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RESEARCH ARTICLE

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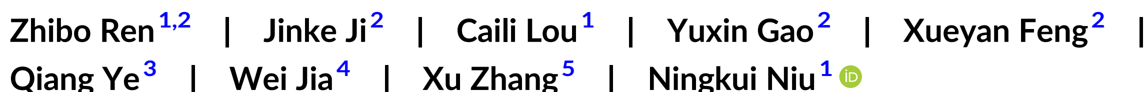
Analysis of the value of potential biomarker S100-A8 protein in the diagnosis and pathogenesis of spinal tuberculosis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Abstract

Objectives: The objective of this study is to evaluate the value of S100-A8 protein as a diagnostic marker for spinal tuberculosis and to explore its role in the potential pathogenesis of spinal tuberculosis (STB).

Methods: The peripheral blood of 100 spinal tuberculosis patients admitted to the General Hospital of Ningxia Medical University from September 2018 to June 2021 were collected as the observation group, and the peripheral blood of 30 healthy medical examiners were collected as the control group. Three samples from the observation group and three samples from the control group were selected for proteomics detection and screening of differential proteins. Kyoto Encyclopedia of Genes (KEGG) was used to enrich and analyze related signaling pathways to confirm the target protein. The serum expression levels of the target proteins were determined and compared between the two groups using enzyme-linked immunosorbent assay (ELISA). Statistical methods were used to evaluate the value of target protein as a diagnostic marker for STB. A macrophage model of *Mycobacterium tuberculosis* infection was constructed and S100-A8 small interfering RNA was used to investigate the molecular mechanism of the target protein.

Results: S100-A8 protein has the value of diagnosing spinal tuberculosis (AUC $= 0.931$, $p < 0.001$), and the expression level in the peripheral blood of the observation group (59.04 ± 19.37 ng/mL) was significantly higher than that of the control group (43.16 ± 10.07 ng/mL) ($p < 0.05$). S100-A8 protein expression showed a significant positive correlation with both CRP and ESR values ($p < 0.01$). Its AUCs for combined bacteriological detection, T-SPOT results, diagnostic imaging, antacid staining results, and pathological results were 0.705 ($p < 0.05$), 0.754 ($p < 0.01$), 0.716 ($p < 0.01$), 0.656 ($p < 0.05$), and 0.681 ($p < 0.01$), respectively. Lack of S100-A8 leads to a significant decrease in the expression levels of TLR4 and IL-17A in infected macrophages.

Conclusion: S100-A8 protein is differentially expressed in the peripheral blood of patients with spinal tuberculosis and healthy individuals and may be a novel candidate biomarker for the diagnosis of spinal tuberculosis. The feedback loop on the

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S100-A8-TLR4-IL-17A axis may play an important role in the inflammatory mechanism of spinal tuberculosis.

KEYWORDS
biomarker, IL-17A, S100-A8, spinal tuberculosis, Toll-like receptor 4

1 | INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB) that poses a serious risk to human health. The 2022 World Health Organization Global Tuberculosis Report shows that the COVID-19 pandemic has reversed years of global progress in the fight against TB and continues to have a devastating impact on TB prevention and control.[1] Osteoarticular TB is a common secondary extrapulmonary TB and spinal tuberculosis (STB) accounts for the highest proportion of systemic osteoarticular TB (more than 60%).[2] STB has an insidious onset, unclear pathogenesis, atypical clinical manifestations, and lack of specificity in imaging and laboratory tests, making early diagnosis and treatment very difficult.[3,4] In addition, the pathogenesis of STB as an inflammatory disease remains unclear, and immune interactions involving multiple cytokines make early intervention challenging.[5] Therefore, it is important to explore potential biomarkers with high specificity and sensitivity for the clinical diagnosis and treatment of STB.

The S100-A8 protein, a major member of the leukocyte protein S100 family, is a calcium-binding protein.[6] Most S100 proteins exist as homodimers; however, some proteins are composed of heterodimers and are encoded by genes clustered in the 1q21 region of human chromosome, including S100-A8/S100-A9.[7] It has been shown that S100-A8 protein has an important role in tumorigenesis, development, and metastasis, and its involvement as a pro-inflammatory factor in early arthritis and autoimmune diseases has also been demonstrated.[8,9] The interleukin 17 (IL-17) family, a subset of cytokines consisting of IL-17A-F, plays crucial roles in the development of inflammatory diseases and IL-17A is the signature cytokine produced by T helper 17 (Th17) cells.[10] Previous studies have shown that S100-A8 is an endogenous activator of Toll-like receptor 4 (TLR4), leading to increased expression of IL-17 through TLR4 signaling, thereby affecting systemic autologous immunity.[11,12] Revealing the TLR4-IL-17A signaling axis is crucial to help understand the underlying inflammatory mechanisms of STB.

In this study, we screened and validated the differential expression of S100-A8 in the peripheral blood of STB patients and healthy individuals by proteomic techniques, evaluated its potential as a diagnostic biomarker for STB by analyzing its correlation with the clinical characteristics of STB patients, and explored its mediated TLR4/IL-17A signaling pathway at the cellular level. It is hoped to provide reference and ideas for the diagnosis and treatment of STB.

2 | MATERIALS AND METHODS

2.1 | Study object

The peripheral blood of 100 patients diagnosed with spinal tuberculosis from Ningxia Medical University General Hospital from September 2018 to June 2021 was collected as the observation group, and the peripheral blood of 30 healthy persons undergoing physical examination was collected as the control group. The observation group included 50 males and 50 females, aged 18–77 years (49.5 ± 16.3 years); the control group included 12 males and 18 females, aged 40–72 years (53.4 ± 9.7 years). There was no statistically significant difference in age and gender between the two groups ($p > 0.05$). The experimental protocol of this study was reviewed and approved by the Ethics Committee of Ningxia Medical University General Hospital (Ethics number: KYLL-2021-932).

Inclusion criteria for STB patients (observation group): (1) STB confirmed by clinical manifestations, laboratory, imaging, bacteriological, and pathological examinations; (2) not taking hormone or drug treatment recently; and (3) clinical information is complete. Exclusion criteria: (1) patients with active pulmonary tuberculosis, diabetes, malignant tumors and infectious diseases; (2) patients whose recent medication may cause changes in blood routine values; (3) women in the menstrual cycle; (4) suppurative spine Spondylitis, Brucella spondylitis, and other spinal lesions caused by other pathogenic bacterial infections; (5) incomplete information or refusal to participate in the investigation and research; and (6) recurrent cases.

Inclusion criteria for healthy subjects (control group): (1) in good health and have not taken immune activating or suppressive drugs in the past month; (2) no history of viral or bacterial infection in the past 2 weeks; and (3) no abnormal symptoms in physical examination, routine blood tests such as red blood cells, monocytes, and granulocytes were within the normal range. Exclusion criteria: (1) people with chronic diseases such as hypertension, diabetes, malignant tumors, and tuberculosis; (2) people who have recently taken medication that may cause changes in blood routine values; (3) women who are in the menstrual cycle; and (4) incomplete information or refusal to participate in the investigation and research.

2.2 | Clinical data collection of patients

General clinical data and information on pathological characteristics of STB patients were collected, including age, gender, disease duration,

laboratory tests (blood routine, CRP, ESR, etc.), bacterial culture, T-SPOT, pathology, and imaging data. The specific STB imaging diagnostic criteria: paradiscal involvement, >50% vertebral destruction, subligamentous spread of infection to three or more contiguous vertebral levels, large abscess with smooth thin wall, and calcification with in paravertebral soft tissue.[13]

2.3 | Proteomics detection and analysis

Three samples from the observation group and three samples from the control group were selected respectively, and Tandem Mass Tag (TMT) quantitative proteomics was performed using a high-resolution mass spectrometer Q-Exactive plus. The mass spectrometry scan was followed by analysis of the differential protein data using the mass spectrometry analysis software Mascot 2.6 and Proteome Discoverer 2.2, in which a total of 16 differential proteins were screened. The preliminary screening conditions were fold change ≥1.2 times and $p < 0.05$, Cluster 3.1 and GraphPad Prism 10.1.0 were used to plot the differential protein cluster analysis and volcano distribution.

2.4 | ELISA validation of differential protein S100-A8

We selected 50 cases in the observation group and 30 cases in the control group for peripheral blood to verify the differences of S100-A8. The 3–5 mL of whole blood was collected from patients with spinal tuberculosis and healthy individuals and centrifuged at 3000 rpm/min for 10 minutes within 1 h. The upper serum was stored at −80°C and then subjected to enzyme-linked immunosorbent assay (ELISA). The ELISA kit (S100-A8 antibody kit, Beijing Xinbosheng Technology & Biology Co., Ltd.) was used according to the instructions.

2.5 | Cells culture and transfection

Human monocytes THP-φ were cultivated within 1640 medium that contained 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) under 37°C and 5% CO2 conditions. At about 80% confluence, THP-φ cells were moved to a 6-well plate, Pharmaceutical Manufacturers Association (PMA) was added to induce macrophages, and Bacillus Calmette Guerin (BCG) was used to simulate the infection state of MTB. S100-A8 siRNA (Shanghai Jima Pharmaceutical Biotechnology Co, Ltd.) was transfected, and the groups were as follows: control group, BCG group, control with S100-A8 siRNA group, and BCG with S100-A8 siRNA group.

2.6 | Western blotting (WB) assay

Macrophages were lysed and proteins were extracted, and the total protein concentration was detected using the BCA protein assay kit (Beyotime, Shanghai, China). The 30 μg total proteins were subject to 10% or 15% SDS-PAGE separation, followed by transfer onto PVDF membranes and incubated with 5% skim milk for 2 h. Membranes were incubated with primary antibodies overnight at 4°C. After washing by PBS thrice, membranes were incubated with HRP-labeled goat anti-rabbit secondary antibody under ambient temperature for 2 h. Enhanced chemiluminescence was utilized to detect protein blots. Blot gray values were assessed using Image J software.

2.7 | Fluorescence in situ hybridization (FISH)

This work cultured THP-φ cells with treatment at 37°C and 5% CO2 in 6-well plates. A coverslip was pre-placed on the bottom of each well. All cells were fixed with 4% paraformaldehyde for 15 minutes. DAPI was applied for nuclear staining. After processing, photographs were taken using the fluorescence microscope (Leica, Germany).

2.8 | Statistical methods

Each experimental procedure was carried out in triplicate. The results were represented by mean ± SD and analyzed by SPSS 26.0 and GraphPad Prism 10.1.0. T-test was used to analyze the significance of the differences between their general and statistical data; the correlation between serum S100-A8 protein expression levels and other count data was analyzed by curve regression and Spearman test; the ROC curve was used to evaluate the sensitivity and specificity of S100-A8 protein in STB diagnosis, and the area under the curve (AUC) ≥ 0.6 indicated diagnostic value. The differences were considered statistically significant at $p < 0.05$ for each statistical method.

3 | RESULTS

3.1 | Screening and validation of S100-A8

Under the preliminary screening conditions, a total of 16 differentially expressed proteins were identified, including 11 up-regulated proteins and 5 down-regulated proteins in STB peripheral blood. Cluster analysis and volcano plot showed differentially expressed proteins between the two groups (Figure 1A,D). Combining the relevant signaling pathways screened (Figure 1E) and the specificity and stability of protein expression, it was finally determined that S100-A8 met the screening requirements. The peripheral blood of 50 STB patients and 30 healthy subjects was verified by ELISA, and it was found that the expression of S100-A8 protein in STB was significantly higher than that in healthy subjects ($p < 0.001$), as shown in Figure 1B. ROC curve analysis showed that the AUC of S100-A8 in the peripheral blood of STB patients was 0.931 (AUC >0.6 has diagnostic value, $p = 0.0001$), as shown in Figure 1C. Relevant results suggest that S100-A8 protein has diagnostic value in STB peripheral blood and has the potential to become a marker for STB detection.

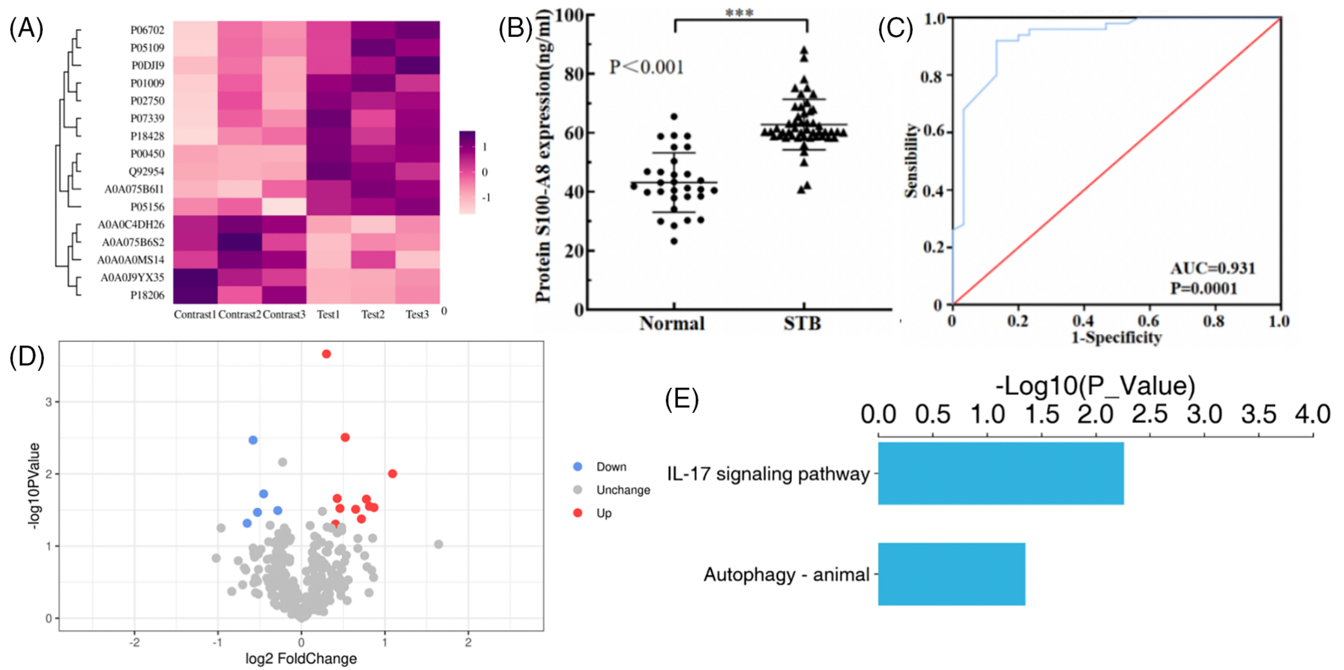


FIGURE 1 Screening of S100-A8 and validation of its expression in peripheral blood of STB patients. (A) Cluster analysis diagram of differential proteins. (B) Detection of S100-A8 protein expression in peripheral blood of two groups by ELISA. (C) ROC curve result of S100-A8 in STB examination. (D) Volcano distribution diagram of differential proteins. (E) KEGG signaling pathway enrichment map of differential proteins.

TABLE 1 Statistical results of laboratory tests in both groups.

Item ($\bar{x}$ ± s)	Control group (n = 30)	Observation group (n = 100)
S100A8 (ng/ml)	43.16 ± 10.07	59.04 ± 19.37
CRP (mg/L)	1.55 ± 0.68	24.92 ± 23.69
ESR (mm/h)	9.27 ± 5.11	40.48 ± 28.71
WBC (*10^9/L)	6.66 ± 1.30	6.61 ± 1.84
NEUT%	68.38 ± 4.99	64.72 ± 10.93
LYM%	25.88 ± 7.93	24.13 ± 9.53

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LYM, lymphocyte; NEUT, neutrophilic granulocyte; WBC, white blood cell.

3.2 | Laboratory tests

The S100-A8 protein expression in the peripheral blood of control group was low and relatively stable (43.16 ± 10.07), compared with a significantly higher level in the observation group (59.04 ± 19.37), as shown in Table 1. There was no statistical difference in white blood cells, neutrophils, and lymphocytes between the two groups ($p > 0.05$). However, the ESR and CRP measurements were significantly higher in observation group ($p < 0.05$).

3.3 | Analysis of the differences of S100-A8 in each clinical subgroup of STB patients

The independent sample T-test was used to analyze the differences in peripheral blood S100-A8 protein expression levels among different clinical subgroups of STB patients. The results showed that the peripheral blood S100-A8 protein expression levels were not statistically significant ($p > 0.05$) in age, gender, disease duration, leukocyte values, lymphocyte values, and neutrophil values in the clinical subgroups of STB, and differed significantly ($p < 0.05$) in diagnostic imaging, and in hematocrit, C-reactive protein, T-SPOT, pathology results, antacid staining results, and The differences were highly significant ($p < 0.01$) in bacteriological tests, as shown in Table 2. The results suggest that S100-A8 has the potential to become an early diagnostic marker for STB.

3.4 | Correlation of S100-A8 protein and quantitative clinical data of STB patients

Curvilinear regression analysis showed that the relative expression of S100-A8 in peripheral blood showed a significant positive correlation with CRP and ESR levels ($p < 0.01$), while there was no correlation with changes in the relative levels of leukocytes, disease process, neutrophils, and lymphocytes ($p > 0.05$), as shown in Figure 2A–F. This conclusion was further verified by bivariate Spearman correlation analysis, which the expression of S100-A8 protein showed a significant positive correlation with both CRP and ESR (correlation coefficients are 0.621 and 0.681, respectively, $p = 0.0001$); while there was no correlation with the changes in the relative values of leukocyte leukocytes, disease duration, neutrophils, and lymphocytes ($p > 0.05$), as shown in Figure 2G. The results suggest that the co-testing of S100-A8 protein with CRP and ESR is beneficial for improving the stability of STB early diagnosis.

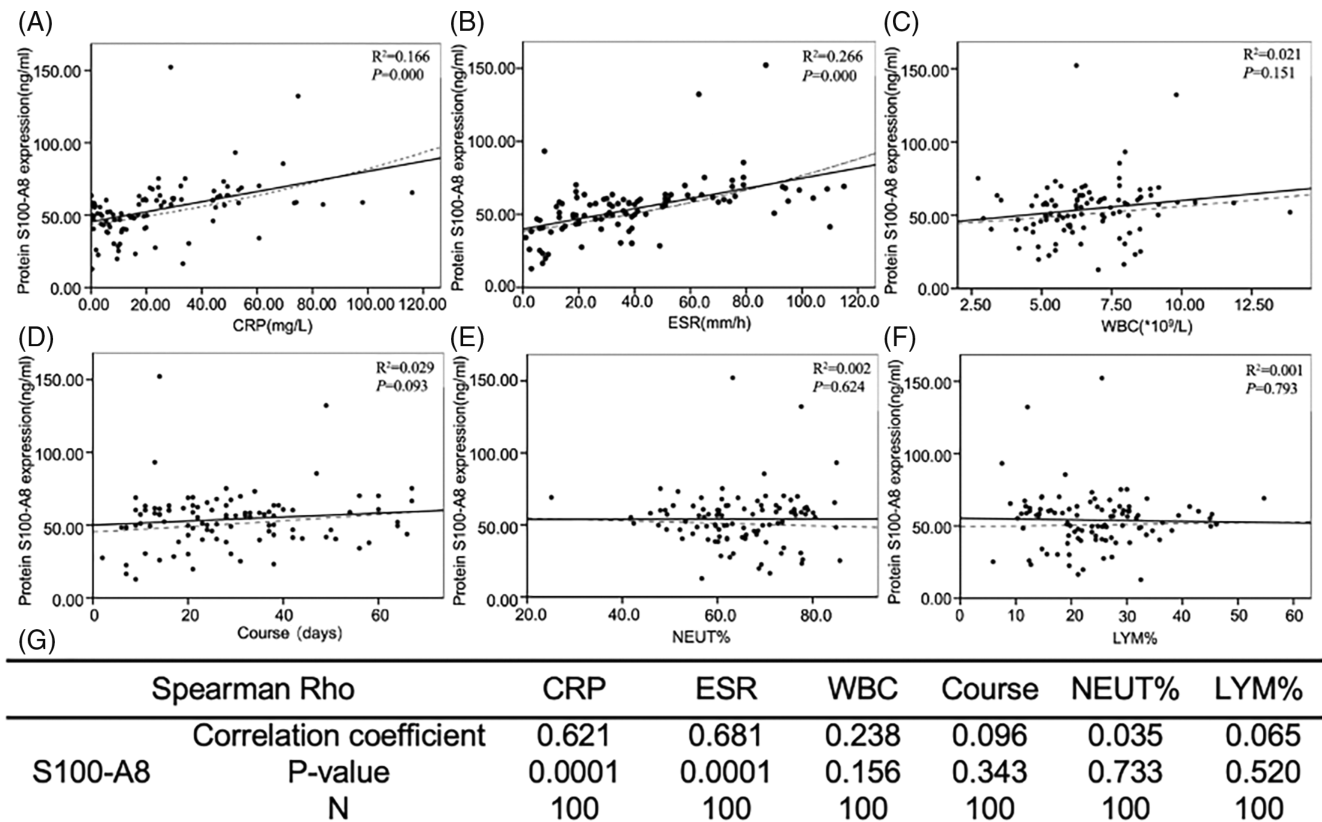
TABLE 2 Difference analysis of S100-A8 protein in various clinical data of STB patients.

Item	*n*	S100-A8 ($\bar{x} \pm s$)	*t* value	*p* value
Age (years)			0.150	0.881
>50	49	53.74 ± 21.81		
≤50	51	54.33 ± 16.92		
Gender			−0.651	0.517
Men	50	52.78 ± 19.15		
Women	50	55.30 ± 19.71		
Course (days)			−0.107	0.915
>14	77	54.15 ± 15.88		
≤14	23	53.66 ± 28.58		
WBC values			−1.846	0.068
Normal	91	52.93 ± 18.29		
Abnormal	9	65.27 ± 26.94		
ESR values			−4.172	0.000
Normal	22	39.94 ± 16.33		
Abnormal	78	58.02 ± 18.36		
CRP values			−7.266	0.000
Normal	15	26.89 ± 7.25		
Abnormal	85	58.83 ± 16.69		
LYM%			−1.445	0.152
Normal	67	52.09 ± 18.29		
Abnormal	33	58.01 ± 21.13		
NEUT%			−1.988	0.051
Normal	78	52.02 ± 18.50		
Abnormal	22	61.18 ± 21.11		
T-SPOT positive			−3.007	0.003
Yes	80	56.84 ± 18.41		
No	20	42.83 ± 19.51		
Imaging diagnosis positive			−2.636	0.010
Yes	76	56.83 ± 19.68		
No	24	45.22 ± 15.65		
Pathology positive			−3.027	0.003
Yes	73	57.46 ± 19.40		
No	27	44.77 ± 16.28		
AFS positive			−3.140	0.002
Yes	19	47.67 ± 15.22		
No	81	36.49 ± 5.79		
Bacteriology positive			−7.920	0.000
Yes	16	81.63 ± 24.95		
No	84	48.78 ± 12.67		

Note: Normal: Test results within the normal range. Abnormal: Test results above or below the normal range.
Abbreviation: AFS, Acid Fast Stain.

3.5 | The diagnostic value of S100-A8 protein combined with the qualitative diagnostic index of STB

The diagnostic value of peripheral blood S100-A8 protein in bacteriological tests, T-SPOT results, diagnostic imaging, antacid staining results, and pathological results of STB patients was analyzed by ROC curve. The results showed that the AUC of S100-A8 protein in bacteriological tests, T-SPOT results, diagnostic imaging, antacid staining results, and pathological results of STB patients in this group were 0.705 ($p = 0.01$*), 0.754 ($p = 0.000$**), 0.716 ($p = 0.001$**), 0.656



Spearman Rho		CRP	ESR	WBC	Course	NEUT%	LYM%
S100-A8	Correlation coefficient	0.621	0.681	0.238	0.096	0.035	0.065
	P-value	0.0001	0.0001	0.156	0.343	0.733	0.520
	N	100	100	100	100	100	100

FIGURE 2 Correlation analysis between S100-A8 protein and quantitative data of STB patients. (A–F) Curvilinear regression analysis of the correlation between S100-A8 expression levels and the relative values of CRP, ESR, leukocytes, disease duration, neutrophils and lymphocytes in STB patients. (G) Bivariate Spearman correlation analysis.

($p = 0.032$*), and 0.681 ($p = 0.006$**), respectively (Figure 3). The results suggest that peripheral blood S100-A8 protein has the potential to be a molecular marker for bacteriological detection, positive T-SPOT, diagnostic imaging, positive antacid staining, and adjunctive peripheral blood molecular marker for pathological findings.

3.6 | S100-A8 protein enhances macrophage inflammatory response after BCG infection through the TLR4-IL-17A signaling pathway

According to the concentration gradient and time gradient of BCG simulating the infection state of MTB, the optimal concentration of BCG acting on THP cells is 8MOI, and the optimal time is 24 h (Figure 4A,B). Under these conditions, the expression levels of related cytokines in four groups were explored to elucidate the mechanism of S100-A8 in the inflammatory response of STB. As shown in Figure 4C, the expression of S100-A8, TLR4, and IL-17A increased after BCG infected macrophages, while after adding S100-A8 siRNA, the expression of the three factors significantly decreased ($p < 0.05$). Next, the downstream and peripheral related cytokines were verified, and it was found that after BCG-infected macrophages, the expression levels of NFκB1, NFκB2, IL-6, and TNF-α were significantly increased; after interfering with S100-A8, NFκB1, NFκB2, and IL-6 expression levels were significantly reduced ($p < 0.05$), but there was no significant difference in TNF-α expression levels ($p > 0.05$). In addition, there was no significant change in the expression of TLR2 and TLR9 before and after BCG infection and interference with S100-A8 ($p > 0.05$) (Figure 4D).

For the core molecules S100-A8, TLR4, and IL-17A in the signaling pathway axis, we used FISH to further verify the expressions and localization. The results showed that after BCG infection of macrophages, the mean fluorescence intensity (MFI) of the three factors was significantly enhanced, while after the addition of S100-A8 siRNA, the MFI was significantly weakened ($p < 0.05$) (Figure 5A–D). This is consistent with the results of western blotting assay, further indicating that S100-A8 protein activates TLR4 to mediate the downstream IL-17 signaling pathway and increase the inflammatory response after BCG infects macrophages.

4 | DISCUSSION

The pathogenesis of STB is still unclear, and the insidious onset and atypical clinical manifestations undoubtedly increase the difficulty of early diagnosis and treatment.[14,15] Despite significant advances in treatment options, tools, policy interventions, and public awareness over the past decades, TB remains one of the major challenges in

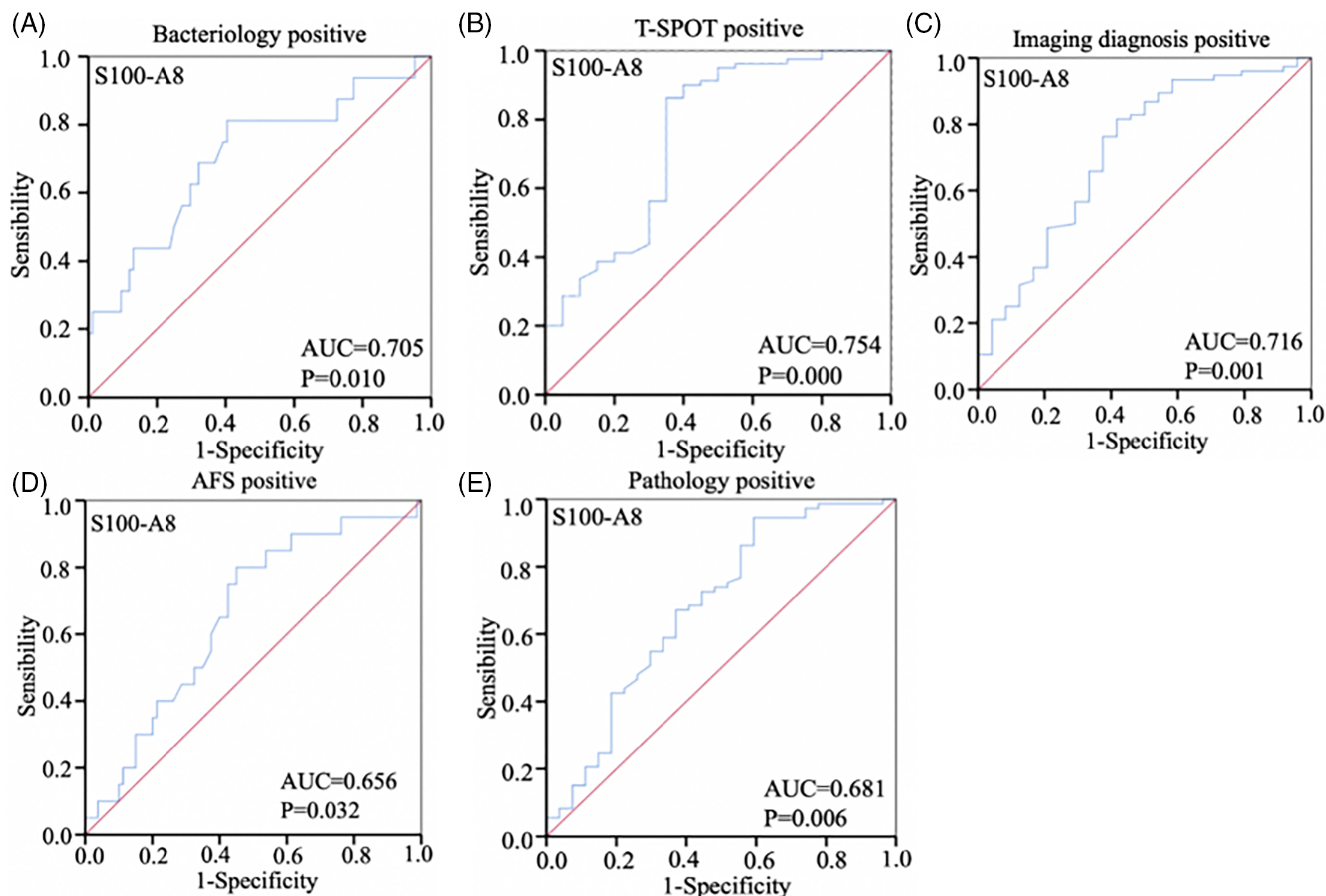


FIGURE 3 ROC curve analysis of the significant difference in the diagnostic value of S100-A8 protein in each clinical subgroup. (A–E) ROC curve analysis of S100-A8 combined with pathology, bacterial culture, T-SPOT, imaging and AFS for diagnosis of STB.

global health care.[16] Bone and joint TB is the most common secondary extra-pulmonary TB, with STB ranking first.[2] STB is mainly caused by MTB in the lung or gastrointestinal tract through blood transmission and MTB resides in the vertebral body by forming tuberculous granulomas.[17] In recent years, numerous studies have shown that the development of STB is associated with a variety of cells and cytokines and is a process involving multiple signaling pathways.[18] Therefore, it is important to further define the pathogenesis of STB and find valuable diagnostic markers or therapeutic target to improve its early diagnosis and treatment.

S100 is a family of 10–20 kDa acidic proteins that are unique to spinal animals.[19] Previous studies have shown that it is closely associated with the development of inflammation and tumors.[20] Kang et al.[8] found significantly higher levels of S100-A8/A9 in the serum and synovial fluid of patients with rheumatoid arthritis than in patients with osteoarthritis or other inflammatory arthritis, not only suggesting that S100-A8/A9 could be a biomarker of diagnostic value but also providing a reference for the application of other spinal infectious diseases, such as STB. During chronic TB development, Scott et al.[21] found that S100-A8/A9 mediated neutrophil accumulation during the development of chronic tuberculosis and confirmed its role in differentiating active tuberculosis from the latent phase of asymptomatic tuberculosis infection. Besides, Wang et al.[22] found that during inflammation, S100-A8/A9 is actively released and plays an important role in regulating the inflammatory response by stimulating leukocyte recruitment and inducing cytokine secretion. These properties make S100-A8 protein not only a candidate biomarker for diagnosis and follow-up but also likewise has the potential to become a predictor for the treatment of inflammation-related diseases.

In this study, proteomics technology was used to screen the differentially expressed proteins in the peripheral blood of STB patients and normal people, and a total of 16 differentially expressed proteins were screened under the initial screening conditions. Based on the differential ploidy ≥1.2 times and the stability difference of expression during the validation process, combined with the screened STB-related signaling pathways, the S100-A8 protein was finally determined to meet the screening requirements. ROC curve analysis indicated that S100-A8 protein is of great value for STB diagnosis. Although S100-A8 protein has been reported as a diagnostic marker in inflammation and tumors, the present study revealed for the first time a stability difference in S100-A8 protein expression in STB.

After determining the diagnostic value of S100-A8 protein in the peripheral blood of STB, we further explored its correlation with the clinical characteristics of STB patients. The results excluded the

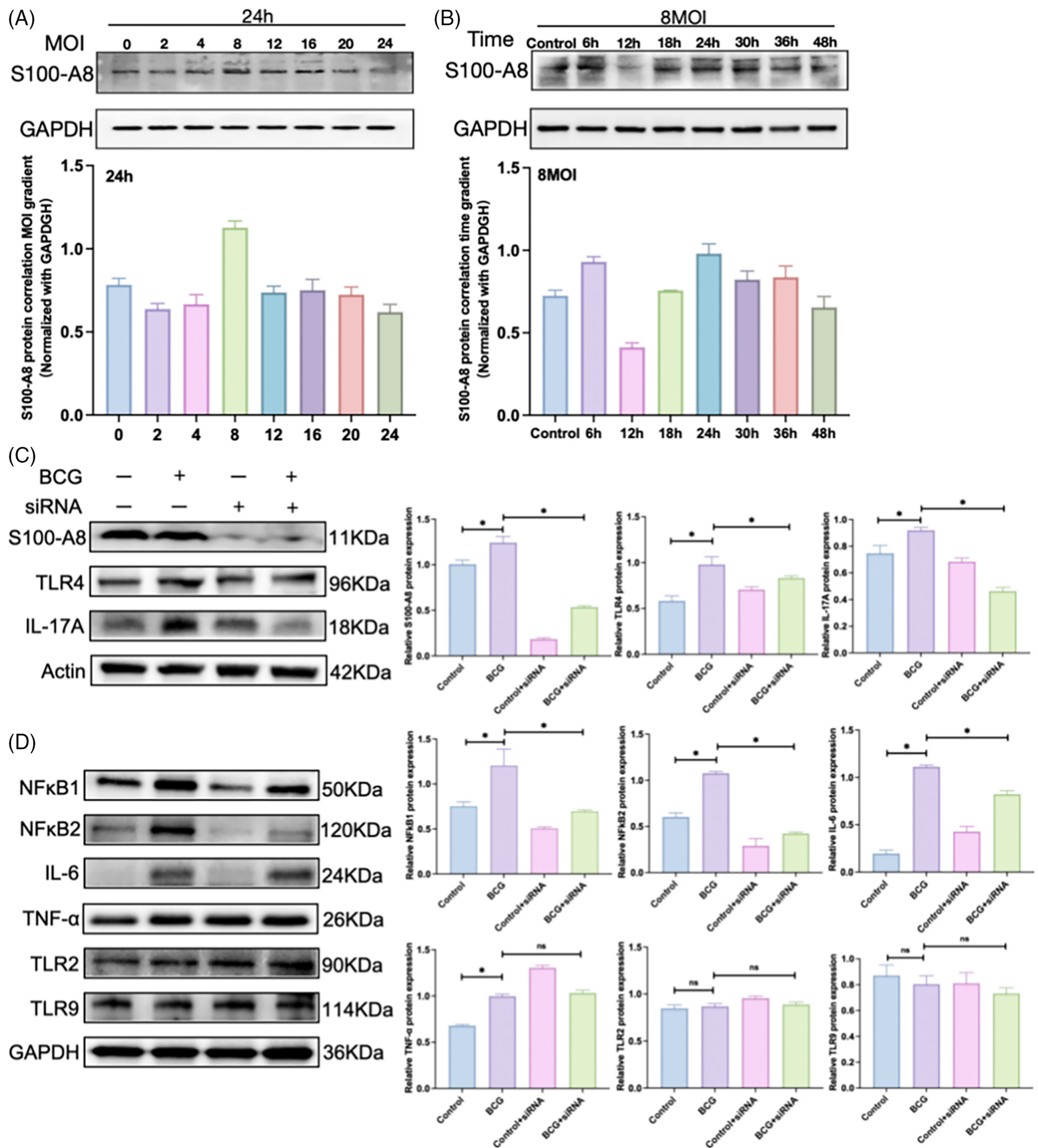


FIGURE 4 Different protein expression levels in macrophages. (A) Concentration gradient of BCG infected macrophages. (B) Time gradient of BCG infected macrophages. (C) Protein expression of core cytokines in four groups. (D) Protein expression of related cytokines in four groups.

differences in protein expression levels of S100-A8 in peripheral blood and age, sex, disease duration, leukocyte value, lymphocyte value, and neutrophil value, making it more reliable when combined with conventional STB diagnostic indicators. The S100-A8protein is not released simultaneously with the neutrophil granule content but is released upon the formation of neutrophil extracellular traps,[7] which may have affected the expression difference to some extent. To further clarify the diagnostic value of the S100-A8 protein, we specifically analyzed its correlation with the clinical data of STB patients. The results showed that the expression of S100-A8 protein in peripheral blood was significantly and positively correlated with the levels of CRP and ESR in our data ($p < 0.01$). CRP and ESR are often used in

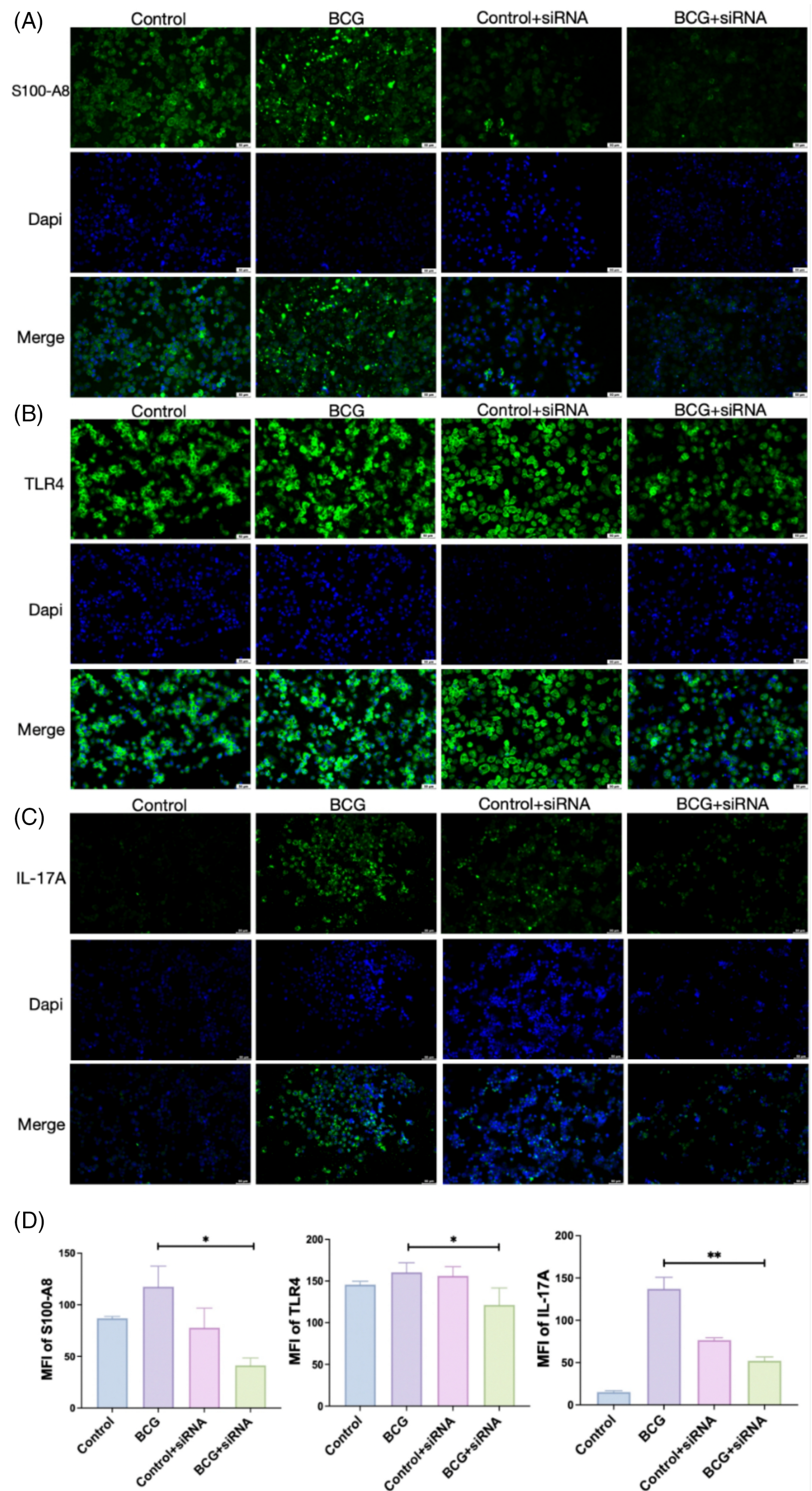


FIGURE 5 Expression and localization of core cytokines in macrophages by FISH. (A) Expression and localization of S100-A8 in macrophages. (B) Expression and localization of TLR4 in macrophages. (C) Expression and localization of IL-17A in macrophages. (D) MFI statistical results of three cytokines.

the assessment of STB as common inflammatory indicators for diagnosing infection and assessing the severity of infection.[23] Therefore, we hypothesized that the synergistic detection of S100-A8 protein with CRP and ESR would improve the early diagnosis and treatment of STB. Bacteriological tests are the gold standard for the diagnosis of STB but still have some limits, such as time consuming and sensitivity limitations.[15] ROC curve analysis further confirms the value of S100-A8 protein in the diagnosis of STB. This is consistent with the conclusion of Grandjean et al.[24] in TB. T-SPOT combined with antacid staining for STB has high sensitivity and specificity for rapid diagnosis of STB, which can significantly improve the detection efficacy.[25] The AUCs of positive T-SPOT and antacid staining results in this group also indicated that when the S100-A8 protein is combined with T-SPOT and antacid staining, it is expected to improve the early diagnostic detection rate of STB. MRI and CT examinations have shown good results for disc involvement, adnexal invasion, and spinal stenosis in STB patients, but STB cannot be confirmed by any single imaging method.[13] The AUC of S100-A8 protein in this group indicated that its combined diagnosis of STB with imaging findings has some clinical significance. The results of the above ROC curve analysis suggest that S100-A8 protein may be a biomarker for the joint diagnosis of STB with bacteriological tests, positive T-SPOT, diagnostic imaging, positive antacid staining, and pathological findings.

Toll-like receptor 4 is a microbe-associated molecular pattern receptor well known for its sensitivity to bacterial lipopolysaccharides (LPS). In macrophages and dendritic cells, LPS-mediated activation of TLR4 triggers the biosynthesis of diverse mediators of inflammation, such as TNF-α and IL6, and activates the production of co-stimulatory molecules required for the adaptive immune response.[26] Previous research has shown that TLR4 serves as an upstream signal of the Th17-IL-17 axis,[27] which is consistent with our results. As an attenuated vaccine, BCG activates the immune system and can be used to construct models of MTB infection.[28] In our BCG-infected macrophage model, increased expression of S100-A8 protein activated the TLR4 receptor, which in turn caused an increase in the expression of downstream IL-17A. Interestingly, the increase in IL-17 stimulates neutrophils to release S100A8,[29] thereby forming a positive cascade feedback. In addition, after adding S100-A8 siRNA, we found that the expression levels of TLR4 and IL-17A decreased, which further illustrates the role of the TLR4-IL-17A signaling pathway axis in the pathogenesis of S100-A8-mediated spinal tuberculosis. During this process, we also found that the expression levels of TLR2 and TLR9 did not change significantly before and after, indicating the role of the TLR4 signaling pathway as the main mediator. The NFκB non-canonical pathway and IL-6, which are downstream of TLR4, also play an important role in this inflammatory mechanism. However, the expression of TNF-α did not decrease significantly after interfering with S100-A8, suggesting that it may be involved in other signaling pathways in the inflammatory response of STB.

In conclusion, S100-A8 protein is differentially expressed in peripheral blood of STB and has the potential to become a biomarker for clinical diagnosis of STB. The feedback loop on the S100-A8-TLR4-IL-17A axis may play an important role in the inflammatory mechanism of spinal tuberculosis, further studies are needed to clarify the function of S100-A8 protein in the pathogenesis of STB.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the interpretation of data, drafting, and/or revising the manuscript and gave final approval for submission.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All the data and material involved in the current study are available from the corresponding author on reasonable request.

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