Globotriaosylceramide as a potential biomarker for auxiliary detection of lower respiratory tract infections of *Pseudomonas aeruginosa*

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Abstract. Lower respiratory tract infections (LRTIs) caused by *Pseudomonas aeruginosa* (PA) are a significant health concern, notably among vulnerable populations. The glycosphingolipid receptor globotriaosylceramide (Gb3) has been implicated in PA pathogenicity, however, its clinical implications remain underexplored. The present study aimed to investigate the clinical value of Gb3 concentrations in serum and bronchoalveolar lavage fluid (BALF) as a biomarker for PA-induced LRTIs. In the current prospective study, 54 PA-infected patients and 54 healthy individuals were enrolled as controls. Gb3 levels were measured using a Gb3 ELISA kit and the levels of inflammatory markers were assessed. The diagnostic accuracy of Gb3 was evaluated using receiver operating characteristic (ROC) curve analysis. The patients with PA-induced LRTIs exhibited significantly higher Gb3 concentration levels in both serum and BALF compared with those noted in healthy controls, with more pronounced elevations noted in BALF. The area under the ROC curve was 0.899 for serum Gb3 and 0.812 for BALF Gb3, indicating high sensitivity and specificity for diagnosis of PA infection. Gb3 levels were also found to be correlated with C-reactive protein and procalcitonin levels, suggesting its potential in reflecting infection severity. Overall, the present findings revealed a significant association between Gb3 levels and PA-induced LRTIs, proposing Gb3 as a promising biomarker for early detection and diagnosis. Further research is warranted to validate the role of Gb3 in various patient populations and to explore its dynamics over the course of infection.

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Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative bacterium known as an opportunistic pathogen, commonly found in water, soil and on human skin (1-4). It poses a significant risk to individuals with weakened immune systems, causing serious infections that are difficult to treat due to their inherent resistance to antibiotics (1-4). Lower respiratory tract infections (LRTIs) caused by PA are particularly concerning, as they can lead to severe conditions, such as pneumonia, which may be life-threatening if untreated (5-9). Patients who are immunocompromised, have cystic fibrosis, or suffer from burn wounds, are notably vulnerable to PA-induced LRTIs (5-9).

Globotriaosylceramide (Gb3) is a glycosphingolipid found in cell membranes that plays a crucial role in various medical conditions (10,11). It was initially recognized in the context of Fabry disease, and its significance has expanded to include tumor biology and infectious diseases (12-16). Gb3 serves as a receptor for specific bacterial toxins associated with gastrointestinal infections, such as enterotoxigenic and Shiga toxin-producing *Escherichia coli* infections (16-19).

In the context of PA, previous research studies have identified the importance of Gb3 in the uptake of the bacterium by host cells (10,11). Gb3 acts as a key receptor by interacting with the bacterial lectin LecA on host cell surfaces (10,11). This interaction activates the 'lipid zipper mechanism', facilitating bacterial adhesion, internalization and progression of the infection (20,21). It forms a plasma membrane domain enriched in Gb3, cluster of differentiation (CD)59, phosphatidylinositol (3,4,5)-triphosphate and flotillin, which facilitates the efficient uptake of PA strain PAO1 into the host cell (22,23). The depletion of flotillins and CD59 from the host cells significantly reduces PA invasiveness, highlighting the importance of the Gb3-enriched domain in bacterial invasion (23,24). However, the concentration of Gb3 in the context of PA infections remains underexplored and its clinical relevance is not well defined.

Despite the recognized role of Gb3 in PA pathogenicity at cellular and molecular levels, there is a scarcity of clinical data regarding Gb3 levels in patients with PA infections. This

scarcity can be attributed to several factors. Firstly, the measurement of Gb3 requires specialized laboratory techniques and equipment, such as ELISA assays, high-performance liquid chromatography and mass spectrometry, which are not widely available in various clinical settings, limiting large-scale studies (25). Secondly, research on Gb3 as a biomarker for PA infections is relatively new. While Gb3 has been extensively studied in the context of Fabry disease and certain cancer types, its specific role in PA-induced LRTIs remains underexplored. Lastly, the involvement of Gb3 in multiple pathological processes, including other bacterial infections and cancer progression, complicates its measurement and interpretation. This complexity necessitates careful study design and analysis, which may have deterred extensive clinical research in this area (26). These challenges have limited the broad application of Gb3 measurements in clinical practice and underscore the necessity for further research to assess the role of Gb3 in PA infections.

In the present study, the concentration levels of Gb3 in bronchoalveolar lavage fluid (BALF) and serum samples from patients with PA infection were quantified and compared with the levels noted in healthy individuals. It is proposed that elevated Gb3 levels are associated with PA infections and may serve as a valuable biomarker for detecting and assessing the severity of these infections.

Materials and methods

Ethics statement. The present study received approval (approval no. 2024-045) from the Ethics Committee of The Affiliated People's Hospital of Ningbo University (Ningbo, China). Written informed consent was obtained from all participants or their guardians, adhering to the principles outlined in the Declaration of Helsinki.

Participant selection. The present prospective study was conducted from 1 June 2024 to 10 September 2024, at The Affiliated People's Hospital of Ningbo University. A total of 108 participants were included and divided into two groups as follows: Group PA included 54 patients with PA-induced LRTIs (30 males and 24 females, mean age 67.54±10.33 years; Table I) and Group H included 54 healthy individuals (31 males and 23 females, mean age 51.47±7.71 years; Table I) without chronic medical history or current medication use, selected from the physical examination center of The Affiliated People's Hospital of Ningbo University. The exclusion criteria included patients on statins, aspirin, inhaled corticosteroids, long-term macrolides or those who received anti-infective medication prior to admission or could not tolerate bronchoscopy and lavage.

The diagnosis of PA-induced LRTIs followed the Guidelines for Chinese Expert Consensus on the Management of Lower Respiratory Tract Infections of *Pseudomonas aeruginosa* in Adults (2022 revision) (27). Diagnosis was based on clinical symptoms, such as pneumonia, tracheobronchitis, lung abscess, or empyema, along with isolation of PA from qualified lower respiratory tract specimens and identification of high-risk factors for acute PA infection.

Diagnostic procedures. All participants underwent diagnostic tests, including blood tests [white blood cell (WBC) counts, neutrophil (NEU) counts, lymphocyte counts, monocyte counts, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP) and procalcitonin (PCT) levels], computed tomography (CT) scans and bronchoscopies. For patients with PA-induced LRTIs, peripheral blood samples were collected on the 1st and 7th days following diagnosis. BALF was collected on the 1st day post-diagnosis from the infected lung segments identified by chest CT scans.

Gb3 level measurement. Peripheral blood and BALF samples were collected and centrifuged within 3 h at 800 x g for 10 min at room temperature (~20˚C). The supernatants of BALF and serum were separated and stored at -80˚C for subsequent analysis. Gb3 levels were measured using the Gb3 ELISA kit (cat no. BLL104146E9; Baililai Biological), according to the manufacturer's protocol.

Statistical analysis. A power analysis was conducted prior to the study to determine the necessary number of participants to achieve adequate statistical power. Based on preliminary data, the positive rate of Gb3 in the Group PA (case group) was anticipated to be 93.3, and 40.0% in the Group H (control group). The sample size calculation formula for matched case-control studies was utilized: $n=2pq \times (Z_\alpha + Z_\beta)^2/(\rho_1-\rho_0)^2$, where $\alpha=0.05$ (two-sided significance level), $Z_\alpha=1.96$ (Z-score corresponding to α), $\beta=0.2$ (for a power of 80%), $Z_\beta=0.842$ (Z-score corresponding to β), $\rho_0=0.4$ (positive rate in the control group), $\rho_1=0.933$ (positive rate in the case group), $P=(\rho_0+\rho_1)/2=0.665$, $q=1-P=0.335$.

Substituting these values into the formula, $n=12.27$, ~13 was obtained. This calculation indicated that at least 13 matched pairs were needed to achieve 80% power at a significance level of 0.05. In the present study, 54 paired samples were initially included. After propensity score matching (PSM) for age and body mass index (BMI), 15 matched pairs were selected for demographic and laboratory result analysis. This number exceeds the calculated requirement and ensures sufficient statistical power. The match was performed using a nearest neighbor algorithm with a 1:1 ratio and a caliper value of 0.03.

Categorical variables are presented as numbers or percentages. Categorical variable comparisons between groups were conducted using the Chi-square test. The Kolmogorov-Smirnov test was used to assess the distribution of continuous variables. Continuous variables with normal distributions are reported as the mean ± standard deviation, while those with non-normal distributions are reported as the median and interquartile range. Group comparisons of non-normally distributed continuous variables were carried out using the Wilcoxon signed rank test. Correlations between variables, including Gb3 levels, CRP and PCT, were assessed using Spearman's rank correlation coefficients. Univariate and multivariate logistic regression analyses were performed to evaluate the association between Gb3 levels and the incidence of PA.

Prediction accuracy was evaluated using receiver operating characteristic (ROC) curves, with the area under the ROC curve (AUC) values calculated for each parameter. Prediction rates were compared using the log-rank test. All statistical analyses were performed using SPSS software version 26.0 (IBM Corp.), GraphPad Prism software version 9.3.1 (GraphPad Software; Dotmatics), MedCalc version

Table I. Clinical characteristics of patients with PA-induced lower respiratory tract infections and healthy controls.

	Unmatched cohort				Matched cohort			
Characteristics	PA (n=54)	Healthy (n=54)	t/z/χ^2	P-value	PA (n=15)	Healthy (n=15)	t/z/χ^2	P-value
Age, years	67.54±10.33	51.47±7.71	7.439	<0.001	54.87±7.75	54.67±7.59	0.868	0.870
BMI, kg/m^2	21.15±3.91	23.57±1.63	3.539	<0.001	21.27±4.77	23.58±1.86	1.750	0.097
Male, n (%)	30 (55.6)	31 (57.4)	3.569	0.312	10 (66.7)	7 (46.7)	1.942	0.379
Chronic obstructive pulmonary diseases, n (%)	16 (29.6)	0 (0)	N/A	N/A	3 (20)	0 (0)	N/A	N/A
Bronchiectasia, %	30 (55.6)	0 (0)	N/A	N/A	5 (33.3)	0 (0)	N/A	N/A
Diffuse panbronchiolitis, n (%)	1 (1.8)	0 (0)	N/A	N/A	0 (0)	0 (0)	N/A	N/A
Interstitial lung disease, n (%)	2 (3.7)	0 (0)	N/A	N/A	0 (0)	0 (0)	N/A	N/A

Categorical variables are expressed as numbers (percentages); continuous variables with a normal distribution are expressed as the mean ± SD; continuous variables with a normal distribution are expressed as the median (interquartile range). N/A, not applicable.

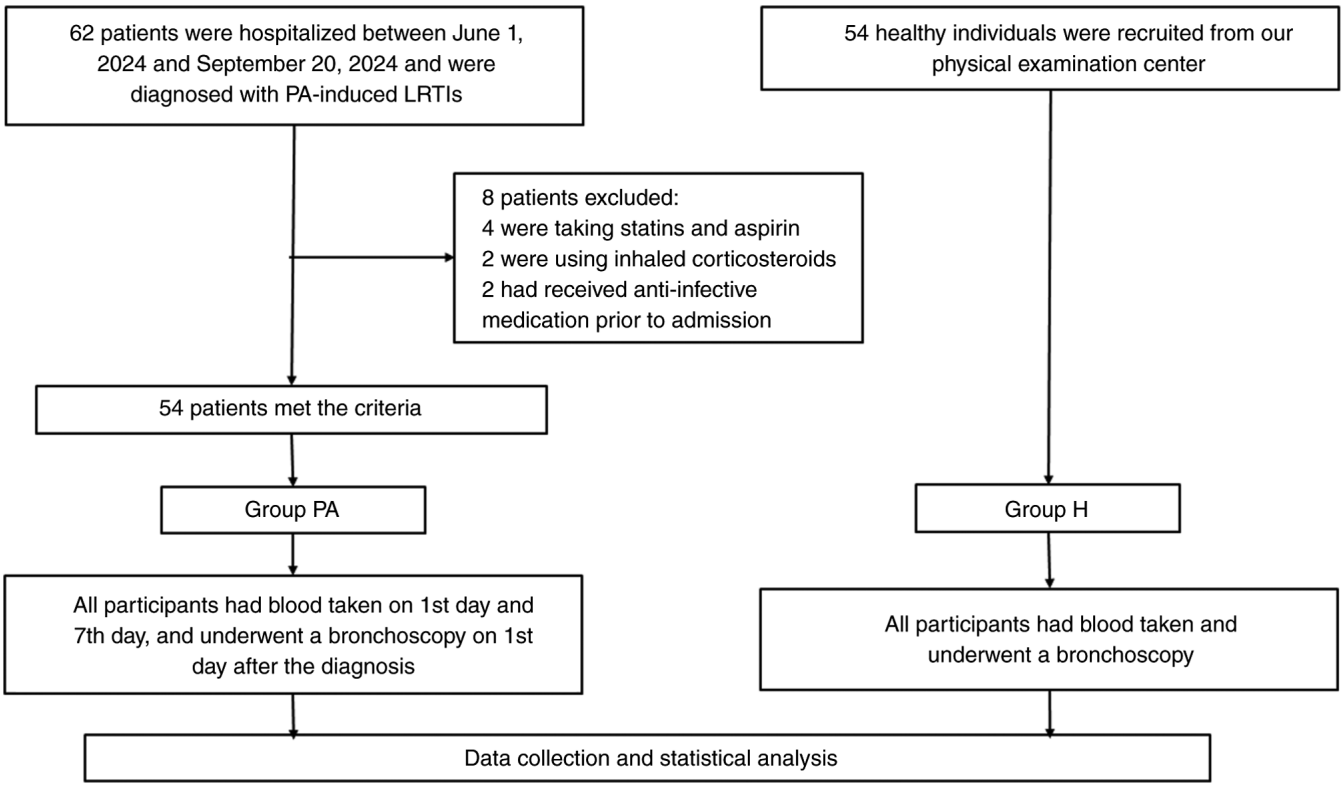

Figure 1. Flow diagram of patient selection and grouping in the present study. PA, *Pseudomonas aeruginosa*; LRTIs, lower respiratory tract infections.

22.026 (MedCalc Software Ltd.) and R software version 4.1.0 (https://www.R-project.org/). A P<0.05 was considered to indicate a statistically significant difference.

Results

Participant composition. As revealed in Fig. 1, 62 patients were initially considered eligible between June 1, 2024 and September 20, 2024. A total of 8 patients were excluded for the following reasons: 4 patients were on statins and aspirin, 2 patients were using inhaled corticosteroids and 2 patients had received anti-infective medication prior to admission. A total of 54 patients with PA-induced LRTIs (Group PA) were included in the study. Additionally, 54 healthy individuals without chronic medical history or current medication use were selected from the physical examination center to form the control group (Group H). This resulted in a total of 108 participants in the present study.

Among the 54 participants in Group PA, 16 patients (29.6%) were diagnosed with chronic obstructive pulmonary diseases, 30 patients (55.6%) had bronchiectasis, 1 patient (1.8%) had diffuse panbronchiolitis and 2 patients (3.7%) had interstitial lung disease, as shown in Table I.

Table II. Laboratory results of patients with PA-induced lower respiratory tract infections and healthy controls.

Laboratory results	PA[a] (n=15)	Healthy (n=15)	t/z/χ^2	P-value
White blood cell count, x10^9/l	14.90 (10.68, 20.63)	6.50 (6.00, 7.30)	3.776	<0.001
Neutrophil count, x10^9/l	12.96 (9.57, 17.68)	4.40 (3.64, 5.18)	4.015	<0.001
Lymphocyte count, x10^9/l	0.75 (0.54, 1.82)	1.80 (1.37, 1.93)	1.922	0.057
Monocytes, x10^9/l	0.61 (0.35, 0.78)	0.47 (0.40, 0.64)	0.809	0.425
Neutrophil-to-lymphocyte ratio	12.59 (7.90, 21,13)	1.85 (2.02, 3.42)	4.102	<0.001
C-reactive protein, mg/l	40.20 (33.30, 74.70)	N/A	N/A	N/A
Procalcitonin, ng/ml	0.53 (0.35, 0.88)	N/A	N/A	N/A
Serum Gb3, ng/ml	380.95 (330.38, 538.17)	300.84 (259.89, 326.79)	3.754	<0.001
Bronchoalveolar lavage fluid Gb3, ng/ml	222.82 (209.69, 237.73)	155.17 (112.77, 197.09)	3.754	<0.001

Categorical variables are expressed as n (%); continuous variables with a normal distribution are expressed as the mean ± SD; continuous variables with a normal distribution are expressed as the median (interquartile range). [a]The samples were obtained on the 1st day of testing after diagnosis. PA, *Pseudomonas aeruginosa*; Gb3, globotriaosylceramide.

Table III. Correlation between Gb3 levels and changes in serum CRP and PCT levels.

	Serum day 7 Gb3		Gb3 change	
Changes in serum levels	r_s	P-value	r_s	P-value
CRP	-0.375	0.168	-0.211	0.451
PCT	-0.736	0.002	-0.507	0.054

Gb3, globotriaosylceramide; CRP, C-reactive protein; PCT, procalcitonin; r_s, Spearman's rank correlation coefficient.

Basic characteristics of participants. The clinical characteristics of Group PA and Group H are presented in Table I. In the unmatched cohort, significant differences were observed between the two groups in terms of age and BMI. The mean age of Group PA was significantly higher than that of Group H (67.54±10.33 years vs. 51.47±7.71 years; P<0.001). Similarly, the mean BMI of Group PA was significantly lower than that of Group H (21.15±3.91 kg/m^2 vs. 23.57±1.63 kg/m^2; P<0.001). Due to these significant differences in age and BMI, which could act as confounding factors, PSM was performed between Groups PA and H to create a matched cohort for further analysis. This process resulted in 15 matched pairs for follow-up analysis (as matched cohorts in Table I).

Gb3 levels and inflammatory indices of participants. The results indicated that both Gb3 levels and the inflammatory index were increased in Group PA compared with those noted in Group H (P<0.001; Table II). Specifically, on day 1, the median serum Gb3 level in Group PA was significantly higher than that in Group H [380.95 ng/ml (IQR, 330.38-538.17 ng/ml) vs. 300.84 ng/ml (IQR, 259.89-326.79 ng/ml); P<0.001; Table II]. Similarly, the median BALF Gb3 level was significantly higher in Group PA compared with Group H [222.82 ng/ml (IQR, 209.69-237.73ng/ml)vs.155.17ng/ml(IQR,112.77-197.09ng/ml); P<0.001; Table II and Fig. 2A and B].

When comparing serum and BALF Gb3 levels within each group, serum Gb3 levels surpassed BALF Gb3 levels in both Group PA and Group H (P<0.001; Fig. 2C). No significant difference was noted in serum Gb3 levels between days 1 and 7 post-diagnosis in Group PA (P=0.865; Fig. 2D).

In addition to Gb3 levels, inflammatory indices were markedly elevated in Group PA. Specifically, the median WBC count was significantly higher in Group PA compared with Group H [14.90x10^9/l (IQR, 10.68-20.63x10^9/l) vs. 6.50x10^9/l (IQR, 6.00-7.30x10^9/l), P<0.001; Table II]. The median NEU count in Group PA was significantly higher than that in Group H [12.96x10^9/l (IQR, 9.57-17.68x10^9/l) vs. 4.40x10^9/l (IQR, 3.64-5.18x10^9/l); P<0.001; Table II]. Additionally, the NLR was significantly higher in Group PA compared with Group H [median, 12.59 (IQR, 7.90-21.13) vs. 1.85 (IQR, 2.02-3.42); P<0.001; Table II].

Further analysis indicated a negative correlation between serum Gb3 levels on day 7 and the changes in serum PCT levels over the 7 days post-diagnosis (r_s=-0.736; P=0.002; Table III). A similar trend was observed between changes in Gb3 levels during the observation period and changes in PCT levels (r_s=-0.507; P=0.054; Table III). However, the correlation between serum Gb3 levels on day 7 and CRP changes was not statistically significant (r_s=-0.375; P=0.168; Table III). Similarly, the correlation between Gb3 changes and CRP changes was not statistically significant (r_s=-0.211; P=0.451; Table III). In addition, a significant correlation between serum Gb3 and BALF Gb3 concentration levels

Table IV. Binary logistic regression analysis of the incidence of *Pseudomonas aeruginosa*-induced lower respiratory tract infections.

	Univariate analysis		Multivariate analysis	
Variable	OR (95% CI)	P-value	OR (95% CI)	P-value
Serum Gb3	1.045 (1.009-1.083)	0.015	1.048 (1.010-1.087)	0.013
BALF Gb3	1.050 (1.016-1.085)	0.004	1.056 (1.016-1.098)	0.006

The age and BMI were adjusted through propensity score matching and included as covariates in the logistic regression model; Gb3, globotriaosylceramide; BALF, bronchoalveolar lavage fluid; OR, odds ratio.

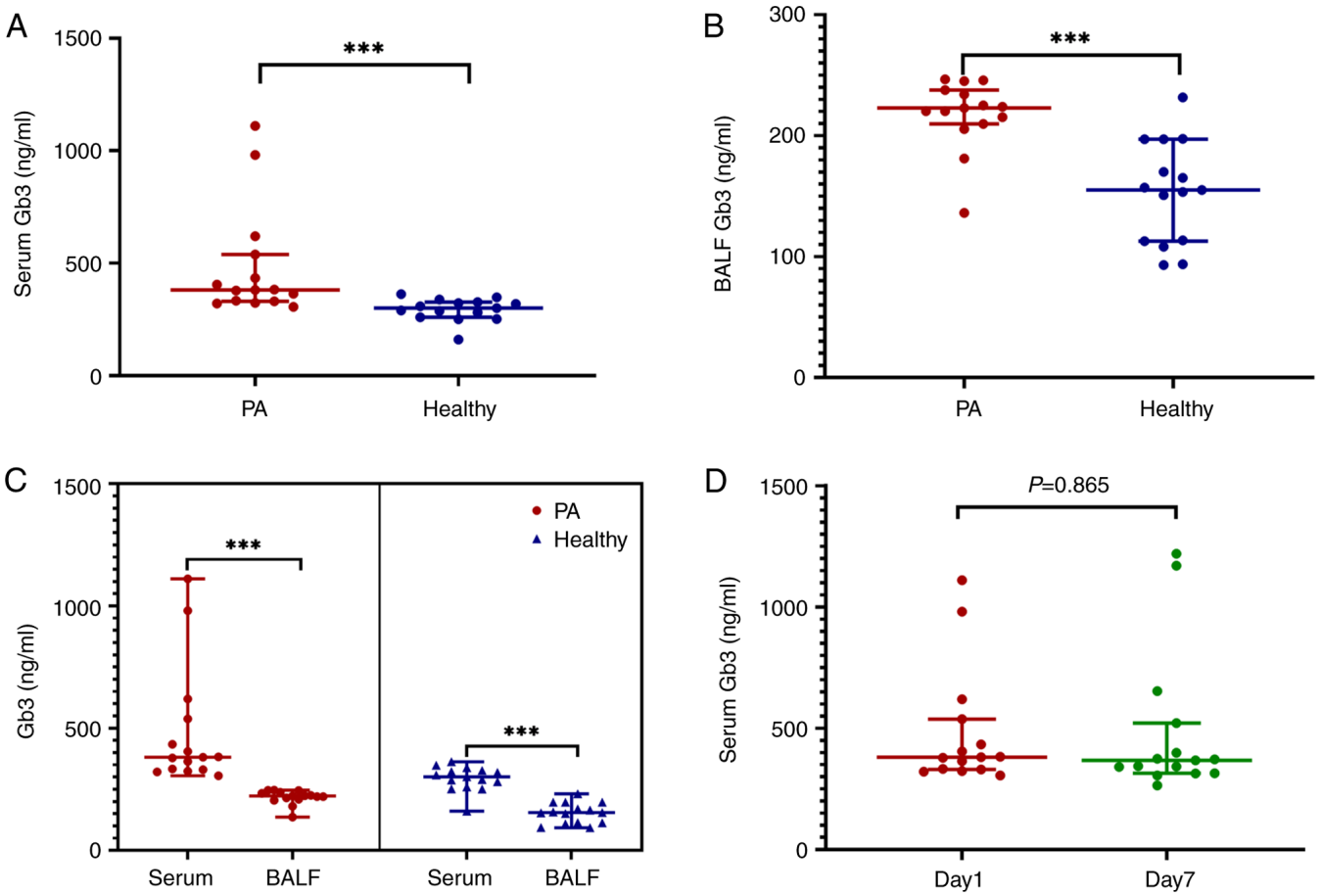

Figure 2. Comparison of serum Gb3 and BALF Gb3 levels between patients with PA induced-LRTIs and healthy individuals. (A) Serum Gb3 levels in healthy individuals and patients with PA induced-LRTIs on the first day following diagnosis. (B) BALF Gb3 levels in healthy individuals and patients with PA induced-LRTIs on the first day following diagnosis. (C) Serum and BALF Gb3 levels in healthy individuals (blue triangles) and patients with PA induced-LRTIs (red circles) on the first day following diagnosis. (D) Serum Gb3 levels in patients with PA-LRTI on the 1st and 7th days following diagnosis. ***P<0.001. Gb3, globotriaosylceramide; BALF, bronchoalveolar lavage fluid; PA, *Pseudomonas aeruginosa*; LRTIs, lower respiratory tract infections.

was observed, as well as a strong association with CRP and PCT levels in patients on day 1 post-diagnosis (Fig. 3).

Predictive value of serum and BALF Gb3 levels. Univariate logistic regression analysis identified serum Gb3 [Odds ratio (OR), 1.045; 95% confidence interval (CI), 1.009-1.083; P=0.015; Table IV] and BALF Gb3 (OR, 1.050; 95% CI, 1.016-1.085; P=0.004; Table IV) as risk factors for PA infections. Multivariate logistic regression analysis further confirmed that serum Gb3 (OR, 1.048; 95% CI, 1.010-1.087; P=0.013; Table IV) and BALF Gb3 (OR, 1.056; 95% CI, 1.016-1.098; P=0.006; Table IV) as independent risk factors for PA infections. ROC curve analysis was conducted to assess the predictive performance of serum and BALF Gb3 levels in differentiating between Groups PA and H. The AUC for serum Gb3 levels was 0.899 (95% CI, 0.814-0.955; P<0.001; Table V), with a sensitivity of 87.04% and a specificity of 80.65%. For BALF Gb3 levels, the AUC was 0.812 (95% CI, 0.711-0.889; P<0.001; Table V), with a sensitivity of 72.22% and a specificity of 73.33%.

Table V. AUC values and thresholds of different parameters for predicting the incidence of *Pseudomonas aeruginosa*-induced lower respiratory tract infections.

		95% CI					
Variable	AUC	Lower limit	Upper limit	Sensitivity (%)	Specificity (%)	Threshold	P-value
Serum Gb3, ng/ml	0.899	0.814	0.955	87.04	80.65	>322.70	<0.001
Bronchoalveolar lavage fluid Gb3, ng/ml	0.812	0.711	0.889	72.22	73.33	>165.15	<0.001

AUC, area under the curve; Gb3, globotriaosylceramide.

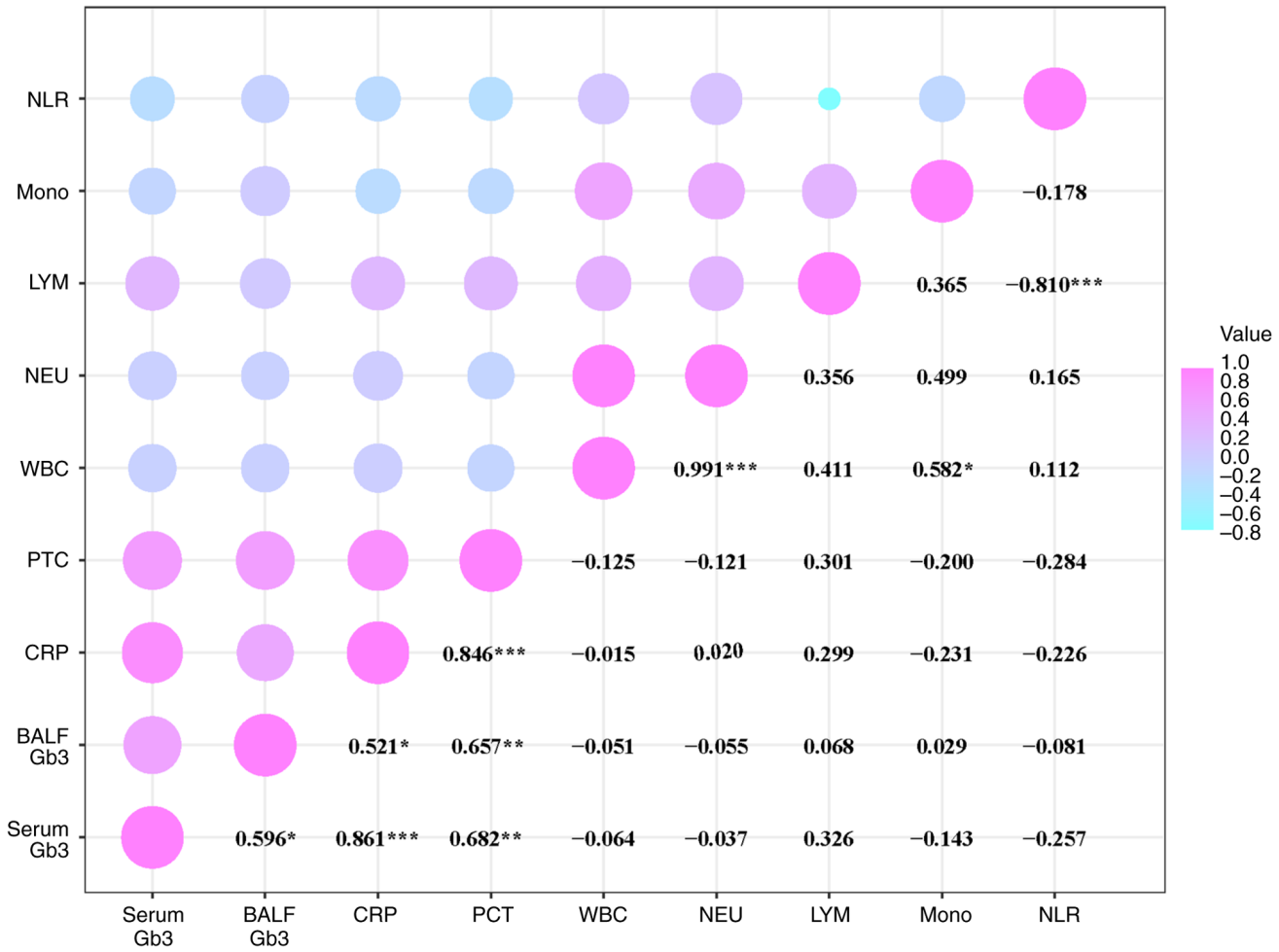

Figure 3. Correlation analysis of Gb3, CRP and PCT levels in patients with PA-LRTIs. The horizontal and vertical axes represent different variables. The numerical values correspond to the Spearman's rank correlation coefficients between the pairs of variables. The size of the bubbles reflects the magnitude of these coefficients, while the color indicates the nature of the correlation: Violet signifies a positive correlation, and blue denotes a negative correlation (*P<0.05; **P<0.01; ***P<0.001). Gb3, globotriaosylceramide; CRP, C-reactive protein; PCT, procalcitonin; PA, *Pseudomonas aeruginosa*; LRTIs, lower respiratory tract infections; BALF, bronchoalveolar lavage fluid; WBC, white blood cell count; NEU, neutrophil count; LYM, lymphocyte count; NLR, neutrophil-to-lymphocyte ratio.

Subsequently, a log-rank test was used to compare the ROC curves of patients with PA-induced LRTIs and those of healthy individuals. The combined use of serum and BALF Gb3 levels indicated the highest predictive capability (Fig. 4). Serum Gb3 alone demonstrated strong predictive ability, followed by BALF Gb3 (Fig. 4). However, the difference between serum Gb3 and BALF Gb3 levels was not statistically significant (P=0.099; Fig. 4).

Discussion

In the present study, it was found that patients with PA-induced LRTIs had significantly higher concentrations of Gb3 in both serum and BALF samples on the first day following diagnosis compared with healthy controls. This increase (notably in BALF) suggests that Gb3 may serve as a specific biomarker

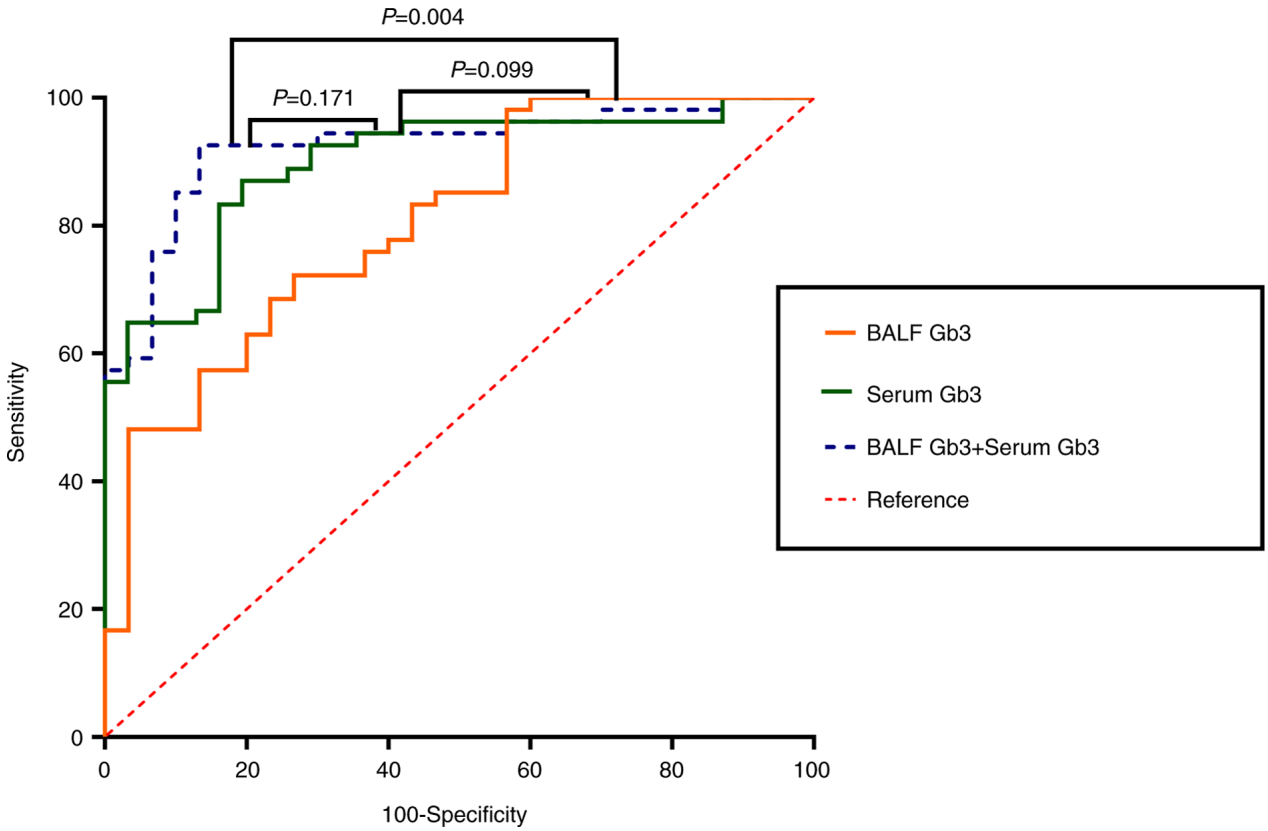

Figure 4. ROC curve analysis demonstrates the predictive performance of Gb3 in distinguishing between patients with PA-LRTI and healthy individuals. ROC, receiver operating characteristic; Gb3, globotriaosylceramide; PA, *Pseudomonas aeruginosa*; LRTI, lower respiratory tract infection; BALF, bronchoalveolar lavage fluid.

for PA-induced LRTIs. Multivariate logistic regression analysis identified Gb3 levels as independent risk factors for PA infection, highlighting the potential predictive role of Gb3 in the disease. ROC curve analysis further supported the clinical utility of Gb3, with serum levels indicating high sensitivity and specificity for PA-induced LRTIs. Although BALF Gb3 also exhibited significant predictive capacity, it was slightly less effective than serum Gb3. Notably, the combination of serum and BALF Gb3 levels provided the strongest predictive capabilities, indicating a synergistic effect in diagnosing PA-induced LRTIs.

The higher predictive ability of serum Gb3 compared with BALF Gb3 may be attributed to several factors. Gb3 is expressed on various immune cells, such as dendritic cells and macrophages, which are more prevalent in the systemic circulation than in the alveoli (28,29). During inflammatory responses, the activation and mobilization of these immune cells can lead to increased Gb3 levels in the serum, reflecting both local pulmonary and systemic immune reactions to PA infection (28,29). In contrast to these observations, BALF-based analysis of Gb3 levels is confined to the lung environment and may not reflect systemic changes (28,29). Furthermore, the bronchoalveolar lavage procedure can dilute the concentration of Gb3 in BALF due to the instillation and retrieval of fluid, potentially reducing its measurable levels and predictive capacity. The differences in Gb3 metabolism and turnover rates between the bloodstream and lung tissue may also contribute to higher and more stable Gb3 levels in serum (28,29). These factors suggest that serum Gb3 may serve as a more robust and reliable biomarker for PA-induced LRTIs compared with BALF Gb3.

Despite the slightly lower predictive ability of BALF Gb3, the elevated levels observed reinforce the association between Gb3 and PA infections in the lung environment, which is the primary site of infection. Increased Gb3 levels in the lungs may provide more binding sites for PA, promoting the initial bacterial adhesion and internalization. In addition, elevated Gb3 levels could affect the immune response of the host, potentially affecting B-cell activation and antibody production. In PA infections, high Gb3 levels may alter the immune response, impacting the ability of the body to clear the pathogen.

The marked difference in Gb3 levels between patients with PA and healthy controls suggests that Gb3 could serve as an auxiliary marker for PA-induced LRTIs. Implementing Gb3 measurement could aid in the early identification and clinical assessment of these infections, which is crucial for initiating prompt and effective treatment. Given the prognostic significance of BALF Gb3 levels, timely bronchoscopy with alveolar lavage may provide valuable insights into the infection and guiding management.

The present research study demonstrated an optimal correlation between Gb3 and these markers on the 1st day post-diagnosis, suggesting that Gb3 may reflect infection severity. CRP and PCT are general markers of inflammation commonly used to evaluate suspected infections (30-33). The

combination of Gb3 with these markers could provide a more comprehensive assessment of the initial condition of a patient.

No significant differences were observed in serum Gb3 levels between pre-treatment and post-treatment periods. This lack of change may be due to an insufficient observation time or the poor overall health of the patients. A previous study by Müller *et al* (34) reported that lysophosphatidylcholine levels significantly decrease during the acute phase of pneumonia (within 48 h), with normalization potentially taking up to 60 days post-treatment (34). The study of Nan *et al* (35) has also shown a significant association between lipid levels and infection severity, indicating that the effects of treatment on lipid levels may not be immediately apparent. In the present study, a negative correlation was observed between serum Gb3 concentrations and the changes in serum CRP and PCT levels on days 1 and 7. This suggests that high Gb3 levels are associated with a slower decrease in these inflammatory markers, potentially indicating longer recovery times.

The present study exhibits several limitations that need to be addressed prior to the applications of these findings in clinical diagnosis. The relatively small sample size, single-center design and the presence of underlying conditions in various patients may affect the generalizability of the current findings. Large-scale, multicenter clinical trials involving more diverse patient populations are necessary to validate these findings and ensure broader clinical applicability. In addition, the changes in BALF Gb3 concentration levels were not monitored over time. Understanding the temporal patterns of Gb3 levels is crucial for assessing its role as a biomarker throughout the infection process, including disease progression and response to treatment. Long-term follow-up studies are required to better elucidate these dynamics.

While it was found that Gb3 levels were significantly elevated in patients with PA-induced LRTIs, Gb3 has also been shown to be implicated in infections caused by other bacterial pathogens, such as Shiga toxin-producing *Escherichia coli* (16-19). This suggests that elevated Gb3 levels may not be exclusive to PA infections, potentially limiting the specificity of Gb3 as a biomarker for PA-induced LRTIs. Consequently, the diagnostic utility of Gb3 may be affected by its involvement in other bacterial infections and elevated Gb3 levels could indicate infections beyond PA. Future research should determine whether the increase in Gb3 levels is specific to PA-induced LRTIs or represents a general response to certain bacterial pathogens.

Finally, there is currently no standardized method for measuring Gb3 levels or established clinical cutoff values. The measurement of Gb3 requires specialized laboratory techniques and equipment, which may limit its practicality in routine clinical settings (25,26). Developing reliable, reproducible measurement techniques and determining clinically relevant thresholds are essential steps prior to the implementation of Gb3 as a diagnostic biomarker. By acknowledging these limitations, the present study aims to guide future research efforts to address these challenges and facilitate the practical application of Gb3 in diagnosing PA-induced LRTIs.

In conclusion, the present study highlights a significant association between elevated levels of Gb3 and PA-induced LRTIs. Patients with PA-induced LRTIs exhibited significantly higher Gb3 concentration levels in both serum and BALF compared with those of healthy controls, with serum Gb3 levels indicating the highest predictive capability. These findings suggest that Gb3 may serve as a promising biomarker for the early detection and diagnosis of PA-induced LRTIs. Although Gb3 indicates a potential as a diagnostic tool, further research is necessary to validate its specificity for PA infections and to overcome current limitations prior to its clinical application. By addressing these challenges, Gb3 can contribute to improved diagnostic accuracy and patient outcomes in PA-induced LRTIs.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

LY conceived the study and supervised the research. TX drafted the manuscript. JL, YD and ZZ acquired and analyzed the data. TX, JL, YD and ZZ participated in data interpretation. TX and LY confirm the authenticity of all the raw data. All authors provided critical revision of the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study received the ethics approval (approval no. 2024-045) of biomedical research involving humans from the Ethics Committee of The Affiliated People's Hospital of Ningbo University (Ningbo, China). Written informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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