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Eicosanoids as Risk and Prognostic Factors for Acute Respiratory Distress Syndrome in Sepsis Patients

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Abstract

Although a number of studies have reported elevated levels of eicosanoids in acute lung injury with sepsis, the possibility that eicosanoids may act as risk and prognostic factors for sepsis patients who develop acute respiratory distress syndrome (ARDS) remains poorly studied. To clarify this aspect, we measured the levels of eicosanoids and used logistic regression analysis and receiver operating characteristic (ROC) curves to investigate whether eicosanoids could act as risk and prognostic factors for sepsis patients who develop ARDS. We conducted a case-control study comparing 13 sepsis patients with ARDS and 23 sepsis patients without ARDS. The plasma levels of leukotriene B_4 (LTB_4), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) were measured by radioimmunoassays as substitutes for the plasma levels of PGI_2 and TXA_2, which are unstable. The levels of eicosanoids in sepsis patients with ARDS were significantly higher than those in sepsis patients without ARDS. Logistic regression analysis revealed that LTB_4 was the only risk factor for sepsis patients with ARDS (odds ratio, 1.10; P=0.02). The area under the ROC curve values for all eicosanoids were significantly greater than 0.5 (P<0.001), and the likelihood value for the TXB_2 levels was higher than those of the other eicosanoids. We conclude that LTB_4 may be an important risk factor for sepsis patients with ARDS, while TXA_2 may be an important prognostic factor for sepsis patients with ARDS.

Keywords: Leukotriene B_4; Thromboxane A_2; Prostaglandin I_2; Lung injury; Logistic regression analysis; Receiver operating characteristic curve

Introduction

Acute respiratory distress syndrome (ARDS) is known to be associated with systemic inflammatory response syndrome (SIRS) through sepsis, and the inflammatory response is produced by inflammatory cells and/or endotoxins. Various inflammatory mediators lead to a critical event in the development of organ injury in the microvessels of organs, especially the lungs. Leukotrienes, thromboxanes and prostaglandins, collectively referred to as eicosanoids, have been reported to be involved in sepsis [1-4], burns [5] and ARDS [6,7]. Leukotrienes contribute to pulmonary edema by inducing neutrophil influx and activation and increasing vascular permeability. Leukotriene B_4 (LTB_4), which is derived by 5-lipoxygenation of arachidonic acid, is synthesized by neutrophils, monocytes, macrophages and endothelial cells, and promotes neutrophil attachment to the vascular endothelium and lung injury [8]. Thromboxane A_2 (TXA_2) mediates the aggregation and release mechanism of platelets and is also a potent vasoconstrictor [9], while prostaglandin I_2 (PGI_2) is a potent endogenous inhibitor of platelet aggregation and a vasodilator [10]. Thus, eicosanoids are involved in the pathological process of lung injury. However, few studies have clinically and systematically investigated the pathophysiological changes in sepsis patients who develop ARDS. In the present study, we measured the plasma levels of the eicosanoids LTB_4, TXB_2 and 6-keto-$PGF_{1\alpha}$ and used logistic regression analysis and receiver operating characteristic (ROC) curves to determine which eicosanoids act as risk and prognostic factors for sepsis patients who develop ARDS.

Materials and Methods

After obtaining informed consent from the patients or their families and approval to conduct the study from the Ethics Committee of our hospital, 36 adult sepsis patients were enrolled and assigned into 23 ARDS-free patients (14 males and 9 females; mean age, 57.78 years; age range, 51.08-64.48 years) and 13 ARDS patients (10 males and 3 females; mean age, 59.23 years; age range, 49.51-68.96 years). There were no significant differences between the groups regarding age

or sex. The criteria for the diagnosis of ARDS were: PaO_2/FiO_2 (P/F ratio) ≤ 200 mmHg; absence of cardiac failure; pulmonary edema-like shadows on a chest X-ray; and pulmonary artery wedge pressure ≤ 18 mmHg when measured [11]. The criterion for the diagnosis of sepsis was a systemic response to infection, manifested by two or more of the following conditions: body temperature >38°C or <36°C; heart rate>90 beats/minute; respiratory rate>20 breaths/minute or $PaCO_2$<32 mmHg; and white blood cell count>12,000/mm^3 or<4000/mm^3, or >10% immature (band) forms [12]. All the patients in the ARDS group had been endotracheally intubated and were on mechanical ventilation in the synchronized intermittent mandatory ventilation mode under intravenous sedation with midazolam 3-4 mg•h^{-1} and buprenorphine 0.03 mg/h.

The methods for measuring the plasma eicosanoid levels were reported previously [5]. Briefly, blood samples were obtained from the ARDS patients within 24 h of ARDS onset and from the ARDS-free patients on the day of admission to the intensive care unit. All blood samples were collected into tubes containing indomethacin-supplemented 4.5 mM ethylenediaminetetraacetic acid (EDTA) and centrifuged at 3000 rpm for 15 min to obtain the plasma. For the LTB_4 assay, the plasma was mixed with a mixture of ethyl acetate and methanol (2:1) and centrifuged at 3000 rpm for 15 min to obtain the supernatant. All plasma samples were stored at -80°C until analysis. LTB_4, 6-keto-$PGF_{1\alpha}$ and TXB_2 were measured by radioimmunoassays (MEN Research Products, DuPont, Boston, MA). The normal ranges

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for the levels of LTB_4, 6-keto-$PGF_{1\alpha}$ and TXB_2 were 77.0 ± 15.7, 12-33 and 14-50 pg/ml, respectively. ROC curves for the eicosanoids were constructed for the sepsis patients with ARDS. We plotted the true-positive rate (sensitivity) on the vertical axis of the graph against the false-positive rate (1-specificity) on the horizontal axis for each threshold. An ideal test should have high sensitivity and low (1-specificity), and therefore produce a curve close to the upper left corner of the graph. The best cut-off points for eicosanoids as prognostic factors were identified as those values that simultaneously maximized the sensitivity and specificity. The likelihood ratios (LRs), defined as the ratio of the frequency of a finding among the diseased patients (true-positive rate) to the frequency of the finding among the non-diseased patients (false-positive rate), and the sensitivity/(1-specificity) ratios were also calculated. A true diagnostic test usually has an LR>10, while an exclusion test has an LR<0.1. The data are reported as means and 95% confidence intervals (CIs). Mann-Whitney tests were used for comparisons of the eicosanoid levels between ARDS and ARDS-free patients. All analysis were performed using the StatView J4.5 software (Abacus Concepts, Berkeley, CA). The ROC curves and logistic regressions were analyzed using SPSS (Chicago, IL). Values of P<0.05 were considered statistically significant.

Results

Table 1 compares the eicosanoid levels in the ARDS and ARDS-free patients. The levels of all three factors were significantly higher in the ARDS patients than in the ARDS-free patients (LTB_4, P<0.0001; TXB_2, P<0.0001; 6-keto-$PGF_{1\alpha}$, P<0.001). Table 2 shows the logistic regression analysis for the eicosanoids in the ARDS patients. The values of the odds ratios for LTB_4, TXB_2 and 6-keto-$PGF_{1\alpha}$ were 1.10 (95% CI, 1.01-1.19; P=0.02), 1.04 (95% CI, 0.98-1.11; P=0.17) and 0.99 (95% CI, 0.83-1.19; P=0.95), respectively. Table 3 and Figure 1 show the ROC curves for the eicosanoids generated for the ARDS patients. The area under the ROC curve values for LTB_4, TXB_2 and 6-keto-$PGF_{1\alpha}$ were 0.933 (95% CI, 0.856-1.010; P<0.001), 0.905 (95% CI, 0.728-1.000; P<0.001) and 0.878 (95% CI, 0.485-0.990; P<0.001), respectively. Table 4 shows the best cut-off points, sensitivities, specificities and LR values. The LR values for LTB_4, TXB_2 and 6-keto-$PGF_{1\alpha}$ were 7.95, 14.30 and 12.37, respectively.

Eicosanoids	ARDS (-)	ARDS (+)	P value
LTB4 (pg.ml⁻¹)	62.7 (52.0-73.4)	118.3 (101.8- 134.8)	<0.0001
TXB2 (pg.ml⁻¹)	38.1 (26.5-49.7)	94.2 (71.0 - 117.3)	<0.0001
6-keto-$PGF_{1\alpha}$ (pg.ml⁻¹)	15.6 (11.7-19.6)	32.2 (25.2 - 39.3)	0.0002
Values are expressed as mean (95% confidential interval)			

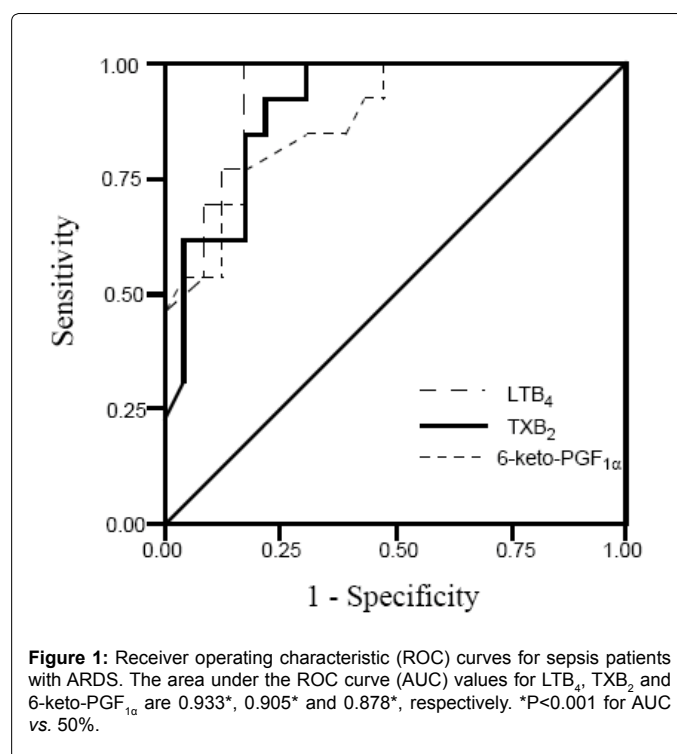**Table 1:** Comparisons of eicosanoids in the ARDS and ARDS-free sepsis patients.

Eicosanoids	Odds Ratio	95% Confidence Interval	P value
LTB_4	1.10	1.01-1.19	0.02
TXB_2	1.04	0.98-1.11	0.17
6-keto-$PGF_{1\alpha}$	0.99	0.83-1.19	0.95

Table 2: Logistic regression analysis of eicosanoids for ARDS in sepsis patients.

Eicosanoids	AUC	95% Confidence Interval	P value*
LTB_4	0.933	0.856-1.010	<0.001
TXB_2	0.905	0.728-1.000	<0.001
6-keto-$PGF_{1\alpha}$	0.878	0.485-0.990	<0.001
ROC: Receiver Operating Characteristic, AUC: Area Under the Curve, *Comparison with AUC=0.5			

Table 3: ROC curve analysis of eicosanoids for ARDS in sepsis patients.

Figure 1: Receiver operating characteristic (ROC) curves for sepsis patients with ARDS. The area under the ROC curve (AUC) values for LTB_4, TXB_2 and 6-keto-$PGF_{1\alpha}$ are 0.933*, 0.905* and 0.878*, respectively. *P<0.001 for AUC *vs.* 50%.

Eicosanoids	Cut-off Point	Sensitivity	Specificity	LR
LTB_4 (pg.ml⁻¹)	103.5	0.69	0.91	7.95
TXB_2 (pg.ml⁻¹)	83	0.62	0.96	14.3
6-keto-$PGF_{1\alpha}$ (pg.ml⁻¹)	35	0.54	0.96	12.37
LR: Likelihood Ratio				

Table 4: Best cut-off points.

Discussion

We measured the plasma levels of three eicosanoids in sepsis patients, and determined whether any of these eicosanoids could act as risk and prognostic factors for sepsis patients who develop ARDS. Our study had four main findings: 1) the levels of all three eicosanoids were significantly higher in the ARDS patients than in the ARDS-free patients; 2) only LTB_4 was a risk factor for sepsis patients with ARDS; 3) all three eicosanoids were specific factors for ARDS; and 4) the likelihood value of the TXB_2 levels was higher than those of the other eicosanoids.

The present study provides information that will be useful for elucidating the process and mechanism of lung injury and for establishing therapeutic strategies in the future. Receptor antagonist and/or synthetase inhibitor analysis have been carried out in patients with ARDS. Thromboxane synthesis inhibitors and/or antagonists improved the acute cardiopulmonary effects of bolus endotoxin [13-15] and increased patient survival [16-18]. The thromboxane A_2 synthesis inhibitor ketoconazole was found to significantly reduce the rate of ARDS development in sepsis patients and decrease their mortality rate [19]. In contrast, ketoconazole was not found to reduce the mortality or duration of mechanical ventilation in a randomized trial [20]. The beneficial effects of ketoconazole may be attributed to the inhibition of leukotriene formation as well as thromboxane A_2 synthesis [7]. The results of the present study revealed that the likelihood value of the TXB_2 levels was higher than those of the other eicosanoids examined,

and that TXA_2 is a prognostic factor for sepsis patients with ARDS rather than a factor for treatments.

Amat et al. [7] analyzed the leukotrienes LTB_4, LTC_4 and LTD_4, and reported that only the plasma concentration of LTB_4 on day 1 was a prognostic marker for ARDS, and that LTB_4 and interleukin-8 together could be useful markers of the mortality rate. Leukotriene antagonists, such as ONO-1078 [21], LY255283 [22] and ICI 198,615 [23], seemed to ameliorate the clinical outcome in models of LPS-induced acute lung injury. These antagonists reduced pulmonary extravascular water contents and bronchoalveolar lavage fluid protein concentrations. Moreover, cytosolic phospholipase A_2 ($cPLA_2$) is important in the pathogenesis of sepsis-induced ARDS, and LTB_4 is probably the major mediator of polymorphonuclear neutrophil infiltration among the $cPLA_2$ products [24,25]. The results of the present study indicate that LTB_4 is a factor for ARDS treatments in sepsis patients rather than a prognostic factor. However, few randomized trials of LTB_4 antagonists have been carried out in sepsis patients with ARDS.

Conclusion

In conclusion, among the eicosanoids examined, LTB_4 may be an important risk factor for sepsis patients who develop ARDS, while TXA_2 may be an important prognostic factor for sepsis patients who develop ARDS. Molecular biological studies and large randomized trials are needed to confirm these findings.

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