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Serum High-Mobility Group Box 1 and Disease Severity in Pulmonary Tuberculosis: A Cross-Sectional Study

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ABSTRACT: High-mobility group box 1 is released during inflammation and tissue injury, but its relationship with pulmonary tuberculosis severity remains unclear. This study evaluated its associations with Respiratory manifestations, sputum smear grade, and chest radiographic extent. This cross-sectional study consecutively recruited 92 adults with bacteriologically confirmed pulmonary tuberculosis at a tertiary referral hospital in Makassar, Indonesia, from May to June 2026. Respiratory manifestations were classified as cough alone, cough with dyspnea, cough with hemoptysis, or cough with both hemoptysis and dyspnea. Pretreatment sputum acid-fast bacilli smears were graded as 1+, 2+, or 3+, and chest radiographic disease was classified as minimal or extensive. Serum high-mobility group box 1 concentrations were measured using a quantitative sandwich enzyme-linked immunosorbent assay. Data were analyzed using the Kruskal–Wallis test with Bonferroni-adjusted pairwise comparisons, the independent-samples t test, Spearman's rank correlation, and multiple linear regression. Serum high-mobility group box 1 differed across Respiratory manifestation groups and sputum smear grades (both $P<0.001$). Sputum smear grade correlated strongly with serum concentrations ($\rho=0.841$, $P<0.001$). Concentrations were higher in extensive than minimal radiographic disease (41,708.55±7,120.62 versus 29,597.53±6,225.99 pg/mL; $P<0.001$). After adjustment, Respiratory manifestations and sputum smear grades 2+ and 3+ remained associated with higher concentrations, whereas radiographic extent did not. Serum high-mobility group box 1 was independently associated with Respiratory manifestations and sputum smear grade. Its association with radiographic extent was attenuated after adjustment. Further studies should assess its potential as a disease-activity biomarker.

1. INTRODUCTION

Tuberculosis remains a major cause of infectious disease morbidity and mortality worldwide [1], with pulmonary tuberculosis serving as the principal source of person-to-person transmission [2]. Despite the availability of effective treatment, many pulmonary tuberculosis survivors experience persistent respiratory symptoms, impaired pulmonary function, or structural lung abnormalities [3]. Although microbiological testing is essential for bacteriological confirmation, pulmonary tuberculosis has a heterogeneous clinical presentation [4]. Patients may differ substantially in respiratory manifestations, sputum bacillary burden, and the extent of radiographic lung involvement. Some patients may even have bacteriologically confirmed disease with few recognized symptoms and limited radiographic abnormalities [5,6]. This heterogeneity reflects complex interactions among

Mycobacterium tuberculosis, host immune responses, bacterial persistence, and tissue-damaging inflammation [2,7]. Biomarkers reflecting these processes may therefore improve understanding of the biological variation observed in pulmonary tuberculosis.

High-mobility group box 1 (HMGB1) is a highly conserved nonhistone nuclear protein involved in chromatin organization, DNA repair, transcription, and other DNA-dependent processes. During infection, inflammation, or cellular stress, HMGB1 may be actively secreted by stimulated immune cells or passively released from damaged and necrotic cells [8,9]. Extracellular HMGB1 functions as a damage-associated molecular pattern by interacting with pattern-recognition receptors, including Toll-like receptors and the receptor for advanced glycation end products. These interactions activate inflammatory pathways, including nuclear factor kappa B signaling, and promote cytokine production and leukocyte recruitment [9]. HMGB1 may therefore provide a biological link between cellular injury and the amplification of local and systemic inflammation.

Pulmonary tuberculosis represents a plausible setting for increased HMGB1 release. Activation and infection of alveolar macrophages, granuloma formation, dysregulated cell death, caseous necrosis, and destruction of the pulmonary parenchyma are characteristic features of progressive disease [2,7]. These pathological processes may stimulate both the active secretion of HMGB1 by immune cells and its passive release from damaged pulmonary tissue [8,9]. However, human evidence remains limited. A small case–control study involving patients with intrathoracic lymphadenopathy found higher serum HMGB1 concentrations in patients with tuberculosis and sarcoidosis than in healthy controls, although the estimates became unstable after multivariable adjustment [10]. In a separate prospective cohort, sputum HMGB1 correlated with bacillary load, decreased during antituberculosis treatment, and showed potential for evaluating culture conversion and treatment failure [11]. Nevertheless, sputum and serum HMGB1 represent biologically and analytically distinct compartments, and findings obtained from sputum cannot be assumed to apply directly to circulating HMGB1.

Respiratory manifestations, sputum smear grade, and radiographic extent represent complementary dimensions of pulmonary tuberculosis. Respiratory manifestations describe the clinical expression of disease, whereas sputum smear grade provides a semiquantitative estimate of acid-fast bacillary burden and is associated with pretreatment infectiousness. However, smear grade does not directly quantify viable bacilli or individual transmission events [12]. Chest radiographic extent reflects the distribution of structural pulmonary involvement and has been associated with time to culture positivity, culture conversion, and treatment outcomes [5,13]. The correspondence among clinical manifestations, microbiological burden, and radiographic involvement is nevertheless incomplete, as demonstrated by bacteriologically confirmed subclinical disease and differences in radiographic severity among patients with similar clinical presentations [5].

Evidence simultaneously relating serum HMGB1 concentrations to clinical, microbiological, and radiographic characteristics in bacteriologically confirmed pulmonary tuberculosis remains lacking. Therefore, this study aimed to evaluate the associations of serum HMGB1 concentrations with respiratory manifestations, sputum smear grade, and chest radiographic extent in adults with bacteriologically confirmed pulmonary tuberculosis. We hypothesized that serum HMGB1 concentrations would differ across respiratory manifestation groups and increase with higher sputum smear grades and more extensive radiographic involvement.

2. MATERIALS AND METHODS

2.1 Study Design and Participants

This analytical cross-sectional study was conducted from May to June 2026 at Dr. Wahidin Sudirohusodo General Hospital, a tertiary referral hospital in Makassar, Indonesia. The study evaluated the associations of serum high-mobility group box 1 (HMGB1) concentrations with respiratory manifestations, sputum acid-fast bacilli smear grade, and chest radiographic extent in adults with pulmonary tuberculosis. Clinical assessment, chest radiography, and sputum examination were performed during the initial diagnostic evaluation. Sputum specimens were collected before the initiation of antituberculosis treatment, whereas serum samples were collected either before or within 14 days after treatment initiation.

Adults with pulmonary tuberculosis were consecutively recruited. Participants were eligible if they were aged ≥18 years; had clinical, radiographic, and bacteriological findings consistent with pulmonary tuberculosis; had a positive pretreatment sputum smear; underwent serum collection before or within 14 days after treatment initiation; had complete study data; and provided written informed consent. Patients were excluded if they had human immunodeficiency virus infection or severe immunosuppression; active malignancy or a history of chemotherapy; prolonged systemic immunosuppressive therapy; severe nontuberculous infection or sepsis; major trauma or surgery within the preceding month; end-stage kidney disease;

decompensated liver cirrhosis; or another severe inflammatory condition considered likely to affect circulating HMGB1 concentrations.

The sample size was calculated based on the correlation between serum HMGB1 concentration and sputum smear grade. Because no directly comparable correlation estimate was available from previous studies, a moderate anticipated correlation coefficient of 0.30 was assumed based on Cohen's effect-size convention [14]. Using Fisher's z transformation, a two-sided significance level of 5%, and 80% statistical power, the minimum required sample size was 85 participants. A total of 92 participants were recruited to allow for potentially incomplete or unusable data.

The study protocol was approved by the Health Research Ethics Committee of Hasanuddin University–Dr. Wahidin Sudirohusodo General Hospital (no. 604/UN4.6.4.5.31/PP36/2026; May 4, 2026). All participants provided written informed consent before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki. Study data were coded and stored without direct personal identifiers.

2.2 Clinical, Microbiological, and Radiographic Assessment

Data were collected using a standardized case-report form and review of participants' medical records. Variables included age, sex, history of contact with a person with tuberculosis, respiratory manifestations, sputum smear grade, chest radiographic extent, treatment status at the time of blood collection, and serum HMGB1 concentration.

Respiratory manifestations were classified into four mutually exclusive groups: cough alone, cough with dyspnea, cough with hemoptysis, and cough with both hemoptysis and dyspnea. Pretreatment sputum smears were graded as 1+, 2+, or 3+ according to the World Health Organization/International Union Against Tuberculosis and Lung Disease grading system.

Posteroanterior chest radiographs obtained during the initial diagnostic evaluation were categorized as showing minimal or extensive disease. Minimal disease was defined as limited pulmonary infiltrates without cavitation or marked tissue destruction, whereas extensive disease was defined as widespread infiltrates, extensive consolidation, cavitation, and/or substantial destruction of the pulmonary parenchyma. Radiographic classification was based on the routine clinical interpretation documented before serum HMGB1 results were available.

2.3 Serum Collection and HMGB1 Measurement

Peripheral venous blood was collected into serum-separator tubes and allowed to clot before centrifugation for 20 min at approximately 1000 × g. The separated serum was aliquoted and stored at −20°C or lower until analysis, and repeated freeze–thaw cycles were avoided.

Serum HMGB1 concentrations were measured using a quantitative sandwich enzyme-linked immunosorbent assay according to the manufacturer's instructions (Human HMG1/HMGB1 ELISA Kit, LifeSpan BioSciences, Seattle, WA, USA; catalog no. LS-F23444). Optical density was measured at 450 nm, and HMGB1 concentrations were calculated from the standard curve and expressed in pg/mL. Samples exceeding the calibration range were diluted, and final concentrations were corrected using the appropriate dilution factor. Laboratory personnel performing the assays were blinded to participants' respiratory manifestations, sputum smear grades, and radiographic findings.

2.4 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 30.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages. Continuous-variable distributions were assessed graphically and using the Shapiro–Wilk test and were summarized as mean ± standard deviation or median with first and third quartiles, as appropriate.

Differences in serum HMGB1 concentrations across respiratory manifestation groups and sputum smear grades were evaluated using the Kruskal–Wallis test. When the overall test was statistically significant, pairwise comparisons were performed with Bonferroni adjustment. The association between ordinal sputum smear grade and serum HMGB1 concentration was assessed using Spearman's rank correlation coefficient. Serum HMGB1 concentrations were compared between minimal and extensive radiographic groups using the independent-samples t test after assessment of homogeneity of variance, with the mean difference and corresponding 95% confidence interval reported.

Multiple linear regression analysis was performed to identify factors independently associated with serum HMGB1 concentration. Age, sex, respiratory manifestation group, sputum smear grade, and radiographic extent were entered simultaneously into the model. Male sex, cough alone, sputum smear grade 1+, and minimal radiographic disease were used as reference categories. Results were reported as unstandardized regression coefficients with 95% confidence intervals and P values. Model assumptions and influential observations were assessed using residual plots, normal probability plots, variance inflation factors, standardized residuals, leverage values, and Cook's distance. All statistical tests were two-sided, and $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Results

A total of 92 patients with pulmonary tuberculosis were included in the analysis, and no data were missing. The mean age was 49.16±16.87 years, and 69 patients (75.0%) were male. A history of contact with a person with tuberculosis was reported by 37 patients (40.2%). Cough with dyspnea was the most frequent respiratory manifestation, occurring in 48 patients (52.2%). Minimal radiographic disease was observed in 49 patients (53.3%), and sputum smear grade 1+ was the most frequent smear category, occurring in 47 patients (51.1%). The mean serum high-mobility group box 1 (HMGB1) concentration was 35,258.11±8,986.79 pg/mL (Table 1).

Table 1. Characteristics of the study participants (n=92)

Characteristic	Value
Age, years	49.16 ± 16.87
Sex	
Male	69 (75.0)
Female	23 (25.0)
Known tuberculosis contact	
No	55 (59.8)
Yes	37 (40.2)
Respiratory manifestations	
Cough alove	18 (19.6)
Cough with dyspnea	48 (52.2)
Cough with Hemoptysis	22 (23.9)
Cough with both Hemoptysis and dyspnea	4 (4.3)
Radiographic extent	
Minimal lesions	49 (53.3)
Extensive lesions	43 (46.7)
Sputum smear grade	
Grade 1+	47 (51.1)
Grade 2+	19 (20.7)
Grade 3+	26 (28.3)
Serum HMGB1, pg/mL	35,258.11 ± 8,986.79

Note: Data are presented as mean ± SD or number (%). HMGB1, high-mobility group box 1.

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Serum HMGB1 concentrations differed significantly across the four respiratory manifestation groups (Kruskal–Wallis H[3]=26.985, P<0.001). Bonferroni-adjusted pairwise comparisons showed significantly higher concentrations in patients with cough with dyspnea, cough with hemoptysis, and cough with both hemoptysis and dyspnea than in those with cough alone. No significant differences were observed among the remaining respiratory manifestation groups (Tables 2 and 3).

Table 2. Serum HMGB1 concentrations according to Respiratory manifestations, sputum smear grade, and radiographic extent

Variable	Serum HMGB1, pg/mL	P value
Respiratory manifestations		<0.001[a]
Cough alone (n=18)	25,315.06 (22,889.95–28,296.38)	
Cough with dyspnea (n=48)	32,766.86 (29,717.57–41,676.27)	
Cough with Hemoptysis (n=22)	40,511.16 (34,555.75–45,628.65)	
Cough with both Hemoptysis and dyspnea (n=4)	48,271.42 (30,827.25–55,785.17)	
Sputum smear grade		<0.001[a]
Grade 1+ (n=47)	28,886.42 (24,825.85–31,922.41)	
Grade 2+ (n=19)	38,252.48 (35,614.50–40,750.65)	
Grade 3+ (n=26)	44,624.14 (43,494.38–47,034.18)	
Radiographic extent		<0.001[b]
Minimal lesions (n=49)	29,597.53 ± 6,225.99	
Extensive lesions (n=43)	41,708.55 ± 7,120.62	

Notes: Data are presented as median (Q1–Q3) or mean ± standard deviation, as appropriate. [a] Kruskal–Wallis test. [b] Independent-samples *t* test. HMGB1, high-mobility group box 1.

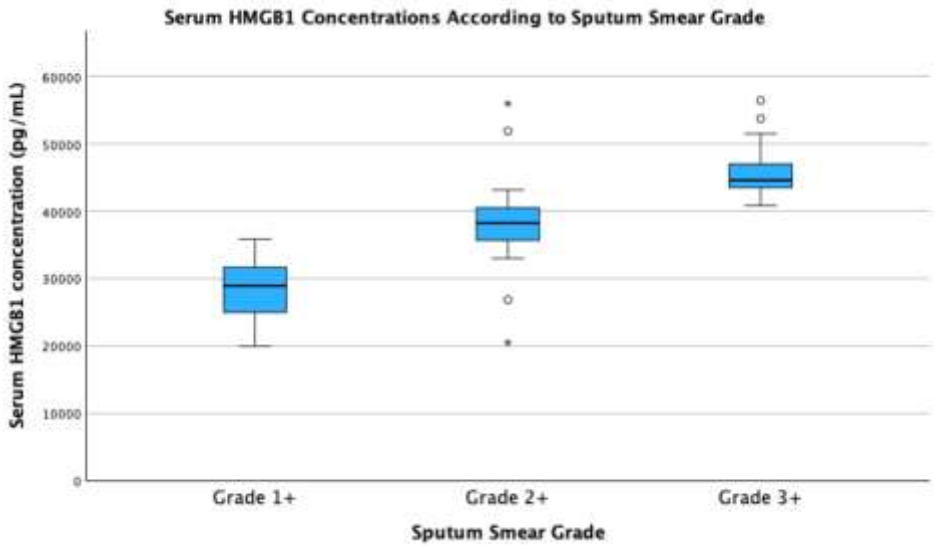

Figure 1. Serum high-mobility group box 1 concentrations according to sputum smear grade.

Serum HMGB1 concentrations also differed significantly across sputum smear grades 1+, 2+, and 3+ (Kruskal–Wallis H[2]=64.384, P<0.001), with progressively higher concentrations at increasing smear grades. All pairwise comparisons remained significant after Bonferroni adjustment. Sputum smear grade showed a strong positive correlation with serum HMGB1 concentration (Spearman ϱ=0.841, P<0.001) (Tables 2 and 3; Figure 1).

Table 3. Bonferroni-adjusted pairwise comparisons of serum HMGB1 concentrations

Variable and pairwise comparison	Adjusted *P* value
Respiratory manifestations	
Cough alone vs cough with dyspnea	0.005*
Cough alone vs cough with hemoptysis	<0.001*
Cough alone vs cough with both hemoptysis and dyspnea	0.012*
Cough with dyspnea vs cough with hemoptysis	0.076
Cough with dyspnea vs cough with both hemoptysis and dyspnea	0.824
Cough with Hemoptysis vs cough with both hemoptysis and dyspnea	1.000
Sputum smear grade	
Grade 1+ vs grade 2+	<0.001*
Grade 1+ vs grade 3+	<0.001*
Grade 2+ vs grade 3+	0.020*

Note: Pairwise comparisons were performed after statistically significant Kruskal–Wallis tests. *P* values were adjusted using the Bonferroni correction. *Statistical significance at $P < 0.05$. HMGB1, high-mobility group box 1.

Patients with extensive radiographic disease had higher serum HMGB1 concentrations than those with minimal disease (41,708.55±7,120.62 vs. 29,597.53±6,225.99 pg/mL). The mean difference was 12,111.02 pg/mL (95% confidence interval, 9,346.87–14,875.17; t[90]=8.705; P<0.001) (Table 2).

The multiple linear regression model was statistically significant and explained 78.7% of the variance in serum HMGB1 concentration (R^2=0.787; adjusted R^2=0.767; F[8,83]=38.442; P<0.001) (Table 4). After adjustment for age, sex, respiratory manifestation group, sputum smear grade, and radiographic extent, cough with dyspnea, cough with hemoptysis, cough with both hemoptysis and dyspnea, and sputum smear grades 2+ and 3+ remained significantly associated with higher serum HMGB1 concentrations.

Table 4. Multivariable associations with serum HMGB1 concentrations

Predictor	Adjusted B, pg/mL	95% CI	*P* value
Age, per 1-year increase	6.88	−50.05 to 63.81	0.811
Female vs male	−1,525.38	−3,766.19 to 715.43	0.179
Respiratory manifestations			
Cough with dyspnea vs cough alone	3,420.47	768.46 to 6,072.49	0.012*
Cough with Hemoptysis vs cough alone	8,093.33	5,122.09 to 11,064.56	<0.001*
Cough with both Hemoptysis and dyspnea vs cough alone	7,441.87	2,235.98 to 12,647.76	0.006*
Sputum smear grade			

Grade 2+ vs grade 1+	5,632.76	2,415.97 to 8,849.56	<0.001*
Grade 3+ vs grade 1+	13,750.34	10,518.22 to 16,982.47	<0.001*
Radiographic extent			
Extensive vs minimal lesions	2,223.53	−649.50 to 5,096.56	0.128

Note: B represents the adjusted mean difference in serum HMGB1 concentration estimated using multiple linear regression. All variables shown were entered simultaneously into the model. Reference categories were male sex, cough alone, sputum smear grade 1+, and minimal radiographic lesions. Model $R^2 = 0.787$; adjusted $R^2 = 0.767$; $F(8,83) = 38.442$; $P < 0.001$. Variance inflation factors ranged from 1.128 to 2.617. *Statistical significance at $P < 0.05$. CI, confidence interval; HMGB1, high-mobility group box 1.

Compared with patients presenting with cough alone, adjusted serum HMGB1 concentrations were higher by 3,420.47 pg/mL in patients with cough with dyspnea, 8,093.33 pg/mL in those with cough with hemoptysis, and 7,441.87 pg/mL in those with cough with both hemoptysis and dyspnea. Compared with sputum smear grade 1+, adjusted concentrations were higher by 5,632.76 pg/mL for grade 2+ and 13,750.34 pg/mL for grade 3+.

Age, sex, and radiographic extent were not significantly associated with serum HMGB1 concentration in the adjusted model. Thus, the association between radiographic extent and serum HMGB1 observed in the unadjusted analysis was attenuated after adjustment. Variance inflation factors ranged from 1.128 to 2.617, and the maximum Cook's distance was 0.613. No observations were excluded from the regression analysis.

3.2 Discussion

This study demonstrated three principal findings. First, serum high-mobility group box 1 (HMGB1) concentrations differed across respiratory manifestation groups and were lowest among patients presenting with cough alone. Second, HMGB1 concentrations increased progressively across sputum smear grades and showed a strong positive correlation with smear grade. Third, patients with extensive radiographic disease had higher HMGB1 concentrations than those with minimal disease in the unadjusted analysis, but this association was attenuated after adjustment for respiratory manifestations and sputum smear grade. Overall, circulating HMGB1 was most consistently associated with smear-defined bacillary burden, whereas its relationships with Respiratory manifestations and radiographic extent appeared to represent partially overlapping dimensions of pulmonary tuberculosis severity.

HMGB1 is a chromatin-associated nuclear protein involved in DNA organization, transcription, and repair. During cellular activation or stress, it may be actively secreted through nonclassical pathways, whereas necrosis and other forms of membrane-disruptive cell death permit its passive extracellular release. Extracellular HMGB1 acts as a damage-associated molecular pattern through receptors such as the receptor for advanced glycation end products and Toll-like receptor 4. Activation of these receptors stimulates nuclear factor kappa B and mitogen-activated protein kinase signaling, thereby promoting cytokine and chemokine production, leukocyte recruitment, and inflammatory amplification [8,9]. The biological effects of HMGB1 also depend on its post-translational modifications and redox state. Consequently, measurement of total serum HMGB1 does not distinguish between its chemotactic, cytokine-inducing, and biologically inactive molecular forms.

Several pathological features of pulmonary tuberculosis provide plausible sources of extracellular HMGB1. Infection and activation of alveolar macrophages, granuloma remodeling, oxidative stress, neutrophil recruitment, extracellular-matrix degradation, and necrotic cell death contribute to progressive pulmonary injury [2,15–17]. Persistent bacillary stimulation may promote active HMGB1 secretion by macrophages and recruited leukocytes, whereas caseous necrosis and destruction of the pulmonary parenchyma may cause passive release from damaged cells. Circulating HMGB1 should therefore be interpreted as a marker associated with inflammatory and tissue-injury activity rather than as a tuberculosis-specific molecule.

Patients presenting with cough alone had lower HMGB1 concentrations than those with cough with dyspnea, cough with hemoptysis, or cough with both hemoptysis and dyspnea. Dyspnea may accompany impaired ventilation, altered gas exchange, extensive inflammatory infiltration, or loss of functional lung tissue. Hemoptysis may arise from airway, cavitary, or parenchymal injury involving inflamed bronchial vessels [6,16]. These processes may plausibly be accompanied by greater

cellular injury and HMGB1 release. Nevertheless, the manifestation groups used in this study represented clinical phenotypes rather than a validated ordinal severity scale. Bacteriologically confirmed pulmonary tuberculosis may occur with few or unrecognized symptoms despite radiographic abnormalities and, in some cases, smear positivity [18,19]. Clinical severity and radiographic involvement may also be discordant, and different clinical phenotypes may reflect distinct rather than uniformly progressive inflammatory patterns [20]. Because differences among the three more complex manifestation groups were not significant after Bonferroni adjustment, the findings support variation in HMGB1 across clinical presentations but not a stepwise increase according to symptom complexity.

The clearest finding was the graded association between serum HMGB1 and sputum smear grade. Smear microscopy provides a semiquantitative estimate of acid-fast bacillary burden, and its relationships with Xpert MTB/RIF Ultra cycle-threshold values and culture time to positivity support its use as an indirect indicator of sputum bacillary load [21]. Pretreatment smear positivity and higher smear grade are also associated with greater transmission risk. However, smear microscopy does not distinguish viable from nonviable bacilli and does not directly quantify individual infectiousness [12]. Transmission is additionally influenced by lesion–airway communication, cough strength, aerosol production, treatment status, host characteristics, and environmental conditions. Viable Mycobacterium tuberculosis may also be aerosolized during tidal breathing, indicating that cough and sputum burden represent only part of transmission biology [22]. The present results should therefore be interpreted primarily as an association between HMGB1 and smear-defined bacillary burden rather than direct evidence of infectiousness.

A greater bacillary burden may produce more sustained antigenic stimulation, macrophage activation, cytokine release, neutrophilic inflammation, and cellular injury, thereby increasing extracellular and circulating HMGB1 concentrations [2,15,17]. Once released, HMGB1 may further amplify inflammatory signaling. However, the cross-sectional design cannot determine whether increased HMGB1 contributes to inflammatory persistence, results from tissue injury, or reflects both processes.

The present findings are consistent with those of Kamolratanakul et al. [11], who reported that sputum HMGB1 correlated with bacillary load, decreased during antituberculosis treatment, and showed potential for assessing culture conversion and treatment failure. Our results provide complementary evidence that circulating HMGB1 is associated with smear-defined bacillary burden. Nevertheless, serum and sputum represent biologically and analytically distinct compartments. Sputum HMGB1 may primarily reflect airway-localized inflammation and tissue injury, whereas serum HMGB1 may reflect systemic inflammatory spillover or release from multiple tissues. Measurements from these compartments should therefore not be regarded as interchangeable.

The association between HMGB1 and sputum smear grade remained significant after adjustment for age, sex, respiratory manifestations, and radiographic extent, suggesting that it was not fully explained by these measured characteristics. However, smear microscopy is affected by specimen quality, sampling variation, smear preparation, staining procedures, and reader interpretation [21]. Further studies should compare serum HMGB1 with more quantitative microbiological indicators, including culture time to positivity, molecular bacterial-load measurements, viable bacillary assays, and serial microbiological responses during treatment.

Extensive radiographic disease was associated with higher serum HMGB1 concentrations in the unadjusted analysis. Greater radiographic involvement may represent a larger volume of inflamed or damaged pulmonary tissue, including consolidation, cavitation, necrosis, and airway destruction. Neutrophil-derived matrix metalloproteinases, S100 proteins, and other inflammatory mediators have been associated with radiographic lung damage and subsequent recovery [23–25]. Quantitative radiographic extent has also been associated with culture conversion and treatment outcomes, whereas advanced radiographic disease in subclinical tuberculosis has been linked to shorter culture time to positivity [5,13].

The attenuation of the radiographic association after multivariable adjustment should not be interpreted as evidence that no biological relationship exists. Respiratory manifestations, sputum smear grade, and radiographic extent may capture overlapping aspects of an underlying disease process, thereby reducing their independent contributions when entered simultaneously into the same model. In addition, categorizing radiographic disease as only minimal or extensive may have obscured differences in lesion volume, cavitation, anatomical distribution, and tissue destruction. Persistent structural and functional abnormalities after microbiological cure further demonstrate that pulmonary tissue injury and bacillary burden are related but nonidentical dimensions of disease [3].

Age and sex were not independently associated with HMGB1 concentrations. Nevertheless, residual confounding by nutritional status, smoking, diabetes, duration of illness, medication exposure, organ dysfunction, and unrecognized inflammatory conditions remains possible. In an exploratory study of intrathoracic lymphadenopathy, serum HMGB1 concentrations were elevated in both tuberculosis and sarcoidosis compared with healthy controls, although their independent discriminatory contribution was uncertain after adjustment [10]. Despite differences between that population and patients with active parenchymal pulmonary tuberculosis, these findings reinforce the interpretation of HMGB1 as a nonspecific inflammatory and tissue-injury marker rather than a disease-specific biomarker.

This study has several strengths, including consecutive recruitment, complete data, assessment of clinical, microbiological, and radiographic domains during the initial diagnostic period, blinding of laboratory personnel to clinical findings, and multivariable analysis. However, several limitations should be considered. The cross-sectional and single-center design prevents causal inference and may limit generalizability. Only patients with smear-positive pulmonary tuberculosis were included, and the findings may not apply to patients with smear-negative, paucibacillary, extrapulmonary, or subclinical disease. The subgroup presenting with cough, hemoptysis, and dyspnea was small, which may have reduced the precision of comparisons. Radiographic extent was classified using a binary routine clinical assessment rather than a validated quantitative scoring system. Quantitative microbiological measurements, inflammatory cytokines, and HMGB1 redox isoforms were not assessed. In addition, serum was collected either before or within 14 days after treatment initiation, and early treatment-related changes may have influenced HMGB1 concentrations. Residual confounding and the absence of longitudinal measurements also limit conclusions regarding prognostic utility and changes during treatment.

4. CONCLUSION

Serum HMGB1 concentrations differed across respiratory manifestation groups and were independently associated with sputum smear grade in adults with bacteriologically confirmed pulmonary tuberculosis. Although HMGB1 concentrations were higher in patients with extensive radiographic disease in the unadjusted analysis, this association did not persist after multivariable adjustment. These findings support serum HMGB1 as a candidate marker of clinical and smear-defined bacteriological disease burden but do not establish tuberculosis specificity, causality, or prognostic utility. Longitudinal multicenter studies incorporating quantitative microbiological, radiographic, and inflammatory assessments are required to determine its clinical value.

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AUTHOR CONTRIBUTIONS

Azhar: Conceptualization, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Project Administration, Writing—Original Draft.

Jamaluddin Madolangan: Conceptualization, Methodology, Supervision, Validation, Interpretation of Findings, Writing—Review & Editing.

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Rizki Amelia Noviyanthi: Investigation, Data Curation, Literature Review, Visualization, Writing—Review & Editing.

All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The study protocol was approved by the Health Research Ethics Committee of Hasanuddin University–Dr. Wahidin Sudirohusodo General Hospital, Makassar, Indonesia (no. 604/UN4.6.4.5.31/PP36/2026; May 4, 2026). Written informed consent was obtained from all participants before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and institutional restrictions.

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