Clinical Study
Circulating Leukotriene B4 Identifies Respiratory Complications after Trauma

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Background. Leukotriene B4 (LTB4), a proinflammatory lipid mediator correlates well with the acute phase of Acute Respiratory Distress Syndrome (ARDS). Therefore, LTB4-levels were investigated to determine whether they might be a useful clinical marker in predicting pulmonary complications (PC) in multiply traumatized patients. Methods. Plasma levels of LTB4 were determined in 100 patients on admission (ED) and for five consecutive days (daily). Twenty healthy volunteers served as control. LTB4-levels were measured by ELISA. Thirty patients developed PC (pneumonia, respiratory failure, acute lung injury (ALI), ARDS, pulmonary embolism) and 70 had no PC (ØPC).

Results. LTB4-levels in the PC-group [127.8 pg/mL, IQR: 104–200 pg/mL] were significantly higher compared to the ØPC-group on admission [95.6 pg/mL, IQR: 55–143 pg/mL] or control-group [58.4 pg/mL, IQR: 36–108 pg/mL]. LTB4 continuously declined to basal levels from day 1 to 5 without differences between the groups. The cutoff to predict PC was calculated at 109.6 pg/mL (72% specificity, 67% sensitivity). LTB4 was not influenced by overall or chest injury severity, age, gender or massive transfusion. Patients with PC received mechanical ventilation for a significantly longer period of time, and had prolonged intensive care unit and overall hospital stay. Conclusion. High LTB4-levels indicate risk for PC development in multiply traumatized patients.

1. Introduction

Trauma patients are at high risk of developing respiratory complications such as pneumonia, respiratory failure, Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS), and pulmonary embolism. Following multiple organ failure (MOF) and sepsis, respiratory complications are among the most common causes of morbidity and mortality for trauma patients surviving the initial postinjury phase [1–5]. The overall mortality from ARDS is still up to 50% [6–8]. Multiply traumatized patients have shown 10% mortality following ALI [9]. Approximately 20% of major trauma admissions develop ARDS or ARDS like pulmonary dysfunction. This represents one of the most frequent complications in these patients and is the major contributor to morbidity and mortality in trauma patients [3, 10, 11].

Several airway diseases including ALI/ARDS are closely associated with neutrophil infiltration of the airway wall [12]. Neutrophils release a variety of oxidants, as well as degradative and proteolytic enzymes, which induce lung inflammation with subsequent airway remodelling, microvascular damage, and lung tissue injury [12–14]. Persistence of neutrophils in the lungs is an important contributing factor to poor survival [15, 16].

Leukotriene (LT) B4 is a proinflammatory lipid mediator derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism [17–20]. LTB4 is a potent chemoattractant which also exerts leukocyte activating abilities and plays a crucial role in neutrophil migration [21–24]. LTB4 induces neutrophil adherence to endothelial cells, promotes chemotaxis, stimulates the generation and release of oxidants, and increases 5-LO activation in neutrophils, resulting in enhanced LTB4 synthesis [17, 18, 25]. Patients with pulmonary disease have elevated levels of LTB4 indicating its proinflammatory role [25–27]. LTB4 concentrations are enhanced in bronchoalveolar lage (BAL) fluid of ALI/ARDS and chronic obstructive pulmonary disease (COPD) patients [27, 28]. Recently, it has been reported that LTB4 and its metabolites, due to a “priming” effect on neutrophils, plays an important role in the development
of polymorphonuclear-neutrophils-(PMN-) induced lung injury [29]. The priming effect of sequestered neutrophils in the lungs leads to their “hyperfunction.” This results in an exaggerated inflammatory cell response to a secondary stimulus potentially inducing lung complications [30–32]. Early identification of high-risk patients for respiratory complications after trauma is important in determining subsequent treatment. The potential prognostic role of LT4 in major trauma patients, suffering lung complications in a later postinjury phase, remains unclarified.

We hypothesize that high levels of LT4 in the plasma of multiply traumatized patients indicate not only a strong proinflammatory response, but may also serve to identify patients at risk for imminent lung complications.

2. Methods

2.1. Ethics. This study was performed in the Goethe University Hospital with ethical approval (167/05, in accordance with the Declaration of Helsinki and following STROBE-guidelines) [33]. All patients signed the informed consent forms themselves or informed consent was obtained from relatives in accordance with ethical standards.

2.2. Patients. Inclusion criteria consisted of a history of acute blunt or penetrating trauma with an Injury Severity Score (ISS) ≥ 16 in any patient between 18 and 80 years of age. Burns, concomitant acute myocardial infarction, and/or lethal injury were exclusion criteria.

Blood samples were obtained from 100 multiply traumatized patients on admittance to the emergency department (ED), and daily for 5 days following the trauma. Upon arrival at the ED, vital signs were documented. Trauma severity was scored using the Abbreviated Injury Scale (AIS) [34–36]. In addition, ISS was calculated [37]. Patients with an ISS from 16–24 were classified as substantially injured patients, patients with an ISS from 25–39 were substantially/severely injured patients and patients with an ISS ≥ 40 were considered severely injured patients.

Pulmonary complications were defined as nosocomial pneumonia, ALI/ARDS, pulmonary embolism, and/or respiratory failure as described below. Pneumonia was defined by radiologic, clinical, and bacteriologic findings with the presence of new pulmonary infiltrates and at least one of the following criteria: positive blood culture, BAL, and/or sputum culture [38]. Lung injury was assessed using the American-European Consensus Conference criteria for ARDS [39]. Pulmonary embolism was diagnosed by computed tomography (CT), and pulmonary edema was diagnosed either by CT scan or chest X-ray. Respiratory failure was defined as the need for prolonged weaning or reintubation.

The control group included 20 healthy nonsmoking volunteers with an unknown chronic disease and no history of abdominal trauma or abdominal surgery within the past 24 months.

2.3. Blood Processing and Analysis. Blood samples were collected as early as possible after injury in prechilled ethylenediaminetetraacetic acid (EDTA) vacuum tubes (BD vacutainer, Becton Dickinson Diagnostics, Aalst, Belgium) and kept on ice. Blood was centrifuged at 2000 × g for 15 minutes at 4°C. The supernatant was stored at −80°C until batch sample analysis.

The mean time between the injury and first blood sample taken directly upon admittance to the ED was 83 ± 7 min. Specimens were used for duplicate measurement of LT4 levels. LT4 was determined using a highly specific commercially available ELISA (LT4 Parameter Assay Kit, R&D Systems, Minneapolis, USA) according to the manufacturer’s instructions. The detection limit was 27.6 pg/mL for LT4.

2.4. Statistics. Kolmogoroff-Smirnoff-Lillieford’s test showed that the plasma concentration of LT4 was not Gaussian-distributed. Median LT4 levels for each of the 3 groups were compared using the Kruskal-Wallis test and the post hoc analysis was performed with Dunn’s multiple comparison test. Data are presented as the median (interquartile range, IQR) or mean ± sem unless otherwise stated. A P value < 0.05 was considered statistically significant. Receiver-operator curves were generated to analyze the optimal cutoff levels. GraphPad Prism 5.0 software (GraphPad Software Inc. San Diego, CA) was used to perform the statistical analysis and computations.

3. Results

The total group consisted of 100 patients (24 female, 76 male), 98% suffering from blunt and 2% from penetrating trauma. All patients were substantially injured (ISS: 34.0 ± 1.7). Of these, 30 patients with an ISS of 33.7 ± 1.6 developed secondary pulmonary complications. Seventy patients with an ISS of 34.1 ± 1.3 had no pulmonary complications. The AISchest was comparable in both groups (3.1 ± 0.2 in the ØPC group and 3.5 ± 0.2 in the PC group). Time on mechanical ventilation, length of stay in the ICU and hospital were significantly prolonged in the PC group. Additionally, more patients developed sepsis (P < 0.05), organ failure, and MOF in the PC group. In-hospital mortality was also increased.

Table 1 summarizes general patient characteristics and physiologic parameters in the study population. Table 2 depicts the type, severity, and cause of injury.

Figure 1 shows the distribution of plasma LT4 values in the first sample obtained in the ED and subsequent daily measurements for five consecutive days. Median concentrations (and IQR) of LT4 in trauma patients on admission were significantly increased compared to healthy controls (106.1 (62–159) pg/mL versus 58.4 (36–108) pg/mL, P < 0.05, Figure 1). The LT4 levels on admission were also significantly elevated compared with levels at day 1 until day 5.

To investigate the relation between the injury severity and LT4 concentrations determined in the ED, the study population was subdivided into three groups: seriously injured patients (ISS: 16–24, n = 17), seriously/severely injured patients (ISS: 25–39, n = 54), and severely injured patients (ISS: ≥40, n = 29). Plasma LT4 concentrations in each group were markedly enhanced (112.8 (68–167) pg/mL, 107.6 (48–164) pg/mL, and 105.3 (62–148) pg/mL, resp.) compared with healthy volunteers 58.4 (36–108) pg/mL, but this tendency was not significant (Figure 2(a)).
The severity of chest trauma was assessed using the AIS chest scores. Patients without a relevant chest injury were graded as AIS\textsubscript{chest} = 2 (n = 21). Patients with serious and serious/severe chest injury (AIS\textsubscript{chest} = 3 or 4) occurred most frequently (n = 23, and n = 49, resp.), whereas patients with an AIS\textsubscript{chest} = 5 occurred less often (n = 7). Taken together, LTB4 levels were increased in all four trauma patient groups (AIS\textsubscript{chest} = 2: 113.9 (61–162), AIS\textsubscript{chest} = 3: 105.8 (47–200), AIS\textsubscript{chest} = 4: 107.7 (64–156), and AIS\textsubscript{chest} = 5: 94.9 (65–133) pg/mL) compared with healthy volunteers, but this difference was not significant (Figure 2(b)).

However, comparing LTB4 levels taken in the ED of those patients who developed pulmonary complications (n = 30) following injury with those patients who did not develop pulmonary complications (n = 70) and healthy volunteers revealed a significant difference (127.8 (104–200) versus 94.9 (65–133) pg/mL).
Mediators of Inflammation

4. Discussion

Respiratory complications, such as pneumonia, respiratory failure, ALI/ARDS, and pulmonary embolism are, next to MOF and sepsis, among the most common causes of late morbidity and mortality after trauma [1–5, 40]. An increased rate of pulmonary complication in severely injured trauma patients is closely associated with an excessive systemic and local inflammatory response including neutrophil influx [12, 40–42]. LT4B represents a potent neutrophil chemoattractant and enhanced LT4B levels are associated with pulmonary disease [25–28]. Despite the close association with airway disease, it remains unclear whether LT4B is a reliable parameter for early identification of high-risk patients for pulmonary complications after multiple trauma.

This study shows that multiply traumatized patients with high LT4B levels (cutoff at 109.6 pg/mL) in the initial phase are at high risk to develop posttraumatic pulmonary complications. The association of increased LT4B in BAL fluid of patients with ALI/ARDS and COPD has previously been reported [27, 28]. The majority of clinical studies focused on the proinflammatory role of LT4B in neutrophil infiltration and the subsequently induced lung injury in ICU patients [27–32]. The decisive role of neutrophils in several airway diseases, including ALI/ARDS, has been described [12–14]. Their persistence in the lungs is closely associated with poor survival [15, 16].

LT4B is biosynthesized from arachidonic acid by the action of cytosolic phospholipase A2, 5-LO together with 5-LO-activating protein (FLAP) and leukotriene A4 hydrolase [43]. 5-LO activity is considered a key factor in LT4B biosynthesis. LT4B levels have been shown to correlate with tumor necrosis factor alpha levels and the number of neutrophils recovered from the BAL fluid of patients with ARDS [44]. Furthermore, LT4B and its metabolites have been shown to cause increased neutrophil adherence to the pulmonary endothelial cell surface, reflecting neutrophil sequestration in the lung and the capillary bed and increasing vascular permeability [17, 29, 45, 46]. Inhibiting the 5-LO rate-limiting enzyme in LT4B biosynthesis by intratracheal application of IL-8 has been shown to prevent lung injury and perfuse LT4B increase in the lungs. Neutrophil chemotaxis in vitro was also inhibited [47]. Therefore, it might be concluded that the impact of enhanced systemic LT4B concentrations in trauma patients presenting at the ED may reflect an ongoing systemic inflammation. This, in turn, may lead to the development of pulmonary complications. Therefore, the predictive relevance of LT4B should be considered in this highly heterogeneous group of patients. Employing timely appropriate treatment (e.g., kinetic therapy, operative and ventilatory strategies) could thereby improve patient outcome.

Other factors have been reported to be associated with the development of posttraumatic pulmonary complications. The extent of chest trauma has been shown to increase respiratory complications, such as ALI/ARDS [48, 49]. Interestingly, we found no significant correlation between the degree of chest injury assessed by the AIS and the rate of posttraumatic pulmonary complications. This may be due to the study having been conducted at a single clinic with a limited number of patients (n = 100). In a multivariate statistical analysis, we found that the effect of trauma severity on LT4B levels as well as the development of pulmonary complications is considered not significant (data not shown).
Evaluation of data from patients with hypoxemic respiratory failure \( (n = 8) \) has shown that LTB4 levels (at ED) were significantly enhanced in this group compared to healthy volunteers, but did not differ markedly from 22 patients with nonhypoxemic respiratory complication (data not shown). In the present study, the mortality was 6% in a cohort of trauma patients with considerable injury (mean ISS > 33). Blunt trauma as the major type of injury in over 90% of patients was in accordance with other European studies [50, 51]. Dysregulated immune response after trauma has been suggested to contribute to complications, such as sepsis and MOF. The incidence of sepsis and MOF vary strongly in the literature [2, 52–56]. In the present study, sepsis occurred in 16% and MOF in only 4% of patients. In line with the literature, trauma patients with pulmonary complications constitute the majority of patients who develop sepsis and/or MOF [52]. Interestingly, in the present study, patients developing pulmonary complications were not more severely injured than patients without pulmonary complications, as had been expected from previous reports [52]. The clinical course was strongly affected by the presence of respiratory complication with a prolonged ICU stay, days on mechanical ventilation, and longer hospital stay.

Pulmonary complications after severe trauma markedly affect the clinical course. The predisposing factors for these patients at risk are not fully understood and their identification before clinical manifestation of complications remains a challenge. Most clinical scoring systems have been developed to compare populations, while their predictive power is limited. The lung organ failure scoring (LOFS) method has been developed to estimate the risk for pulmonary complications in trauma patients [57]. Its effectiveness still needs to be assessed in prospective clinical studies.

In conclusion, to stratify the risk for later pulmonary complications the results presented here encourage LTB4 assessment early after trauma. In the present study, the LTB4
AUC is quite small. Enhanced patient numbers, especially in the group of patients with pulmonary complications, could strengthen the hypothesis that LTB4 may be of predictive value. While the pathophysiological sequelae of increased LTB4 release is principally understood, identification of relevant effects such as neutrophil adherence or edema formation need demonstration in the clinical setting. Therefore, clinical studies with larger numbers of patients are required to clarify the role of LTB4 in pulmonary complications and resolve its predictive efficacy.

Authors’ Contributions

B. Auner and E. V. Geiger contributed equally in this research.

References


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