High levels of plasma S100A9 at admission indicate an increased risk of death in severe tuberculosis patients

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ABSTRACT

Objective: This study aims to evaluate plasma S100A9 levels in tuberculosis (TB) patients with admission to the ICU as a marker to predict the risk of death for pulmonary severe TB.

Methods: This study enrolled 256 severe TB patients admitted to Beijing Chest Hospital from Jan to Dec 2019. The S100A9 levels were measured by ELISA. Standard clinical parameters were collected. The non-parametric Mann-Whitney test, *t*-test, and chi-square test were applied to statistical comparison. A multivariable analysis was performed to identify risk factors for death.

Results: The plasma S100A9 levels were higher in non-survivors (25.88, 16.77–44.64) compared to survivors (15.51, 13.67–19.94). S100A9 performed better than Acute Physiology and Chronic Health Evaluation (APACHE II) score in predicting death, with AUC of 0.725, sensitivity of 65.5%, and specificity of 80.3%. By combining APACHE II score together with the S100A9 levels we got an AUC of 0.754 (95% CI 0.68 to 0.82) in predicting death. Lastly, S100A9 levels were significantly higher in patients with APACHE II score >17.5, sputum smear-positive, early death, and high cavitary lesions numbers, all of which were related to TB progression.

Conclusion: Measurement and monitoring levels of plasma S100A9 in severe TB patients could facilitate the evaluation of patients with high risk at the early stage, which may help to improve the treatment outcome for TB patients.

1. Introduction

Tuberculosis (TB) is a leading cause of death in humans due to an infectious agent (*Mycobacterium tuberculosis, Mtb*) and it remains a major public health burden in developing countries [1,2]. Disease progression is one of the most predominant causes of TB mortality and the mortality rate of patients with severe pulmonary TB is as high as 69.80% [3]. Severe TB patients had poor outcomes with no standard evaluation criteria, especially TB patients combined with respiratory failure [4]. Respiratory failure is a common disorder in the intensive care unit (ICU) and it is associated with high mortality and morbidity. Although TB cases requiring ICU represent only 1–3% of all hospital admissions for TB, in-hospital TB mortality rates associated are higher (range 29–83%) than for other infections [5].

Pulmonary TB reflects a complex interplay that exists between distinctive components of the innate and acquired immune responses, as well as with the pathogen itself. A previous study reported the use of Acute Physiology and Chronic Health Evaluation (APACHE) II score in guiding the treatments for critically ill TB patients [6]. Previous studies have reported the application of some proteins as biomarkers [7,8] such as interleukin 6 or Hemoglobin A1c, which were related to TB severity. However, there was no standard criteria to monitor the severity and prognosis of patients with TB.

S100A9 is a calcium-binding protein and was originally regarded as a regulator of the immune response and a mediator of the inflammatory process [9]. Recent studies have focused on tracing S100A9 as a novel diagnostic and prognostic biomarker in TB. The levels of S100A9 have been employed for indicating inflammation in TB patients [10]. Our previous study also found that the plasma S100A9 was associated with the development of TB and has a potential to distinguish between different stages of TB [11]. However, S100A9 has not yet been evaluated as a biomarker for predicting outcomes in pulmonary TB patients.

Biomarkers for admission to ICU help to predict the outcome of TB patients and avoid overuse of costly adjuvant treatments.

To fill that paucity, in this study we measured the plasma S100A9 levels in severe pulmonary TB patients at admission to ICU along with standard clinical parameters and APACHE II scores, analyzed the association with the survival of the patients, and compared their performance in predicting TB death, which may facilitate the evaluation of patients with high risk at the early stage and help to improve the treatment outcome for TB patients.

2. Methods

2.1. Patient admission and sample collection

The project was authorized by the Ethical Committees of the Chinese Clinical Trial Registry, the number of the approval: ChiCTR1800020378 and informed consents were obtained from all participants and/or their legal guardians. Patients were prospectively recruited and enrolled by the staff at the Beijing Chest Hospital affiliated to Capital Medical University (Beijing, China) between Jan and Dec 2019. The present study was conducted according to the principles of the Declaration of Helsinki.

The patient sample size was determined based on the categorical test's statistical calculation to obtain a minimum of 40 samples. In the present study, we set the smallest sample size to 50 considering a 20% non-response rate. The cohort selection of patients was based on the following criteria: (i) written informed consent from the patients or their legally authorized surrogate to be used for research purposes; (ii) diagnosis of active tuberculosis [12]; (iii) three or more lung lobe alterations were observed in a chest computed tomography (CT) scan [11] (iv) accompanied respiratory failure [13]; (v) well-traceable blood withdrawn in the first 24 h post-admission in ICU with plasma frozen at −80 °C. The exclusion criteria were: (i) patients younger than 18 years old; (ii) non-tuberculous mycobacterial disease; (iii) incomplete clinical data; (iv) use immune-suppressive drugs; (v) HIV infection (Our hospital refer HIV-positive patients to HIV specialized hospital). The enrolled patients were further divided into survivor and non-survivor groups based on the outcome of patients in ICU. The non-survivor group was divided into the early death group (before day 14) and the late death group (after day 14) [14]. We also recruit an age-matched group of pneumonia patients with respiratory failure (n = 18), who had signs and symptoms of lower respiratory tract infection and received a chest radiograph for clinical diagnosis of pneumonia [15], and had no exposure to *Mtb,* a negative tuberculin skin test and T-SPOT.TB test result. An age-matched group of healthy volunteers (n = 40) were recruited from a population who had no exposure to *Mtb*, a negative tuberculin skin test and T-SPOT. TB test result, a normal chest CT and no clinical evidence of any other diseases. All blood samples from each group were collected from venous catheters into tubes containing EDTA. Blood samples were centrifuged at 1400*g* for 5 min at 4 °C and the supernatant plasma was stored at −80 °C.

2.2. ELISA of S100A9

Enzyme-linked immunosorbent assay (ELISA) was performed to measure the levels of S100A9 protein in plasma using a commercially available kit (Jiangsu Jingmei Biological Technology Co., Ltd. China; the detection limit was 0.2 μg/L). Briefly, high-binding 96-well microplates were coated overnight at 20 °C with anti-S100A9 mAb diluted in 50 mM phosphate buffer (5 μg/mL, pH 7.4) and then saturated with casein buffer for storage at 4 °C until use. The wells were washed 5 times with 50 mM potassium phosphate/0.02% Tween-20 before the addition of 100 μL of human plasma or recombinant S100A9 proteins diluted in casein buffer. After one-hour incubation at room temperature, the plates were washed 5 times and 100 μL of biotinylated anti-S100A9 mAb (100 ng/mL) was added for one-hour treatment. After that, the wells were washed and 100 μL of streptavidin poly-HRP was added to the wells for

30 min. The wells were then washed 5 times and 100 μL of Ultra TMB-ELISA Substrate Solution was added. The reaction was stopped after 30 min by addition of 100 μL of 1 M H_2SO_4 and the plate was immediately read at 450 nm using a Tecan microplate reader (Infinite 2000 PRO) (Tecan Group Ltd, Mannedorf, Switzerland).

2.3. Statistical analysis

Statistical analysis was performed with SPSS 26.0 software and GraphPad Prism 7.0. The data were expressed as median values with interquartile range (25–75), mean ± SD or percentage. The non-parametric Mann-Whitney test, *t*-test and chi-square test were applied for comparison. To evaluate the risk factors for death in severe TB patients, we compared clinical variables between the deceased and surviving groups using univariate comparison and subsequent multiple logistic regression. A *p-value* of <0.05 was considered significant. The diagnostic efficiency of S100A9, APACHE II, and their combination were assessed by the receiver operating characteristic (ROC) analysis to compare the area under the curve (AUC) of each biomarker. Meanwhile, the cut-off value of individual biomarker was determined by using ROC curve to predict outcomes with the highest sensitivity and specificity.

3. Results

3.1. Baseline clinical characteristics of the patients

A total of 256 TB patients were enrolled, and 38 patients were excluded due to either insufficient amount of plasma or interaction with immune-suppressive drugs (Fig. 1). Of these patients, a total of 218 patients were categorized into the survivor group (n = 122) and the non-survivor group (n = 96). Among the non-survivors at day 28, we looked at the potential differences in S100A9 between early (before day 14) versus later (after day 14) deaths.

We summarized the demographic characteristics of all TB patients and the control group as in Table 1. The median age of all TB patients was 57.25 ± 19.69 years, and the male: female ratio was 157:61, with an average length of stay in ICU at 13.94 ± 12.57 days. There is no difference between TB patients and the control group in terms of age and gender. The mortality rate of TB patients in ICU was 44.04% (96 of 218). The overall clinical characteristics of TB patients are as follows: 73.85% were mechanically ventilated and 6.42% had renal replacement therapy. There are 26.62% severe TB patients not receiving anti-TB treatment because they suffered hepatic insufficiency which made them unable to stand the side-effects of anti-TB drugs. Among the cohort, the non-survival group and survival group patients were similar in age, gender, complications, comorbidities and TB characteristics (*p-value* > 0.05). Compared with the non-survival group, levels of C-reactive protein, the proportion of patients with mechanical ventilation and the APACHE II score in the survival group was statistically lower, and the levels of albumin in the survival group were statistically higher, (*p-value* < 0.05).

In multivariable analyses, Albumin (hazard ratio 1.106, 95% CI: 1.031–1.183, *p-value* = 0.005), Apache II score (hazard ratio 0.830, 95% CI: 0.755–0.913, *p-value* < 0.001) and S100A9 (hazard ratio 0.944, 95% CI: 0.921–0.968, *p-value* < 0.001) were independent risk factors for death of severe TB patients (Table2).

3.2. Plasma S100A9 levels in TB patients

The median values for S100A9 in sever TB group in admission to ICU, pneumonia patients with respiratory failure group and control group was presented in Table 3. The differences between the Healthy control group and the total cohort group (*p-value* < 0.001), the Healthy control group and pneumonia with respiratory failure group (*p-value* = 0.018), were statistically significant. However, although the S100A9 levels in TB patients were higher than pneumonia patients, there was no significant
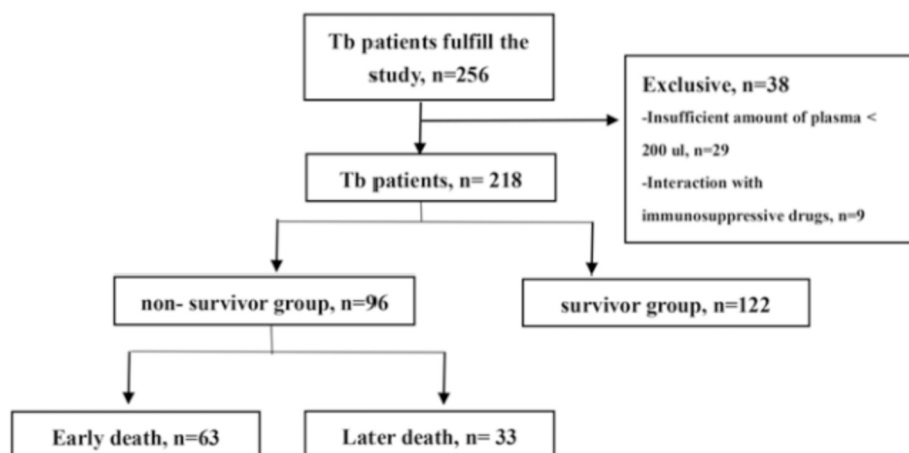
Fig. 1. Schematic representation of TB patients.

Table 1
Patients characteristics and clinical presentation of study population.

	Healthy Control (n = 40)	Total cohort (n = 218)	Survivors (n = 122)	Non-survivors (n = 96)	Statistical value*	p-value*	Statistical value #	p-value#
Demographic parameters								
Age (mean ± SD)	57.17 ± 19.88	57.25 ± 19.69	57.76 ± 20.10	56.68 ± 19.25	0.024[a]	0.981	0.405[a]	0.686
Male, n (%)	29 (72.50)	157 (72.02)	91 (74.59)	66 (68.75)	0.017[b]	0.897	0.643[b]	0.422
Comorbid diseases								
Chronic obstructive pulmonary disease, n (%)	NA	38 (17.43)	24 (19.67)	14 (14.58)	NA	NA	0.645[b]	0.421
Diabetes mellitus, n (%)	NA	69 (31.65)	40 (32.79)	29 (30.21)	NA	NA	0.067[b]	0.795
Hypertension, n (%)	NA	51 (23.39)	25 (20.49)	26 (27.08)	NA	NA	0.433[b]	0.511
Complications								
Cardiac insufficiency, n (%)	NA	36 (16.51)	19 (15.57)	17 (17.71)	NA	NA	0.057[b]	0.812
Hepatic insufficiency, n (%)	NA	85 (38.99)	43 (35.24)	42 (43.75)	NA	NA	1.295[b]	0.255
Renal insufficiency, n (%)	NA	45 (20.64)	23 (18.85)	22 (22.92)	NA	NA	0.473[b]	0.492
Tuberculosis characteristics								
Miliary TB, n (%)	NA	32 (14.68)	18 (14.75)	14 (14.58)	NA	NA	0.079[b]	0.778
Cavitation, n (%)	NA	79 (36.24)	44 (36.07)	35 (36.46)	NA	NA	0.007[b]	0.934
Sputum smear positive, n (%)	NA	169 (77.53)	92 (75.41)	77 (80.21)	NA	NA	1.698[b]	0.193
GeneXpert MTB/RIF, n (%)	NA	212 (97.25)	119 (97.54)	93 (96.88)	NA	NA	0.014[b]	0.905
Biological parameters								
White cell count (×10^9/l, mean ± SD)	NA	12.70 ± 7.31	12.41 ± 6.99	13.07 ± 7.70	NA	NA	0.662[a]	0.509
Hemoglobin (g/L, mean ± SD)	NA	102.67 ± 24.00	104.39 ± 25.67	100.47 ± 21.50	NA	NA	1.266 [a]	0.207
Albumin (g/L, mean ± SD)	NA	27.04 ± 4.85	27.84 ± 5.09	26.02 ± 4.35	NA	NA	2.749 [a]	**0.006**
Creatinine (μmol/l, mean ± SD)	NA	108.34 ± 83.38	104.75 ± 81.54	112.89 ± 85.89	NA	NA	0.677 [a]	0.499
C-reactive protein (mg/l, mean ± SD)	NA	91.42 ± 60.77	83.02 ± 52.87	101.91 ± 68.12	NA	NA	2.298 [a]	**0.023**
Treatment								
Anti-TB Treatment in ICU, n (%)	NA	160 (73.39)	92 (75.41)	68 (70.83)	NA	NA	0.365[b]	0.545
Mechanical ventilation, n (%)	NA	161 (73.85)	81 (66.39)	80 (83.33)	NA	NA	7.131[b]	**0.008**
CRRT, n (%)	NA	14 (6.42)	6 (4.92)	8 (8.33)	NA	NA	0.552[b]	0.458
Score								
APACHE II score (mean ± SD)	NA	20.09 ± 3.42	19.35 ± 3.37	21.02 ± 3.27	NA	NA	3.690 [a]	**<0.001**
SOFA score (mean ± SD)	NA	8.91 ± 1.97	8.75 ± 1.78	9.13 ± 2.19	NA	NA	1.413 [a]	0.159
S100A9 concentrations								
S100A9 (μg/L, median and interquartiles)	8.62 (7.40, 13.66)	17.98 (14.01, 27.69)	15.51 (13.67, 19.94)	25.88 (16.77, 44.64)	6.888[c]	**<0.001**	5.695[c]	**<0.001**

Data were expressed in median and interquartiles (IQ), percentage or mean ± SD.
a: *t* test; b: Chi-square test; c: Mann-Whitney test.
*: control vs total cohort, #: survivors vs non-survivors.

difference between them (*p-value* > 0.05), which will be verified using larger sample size in our future work.

The median value of S100A9 was presented in Table 4 and Fig. 2. Compared to the age-matched control group, the whole cohort of TB patients had significantly higher levels of S100A9 (*p-value* < 0.001). Within the cohort of those patients, the non-survivors had dramatically higher levels of S100A9 than the survivors (*p-value* < 0.001) (Table 4, Fig. 2). The non-survivors also had a significantly higher APACHE II

Table 2
Multivariable logistic regression analyses for risk factors for death of severe TB patients.

Variable	B	S.E.	Wald	Significance.	OR	95% C.I.	
						Lower	Upper
Albumin	0.099	0.035	7.973	**0.005**	1.105	1.031	1.183
S100A9	−0.057	0.012	21.201	**<0.001**	0.944	0.921	0.968
APACHE II score	−0.186	0.049	14.655	**<0.001**	0.830	0.755	0.913

S.E: Standard Error.
OR: odds ratio.
CI: confidence interval.
All of the above analyzed results are from one logistic regression model.

Table 3
Comparison of the median values for S100A9 in severe TB group in admission to ICU, pneumonia patients with respiratory failure group and healthy control group.

	Healthy control (n = 40)	Pneumonia with respiratory failure (n = 18)	Total cohort (n = 218)	Statistical value[1]	*p-value*[1]	Statistical value[2]	*p-value*[2]	Statistical value[3]	*p-value*[3]
S100A9 (μg/L, median and interquartiles)	8.62 (7.40, 13.66)	15.23 (10.89, 23.01)	17.98 (14.01, 27.69)	6.888	**<0.001**	2.361	**0.018**	0.843	0.399

Data were expressed in median and interquartiles (IQ).
[1]: control vs total cohort, [2]: control vs pneumonia, [3]: pneumonia vs total cohort
The differences between compared groups were analyzed using one-way ANOVA + Tukey's multiple comparisons test.

Table 4
Comparison of the median values for S100A9, albumin and APACHE II score in survivors, non-survivors before day 14 and non-survivors at day 28.

	Healthy Control (n = 40)	Total cohort (survivor + death) (n = 218)	Survivors (n = 122)	Non-survivors (n = 96)	Early death n = 63	Late death n = 33	*p-value*[1]	*p-value*[2]	*p-value*[3]
APACHE II score	NA	20.09 ± 3.42	19.35 ± 3.37	21.02 ± 3.27	21.11 ± 3.29	20.85 ± 3.26	NA	**<0.001**	0.710
Albumin	NA	27.04 ± 4.85	27.84 ± 5.09	26.02 ± 4.35	27.05 ± 1.06	25.75 ± 5.44	NA	**0.006**	0.425
S100A9 (μg/L)	8.62 (7.40, 13.66)	17.98 (14.01, 27.69)	15.51 (13.67, 19.94)	25.88 (16.77, 44.64)	26.66 (20.20, 44.54)	20.46 (14.01, 49.22)	<0.001	**<0.001**	**0.041**

Data were expressed in median and interquartiles (IQ) or mean ± SD. (Mann-Whitney test or *t* test).
p-value[1]: control vs Total cohort, *p-value*[2]: survivors vs non-survivors, *p-value*[3]: Early death vs Late death.
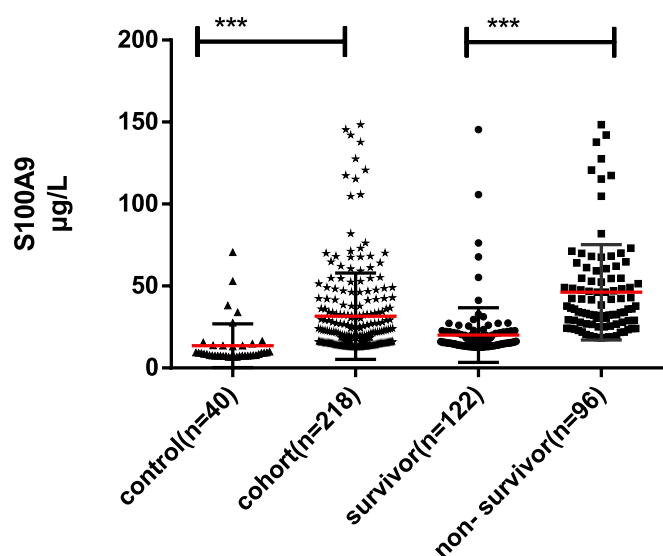

Fig. 2. Plasma S100A9 levels in controls, total cohort, survivors and non-survivors. Plasma S100A9 levels were analyzed using ELISA. Mann-Whitney test. Asterisk indicate *p-value* and *** means *p* < 0.001.

there could be undergoing different molecular mechanisms between early and late deaths (>day 14). Table 3 compared the median APACHE II scores, albumin and S100A9 levels in patients who died earlier with those died later. The early death group had higher S100A9 levels than the late death group (*p-value* < 0.05), although there was no difference between the two groups' APACHE II score and albumin levels (*p-value* > 0.05).

3.3. Prediction values of plasma S100A9 levels albumin and APACHE II score

To evaluate the potential of S100A9 in predicting the death in TB patients, the optimal cut-off value for the S100A9 values, albumin and APACHE II score was estimated using the ROC curve, as illustrated in Fig. 3. S100A9 had an area under the ROC curve (AUC) of 0.727 (95% CI 0.65 to 0.80) in predicting death. A cut-off value of 21.69 μg/L had a sensitivity of 65.5% and specificity of 80.3% in predicting death. Plasma S100A9 levels above this cut-off value were hereafter referred to as high and below this value are referred to as low. APACHE II score had an AUC of 0.658 (95% CI 0.59 to 0.73) in predicting death. A cut-off value of 17.5 had a sensitivity of 86.5% and specificity of 46.7% in predicting death. Albumin had an AUC of 0.407 (95% CI 0.33 to 0.48) in predicting death. A cut-off value of 21.45 g/L had a sensitivity of 93.8% and specificity of 9.9% in predicting death. We combined APACHE II score ≥17.5 together with a high S100A9 levels in predicting death. The combination had the highest AUC of 0.754 (95% CI 0.68 to 0.82) in predicting death with 57.3 % sensitivity and 93.4% specificity (Table 5, Fig. 3).
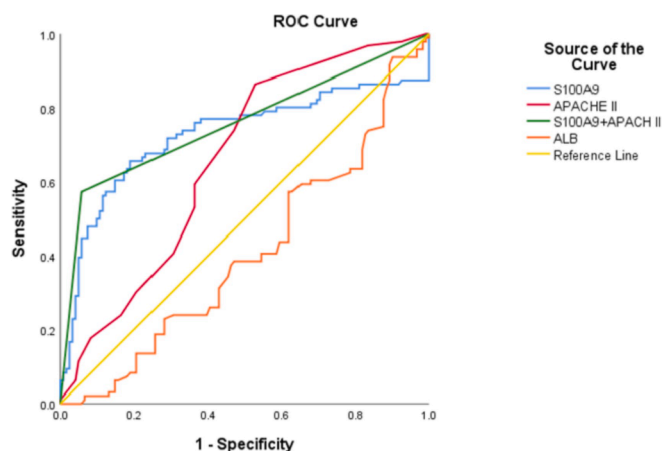
score than the survivors (*p-value* < 0.001), but showed a significantly lower albumin than the survivors (*p* = 0.006) (Table 4).

The majority of deaths occurred before day 14 (63/96) suggesting

Fig. 3. Receiver operating characteristic (ROC) curves in different groups. ROC curves on plasma S100A9 levels. APACHE II score and the combination of S100A9 levels ≥21.69 μg/L, albumin ≥21.45 g/L and APACHE II ≥17.5 measured on day of admission to the ICU in relation to case fatality.

3.4. Relationship between S100A9 expression and the severity of TB

Table 6 presents the plasma S100A9 levels stratified by various demographic features, underlying conditions, and characteristics of TB. The levels of S100A9 were statistically significantly higher in the following conditions: APACHE II score > 17.5, sputum smear-positive, early death (<14 day) and the number of cavitary lesions, among which sputum smear-positive and cavitation are well known to be directly relevant to the severity of TB. The following variables were not associated with S100A9 levels: gender, age over 60 years, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, cardiac insufficiency, hepatic insufficiency, renal insufficiency.

4. Discussion

This is a cohort study to evaluate the expression of plasma S100A9 levels in severe TB patients and to investigate the use of S100A9 as a prognostic marker in addition to the APACHE II score. To our knowledge, our study is the first report to measure plasma levels of S100A9 in the severe TB and assess its association with the related mortality. A notable finding of the present study was that severe TB patients had significantly higher plasma S100A9 levels compared to the healthy control, and the non-survivors of severe TB patients had further elevated plasma S100A9 levels than survivors of severe TB patients. Furthermore, with an optimal cut-off of 21.69 μg/L, S100A9 performed better than APACHE II score in the prediction of death in severe TB patients with a sensitivity of 65.5% and specificity of 80.3%. Moreover, S100A9 expression levels but not APACHE II score were associated with TB severity as indicated by clinical characteristics directly relevant to TB severity. By combining APACHE II score together with the S100A9 levels we got an AUC of 0.754 (95% CI 0.68 to 0.82) in predicting death. Taken

together, our findings suggested that S100A9 expression levels may be a useful indicator for predicting patients outcomes of severe TB.

S100A9 is a major calciumbinding protein in neutrophils and monocytes, and recognized as a damageassociated molecularpattern molecule [16]. Among the members of the calgranulin protein family, S100A9 was shown to be specifically linked to innate immune function. It is released from granulocytes, monocytes, and macrophages in the early differentiation stages [17]. S100A9 activates inflammatory responses by binding to the toll-like receptor 4 and the receptor for advanced glycation end products (RAGE). It also amplifies the stimulatory effect of bacterial products (such as endotoxin or LPS), resulting in the promotion of excessive inflammation and tissue damage. Thus, S100A9 protein has been demonstrated to play a key role in inflammation-related diseases [9].

Previous studies have found that TB patients had higher S100A9 levels than healthy controls [10,18], which was also confirmed by our results. It has been suggested that S100A9 may play a major pathological role in TB by mediating neutrophil accumulation and inflammation [18]. Kundu et al proposed S100A9 a potent biomarker for tuberculosis and diabetes copathogenesis [19]. Scott et al have reported that after Mtb infection, S100A9 expression was required for the accumulation of neutrophils in TB patients [20]. The increased S100A9 expression was also associated with aggravation of lung inflammation induced by injury [21]. Therefore, in the severe TB patients, infection of large areas of lung tissue by *Mtb*, can lead to systemic inflammation and intense cellular stress. It has been suggested that the increased mRNA levels of S100A9 were associated with disease progression and rapidly decreased upon effective anti-TB treatment in TB patients [20]. Ma and his colleagues have reported that higher expression of S100A8/S100A9 genes possibly forms the genomic basis of TB-IRIS in a subset of HIV patients while on highly active antiretroviral therapy [22]. In our study, the non-survivors had higher plasma S100A9 levels than survivors, and further in non-survivor plasma S100A9 levels were higher in early death (<14 days) than that in late death (>14 days). Our results concurred the potential application of S100A9 as an indicator for TB progression, and suggested that the increased S100A9 levels might be related to higher mortality in severe TB cases. But larger cohorts of severe TB patients will be needed to validate this hypothesis in future.

The APACHE II score was given by the sum of the acute physiology score comprising 12 variables, age points and chronic health points. This score was widely used in the validation and prediction of clinical results of severe patients in the ICU, and a previous study implied that this score could be used in TB patients [23]. Higher APACHE II score was associated with more serious conditions, poorer prognosis, and higher mortality rates for patients [6,24]. In our study, both the APACHE II score and levels of S100A9 in pulmonary TB patients with respiratory failure on ICU admission were higher in non-survivors than survivors. However, S100A9 performed better than APACHE II score in predicting death with higher AUC, sensitivity, and specificity based on ROC analysis. ROC is a probability curve and the AUC determined from ROC curve represents the degree or measure of separability. The closer the AUC is to 1, the better the model is to distinguish between survivor and

Table 5
Diagnostic values of plasma S100A9 levels, albumin, APACHE II score and both S100A9 levels ≥21.69 μg/L and APACHE II ≥17.5 measured on day of admission to the ICU in relation to case fatality.

	deceased/ survivors (n)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	AUC with 95% CI	p-value
S100A9	87/131	65.6	80.3	72.4	74.8	0.727 (0.652, 0.802)	<0.001
APACHE II score	70/148	86.5	46.7	56.1	81.4	0.658 (0.586, 0.730)	<0.001
S100A9 > 21.6 μg/L and APACHE II > 17.5	61/157	57.3	93.4	86.7	73.9	0.758 (0.689, 0.826)	<0.001
Albumin	18/200	93.8	9.9	18.8	61.0	0.407 (0.331, 0.482)	<0.001

Table 6

Comparison of the median values for S100A9 stratified by various demographic features, underlying conditions, and characteristics of TB. Data were expressed in median and interquartile (IQ). (Mann-Whitney test).

Characteristics	Plasma S100A9 (µg/L) on day admission to ICU			
	Factor present, median (quartiles)	Factor absent, median (quartiles)	Z	*p-value*
Male	16.87 (14.01, 29.88)	18.87 (14.27, 27.87)	0.856	0.392
Age over 60 years	18.67 (13.62, 27.32)	20.20 (15.04, 28.43)	1.599	0.111
APACHE II score >17.5	20.17 (14.44, 31.57)	16.67 (13.57, 24.33)	4.311	**<0.001**
Chronic obstructive pulmonary disease	18.22 (14.01, 27.69)	16.77 (14.44, 25.88)	0.303	0.762
Diabetes mellitus	18.91 (14.61, 27.47)	16.67 (14.129, 25.88)	0.524	0.600
Hypertension	19.74 (14.01, 39.09)	17.23 (14.17, 26.10)	1.021	0.307
Cardiac insufficiency	16.67 (14.69, 24.07)	19.17 (14.01, 27.69)	0.509	0.611
Hepatic insufficiency	17.23 (13.75, 28.47)	18.71 (14.34, 27.40)	1.305	0.192
Renal insufficiency	16.87 (14.01, 22.27)	18.71 (14.12, 32.09)	0.888	0.374
TB Characteristics				
Sputum smear positive	24.32 (18.17, 43.50)	19.17 (14.13, 29.28)	2.304	**0.023**
Miliary TB	21.22 (14.19, 39.47)	18.46 (15.93, 28.22)	0.829	0.407
Cavitation	20.46 (14.34, 28.47)	16.84 (13.93, 26.01)	2.011	**0.042**
Early death	26.66 (20.20, 44.54)	20.46 (14.01, 49.22)	3.311	**0.001**

non-survivor groups. The AUC of S100A9 in predicting death was 0.725, which was higher than the AUC of APACHE II score and similar with combination of APACHE II score and S100A9 levels in predicting death. There was no difference in APACHE II score between non-survivor of early death (<14 days) and late death (>14 days). However, the S100A9 levels, was significantly higher in the group of early death in comparison with late death. Moreover, we also observed significantly higher levels of S100A9 in the sputum smear-positive and cavitation TB patients, both of which have been used to assess the progression and severity of TB in clinical [25,26]. The release of S100A9 has been shown to result in the induction of metalloproteinases and enabled macrophages autophagy and apoptosis [27,28]. Those foamy macrophages in TB lesion could then form the cavities in lung [29].

Malnutrition leads to a decrease in immunity and *Mtb* is susceptible to infection; on the other hand, increased consumption of tuberculosis leads to malnutrition in the body. Albumin can indicate the nutritional status of patients [30]. A previous cross-sectional study reported, 300 adult TB patients have been surveyed in China, they found that most male (90.8%) and female (58.4%) TB patients had insufficient daily protein intake. Li et al, found that more TB patients had albumin levels <35 g/L [31]. Our results showed that severe TB patients albumin levels were even lower, ≤30 g/L. Remarkably, the albumin was much lower in non-survivors than survivors. In multivariable analyses, albumin was an independent risk factor for death of patients with severe TB, implying that the nutritional status was correlated with poor prognosis, thus demonstrating that albumin might be a good indicator of poor prognosis [32].

There are some limitations in this study. The most important one was the relatively small scale of the study cohort and all participants in the study were enrolled from a single specialized center, which may compromise the reliability and generalizability of our findings. Furthermore, our enrolled participants comprised severe TB patients that accompanied respiratory failure, therefore it needs to be cautious

when extrapolating these results to other TB patients.

5. Conclusions

The elevation of plasma S100A9 levels were associated with a higher risk of death, which had better predictive value than the APACHE II score in severe TB patients. This suggested that measurement of S100A9 levels at the early phase of severe TB patients could improve the evaluation of tissue damage and indicate a higher risk of death. These results may guide the shared decisions between severe TB patients and their clinicians. Evaluation of S100A9 levels may help the management of severe TB patients in the ICU and hospital outcome.

7. Contributions of author

Conceptualization, Qiuyue Liu, Qi Li and Jun Lin; methodology, Qiuyue Liu, and Baojian Luo; software, Qiuyue Liu, and Ru Li; validation, Qi Li, and Ru Li.; formal analysis, Qiuyue Liu and Baojian Luo; investigation, Qi Li; resources, Qiuyue Liu; data curation, Qiuyue Liu; writing—original draft preparation, Qiuyue Liu; writing—review and editing Ru Li, Jun Lin and Lingna Lyu.; visualization, Qiuyue Liu and Ru Li; supervision, Jun Lin; project administration, Baojian Luo; funding acquisition, Qiuyue Liu. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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