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Original Research

Diagnostic value of apoptosis biomarkers in severe sepsis-A pilot study

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Abstract: Severe sepsis is associated with significant mortality and massive immune cell lose, or apoptosis. It is unclear whether plasma apoptosis biomarkers could be used as a diagnostic test for severe sepsis. Forty patients with severe sepsis and 35 healthy controls were enrolled. The percentage and apoptosis of monocytes and lymphocytes were detected by flow cytometric analysis. Plasma levels of tumor necrosis factor (TNF)-α, soluble TNF receptor (sTNFR), soluble Fas (sFas), Fas ligand (FasL), caspase-1, and procalcitonin (PCT) were measured. Plasma caspase-1 level was positively correlated with CD4 lymphocyte apoptosis in controls and patients, and with CD8 lymphocyte apoptosis in all subjects. Plasma FasL level was negatively correlated with CD4 and CD8 lymphocyte apoptosis in all subjects. The sFas/FasL ratio was positively correlated with CD4 and CD8 lymphocyte apoptosis and negatively with monocyte apoptosis in all subjects. Compared with PCT, caspase-1, FasL, and sFas/FasL ratio had better negative predictive value and likelihood ratio for a negative test. PCT had better positive predictive value and likelihood ratio for a positive test. This work demonstrated caspase-1, FasL, and sFas/FasL ratio could be candidates for diagnosis of severe sepsis and their diagnostic value was not inferior to that of PCT.

Key words: Severe sepsis, apoptosis, caspase-1, Fas ligand, sFas/FasL, procalcitonin.

Introduction

Patients with severe sepsis have significant mortality. The mortality rate may decrease if they are early diagnosed and managed adequately (1). Although the 2012 Surviving Sepsis Campaign guidelines suggest utilization of procalcitonin (PCT) or C-reactive protein to diagnose severe sepsis. The utility of PCT levels to distinguish sepsis-induced acute inflammation from other causes of generalized inflammation has not been demonstrated. New biomarkers should be investigated to identify severe sepsis.

Recent studies have shown massive immune cell loss, or apoptosis, in severe sepsis (2,3). Apoptosis is the process of programmed cell death. The signals triggering apoptosis originate from either extracellular (extrinsic) or intracellular (intrinsic) pathways. The extrinsic apoptosis pathway is triggered when ligands [for example, Fas ligand (FasL) or tumor necrosis factor (TNF)-α] bind to cell surface death receptors [for example, Fas or TNF receptor (TNFR)]. Pyroptosis is another form of cell death and initiated by a variety of stimuli, including cytosolic flagellin or type III secretion system rod proteins, phagocytosis of crystals, and opening of pores in the membrane (4). The important mediator is caspase-1, which plays a conservative role as a cell death protease (5,6).

Due to important roles of apoptosis markers in severe sepsis, Huttunen et al. designed a prospective cohort study and found that Fas, FasL, or Fas/FasL ratio did not fill their expections as a prognostic marker (7). However, serum soluble Fas (sFas) levels in patients who later developed sepsis were significantly increased at day 5 and day 9 after trauma, compared with no sepsis patients (8). And sFas levels were positively correlated with Sequential Organ Failure Assessment score at day 1, day 5, and day 9 in septic patients. Thus, soluble apoptosis biomarkers might be a candidate of diagnostic marker although they might not be a good marker for prognosis prediction.

It was still unclear whether plasma-soluble apoptosis biomarkers were correlated with immune-cell apoptosis. If plasma-soluble apoptosis biomarkers had good correlation with immune cell apoptosis, these biomarkers could be used as a diagnostic test for severe sepsis. Thus, we designed a pilot study to test our hypothesis.

Materials and Methods

Participants and definitions

From August 2011 to December 2013, 40 patients who were admitted to a 20-bed intensive care unit (ICU) in a regional teaching referral hospital for severe sepsis were enrolled in this study. Systemic inflammatory response syndrome (SIRS) was defined as two or more of the following criteria: (1) body temperature >38°C or <36°C; (2) respiratory rate >20 breaths/minute; (3) heart rate >90 beats/minute; and (4) white blood count >12,000/μl or <4,000/μl or >10% bands. Sepsis was defined as SIRS according to a confirmed infectious etiology. To validate experimental findings, 23 men and

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12 women visiting our health evaluation center for examinations were enrolled as healthy controls.

Severe sepsis was defined according to the consensus criteria of sepsis with one or more organ dysfunction (9,10), like shock, respiratory failure, acute renal failure, jaundice and thrombocytopenia. Septic shock was defined as sepsis-induced hypotension unresponsive to fluid resuscitation. Respiratory failure was defined as ventilation dysfunction requiring invasive ventilator support. Acute renal failure was defined as a rapid increase in creatinine level (>0.5 mg/dL). Jaundice was defined as hyper-bilirubinemia (total bilirubin >2 mg/dL) while thrombocytopenia was defined as a platelet count <150,000/μL. Disease severity was assessed by the Acute Physiology and Chronic Health Evaluation (APACHE) II score (11).

Standard treatment according to guidelines was provided to all patients (12,13). The Institutional Review Board at Chang Gung Memorial Hospital approved this study (100-0475B, 101-2761C, 102-0135C), and the patients' close family members provided informed consent. Patients who survived longer than 28 days after ICU admission were defined as survivors. All co-morbidities and past histories were recorded.

Plasma and peripheral blood mononuclear cell (PBMC) preparation

Whole blood (10 ml) was obtained from each patient at 08:30 AM within 48 hours of admission to ICU and immediately mixed with heparin. Whole blood from controls was obtained at 08:00-08:30 AM and also immediately mixed with heparin. Plasma samples were obtained from 2 ml of whole blood and stored at -80°C until use. PBMCs were isolated via differential centrifugation over Ficoll-Plaque (Amersham Biosciences, Uppsala, Sweden) from the residual 8 ml of whole blood within 2 h of collection.

Flow cytometric analysis

Half of the PBMCs were suspended in 50 μl of phosphate-buffered saline (PBS) and incubated in the dark for 15 min at room temperature with 10 μl of $CD4_{ECD}$, Annexin V_{PE}, 7-aminoactinomycin D (AAD), $CD11b_{PC7}$, $CD8_{APC}$, $CD3_{Alexa\ Fluor\ 700}$, and $CD14_{APC\text{-}750}$ antibodies. Then, the cells were washed once by 500 μl of PBS and resuspended in 500 μl of PBS. The percentage and apoptosis of immune cells were detected by an eight-color flow cytofluorimeter (Beckman Coulter, CA, USA). T lymphocytes were identified with positive CD3 and CD4 (Figure 1A). $CD8^+$ lymphocytes were identified with positive CD3 and CD8 (Figure 1B). Monocytes were identified with positive CD11b and CD14 (Figure 1C). Apoptosis was identified with positive 7-AAD and annexin V in gated cells.

Measurement of plasma levels of apoptosis biomarkers and PCT

Plasma levels of TNF-α, soluble TNFR (sTNFR), and sFas were measured with a human enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, Vienna, Austria) according to the manufacturer's instructions. Plasma levels of FasL and caspase-1 were measured with human ELISA kits (R&D Systems, Inc., MN, USA) according to the manufacturer's instructions. Plasma PCT level was measured with human ELISA kits (BioVendor, NC, USA) according to the manufacturer's instructions.

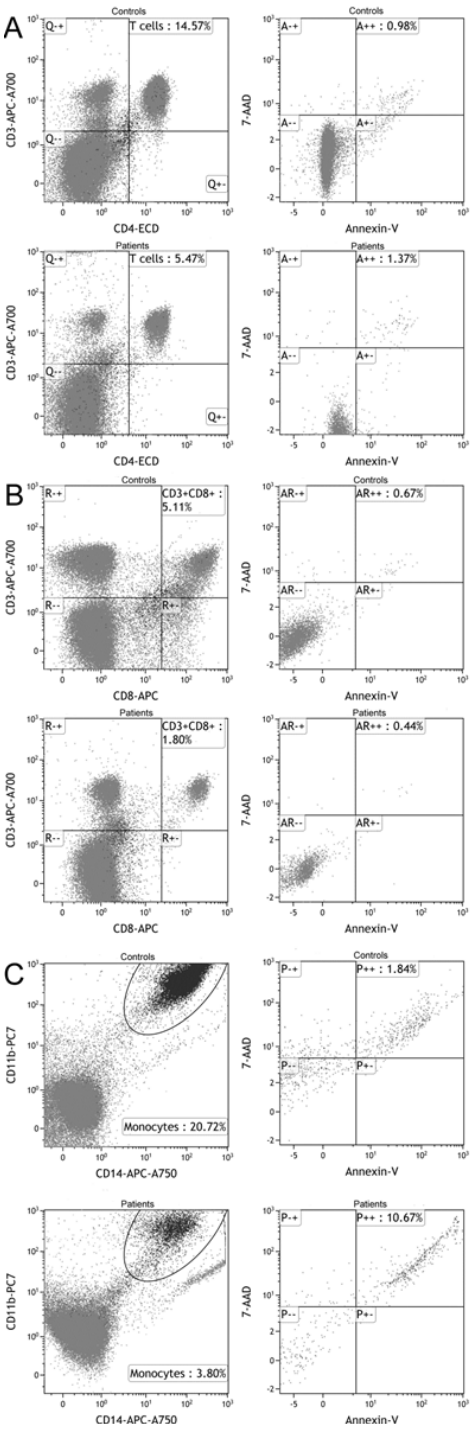

Figure 1. (A) T lymphocytes were identified with positive CD3 and CD4. The percentages of T lymphocytes in peripheral blood mononuclear cells in a control and patient were 14.57 and 5.47%, respectively. Apoptosis was identified with positive 7-aminoactinomycin D (AAD) and annexin V. The percentages of apoptosis in T lymphocytes in a control and patient were 0.98 and 1.37%, respectively. (B) $CD8^+$ lymphocytes were identified with positive CD3 and CD8. The percentages of $CD8^+$ lymphocytes in peripheral blood mononuclear cells in a control and patient were 5.11 and 1.80%, respectively. The percentages of apoptosis in $CD8^+$ lymphocytes in a control and patient were 0.67 and 0.44%, respectively. (C) Monocytes were identified with positive CD11b and CD14. The percentages of monocytes in peripheral blood mononuclear cells in a control and patient were 20.72 and 3.80%, respectively. The percentages of apoptosis in monocytes in a control and patient were 1.84 and 10.67%, respectively.

Statistical analysis

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software V11.0.1 for Windows (SPSS, Inc., IL, USA). Differences between groups for continuous variables were analyzed by Mann-Whitney test while differences for categorical variables were analyzed by the chi-square test or Fisher's exact test. The Spearman rank correlation was used to measure the correlation between plasma apoptosis biomarker levels and apoptosis percentages. A reasonable cutoff value of plasma apoptosis biomarker was chosen according to the receiver operating characteristic (ROC) analysis. A p value <0.05 was considered statistically significant. The cutoff value (2000 pg/ml) of plasma PCT level for severe sepsis was chosen according to clinical practice and suggestion.

Sensitivity and specificity are defined, respectively, as the proportion of patients correctly identified by the test as abnormal and the proportion of healthy subjects correctly identified. The positive predictive value (PPV) is the proportion of patients with severe sepsis among those with positive test results. The negative predictive value (NPV) is the proportion of subjects without severe sepsis among those with negative test results. The likelihood ratio for a positive test result (LR+) is the odds of a positive test result in severe septic patients, versus a positive test result in patients without severe sepsis. The likelihood ratio for a negative test result (LR-) is the odds of a negative test result in patients without severe sepsis, versus a negative test result in severe septic patients.

Results

Of the 40 enrolled subjects with severe sepsis, 35 survived for 28 days and 5 died (Table 1). There were no significant differences in age, gender, APACHE II score, histories, adverse events, and plasma mediator levels between shock and no-shock patients. Patients with severe sepsis had higher age than healthy controls. Plasma TNF-α levels in patients and controls were not detected. Plasma sTNFR, sFas, sFas/FasL ratio, caspase-1 and PCT levels in patients were higher than in controls. Plasma FasL level in patients was lower than in controls.

Correlation between cell apoptosis percentages and apoptosis markers

Compared with the healthy controls, the patients with severe sepsis had a higher white blood cell count and percentage of neutrophils, and lower percentages of lymphocyte and monocytes (Table 2). There was no difference in circulating PBMCs per microliter between patients and controls. Percentages of CD4 lymphocytes, CD8 lymphocytes and monocytes in PBMCs of severe septic patients were lower than those of controls. Patients had higher apoptosis percentage in CD4 and CD8 lymphocytes, compared with controls. The apoptosis of monocytes was similar between patients and controls. There were no differences in cell subsets and apoptosis of PBMCs between shock and no-shock patients.

TNF-α was not used to analyze the correlation with apoptosis due to its level being undetectable in this study. Also, sTNFR was not analyzed in controls due to un-

Table 1. Clinical characteristics and plasma apoptosis markers in participants (number, mean ± standard error mean).

	Shock (n=12)	No shock (n=28)	All patients (n=40)	Controls (n=35)
Age (years old)	69.5 ± 4.2	76.5 ± 2.3	74.5 ± 2.1	57.6 ± 1.3*
Male (%)	7 (58)	21 (75)	28 (70)	23 (66)
APACHE II score	18.7 ± 2.0	17.2 0.8	17.6 ± 0.8	N/A
History (%)				
COPD	2 (17)	9 (32)	11 (28)	0 (0)
Heart failure	2 (17)	2 (7)	4 (10)	0 (0)
Pneumoconiosis	0 (0)	4 (14)	4 (10)	0 (0)
Hypertension	6 (50)	16 (57)	22 (55)	0 (0)
Diabetes mellitus	3 (25)	8 (29)	11 (28)	0 (0)
Old CVA	4 (33)	6 (21)	10 (25)	0 (0)
ESRD	1 (8)	2 (7)	3 (8)	0 (0)
Liver cirrhosis	2 (17)	2 (7)	4 (10)	0 (0)
Active malignancy	1 (8)	3 (11)	4 (10)	0 (0)
Adverse event				
Respiratory failure	12 (100)	22 (79)	34 (85)	N/A
New arrhythmia	0 (0)	2 (7)	2 (5)	N/A
GI bleeding	3 (25)	1 (4)	4 (10)	N/A
Acute renal failure	2 (17)	7 (25)	9 (23)	N/A
Thrombocytopenia	4 (33)	3 (11)	7 (18)	N/A
Jaundice	2 (17)	0 (0)	2 (5)	N/A
Bacteremia	0 (0)	3 (11)	3 (8)	N/A
Mortality (%)	2 (17)	3 (11)	5 (13)	N/A
Plasma level, pg/ml				
TNF-α	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
sTNFR	1467.5 ± 1058.0	517.9 ± 226.9	802.8 ± 352.9	0.0 ± 0.0*
sFas	1356.0 ± 610.6	1388.0 ± 654.5	1378.4 ± 489.0	946.9 ± 417.8
FasL	51.4 ± 11.1	36.7 ± 4.7	41.2 ± 4.7	88.1 ± 5.8*
sFas/FasL	37.4 ± 19.0	57.3 22.6	51.3 ± 16.7	12.0 ± 4.7*
Caspase-1	187.4 ± 34.3	180.8 ± 13.3	182.8 ± 13.6	86.1 ± 5.8*
Procalcitonin	4458.4 ± 1251.2	2815.3 ± 609.8	3308.2 ± 572.0	6.2 ± 6.2*

Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; N/A, not applicable; COPD, chronic obstructive pulmonary disease; CVA, cerebral vascular accident; ESRD, end stage renal disease; GI, gastrointestinal; TNF, tumor necrosis factor; sTNFR, soluble tumor necrosis factor receptor; sFas, soluble Fas; FasL, Fas ligand.

*P < 0.05 compared with patients by Mann-Whitney test.

Table 2. Cell subsets and apoptosis in PBMCs of participants (mean ± standard error mean).

	Shock (n=12)	No shock (n=28)	All patients (n=40)	Controls (n=35)
WBC, $10^3/\mu L$	15.6 ± 2.7	14.6 ± 1.3	14.9 ± 1.2	5.1 ± 0.2*
Neutrophil, %	84.9 ± 2.7	85.0 ± 1.9	85.0 ± 1.5	57.9 ± 1.1*
Lymphocyte, %	9.9 ± 2.5	9.4 ± 1.4	9.6 ± 1.2	32.0 ± 1.1*
Monocyte, %	4.7 ± 0.7	4.5 ± 0.5	4.6 ± 0.4	6.1 ± 0.3*
PBMCs, /μL	2351.8 ± 861.1	1891.2 ± 206.0	2029.4 ± 290.6	1933.5 ± 86.1
Total CD4 lymphocytes in PBMCs, %	2.1 ± 0.6	1.8 ± 0.4	1.9 ± 0.3	10.6 ± 1.5*
Apoptosis in total CD4 lymphocytes, %	2.5 ± 1.4	1.1 ± 0.5	1.5 ± 0.6	0.2 ± 0.1*
Total CD8 lymphocytes in PBMCs, %	1.0 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	6.3 ± 1.1*
Apoptosis in total CD8 lymphocytes, %	2.0 ± 0.7	1.5 ± 0.3	1.6 ± 0.3	0.4 ± 0.1*
Total monocytes in PBMCs, %	5.3 ± 2.7	3.0 ± 0.6	3.7 ± 0.9	7.6 ± 0.7*
Apoptosis in total monocytes, %	8.1 ± 2.5	7.4 ± 1.8	7.6 ± 1.5	5.0 ± 0.9

Abbreviations: PBMCs, peripheral blood mononuclear cells; WBC, white blood cell

*$p < 0.05$ compared with patients by Mann-Whitney test.

detectable level. Plasma caspase-1 level was positively correlated with CD4 lymphocyte apoptosis percentage in controls and patients (Table 3). Plasma caspase-1 level did not show significant correlation with CD8 lymphocyte and monocyte apoptosis pencentages in control and patient groups, but was positively correlated with CD8 lymphocyte apoptosis percentages in all subjects. Plasma FasL level was negatively correlated with CD4 and CD8 lymphocyte apoptosis percentages in all subjects. Plasma sTNFR level was negatively correlated with CD4 and CD8 lymphocyte apoptosis percentages in the patient group. The sFas/FasL ratio was positively correlated with CD4 and CD8 lymphocyte apoptosis percentages and negatively with monocyte apoptosis percentage in all subjects but not in patient or control groups. There was no correlation between plasma sTNFR level and monocytes apoptosis percentage in patient group. Plasma sFas level was not correlated with these 3 types of cell apoptosis percentages in controls, patients and all subjects.

Diagnostic value of apoptosis biomarkers in severe sepsis

Only caspase-1, FasL, and sFas/FasL ratio were used for analysis due to good correlation with cell apoptosis. Figure 2 shows the ROC curves for PCT, caspase-1, FasL, and sFas/FasL ratio. Plasma PCT, caspase-1, and sFas/FasL ratio were positive predictors, and plasma FasL level was a negative predictor. The areas under the curves of PCT, caspase-1, FasL, and sFas/FasL ratio were 0.906, 0.885, 0.136, and 0.819, respectively. Compared with PCT, caspase-1, FasL, and sFas/FasL ratio had better sensitivity and poorer specificity (Table 4). The caspase-1, FasL, and sFas/FasL ratio also had better NPV and LR-. PCT had better PPV and LR+.

Discussion

In this study, plasma caspase-1 levels in patients with severe sepsis were higher than those in controls. This result was similar to Delogu's study, which showed that blood levels of caspase-1 (101.5 +/- 18.2 pg/ml vs 9.09 +/- 2.7 pg/ml, $p < 0.001$) were significantly higher in septic patients vs controls (14). Exline et al. also found septic patients had higher microvesicular caspase-1 activity on day 1, and this persisted on day 3 (15). Microvesiculars isolated from septic patients were able to induce apoptosis in healthy donor lymphocytes. Depletion of microvesiculars greatly diminished this apoptotic signal. Furthermore, our study first found plasma caspase-1 level was significantly positively correlated with apoptosis of CD4 and CD8 lymphocytes. Higher

Table 3. Correlation coefficients between cell apoptosis percentages and plasma apoptosis marker levels.

	CD4 lymphocytes	CD8 lymphocytes	Monocytes
Controls (n=35)			
sTNFR			
sFas	0.320	0.135	0.024
FasL	-0.142	-0.159	-0.007
Caspase-1	0.404*	0.071	-0.305
sFas/FasL	0.174	0.311	-0.101
Patients (n=40)			
sTNFR	-0.403*	-0.353*	-0183
sFas	-0.257	-0.200	-0.029
FasL	0.028	-0.081	0.203
Caspase-1	0.410*	0.184	0.101
sFas/FasL	-0.020	0.087	-0.240
All (n=75)			
sTNFR			
sFas	0.044	0.035	-0.029
FasL	-0.279*	-0.385*	0.045
Caspase-1	0.521*	0.396*	-0.20
sFas/FasL	0.424*	0.331*	-0.391*

Abbreviations: sTNFR, soluble tumor necrosis factor receptor; sFas, soluble Fas; FasL, Fas ligand.

*$p < 0.05$ by Spearman test.

Table 4. Values used to evaluate plasma biomarkers in patients with severe sepsis.

Reliability / (cutoff value, pg/ml)	PCT (≥2000.0)	Caspase-1 (≥125.0)	FasL (≤65.0)	sFas/FasL (≥8.1)
Sensitivity, %	50.0	80.0	80.0	80.0
Specificity, %	100.0	82.9	77.1	85.7
Positive predictive value, %	100.0	84.2	80.0	86.5
Negative predictive value, %	63.6	78.4	77.1	78.9
LR+	∞	4.7	3.5	5.6
LR-	2.0	4.1	3.9	4.3

Abbreviations: PCT, procalcitonin; FasL, Fas ligand; sFas, soluble Fas; LR+, likelihood ratio for a positive test result; LR-, likelihood ratio for a negative test result.

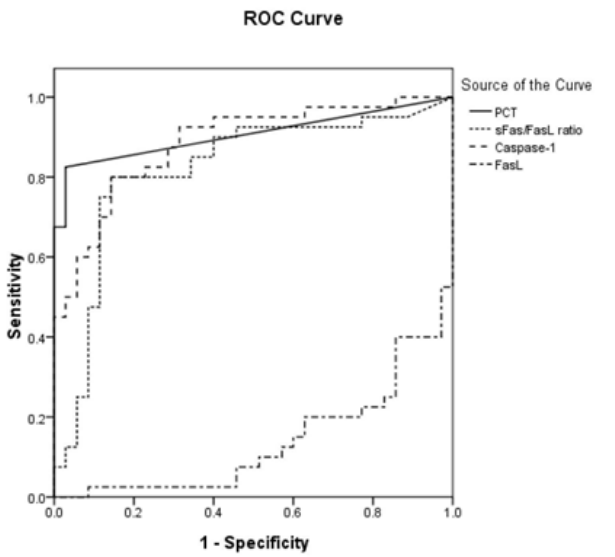

Figure 2. The receiver operating characteristic (ROC) curves showed that procalcitonin (PCT), caspase-1, Fas ligand (FasL), and soluble Fas (sFas)/FasL ration were good predictors of severe sepsis. Plasma PCT, caspase-1, and sFas/FasL ratio levels were positive predictors and plasma FasL level was a negative predictor. The areas under the curves of PCT, caspase-1, FasL, and sFas/FasL ratio were 0.906, 0.885, 0.136, and 0.819, respectively.

plasma caspase-1 levels showed more severe apoptosis in CD4 and CD8 lymphocytes.

Plasma sTNFR levels in patients with severe sepsis were higher than those in controls. This result was similar to De Freitas et al's study (16). However, plasma sTNFR levels in controls in this study were undetectable. The sensitivity of the sTNFR ELISA kit utilized in this work was 53 pg/ml. This might suggest that immune-cell apoptosis in controls is minimal and does not release sTNFR to blood circulation. Because plasma sTNFR levels in controls were undetectable, the correlation statistic between sTNFR level and cell apoptosis percentage cannot be analyzed in controls and all participants by SPSS software. Base on no evidence of correlation between sTNFR level and cell apoptosis percentage, using plasma sTNFR level to diagnose severe sepsis may be unsuitable in a broad population.

Expressions of Fas and FasL on T-cells of patients with SIRS and multiple organ dysfunction syndrome were significantly higher than those of controls (17). In this work, plasma FasL level and percentages of CD4 & CD8 lymphocytes in patients with severe sepsis were lower than controls. Absolute circulatory counts of CD4 and CD8 lymphocytes in patients with severe sepsis were also lower than in controls (2). Plasma FasL level was negatively correlated with CD4 and CD8 lymphocytes apoptosis in this work. All the above findings suggest that lower plasma FasL level in patients might be due to too few lymphocyte counts although of higher expression in T cells. However, De Freitas et al. found the serum level of FasL in severe septic patients was higher than that in healthy controls (16). Thus, more studies are needed to determine plasma FasL level in patients with severe sepsis.

In expectation, sFas/FasL ratio was positively correlated with lymphocyte apoptosis. The cause of negative correlation of sFas/FasL ratio with monocyte apoptosis is unclear. Daigneault et al. found that apoptosis of CD3+ cells in PBMC cultures after bacterium challenge required monocytes to induction (18). Maybe, less monocyte apoptosis induced more lymphocyte apoptosis. Thus, sFas/FasL ratio represented lymphocyte apoptosis level. The cause of negative correlation of sFas/FasL ratio with monocyte apoptosis might just be the result of more intact monocytes to induce lymphocyte apoptosis.

In the diagnostic evaluations, caspase-1, FasL, and sFas/FasL ratio had higher sensitivity but lower specificity than PCT. PCT had the highest PPV (100%), with nearly 64% of NPV. This means that all patients with PCT ≥ 2ng/ml had severe sepsis, but low PCT level could not rule out the diagnosis of severe sepsis. In contrast to PCT, caspase-1, FasL, and sFas/FasL ratio had better balance between PPV and NPV (around >80% and 78%, respectively). However, either PPV or NPV are markedly influenced by the prevalence of severe sepsis in the different study groups. These two diagnostic values may be not suitable for use in general population.

For revising prevalence or prior probability, the LR is used with increasing frequency by clinical physicians. PCT had very high LR+ but relatively low LR-. This made the PCT test unsuitable to rule out severe sepsis. For caspase-1 and sFas/FasL ratio, LR+ and LR- were both greater than 4. This means the number of patients with positive sFas/FasL test had a 5.6-fold ratio to that of patients with negative test for diagnosing severe sepsis. And the number of patients with negative sFas/FasL test had a 4.3-fold ratio to that of patients with positive test to rule out severe sepsis.

To sum up, tests with high PPV or LR+ could be used to diagnose sepsis in a low probability group, such as stable patients in general ward. In this condition, PCT test was better due to having the highest PPV and LR+ in these four tests. In critically ill patients at ICU admission, the probability of severe sepsis is high. Physicians need a test to rule out severe sepsis and prevent antibiotic overuse. In this study, the PCT test was not suitable to rule out severe sepsis. Other tests, like cas-

pase-1, FasL, or sFas/FasL tests, could be used due to their higher NPV and LR-.

There is one limitation in this work. Healthy individuals were used as controls. In clinical practices, severe sepsis should be quickly diagnosed in patients with SIRS because such patients need prescription of broad-spectrum antibiotic as soon as possible. Other critical illnesses, such as severe heart failure, tachyarrhythmia, massive bleeding, etc. do not require antibiotic use. These apoptosis biomarkers should be tested in non-sepsis critically ill patients.

In conclusion, plasma caspase-1 and FasL levels were significantly correlated with apoptosis of CD4 and CD8 lymphocytes. Higher plasma caspase-1 level and sFas/FasL ratio indicated higher apoptosis of CD4 and CD8 lymphocytes. Lower plasma FasL level indicated higher apoptosis of CD4 and CD8 lymphocytes. Furthermore, this work demonstrated that caspase-1, FasL, and sFas/FasL ratio could be candidates for the diagnosis of severe sepsis, and that their diagnostic value was not inferior to PCT. A large-scale study is necessary to confirm their diagnostic value in critically ill patients.

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