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Response of routine inflammatory biomarkers and novel Pancreatic Stone Protein to inhalation injury and its interference with sepsis detection in severely burned patients

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ABSTRACT

Background: Inhalation of thermal and chemical products of combustion evokes an immune response measurable at a systemic level. Inhalation injury related kinetics of currently available inflammatory biomarkers and novel Pancreatic Stone Protein (PSP) as well as their interference with septic events has not been addressed to literature yet.
Methods: Analysis of the influence of inhalation injury and ARDS on biomarker kinetics (PSP, procalcitonin (PCT), C-reactive Protein (CRP), white blood cells (WBC)) in 90 patients admitted to Zurich Burn Center between May 2015 and October 2018 with burns ≥15% total body surface area (TBSA) over 14 days.
Results: Twenty-five (27%) of 90 included patients presented with inhalation injury (median age 52 years [IQR 27], median TBSA 31.5% [IQR 21], mean ABSI-Score 7 ± 3). At admission, only WBC demonstrated significantly higher values in the inhalation injury group ($p = 0.011$). Acute respiratory distress syndrome (ARDS) was present in 32% without association to the severity of inhalation injury ($p = 0.11$). WBC, CRP and PCT failed to delineate inhalation injury related inflammation from septic progression at most time points. PSP was the strongest marker to identify septic patients both by its higher values and steeper increase over time ($p < 0.001$).
Conclusion: Inhalation injury leads to an inflammatory response at a systemic level with alterations of biomarkers. While routine inflammatory markers demonstrated strong interferences between inhalation injury with its associated ARDS and evolving sepsis, PSP reliably identified septic patients in a setting of inflammatory turbulences secondary to inhalation injury.

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1. Introduction

Q3 Inhalation injury represents a concomitant condition in up to one-third of all burn injuries, which harshly increases morbidity and mortality [1]. Its thermal component mainly affects the supraglottic airway, whereas the chemical irritation addresses the whole respiratory tract – inducing cellular damage, changes in regional blood flow and perfusion, airway obstruction, as well as systemic toxicity due to agents such as carbon monoxide and cyanide [2]. The degree of inhalation injury is variable depending on the gas components inhaled, the length of exposure, and individual preexistent lung diseases – and so is the resultant inflammatory response. Serum levels of IL-6, IL-8, TNF alpha and IL-1 receptor antagonist (IL-1RA) increase with inhalation injury severity [3]. Likewise, increased inflammatory cytokines from bronchoalveolar lavage fluid (e.g., IL-4, IL-6, IL-9, IL-15) demonstrated strong correlations with the grade of inhalation injury [4]. The measurement of serum procalcitonin levels at admission proved to be a useful prognostic indicator of the severity of inhalational injury [5].

Acute respiratory distress syndrome (ARDS) is considered a severe complication owing to inhaled smoke and fumes. It is usually defined according to the Berlin definition as acute condition occurring within one week of a clinical event (i.e. inhalation injury) not explained by cardiac failure or fluid overload and associated with hypoxemia ($PaO_2/FiO_2 \leq 300$ mmHG) plus a positive end-expiratory pressure of ≥ 5 cmH_2O [6,7]. Inhalation injury is associated with the occurrence of ARDS in about 40% of intensive care burn patients and is characterized by an explosive acute inflammatory response in the lung parenchyma, leading to alveolar edema, decreased lung compliance and, ultimately, hypoxemia with death [8,9]. Interestingly, the severity of inhalation injury does not correlate with the development of ARDS in burn patients [10]. Still, little is known about the temporal kinetics of established inflammatory biomarkers in response to inhalation injury and ARDS in human burn injuries, such as leucocytes (WBC), C-reactive protein (CRP) and procalcitonin (PCT), as well as Pancreatic Stone Protein (PSP) as an upcoming sepsis biomarker. Pancreatic Stone Protein (PSP) has lately gained increasing attention in critically ill patients as accurate diagnostic and prognostic marker [11]. Originally described as a protein constitutively secreted by pancreatic acinar cells to inhibit growth and nucleation of calcium carbonate crystals, insights from more recent studies suggested PSP as acute phase protein activating neutrophil granulocytes in the early phase of systemic infections and sepsis [12–18]. Our group recently published encouraging results on PSP's excellent accuracy in sepsis prediction in burn patients – outperforming established pro-inflammatory biomarkers such as PCT and CRP [19].

How far inhalation injury and ARDS affect the expression of PSP and currently available biomarkers was to be investigated in the present study. Moreover, we focused on the inflammatory interference of inhalation injury with the diagnosis of infectious/septic events.

2. Methods

2.1. Type of study

Longitudinal, observational study.

2.2. Participants

Between May 2015 and October 2018, patients with burns ≥10% TBSA admitted to our burn center were asked for participation. Affected TBSA was determined using Lund Browder charts. Minimum TBSA of 15% was chosen for the present study based on evidence for systemic stress, impaired immunity and massive fluid shifts [20,21]. Exclusion criteria were age <18 years, current infection at admission, immunosuppressive medication, and burn injuries older than 6 h. All patients received comprehensive oral and written information on the present study and had to sign the informed consent for enrollment. Close relatives and authorized representatives were asked for informed consent by proxy, if the patient was unable to consent due to the extent of the injury. Fig. 1 shows the flow diagram for patient enrollment. Approval was obtained from the Ethics committee of the University of Zurich, Switzerland, on April 20th 2015 (KEK-ZH-No.: 2014-0631). The present study is an ancillary study to the initial

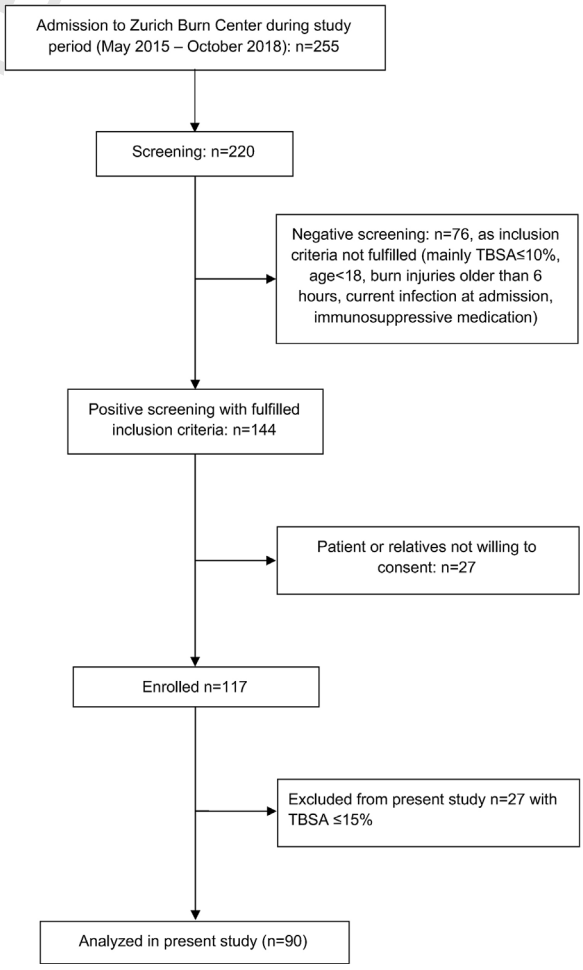

Fig. 1 – Flow diagram of patient enrollment.

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study with the identifier NCT02537821. Study registration: clinicaltrials.gov identifier: NCT02537821.

2.3. Measurement of serum/plasma biomarker concentration

Blood samples for measurement of conventional inflammatory biomarkers (CRP, WBCs and PCT) and PSP were drawn daily at 6 am and starting at admission to our burn center. Leukocyte counts, CRP and PCT levels were directly measured by routine testing at the Institute of Clinical Chemistry, University Hospital Zurich. For subsequent analysis, serum samples were stored at −80 °C. The concentration of PSP/REG Iα was measured with an isoform specific ELISA, which was established in our laboratory [12,22]. Antibodies (affinity-purified IgG) made in guinea pig anti-PSP/REG Iα were diluted 1:500 in Tris-buffered saline (TBS: 10 mM Tris; 0.9% NaCl) and coated on 96-well Maxisorp Nunc plates at room temperature (RT) over night or at 4 °C if incubation lasted longer than one night. Non-adherent antibodies were removed with three washing steps. The wash buffer consisted of TBS and 0.05% Tween. Bovine serum albumin (BSA, tested for low level PSP/REG Iα content) 1% in TBS was used to block the plates for at least 1 h at RT. Serum samples collected from patients were pre-diluted in 1% BSA/TBS and loaded on the plate in duplicates. The standard curve was generated by a dilution series ranging from 4 ng/ml to 0.1 ng/ml, prepared with recombinant PSP. In addition, a blank was added. After 1 h of incubation and three washing steps a secondary antibody, rabbit anti-PSP/REG Iα, 1:500 diluted in 1% BSA/TBS was incubated in the plate. Subsequently, a biotinylated phosphatase antibody 1:6000 diluted in 1% BSA TBS was added for 1 h. After another washing step, the phosphatase substrate (SIGMA Aldrich) was added, which was previously dissolved in Alkaline Phosphatase Buffer according to instructions. PSP was detected photometrically at 405 nm.

2.4. Patient-related data

Besides demographic data (age, gender, body mass index (BMI), comorbidities) and trauma-related data (TBSA, mechanism of injury, inhalation injury), clinical parameters were extracted from the patients' chart retrospectively and collected in the case report form for 14 consecutive days after admission. The latter included: blood count, electrolytes, inflammatory markers (PCT, CRP, PSP). Additionally, vital signs as well as data on circulatory support, ventilation and mental status were collected in order to closely study pathophysiological changes with regard to infection and sepsis. Clinical parameters at the timepoint of blood sampling were chosen for analysis (usually at 6 am daily). All treating physicians were blinded to PSP results whereas they were aware of WBCs, CRP, and PCT values. Primary outcomes of the study were the occurrence of inhalation injury and daily biomarker levels. Secondary outcomes were the occurrence of ARDS and sepsis.

2.5. Definition of inhalation injury, ARDS, infection and sepsis

We used the Abbreviated Injury Score (AIS) for severity grading of inhalation injury [23]. The AIS assigns a severity score from 0 (no injury) to 4 (massive injury) based on the findings at the initial fiberoptic bronchoscopy examination, which was performed by an experienced anesthesiologist at admission. ARDS was defined according to the Berlin definition as acute condition occurring within one week of a clinical event (i.e. inhalation injury) not explained by cardiac failure or fluid overload and associated with hypoxemia ($PaO_2/FiO_2 \leq 300$ mmHG) plus a positive end-expiratory pressure of $\geq$5 cmH_2O [6,7]. By definition, patients could not be diagnosed with ARDS until they required intubation and the fraction of inspired oxygen was precisely known. Moreover, we used the Centers for Disease Control and Prevention (CDC)-definition for hospital acquired infections and the Third International Consensus Definition for Sepsis (Sepsis-3), which characterizes sepsis as life-threatening organ dysfunction caused by a dysregulated host response to a suspected or confirmed infection [24,25]. Organ dysfunction can be identified as an acute change in total Sequential Organ Failure Assessment (SOFA) score $\geq$2 points subsequent to the infection. The corresponding time point of any infection or sepsis was determined by the date of sampling, which subsequently turned out positive. Only microbiologically proven infections were documented and used for the current study.

2.6. Statistical analysis

Based on pre-study observations and considerations, the authors expected higher WBC levels in the inhalation injury group (versus patients without inhalation injury) with an estimated effect size $d = 0.8$. Incidence of inhalation injury was assumed with 1/3 of the whole population. Given $\alpha = 0.05$ and power = 0.9, the a priori sample size calculation for a mean comparison by Mann–Whitney-*U*-Test resulted in ~80 patients ($n_{inhalation} = 20$, $n_{no\text{-}inhalation} = 60$) [26].

Discrete values are expressed as counts with percentages, while continuous variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. Baseline characteristics were compared between groups using Chi-Square test for counts and Mann–Whitney-*U*-Test for continuous data. Biomarker time courses were compared between groups using a linear mixed effects regression model with random intercepts. Linear mixed models have proven superior to traditional repeated-measures ANOVA as they properly take into account interindividual differences in complex longitudinal data [27]. All tests were two tailed; $p < .05$ was considered significant. Data were analyzed using Jamovi (The jamovi project (2019). *jamovi* (Version 1.0.5)) and GraphPad Prism version 6.00 for Macintosh (GraphPad Software, La Jolla, California, USA).

3. Results

3.1. Baseline characteristics of study population

Baseline characteristics of the study population according to the status of inhalation injury are given in Table 1. Ninety severely burned patients (18 female) with a median age of 52 years (IQR 27) and median TBSA of 31.5% (IQR 21) were included. Twenty-five patients (27%) presented with inhala-

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Table 1 – Baseline characteristics in total and according to the state of inhalation injury. Baseline characteristics were compared between groups using Chi-Square test for counts and Mann–Whitney-*U*-Test for continuous data. * indicates $p < 0.05$ and ** indicates $p < 0.01$.

Baseline characteristics	Total	No inhalation injury	Inhalation injury	*p*
Number of patients (*n*, %)	90	65 (73%)	25 (27%) Grade I: *n* = 15 Grade II: *n* = 4 Grade III: *n* = 6 Grade IV: *n* = 0	–
Age (years; median, IQR)	52 (27)	51 (34)	52 (16%)	.800
Gender (*n*, %)	Female: 18 (20%) Male: 72 (80%)	Female: 13 (72%) Male: 52 (72%)	Female: 5 (27%) Male: 20 (27%)	.999
TBSA (%; median, IQR)	31.5 (21)	30 (16)	41 (35)	.038*
Diabetes mellitus (*n*, %)	8/90 (9%)	7/65 (11%)	1/25 (4%)	.290
Hypertension (*n*, %)	18/90 (20%)	15/65 (23%)	3 (12%)	.496
Lung disease (*n*, %)	2/90 (2%)	1/65 (2%)	1/25 (4%)	.633
Liver insufficiency (*n*, %)	4/90 (4%)	4/65 (6%)	–	–
Chronic heart failure (*n*, %)	4/90 (4%)	3/65 (5%)	1/25 (4%)	.722
Chronic renal failure (*n*, %)	–	–	–	–
ABSI (median, IQR)	7.5 (4)	7 (3)	11 (6)	.001**
Baux score (median, IQR)	84 (38)	81 (41)	90 (44)	.013*
Mortality (*n*, %)	14/90 (16%)	6/65 (9%)	8/25 (32%)	.008**
ARDS – induced by inhalation injury (*n*, %)	–	–	8/25 (32%) Grade I: *n* = 3 Grade II: *n* = 1 Grade III: *n* = 4	.110
Pneumonia (*n*; %)	34/90 (38%)	20/65 (31%)	14/25 (56%)	.027*
Other infections (*n*; %)	25/90 (28%)	21/65 (32%)	4/25 (16%)	.011*
Sepsis (*n*; %)	46/90 (51%)	30/65 (46%)	16/25 (64%)	.099
Mechanical ventilation (*n*, %)	72/90 (80%)	47/65 (72%)	25/25 (100%)	.001**
Time of ventilation (h; median, IQR)	96 (192)	72 (156)	192 (252)	.001**
Length of stay (days; median, IQR)	28 (40)	30 (37)	20 (60)	.274
Length of ICU stay (days; median, IQR)	16 (32)	16 (24)	16 (44)	.971

tion injury, 15 of them with grade I, 4 with grade II und 6 with grade III. Except for hypertension (20%), the prevalence of comorbidities was low (<10%) in the present cohort and did not differ between patients with inhalation injury and without ($p > 0.290$). Fourteen patients (16%) died with significantly higher mortality in the inhalation injury group ($p = 0.008$). TBSA and ABSI score differed between the two groups. As inhalation injury contributes as independent factor to the ABSI score, we adjusted all following analyses for TBSA only (correlation TBSA/ABSI $r = 0.82$, $p < 0.0001$). Inhalation injury induced ARDS was diagnosed in 8/25 patients (32%). There was no association between the severity of inhalation injury and occurrence of ARDS ($p = 0.11$). More than half of the patients (56%) with inhalation injury exhibited a pneumonia during the first two weeks, whereas patients without smoke inhalation showed pulmonary infection in 31% ($p = 0.027$). Likewise, septic pneumonia occurred more often in patients with inhalation injury as compared to those without (48% vs. 27%; $p = 0.031$). Overall septic progression was present in 50% of the patients with a not significant trend to a higher incidence in patients with inhalation injury (64%) as opposed to patients without smoke inhalation (45%, $p = 0.099$). The number and site of infections are summarized in Table 2, the isolated microorganisms per patient are given as supplemental material (S1). All patients with inhalation injury were mechanically ventilated and demonstrated a longer duration of ventilation than patients without inhalation injury ($p < 0.001$). The overall length of stay as well as the ICU stay did not differ between the two groups ($p_{LOS} = 0.274$, $p_{ICU\text{-}LOS} = 0.971$).

3.2. *Inflammatory markers at baseline as related to status of inhalation injury and its grades*

Fig. 2 depicts the biomarker levels at admission according to the presence of inhalation injury and its grades. With adjusted TBSA, WBC demonstrated significantly higher values in the inhalation injury group ($p = 0.011$). None of the other biomarkers showed differences between the two groups at admission. Likewise, the severity of inhalation injury was not reflected by an alteration of the biomarker levels ($p > 0.2$).

3.3. *Time course of inflammatory markers as related to the status of inhalation injury*

Fig. 3 illustrates the biomarker levels over 14 days for patients with and without inhalation injury. WBC was significantly elevated in patients with inhalation injury during the first two days after admission ($p_{t0} = 0.011$; $p_{t1} < 0.001$). CRP time course showed no significant difference between the two groups except for day 6 after trauma ($p = 0.026$). In contrast, PCT and PSP demonstrated higher values at several time points with a highly significant interaction for PSP between time and inhalation injury indicated by a steeper increase of patients with inhalation injury as compared to those without ($p < 0.001$).

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Table 2 – Type of infection in total and according to the status of inhalation injury and local infection vs. sepsis.

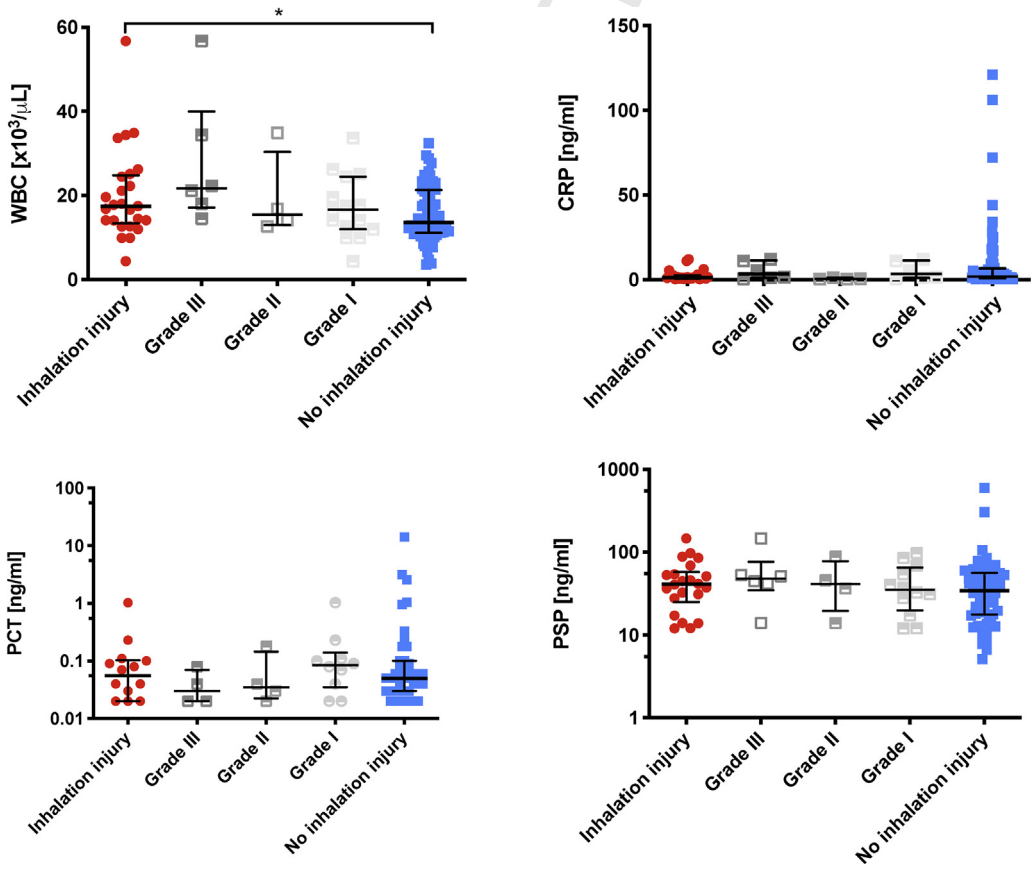
Site of infection	Total (n = 59/90)	No inhalation injury (n = 65)	Inhalation injury (n = 25)	Local infection (n = 13)	Sepsis (n = 46)
Wound infection	12/59 (20%)	9/65 (14%)	3/25 (12%)	3 (23%)	9 (20%)
Pneumonia	34/59 (58%)	20/65 (31%)	14/25 (56%)	4 (31%)	30 (65%)
Urinary tract infection	3/59 (5%)	3/65 (5%)	0	3 (23%)	0
Central line infection	6/59 (10%)	6/65 (9%)	0	2 (15%)	4 (9%)
Bacteremia	4/59 (7%)	3/65 (5%)	1/25 (4%)	1 (8%)	3 (7%)

3.4. *Time course of inflammatory markers as related to the presence of ARDS*

Fig. 4 illustrates the biomarker levels over 14 days for patients with and without ARDS following inhalation injury. WBC was significantly elevated in patients developing ARDS at admission (p = 0.03) with an odds ratio of 1.1 (95%-CI: 0.9; 1.2). CRP values showed no significant difference between the two groups. Similarly, PCT and PSP demonstrated higher values at several later time points (from day 6/7) but were not associated with ARDS, which must occur within the first week as required by the Berlin definition.

3.5. *Time course of inflammatory markers as related to the status of sepsis after smoke inhalation*

Fig. 5 depicts the biomarker time course for patients with inhalation injury and sepsis vs. no sepsis during the first 14 days. WBC, CRP and PCT demonstrated similar kinetics for both groups with no ability to differentiated between them at single days. PSP was the strongest marker to identify septic patients in the setting of exhibited inhalation injury both by its higher values and steeper increase over time ($p < 0.001$). Binary logistic regression with ROC curve analysis yielded AUC results of 0.8–0.9 for day 5–14. Table 3 depicts the AUC values



Q5 Fig. 2 – Levels of biomarkers at baseline according to the status of inhalation injury (red and blue) and its grade I–III (gray) shown as scatter plot with median and interquartile range. Only WBC was significantly increased in patients with inhalation injury (p = 0.011; determined in a linear mixed effect regression model with random intercepts and adjusted for TBSA). The severity of inhalation injury was not reflected by an alteration of the biomarker levels ($p > 0.2$). * indicates $p < 0.05$ and ** indicates $p < 0.01$ (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

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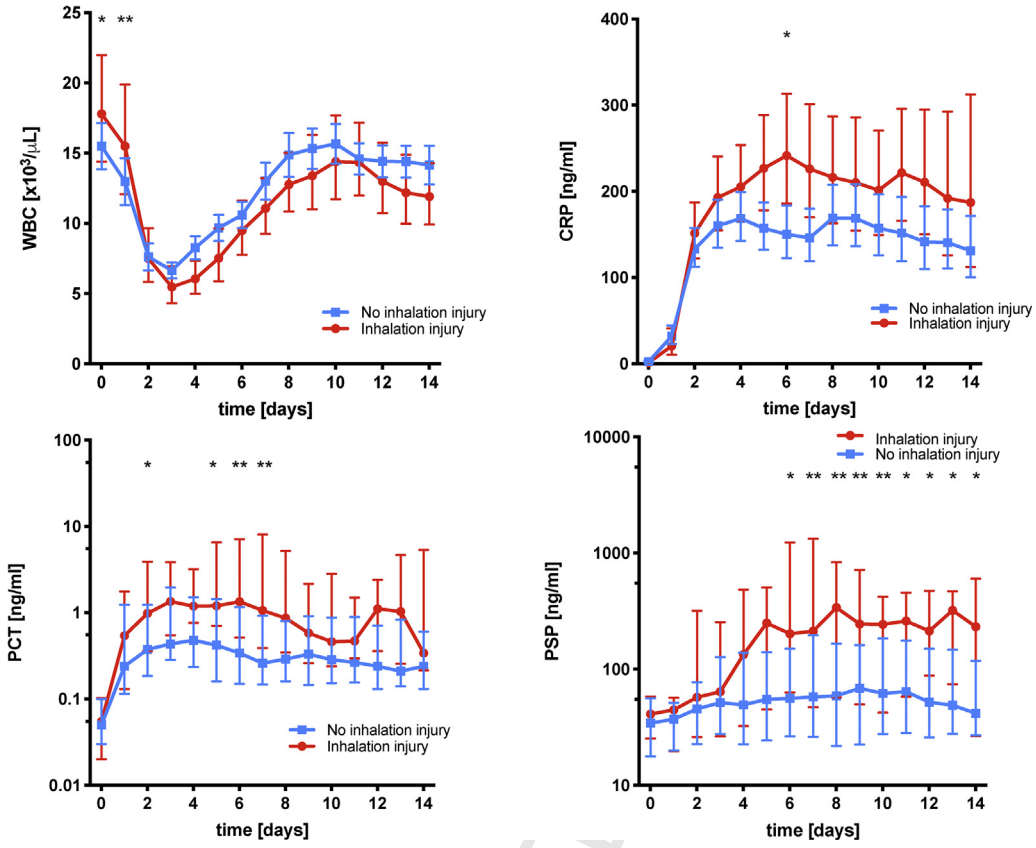

Fig. 3 – Time course of biomarkers over 14 days from admission according to the status of inhalation injury (red and blue) shown as scatter plot with median and interquartile range. WBC values of patients with inhalation injury were elevated in the first two days, whereas PCT and PSP demonstrated higher serum values in the inhalation group at several time points. PSP demonstrated a highly significant interaction between time and inhalation injury ($p < 0.001$; determined in a linear mixed effect regression model with random intercepts and adjusted for TBSA). * indicates $p < 0.05$ and ** indicates $p < 0.01$ (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

for each biomarker and time point with regard to their predictive ability of sepsis in the presence of inhalation injury. In Fig. 6, the corresponding biomarker time courses of patients without inhalation injury (+/− sepsis) was added to Fig. 5 to further illustrate the effect of the inhalation injury. Whereas WBC levels hardly varied across the groups, CRP and PCT time course demonstrated higher levels in patients with inhalation injury and/or septic progression as opposed to patients with an uneventful course (blue line). In contrast to routine inflammatory markers, PSP demonstrated constant levels ranging between 20 and 30 ng/ml for patients without inhalation injury and without sepsis across 14 days. On the other hand, PSP reliably delineated septic courses (red and green line) from non-septic ones (blue and black line).

4. Discussion

The present study investigated the influence of inhalation injury on the expression of routine inflammatory biomarkers (WBC, CRP, PCT) and novel PSP in a cohort of 90 severely burned patients over 14 days. Similarly, the biomarker levels were investigated according to the status of inhalation injury induced ARDS. Additionally, the as yet unclear interference of inhalation injury with sepsis detection was elucidated. This issue is of great importance to burn specialists and intensive care physicians as smoke inhalation injuries accompany about 30% of all burn accidents, but its inflammatory impact has hardly been addressed in literature [28].

In a first step, we demonstrated that inhalation injury triggers an immediate elevation of the leukocyte count (Fig. 2). This instantaneous increase is thought to result from the heat related damage of the tracheobronchial mucosa leading to local chemotaxis and systemic upregulation of leucocytes [29]. Associated release of cytokines is considered to be secondary to complement activation by heat denatured proteins in the lungs. This upregulation of cytokines as immune mediators has been well described in human and animal experiments demonstrating moderate to strong correlations with the severity of inhalation injury [3,4,7,30]. In our study, patients with grade III inhalation injury tended to show higher WBC serum values as compared to patients with inhalation injury grade I and II. Similarly, PCT has been found to be constitutively released secondary to inhalation injury serving

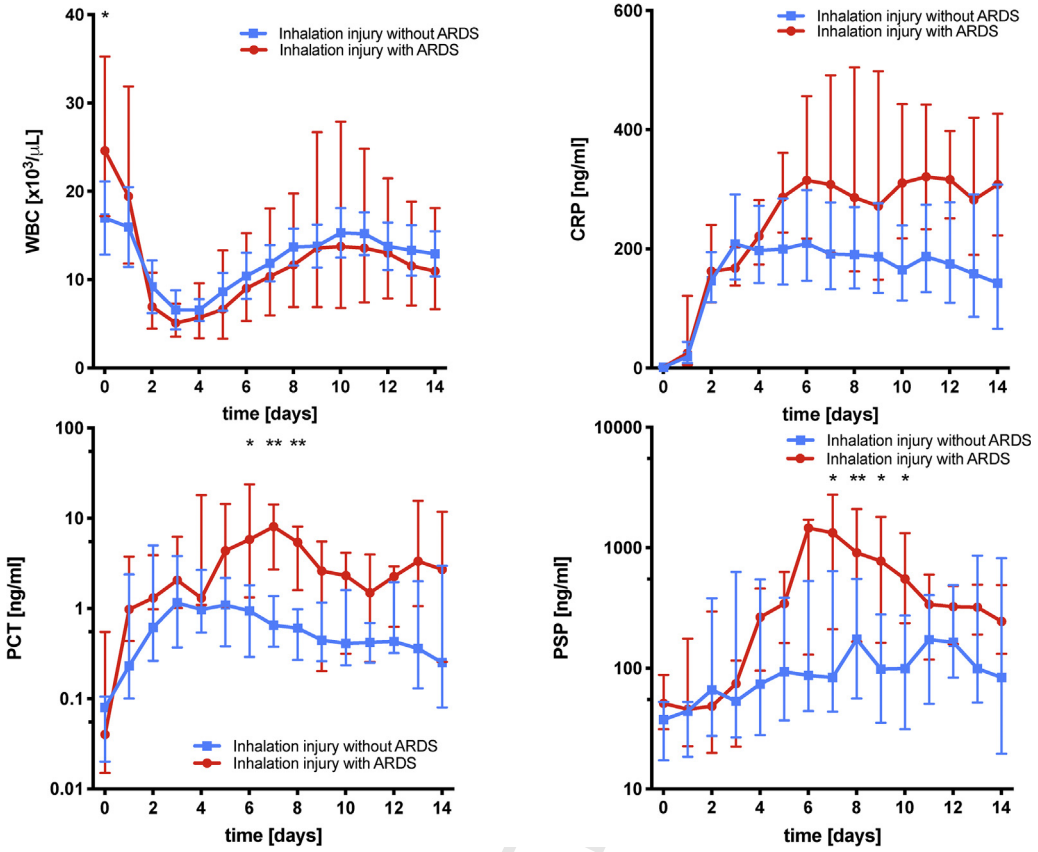

Fig. 4 – Biomarker time course over 14 days according to the status of ARDS following inhalation injury (red and blue). WBC was significantly elevated in patients with ARDS at admission ($p = 0.03$; odds ratio: 1.1 (95%-CI: 0.9; 1.2)). CRP values showed no significant difference between the two groups. Similarly, PCT and PSP demonstrated higher values at several later time points but were not able to predict ARDS at an early time point (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

as useful prognostic indicator of the severity of inhalation injury occurring in burn patients [5]. We did neither observe a difference in PCT, CRP and PSP levels between patients with and without inhalation injury at baseline nor did we find a corresponding correlation with the grade of the inhalation injury. This might be explained by a delayed expression of intrahepatic synthesis of CRP, while PCT and PSP remains rather unaffected by sterile inflammation caused by inhalation injury. This consideration is further elucidated in the following paragraphs.

In a second step, we took a closer look at the temporal kinetics of the four inflammatory markers (Fig. 3). While WBC time course of patients with and without inhalation injury did not vary after the first two days, PCT and PSP demonstrated higher levels in the inhalation injury group over time. Additionally, only PSP demonstrated a highly significant interaction between time and inhalation injury ($p < 0.001$) indicated by the steeper increase of patients with inhalation injury. This is explained by the fact, that patients with inhalation injury are more susceptible to (respiratory tract) infections with potential septic progression than patients without inhalation injury but equal TBSA [31–33]. Indeed, we found a higher incidence of (septic) pneumonia in patients with inhalation injury as opposed to patients without smoke inhalation. Of note, the overall incidence of sepsis in our study was not statically different between the two groups ($p = 0.099$). These findings suggest, that inhalation injury does not necessarily result in sepsis more often in patients with smoke inhalation than in patients without.

The direct lung injury due to inhaled smoke leads to ARDS in about 40% [8]. This kind of "direct ARDS" is to be delineated from ARDS mediated by the inflammatory response associated with the burn injury itself or its infectious complications [8,9]. In the present study, we referred to ARDS, which was directly induced by inhalation injury and occurred within one week after trauma as suggested by the Berlin definition [34]. We observed no association between the severity of inhalation injury and occurrence of ARDS confirming previous findings [10]. Moreover, we found elevated WBC levels at admission in patients exhibiting ARDS with a poor odds ratio of 1.1. The other biomarkers tested showed no significant difference between patients with ARDS and those without across the first week after admission. These findings are concordant with previous studies investigating various putative biomarkers to predict ARDS [35]. Their data have been largely disappointing and the 'troponin' of ARDS remains elusive. During the first week after admission, PSP values (and other biomarkers) insignificantly varied between patients with and without

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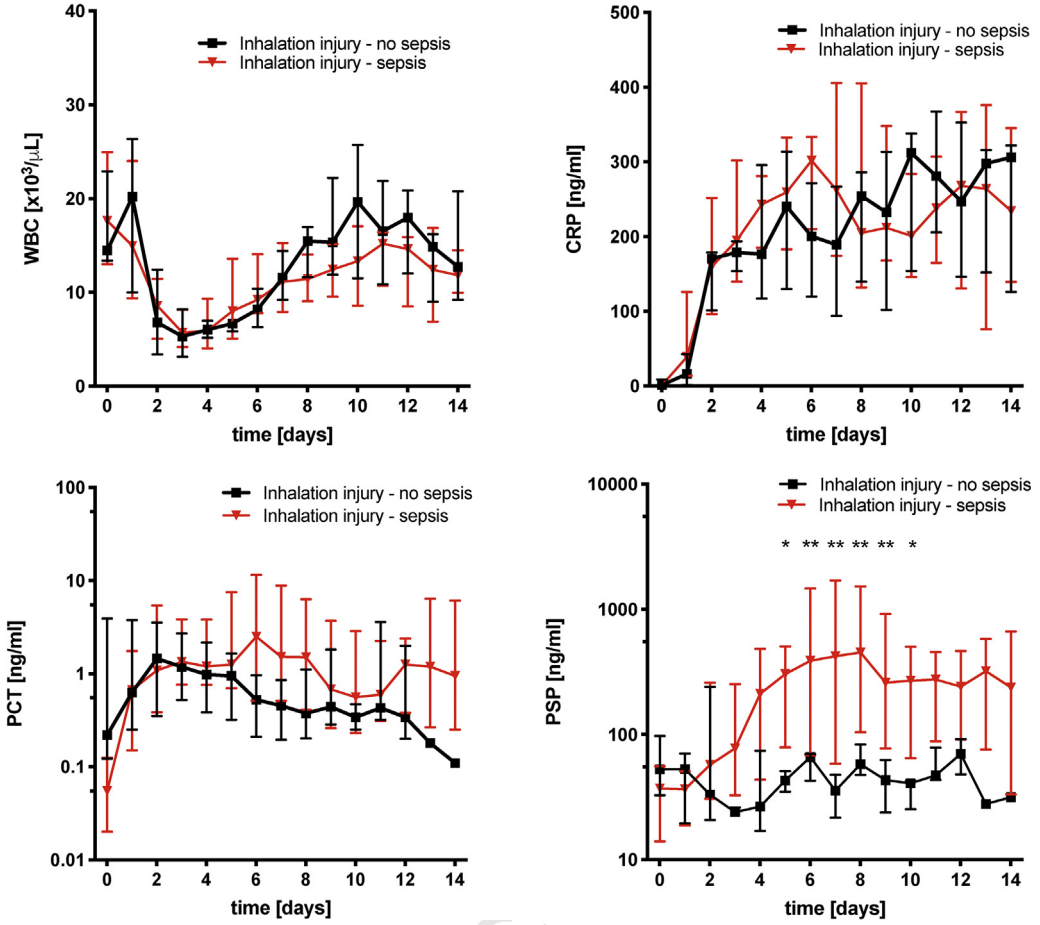

Fig. 5 – Time course of inflammatory markers over 14 days for patients with inhalation injury and according to the status of sepsis. WBC and CRP demonstrated similar kinetics for both groups. PCT differentiated between these two groups at day 6 and 7 ($p = 0.023$). PSP was the strongest marker to identify septic patients in the setting of exhibited inhalation injury both by its higher values and steeper increase over time ($p < 0.001$; determined in a linear mixed effect regression model with random intercepts and adjusted for TBSA). * indicates $p < 0.05$ and ** indicates $p < 0.01$.

ARDS, which is why the later/delayed increase is more likely explained by the septic progression of these patients than by the ARDS itself (6/8 patients [75%] with ARDS became septic). It is to be noted, that the statistical validity within the ARDS subgroup analysis is limited due to the low number of patients ($n = 8$).

To further corroborate the interference between inhalation injury related inflammation and septic events, we focused on the group with inhalation injury +/− sepsis. Interestingly, only PSP demonstrated a significant interaction (time × status of sepsis) meaning that septic patients exhibit a steeper increase over time as compared to non-septic patients in the presence of inhalation injury. Together with a high accuracy of sepsis prediction as demonstrated by the presented curve analysis and previous studies, this is a crucial finding emphasizing the profound involvement of PSP in septic processes [19]. Au contraire, CRP and PCT largely failed to discriminate septic from non-septic patients in the presence of inhalation injury. Additional comparison with patients without inhalation injury demonstrated constant PSP levels ranging between 20 and 30 ng/ml for patients without inhalation injury and without sepsis across 14 days. Again, this finding substantiates the high specificity of PSP for a developing sepsis, which has been observed beyond burns [13,14,16]. For WBCs, CRP and PCT, we observed alterations in non-septic patients with inhalation injury suggesting a certain inflammatory effect caused by the inhalation injury.

According to our results, bedside clinicians may assume an inflammatory impact following inhalation injury irrespective of the patient's TBSA. Leucocytes and CRP did not prove to be helpful for preclinical sepsis detection due to their strong interference with additional inflammatory insults such as inhalation injury. Similarly, PCT seems to be influenced by inhalation injury reducing its usefulness in clinical routine. It demonstrated higher values for septic patients at few time points but lacked a desirable increase over time reflecting the patient's deterioration. As to that, burn specialists might take advantage of novel biomarkers like PSP in terms of its robustness toward inflammatory stressors indicated not only

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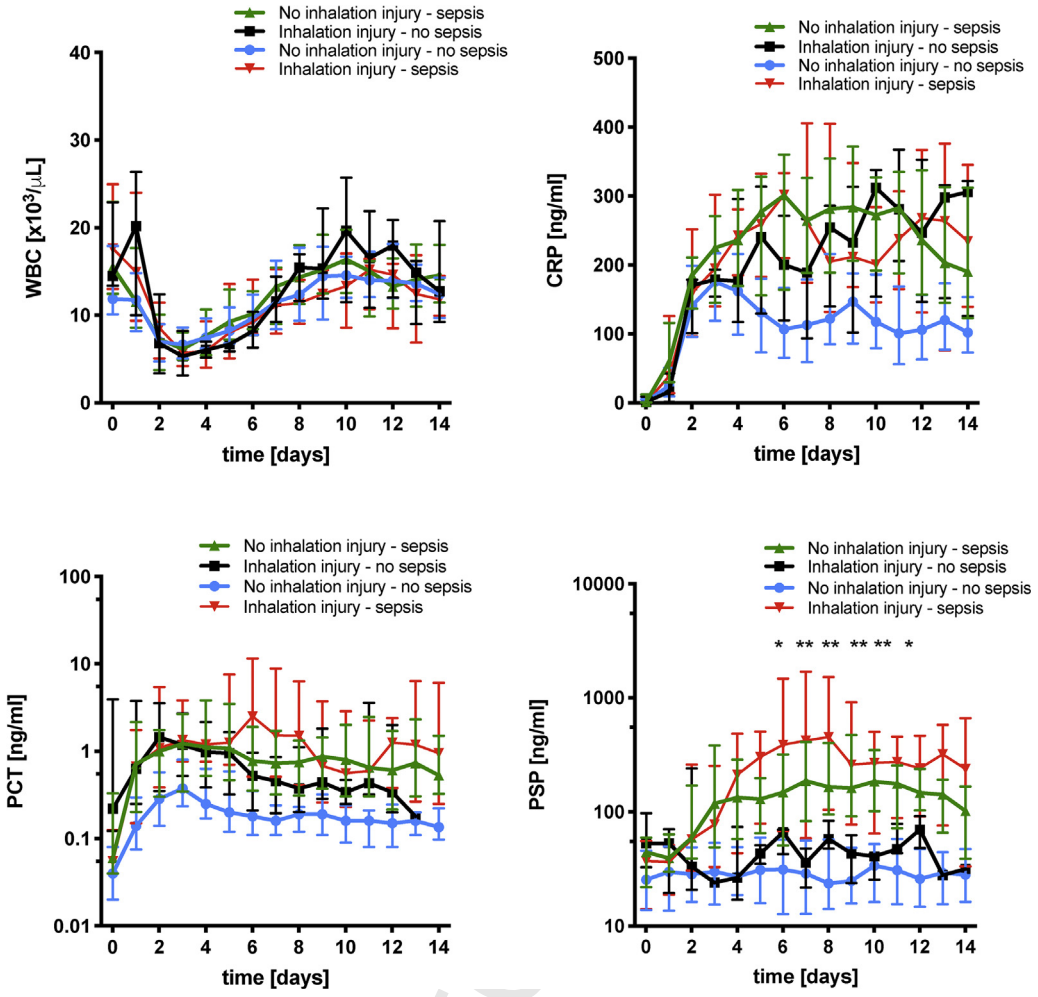

Fig. 6 – Time course of inflammatory markers over 14 days according to the status of inhalation injury and sepsis. WBC demonstrated similar kinetics for all groups. CRP and PCT time course demonstrated higher levels in patients with inhalation injury and/or septic progression as opposed to patients with an uneventful course (blue line). Additionally, WBC and CRP were not able to differentiate between patients developing a septic event and those with no septic event after smoke inhalation (red and black lines). PCT differentiated between these two groups (red vs black line) at day 6 and 7 ($p = 0.023$). PSP was the strongest marker to identify septic patients in the setting of exhibited inhalation injury both by its higher values and steeper increase over time ($p < 0.001$; determined in a linear mixed effect regression model with random intercepts and adjusted for TBSA). * indicates $p < 0.05$ and ** indicates $p < 0.01$ (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 3 – AUC with 95%-CI for each biomarker and time point with regard to their predictive ability of sepsis in the presence of inhalation injury. PSP demonstrates AUCs between 0.80 and 0.94 for day 5–14 (also see Fig. 5).

Biomarker	AUC	95%-CI	Biomarker	AUC	95%-CI
WBC – day 0	0.54	0.30; 0.78	CRP – day 0	0.71	0.49; 0.92
WBC – day 1	0.58	0.32; 0.83	CRP – day 1	0.71	0.48; 0.94
WBC – day 2	0.57	0.27; 0.87	CRP – day 2	0.57	0.33; 0.81
WBC – day 3	0.55	0.21; 0.88	CRP – day 3	0.62	0.38; 0.86
WBC – day 4	0.52	0.27; 0.76	CRP – day 4	0.63	0.30; 0.95
WBC – day 5	0.67	0.44; 0.90	CRP – day 5	0.59	0.31; 0.88
WBC – day 6	0.64	0.35; 0.93	CRP – day 6	0.80	0.60; 1.00
WBC – day 7	0.53	0.27; 0.79	CRP – day 7	0.68	0.42; 0.94
WBC – day 8	0.70	0.43; 0.97	CRP – day 8	0.52	0.22; 0.81
WBC – day 9	0.72	0.44; 0.99	CRP – day 9	0.57	0.25; 0.88
WBC – day 10	0.67	0.35; 0.98	CRP – day 10	0.68	0.34; 1.00
WBC – day 11	0.58	0.24; 0.92	CRP – day 11	0.62	0.33; 0.90
WBC – day 12	0.73	0.47; 0.99	CRP – day 12	0.52	0.23; 0.81

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Table 3 (*Continued*)

Biomarker	AUC	95%-CI	Biomarker	AUC	95%-CI
WBC – day 13	0.51	0.21; 0.81	CRP – day 13	0.56	0.25; 0.87
WBC – day 14	0.60	0.20; 0.99	CRP – day 14	0.55	0.21; 0.88
PCT – day 0	0.88	0.69; 1.00	PSP – day 0	0.70	0.47; 0.94
PCT – day 1	0.60	0.36; 0.84	PSP – day 1	0.58	0.28; 0.87
PCT – day 2	0.54	0.24; 0.84	PSP – day 2	0.81	0.62; 0.99
PCT – day 3	0.58	0.30; 0.85	PSP – day 3	0.79	0.55; 0.99
PCT – day 4	0.63	0.34; 0.91	PSP – day 4	0.75	0.54; 0.96
PCT – day 5	0.69	0.43; 0.96	PSP – day 5	0.81	0.62; 1.00
PCT – day 6	0.80	0.59; 1.00	PSP – day 6	0.94	0.81; 1.00
PCT – day 7	0.83	0.62; 1.00	PSP – day 7	0.78	0.57; 0.98
PCT – day 8	0.77	0.53; 1.00	PSP – day 8	0.90	0.75; 1.00
PCT – day 9	0.59	0.29; 0.89	PSP – day 9	0.90	0.73; 1.00
PCT – day 10	0.63	0.39; 0.88	PSP – day 10	0.81	0.60; 1.00
PCT – day 11	0.50	0.14; 0.86	PSP – day 11	0.82	0.62; 1.00
PCT – day 12	0.69	0.37; 1.00	PSP – day 12	0.86	0.67; 1.00
PCT– day 13	0.79	0.57; 1.00	PSP – day 13	0.85	0.65; 1.00
PCT – day 14	0.83	0.62; 1.00	PSP – day 14	0.80	0.63; 0.98

by its elevated absolute values but also by its solid temporal increase in septic patients.

Elucidating the role of PSP in severely burned patients with respect to the occurrence of sepsis, the present study represents a crucial step on the way to test a potentially helpful biomarker for early sepsis detection. Besides PSP's robustness toward inflammatory stressors such as inhalation injury combined with its excellent predictive power for sepsis, PSP can readily be measured bedside from a drop of whole blood using a nanofluid based assay (abioSCOPE, Abionic SA, Epalinges, Switzerland). This device allows quantifying PSP levels at a picomolar range within 2–5 min and without preanalytical work. Additionally, the ultimate goal in sepsis biomarker research is the development of a score that can be used for sepsis diagnosis and risk stratification [36,37]. Ideally, such a sepsis score comprises a combination of several biomarkers, including proteomic, transcriptomic, and metabolomic candidates as wells as clinical parameters. In our opinion, PSP is a very promising candidate to be included in a score for sepsis in general, and for discrimination of septic complications in patients with underlying systemic inflammation in particular, as indicated by the results of the present study in burn patients. Current and future efforts by our research group head toward this direction.

Although the present study comes up with useful results and is the first to enlighten the impact of inhalation injury on biomarker dynamics in burn patients, several limitations are worth mentioning. Given the single-center design of our study, there is no external validation of our data, which has to be addressed in future work. Measurement of biomarkers was performed only once per 24 h, neglecting potential alterations between these intervals. Moreover, further potential influences on the inflammatory response, e.g. by repetitive surgical procedures, administration of antibiotics or ventilator support, have not been included. Likewise, comorbidities were rare in our cohort (<10%) and therefore not considered in the analyses. Furthermore, the ARDS subgroup was too small ($n = 8$) to present valid test statistics with informative quantification of the inflammatory influence of ARDS on the biomarker time courses. Furthermore, the ARDS subgroup was too small ($n = 8$) to present valid test statistics with informative quantification of the inflammatory influence of ARDS on the biomarker time courses. Lastly, both multiple infection sites and multiple septic episodes were not taken into account, as inflammatory processes with the corresponding alterations of the biomarker levels become even more complex and often vaguely interpretable after a first infectious/septic episode.

5. Conclusion

In conclusion, inhalation injury leads to an inflammatory response at a systemic level with alterations of routine biomarkers – irrespective of the trauma severity. While routine inflammatory markers demonstrated strong interreferences between inhalation injury with its associated ARDS and evolving sepsis, PSP reliably identified septic patients in a setting of inflammatory turbulences secondary to inhalation injury.

Ethics approval

Approval was obtained from the Ethics committee of the University of Zurich, Switzerland on April 20th 2015 (KEK-ZH-No: 2014-0631).

Conflicts of interest and financial disclosure

Rolf Graf is inventor of an assay covered by patent no.: EP 2185937 B2 "METHOD FOR ASSAYING SEPSIS IN HUMANS", which is owned by the University of Zurich (Zurich, Switzerland). This does not alter the authors' adherence to all the policies of Burns on sharing data and materials.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.burns.2020.04.039.

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