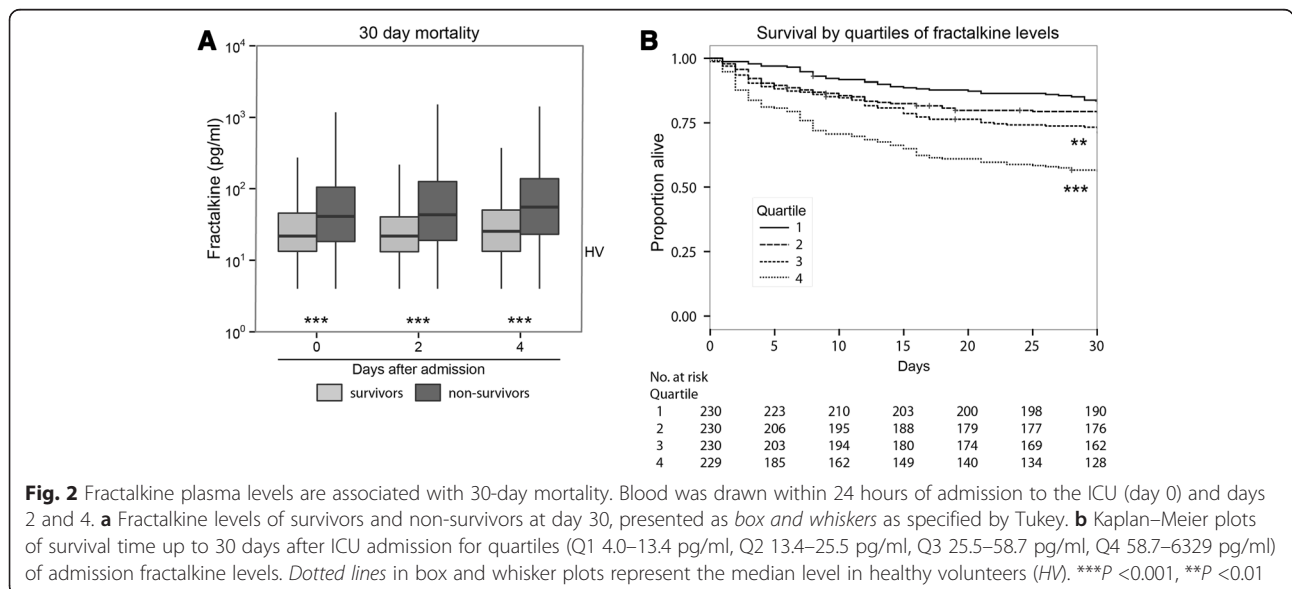
To assess the relationship between fractalkine levels and sepsis mortality, measurements were partitioned in survivors and non-survivors at day 30 after ICU admission. Fractalkine levels on admission and 2 and 4 days thereafter were significantly higher in non-survivors compared to survivors (Fig. 2a,  $P < 0.001$  for all time points). In a sub-analysis (restricted to patients presenting with shock, fractalkine remained significantly associated with mortality (Additional file 2: Figure S2). To further investigate the association between fractalkine and 30-day mortality, admission fractalkine levels were partitioned into quartiles for which log-rank tests were performed. Quartile 3 (50–75 %, 25.5–58.7 pg/ml) and quartile 4 (75–100 %, 58.7–6329.0 pg/ml) of fractalkine levels had statistically significantly reduced survival ( $P < 0.01$  and  $P < 0.001$ , respectively), compared to quartile 1 (Fig. 2b). Admission fractalkine levels remained significantly associated with 30-day mortality after adjustment for age, Charlson comorbidity index, body mass index (BMI), admission type and APACHE IV scores in a logistic regression model (odds ratio (OR), 1.37 (95 % CI, 1.19–1.58) for each log increase in fractalkine,  $P < 0.0001$ ). Similarly, when fractalkine levels were included in a logistic regression analysis together with SOFA scores, the association with 30-day mortality persisted (OR, 1.25, 95 % CI, 1.09–1.44, for each log increase in fractalkine,  $P = 0.001$ ). Moreover, plasma levels on days 2 and 4 remained independently associated with 30-day mortality after adjustment for SOFA scores for those particular days (OR 1.40, 95 % CI 1.20–1.63,  $P = 0.001$  and OR

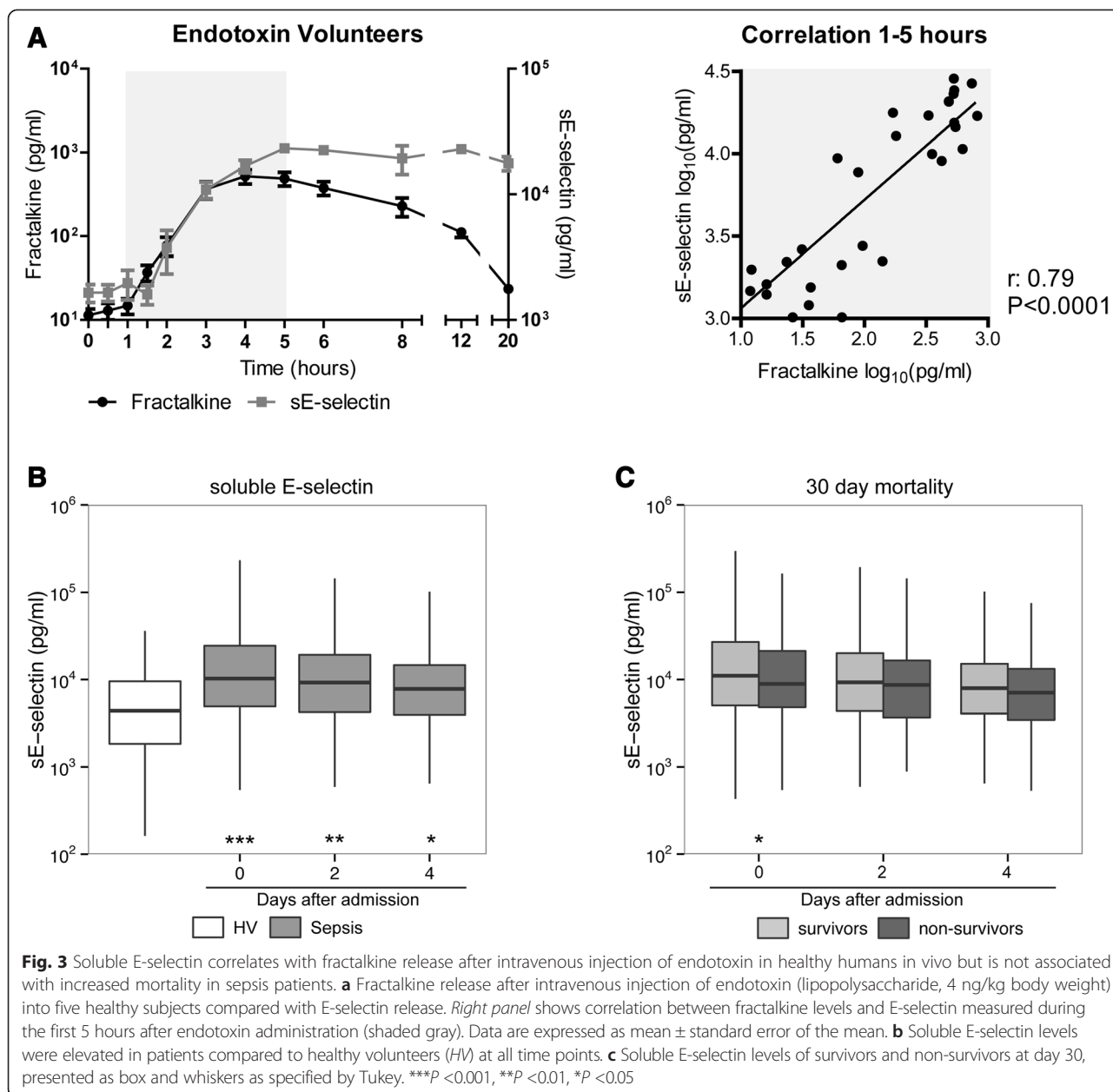
1.31, 95 % CI 1.08–1.59,  $P = 0.006$ , respectively). The ROC AUC of fractalkine was 0.65 (95 % CI 0.61–0.69). The Youden index determined a fractalkine level of 38.2 pg/ml to be the optimal cutoff. At this cutoff, fractalkine had 54 % sensitivity and 71 % specificity for predicting mortality at 30 days after ICU admission. Fractalkine in addition to the APACHE IV score, improved classification of patients who were deceased at 30 days after admission and those who were not; the NRI was 0.27 (95 % CI 0.12–0.41,  $P = 0.0003$ ) and the IDI was 0.02 (95 % CI 0.006–0.02,  $P = 0.002$ ).

The association between elevated fractalkine concentrations and mortality persisted when outcomes were 7-day, 90-day or 1-year mortality (Additional file 3: Figure S3). Together these data suggest that higher fractalkine levels are associated with short-term mortality, and that this association drives differences in long-term mortality.

**Comparison of fractalkine release with that of soluble E-selectin**

Several studies have identified endothelial cells as a major source of fractalkine [4, 30, 31]. Considering that it is difficult to assess whether the endothelium produces fractalkine during human sepsis in vivo, we compared the kinetics of fractalkine release into the circulation relative to that of the established specific endothelial cell activation marker E-selectin [32, 33] in a controlled human setting of systemic inflammation induced by bolus intravenous injection of LPS. Intravenous LPS induced a rise in fractalkine levels with similar kinetics to the increase in soluble E-selectin (Fig. 3a,  $P < 0.0001$ ). The initial increase in soluble E-selectin coincided with the rise in fractalkine levels, and fractalkine and soluble





E-selectin levels measured during the first 5 hours after LPS injection had strong positive correlation (Fig. 3a;  $r = 0.79$ ,  $P < 0.0001$ ). The release of IL-6 occurred faster and was more transient, and IL-6 levels did not correlate with fractalkine levels (Additional file 4: Figure S4). We also measured soluble E-selectin levels in the patient samples used in Figs. 1 and 2. While soluble E-selectin levels were clearly elevated in patients with sepsis when compared with healthy controls (Fig. 3b,  $P < 0.001$ ), confirming previous reports [34, 35], soluble E-selectin was not associated with increased mortality at day 30 (Fig. 3c); admission soluble E-selectin was, in contrast with fractalkine, lower in

non-survivors ( $P = 0.02$ ). The ROC AUC of soluble E-selectin for 30-day mortality was 0.55 (95 % CI 0.51–0.59). Fractalkine outperformed soluble E-selectin in predicting 30-day mortality ( $P = 0.001$ ).

**Elevated plasma fractalkine levels are not specific for infection**

Considerable research has focused on discriminating infectious from non-infectious sources of critical illness. To determine whether fractalkine levels can provide diagnostic value we analyzed fractalkine levels in 344 patients consecutively admitted to the ICU with suspected CAP. Of these, 271 patients were classified as

having CAP (these were also part of the sepsis cohort described above), whereas in 73 patients the CAP diagnosis was retrospectively refuted (no-CAP controls) [17]. Clinical characteristics of patients with and without CAP are depicted in Table 2. These patients were largely similar in demographics, comorbidities, severity of disease and outcome. Patients with CAP more often had shock and had longer lengths of stay in the ICU. The ICU mortality and 30-day mortality did not differ between groups. Fractalkine levels were similar in patients with and without CAP on admission and at 2 and 4 days thereafter (Fig. 4a). Fractalkine levels were higher in patients with CAP and without CAP who presented with shock than in those who did not (Fig. 4b); similarly, fractalkine levels were higher in patients with and without CAP who did not survive until day 30, when compared with those who did (Fig. 4c).

## Discussion

Fractalkine is the only member of the CX3C class of chemokines, and thus far, primarily studied as a potential mediator of chronic inflammatory conditions. We performed a detailed analysis of plasma fractalkine concentrations during the first 4 days after ICU admission in almost 1,200 acutely ill patients to show that elevated plasma fractalkine, while not specific for the presence of infection,

is a sustained marker of disease severity and an adverse outcome in sepsis patients admitted to the ICU.

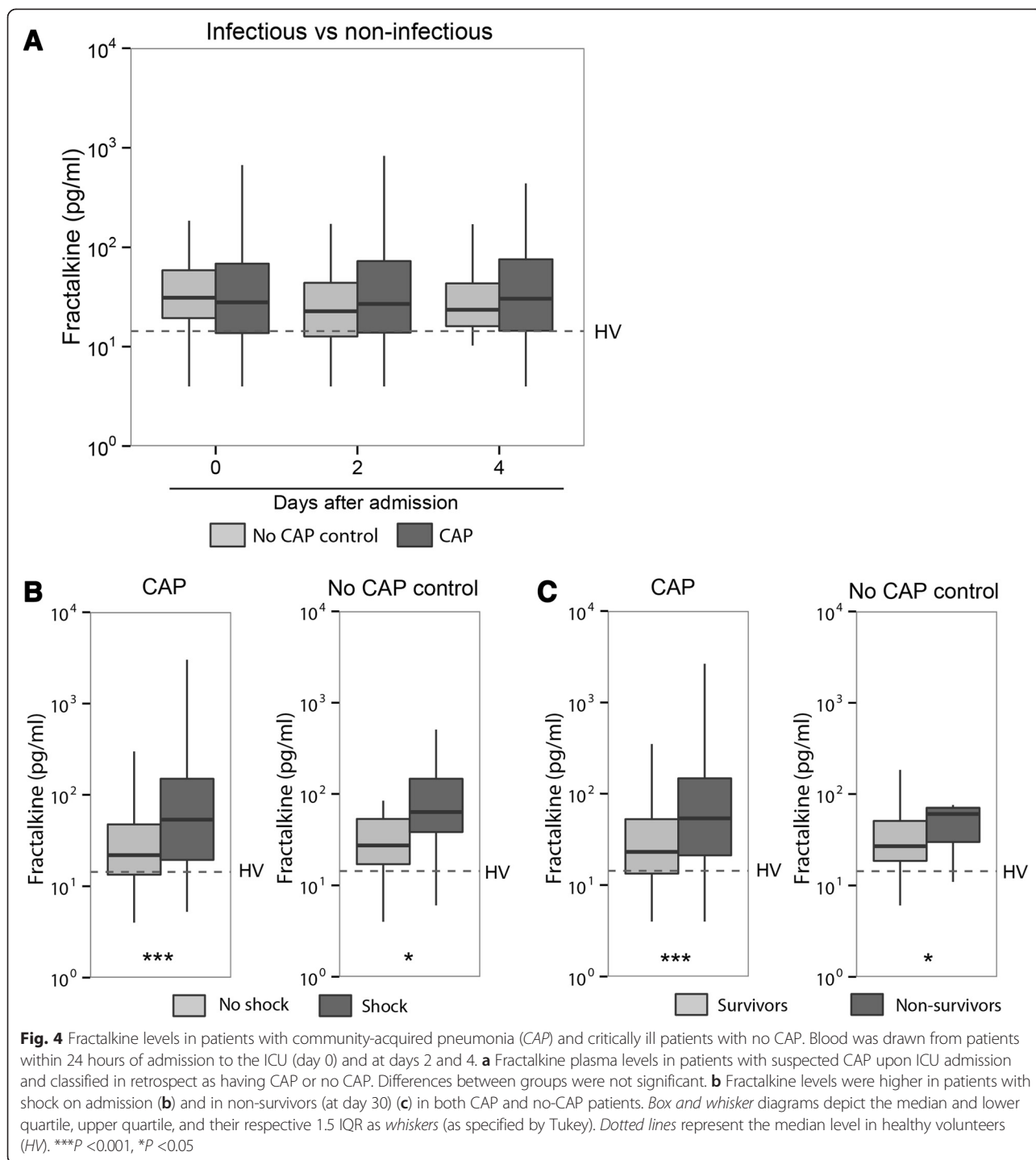
Fractalkine may not be merely a marker of disease severity in sepsis. Animal studies have suggested an important functional role for fractalkine in the pathogenesis of sepsis. In accordance with our human data, abdominal sepsis induced by CLP in mice was associated with elevated plasma fractalkine levels [14, 15]. Elimination of the biological activity of fractalkine, achieved by studying mice deficient for its receptor CX3CR1, has been shown to exaggerate organ damage and increase lethality [13]. During murine abdominal sepsis, fractalkine mRNA levels are elevated in the heart, lung, kidney, and liver, indicating that many organs contribute to elevated plasma concentrations [15].

Although fractalkine can be produced by a variety of cell types, arterial and capillary endothelial cells have been identified as a major source during endotoxemia in rats [31]. In accordance, several studies have reported the capacity of endothelial cells to produce fractalkine [4, 30, 31]. As endothelial cells are not easily accessible for studying production of inflammatory mediators in patients with sepsis, we used the model of human endotoxemia to obtain indirect evidence for the endothelium as an important source of fractalkine, comparing the release of fractalkine with that of E-selectin. We regarded E-selectin a relevant biomarker in this context,

**Table 2** Clinical characteristics and outcome of patients with community-acquired pneumonia (CAP) and no-CAP controls

	CAP n = 271	No CAP n = 73	P
Demographics			
Age, years, mean (SD)	59.7 (16.7)	58.8 (17.2)	0.70
Gender, male, n (%)	155 (57.2)	40 (54.8)	0.79
Race, white, n (%)	236 (87.1)	57 (78.1)	0.04
Body mass index, kg/m <sup>2</sup> , mean (SD)	24.9 (6.0)	26.4 (9.4)	0.21
Charlson score, median (IQR)	4 (2–6)	3 (2–5)	0.07
Severity of disease in first 24 hours			
APACHE IV score, median (IQR)	79 (62–101)	68 (52–99)	0.07
SOFA score, median (IQR) <sup>a</sup>	7 (4–9)	6 (3–7)	0.01
Shock, n (%)	71 (26.2)	10 (13.7)	0.03
Mechanical ventilation, n (%)	203 (74.9)	57 (78.1)	0.66
Renal replacement therapy, n (%)	19 (7)	4 (5.5)	0.78
Outcome			
ICU length of stay, days, median (IQR)	5 (2–11)	2 (1–4)	<0.0001
ICU mortality, n (%)	53 (19.6)	9 (12.3)	0.18
30-day mortality, n (%)	73 (26.9)	16 (21.9)	0.37
90-day mortality, n (%)	92 (33.9)	19 (26)	0.16
1-year mortality, n (%)	121 (44.6)	30 (41.1)	0.50

All patients were treated for suspected CAP upon ICU admission and classified in retrospect as having or not having CAP, as described in "Methods". <sup>a</sup>Central nervous system not included in score. APACHE Acute Physiology and Chronic Health Evaluation, SOFA Sequential Organ Failure Assessment



considering that it is endothelial-cell-specific [36], and that increased endothelial cell expression of fractalkine and E-selectin by inflammatory stimuli is mediated by similar mechanisms involving activation of NF- $\kappa$ B [30, 37]; moreover, the release of both fractalkine and E-selectin from endothelial cells is regulated by members of the family of disintegrins and metalloproteinases [38, 39].

We indeed found remarkably similar kinetics for the appearance of fractalkine and E-selectin in the circulation after intravenous injection of LPS, and fractalkine levels were positively and significantly correlated with E-selectin levels. Together these data suggest endothelial cells are a major source of fractalkine in sepsis. Blood leukocytes are an unlikely source of fractalkine in sepsis,



as whole blood leukocytes did not release fractalkine upon stimulation with Toll-like receptor ligands relevant for common sepsis pathogens (data not shown). In accordance, fractalkine mRNA levels were undetectable in blood leukocytes from a subgroup of 440 sepsis patients in whom the genome-wide transcriptome was assessed (data not shown).

A substantial number of biomarkers have been proposed for the diagnostic stratification of infectious and non-infectious ICU patients [29], most notably procalcitonin [40]. To the best of our knowledge, published data on fractalkine levels in an ICU setting is limited to a single study reporting fractalkine concentrations in 43 patients with septic shock [16]. Considering that circulating fractalkine concentrations are elevated in a variety of chronic non-infectious inflammatory conditions [41–45], we argued that elevated fractalkine levels are likely not specific for the presence of infection upon ICU admission. To address this, we measured fractalkine levels in critically ill patients suspected of having CAP upon ICU admission, who in retrospect were classified as having an infection likelihood of ‘definite’ or ‘probable’ (CAP patients), or ‘none’ (no-CAP patients). The likelihood of the CAP diagnosis was classified by dedicated research physicians by post hoc review of all available clinical, radiological, and microbiological evidence, and using strict diagnostic criteria [17]; the interobserver agreement for the diagnosis and likelihood of CAP was excellent [17]. By using this additional ICU cohort we clearly show that fractalkine cannot be considered a marker for infection in critically ill patients. Also elevated fractalkine levels were associated with increased disease severity and an adverse outcome in patients without CAP.

How could fractalkine levels be of use at the bedside? Many different biomarkers for diagnosis of infection and ICU prognosis have been described in the literature, and none of these are currently used in clinical practice [29]. The challenge for future clinical implementation is to define combinations of biomarkers concurrently measured at the bedside that together with clinical readouts provide useful information for detection of infection and risk stratification [46]. The current study suggests that plasma fractalkine levels could be part of a test aiding in risk stratification during the first days after ICU admission.

## Conclusions

This large observational study suggests that plasma fractalkine, while not specific for infection, is a sustained biomarker of disease severity and outcome in sepsis patients admitted to the ICU, irrespective of the primary source of infection and is likely derived from the activated vascular endothelium.

## Key messages

- Plasma fractalkine is an endothelial-cell-derived biomarker
- Fractalkine levels are elevated in sepsis patients during the first 4 days after ICU admission
- Fractalkine levels are strongly associated with severity of disease and adverse outcome
- Elevated plasma fractalkine levels are not specific for infection in the critically ill

## Additional files

**Additional file 1: Figure S1.** Elevated IL-6, IL-8 and IL-10 levels in sepsis patients. Blood was drawn from patients within 24 hours of admission to the ICU (day 0) and at days 2 and 4. IL-6 (**a**), IL-8 (**b**) and IL-10 (**c**) levels were measured in plasma. *Box and whisker* diagrams depict median and lower quartile, upper quartile, and their respective 1.5 IQR as *whiskers* (as specified by Tukey). *Gray boxes*, sepsis patients (n = 1,103), *open boxes*, healthy volunteers (HV, n = 27). \*\*\*P < 0.001 for sepsis patients compared to HV and for decline over time. (DOC 151 kb)

**Additional file 2: Figure S2.** Fractalkine plasma levels are associated with mortality in patients with shock within 24 hours of admission. Patient blood was drawn within 24 hours of admission to the ICU (day 0) and at days 2 and 4. Fractalkine levels of 30-day survivors and non-survivors in a subgroup of patients admitted with shock. *Box and whisker* diagrams depict median and lower quartile, upper quartile, and their respective 1.5 IQR as *whiskers* (as specified by Tukey). *Dotted lines* in boxes, median level in healthy volunteers (HV). \*\*P < 0.01, \*P < 0.05. (DOC 83 kb)

**Additional file 3: Figure S3.** Elevated fractalkine plasma levels are associated with short- and long-term mortality. Patient blood was drawn within 24 hours of admission to the ICU (day 0) and at days 2 and 4. Fractalkine levels are shown for survivors and non-survivors at day 7 (**a**), day 90 (**b**) and one year (**c**) after ICU admission. *Box and whisker* diagrams depict median and lower quartile, upper quartile, and their respective 1.5 IQR as *whiskers* (as specified by Tukey). *Dotted lines* in boxes, median level in healthy volunteers. \*\*\*P < 0.001. (DOC 81 kb)

**Additional file 4: Figure S4.** Fractalkine compared to IL-6 release after intravenous injection of endotoxin to healthy humans in vivo. Fractalkine release after intravenous injection of endotoxin (lipopolysaccharide, 4 ng/kg body weight) into five healthy subjects compared with IL-6 release. *Right panel*, correlation between fractalkine levels and IL-6 measured during the first 5 hours after endotoxin administration (*gray*). Data are expressed as mean ± standard error of the mean. (DOC 135 kb)

## Abbreviations

APACHE: Acute Physiology and Chronic Health Evaluation; AUC: area under the curve; BMI: body mass index; CAP: community-acquired pneumonia; CI: confidence interval; CLP: cecal ligation and puncture; ICU: intensive care unit; IDI: integrated discrimination improvement; IL: interleukin; LPS: lipopolysaccharide; NRI: net reclassification improvement; OR: odds ratio; ROC: receiver operating characteristic; SOFA: Sequential Organ Failure Assessment; TNF: tumor necrosis factor.

## Competing interests

The authors declare that there are no competing interests.

## Authors' contributions

AJH, MAW, and TvdP designed the study. AJH, MAW, LAVV, BPS, HB, PMCK, and AHZ performed the analyses. AJH, MAW, LAVV, BPS, PMCK, JH, OC, MJS, and MB collected the data. AJH, MAW, and TvdP drafted the manuscript. All authors critically reviewed the manuscript. All authors read and approved the final version of this manuscript.

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