Journal of Inflammation Research Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Immune Markers and Inflammatory Cytokines in Granulomatous Lobular Mastitis: A Case-Control Study

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Purpose: Granulomatous lobular mastitis (GLM) has seen a rising incidence, though its pathogenesis remains unclear, posing challenges for treatment and contributing to high recurrence rates with conventional therapies. While the role of inflammatory and immune factors in GLM has been recognized, a comprehensive clinical evaluation of these markers is still lacking. This study aims to identify potential diagnostic markers and therapeutic targets by comparing immune markers and cytokine levels in GLM patients and healthy controls.

Methods: Conducted at Beijing Hospital of Traditional Chinese Medicine, Capital Medical University from July 2023 to May 2024, this study enrolled 30 GLM patients and 15 healthy female controls in a 2:1 ratio. Serum levels of immune markers and cytokines were analyzed to explore their potential association with GLM.

Results: The study population comprised 30 GLM patients with a mean age of 33.40 ± 4.12 years and 15 healthy female controls with a mean age of 32.13 ± 6.19 years. Significantly elevated levels of C-reactive protein (CRP), Immunoglobulin A (IgA), Complement Component 3 (C3), Complement Component 4 (C4), Compliment Component 1q (C1q), Alpha1-antit-rypsin (AAT), α1-acidglycoprotein (AGP), Anti-histone antibodies (Anti-HIS), Anti-Ro52 antibodies (Anti-Ro52), Anti-double stranded DNA antibodies (Anti-dsDNA), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Tumor Necrosis Factor-α (TNF-α) were observed in GLM patients compared to controls (all $P < 0.05$). Subgroup analysis revealed higher levels of CRP, C3, C1q, AAT, and AGP in patients with larger mass areas and those with erythema nodosum (all $P < 0.05$). No significant differences were found in subgroups based on disease duration or recurrence (both $P > 0.05$).

Conclusion: Serum levels of CRP, IgA, AAT, AGP, Anti-HIS, Anti-Ro52, Anti-dsDNA, C3, C4, C1q, IL-6, IL-10, and TNF-α may serve as diagnostic and prognostic indicators for GLM, with CRP, AAT, AGP, and C1q being particularly indicative of disease severity. These markers offer potential therapeutic targets for GLM.

Keywords: granulomatous lobular mastitis, peripheral serology, immune markers, cytokine

Introduction

Granulomatous mastitis is a chronic inflammatory disease centered on breast lobules with non-caseating necrotizing granulomas as the main pathological feature.[1,2] Despite extensive investigation, the precise etiology of GLM remains elusive, and its pathogenesis is not yet fully understood. Recent research has suggested various potential risk factors, including autoimmune dysregulation, nipple inversion, hormonal imbalances, and localized breast trauma.[3–5] These factors are hypothesized to trigger a type IV hypersensitivity reaction, leading to an imbalance in immune markers and inflammatory mediators. Consequently, treatment approaches include the use of immunosuppressive agents,[6] corticosteroid therapy,[7] and medications that target specific inflammatory pathways.[8–10] However, the key immune markers and cytokines involved in GLM have yet to be clearly identified. The majority of existing literature is based on animal models or limited by retrospective clinical studies, often lacking healthy control groups. To address these limitations, the

Received: 13 September 2024
Accepted: 4 November 2024
Published: 11 November 2024

current study enrolled 30 female GLM patients and 15 healthy female controls in a 2:1 ratio. Both groups underwent assessment of immune markers (IM) and inflammatory cytokines (CK). The IM included complement levels, antinuclear antibodies, immunoglobulins, acute-phase proteins, and rheumatoid factors, while cytokines assessed included interleukins, interferons (INF), and tumor necrosis factors (TNF). This systematic evaluation aimed to identify differences in immunoserology and cytokines between GLM patients and healthy individuals, thereby providing potential diagnostic markers and therapeutic targets for GLM. Futhermore, subgroup analyses of GLM patients with clinical symptoms were conducted to explore the correlation between serum immune markers, cytokines, and disease severity.

Materials and Methods

Patients

The study group consisted of 30 patients with a confirmed diagnosis of granulomatous lobular mastitis (GLM) who visited the Galactophore Department in Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, between July 2023 and May 2024. A control group of 15 healthy female medical examiners was also selected during the same period. The study adhered to the principles of the Declaration of Helsinki, was approved by the Medical Ethics Committee of Beijing Hospital of Traditional Chinese Medicine, Capital Medical University (Approval No. 2023BL02-076-01), and all participants provided informed consent.

Inclusion Criteria

(1) disease onset within the past three months, (2) female gender, (3) confirmation of GLM diagnosis based on pathological findings, and (4) availability of complete clinical data.

Exclusion Criteria

(1) lactating and pregnant women, (2) individuals who had received antibiotic or glucocorticoid treatment in the preceding month, (3) presence of cardiovascular, endocrine, hepatic, renal, hematopoietic, or other systemic diseases, (4) incomplete clinical data pertinent to the study, (5) other autoimmune diseases, and (6) co-occurrence with breast malignant tumors.

Research Methods involved fasting blood sampling in the morning from both groups for serological assessment of immune markers and cytokines.

Healthy Control Group

The healthy control group consisted of female participants who met the following criteria: (1) no history of breast disease, including mastitis or any breast-related conditions; (2) no autoimmune or chronic inflammatory conditions; (3) no active or recent infections (within the past month); (4) no use of immunosuppressive or corticosteroid medications; and (5) good overall health, as confirmed by medical history and clinical examination.

Research Methods

Fasting blood samples were collected in the morning from both groups for serological assessment of immune markers and cytokines. The immune markers and cytokines were detected as follows:

Immune markers: C-reactive protein (CRP), Anti-Streptolysin O (ASO), Immunoglobulin M (IgM), Immunoglobulin-E (IgE), Complement Component 3 (C3), Complement Component 4 (C4), Alpha1-antitrypsin (AAT), Immunoglobulin G (IgG), Immunoglobulin A (IgA), α1-acid glycoprotein (AGP), Complement Component 1q (C1q), Antinuclear antibodies (ANA), Antineutrophil Cytoplasmic antibodies (ANCA), Anti-double stranded DNA antibodies (Anti-dsDNA), Anti-ribonucleoprotein antibodies (Anti-nRNP), Anti-Sm antibodies (Anti-Sm), Anti-SSA antibodies (Anti-SSA), Anti-SSB antibodies (Anti-SSB), Anti- Sc1-70 antibodies (Anti-Sc1-70), Anti-Jo-1 antibodies (Anti-Jo-1), Anti-ribosomal P-protein antibodies (Anti-rRNP), Anti-mitochondrial M2 antibodies (Anti-AMA-M2), Anti-histone antibodies (Anti-HIS), Anti-Centromere Protein-B antibodies (Anti-CENP-B), Anti-Prolife-rating Cell Nuclear Antigen antibodies (Anti-PCNA), Anti-nucleosome antibodies (Anti-ANuA), Anti-Ro52 antibodies (Anti-Ro52), Anti-PM-Sc1 antibodies (Anti-PM-Sc1).

Cytokines:Interleukin-1beta (IL-1β), Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleu-kin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-12p70 (IL-12p70), Inte-rleukin-17 (IL-17), Interferon-α (INF-α), Interferon-γ (INF-γ), Tumor Necrosis Factor-α (TNF-α).

The normal ranges of immune markers and inflammation cytokines can be found in Supplementary material 1. If the value of a indicator was lower than the minimum detection limit, the value of the lower limit of detection was taken (eg, if IL-1β < 3.0, we recorded IL-β = 3.0).

Statistical Methods

The selection of statistical methods is determined by the nature of the data. Normally distributed continuous variables were analyzed using the independent samples *t*-test to assess differences between groups. When data deviate from normality, the non-parametric Wilcoxon rank-sum test (equivalent to the Mann–Whitney *U*-test) is applied. Normally distributed data were reported as the mean ± standard deviation $(\bar{x} + s)$ whereas non-normally distributed data were described using the median (Q2) and interquartile range (IQR) to indicate central tendency and dispersion. Categorical binary data were analyzed using the chi-square test. Data analysis was performed using SPSS software, Version 26.0 (IBM Corp). All p-values reported are two-tailed, with statistical significance defined as $P < 0.05$.

Results

General Information

The study enrolled 45 female participants, with 30 allocated to the GLM group, having a mean age of 33.40 ± 4.12 years, and 15 allocated to the healthy control group, having a mean age of 32.13 ± 6.19 years. Comprehensive data on the health status of the GLM group and the characteristics of their breast conditions are detailed in Tables 1 and 2, respectively.

Table 1 General Characteristics of the GLM Group

Variables	Percentage (%)
Duration of illness (days)	46.47±4.53
Body Mass Index (≥24)	50.00
Lactation obstacles	6.67
Menstrual regularity	16.67
History of breast trauma	26.67
History of non-lactating mastitis	16.67
Prolactin elevation	3.45
Oral sex hormone drugs within three months	3.33

Table 2 Clinical Manifestations of the GLM Group

Variables	Percentage (%)
Breast lumps	100
Redness and swelling of the breast	73.33
Size of the breast lump (cm^2)	44.61±33.28
The extent of breast erythema (cm^2)	29.69±28.79
Changes in skin appearance	73.33
Breast abscess	23.33
Breast ulcer	13.33
Breast pain (%, Score)	100, (4.63±1.85)
Nipple discharge	16.67
Nipple reversion	16.67
Erythema nodosum	13.33

IM and CK Comparison Between GLM Patients and Healthy Controls

In our analysis of the immune markers, levels of CRP, IgA, C3, C4, C1q, AAT, AGP, Anti-HIS, Anti-Ro52, and Anti-dsDNA were significantly higher in GLM patients than in healthy controls ($P < 0.05$, Table 3 and Supplementary Figures A-B). Similarly, IL-6, IL-10, and TNF-α were significantly elevated in GLM patients compared to the control group ($P < 0.05$, Table 4, and Supplementary Figure C).

GLM Subgroup Analysis by Symptoms

To address the immune and cytokine indicators that are significantly different between GLM patients and normal population, we conducted a subgroup analysis of GLM patients using four criteria: 1) large vs small GLM mass size; 2) presence vs absence of erythema nodosum; 3) long vs short disease duration in GLM long/short; and 4) Primary vs recurrent GLM.

In the comparison between the large and small GLM mass groups, significant differences were observed in CRP, C3, C1q, AAT, AGP levels in the large mass group compared to the small mass group ($P<0.05$, Table 5 and Supplementary Figure D). The group with erythema nodosum showed statistically significant differences in in CRP, C1q, AAT, AGP, and Anti-dsDNA levels compared to the group without erythema nodosum ($P<0.05$, Table 6 and Supplementary Figure E). No significant differences were observed between the long and short disease duration groups or the GLM primary and recurrent GLM groups across all evaluated indicators ($P>0.05$, Tables 7, 8 and Supplementary Figure F-G).

Table 3 IM Comparison Between GLM Patients and Healthy Controls

Variables	GLM Patients Q2 (Q1, Q3)	Healthy Controls Q2 (Q1, Q3)	P-value
CRP	10.60(3.15,30.58)	0.60(0.20,1.30)	0.000*
ASO	82.45(34.28,111.88)	53.90(31.10,152.20)	0.800
IgM	1.66(1.26,1.97)	1.50(1.00,1.58)	0.159
IgE	46.20(21.00,213.05)	29.20(8.80,87.00)	0.233
C3	1.41(1.24,1.91)	0.89(0.82,1.09)	0.000*
C4	0.27(0.24,0.31)	0.17(0.15,0.21)	0.000*
AAT	150.85(138.48,176.03)	113.10(109.00,132.30)	0.000*
IgG	13.15±2.90	12.16±1.37	0.125
IgA	2.55±0.90	1.90±0.44	0.012*
AGP	137.39±53.01	63.97±14.65	0.000*
C1q	263.60±57.88	212.47±49.03	0.005*
ANA(P/T)	6/30	1/15	0.245
ANCA(P/T)	1/30	0/15	0.475
Anti-dsDNA	10.00(10.00,10.00)	10.00(10.00,22.08)	0.030*
Anti-nRNP	2.00(1.00,2.25)	2.00(1.00,2.00)	0.662
Anti-Sm	2.00(1.00,2.00)	2.00(0.00,2.00)	0.908
Anti-SSA	2.00(1.00,2.00)	2.00(1.00,3.00)	0.278
Anti-SSB	0.75(1.00,2.00)	2.00(0.00,4.00)	0.474
Anti-Sc1-70	2.00(1.00,2.00)	2.00(1.00,2.00)	0.667
Anti-Jo-1	1.00(0.00,2.00)	2.00(0.00,3.00)	0.164
Anti-rRNP	1.00(0.00,2.00)	1.00(0.00,2.00)	0.781
Anti-AMA-M2	2.00(2.00,4.00)	2.00(2.00,3.00)	0.369
Anti-HIS	0.00(0.00,2.00)	1.00(0.00,2.00)	0.044*
Anti-CENP-B	2.00(1.00,2.00)	2.00(1.00,2.00)	0.713
Anti-PCNA	2.00(0.00,2.00)	2.00(1.00,3.00)	0.422
Anti-ANuA	2.00(0.00,2.25)	2.00(1.00,2.00)	0.990
Anti-Ro52	2.00(1.00,3.00)	1.00(0.00,2.00)	0.012*
Anti-PM-Sc1	2.00(1.00,2.00)	2.00(1.00,3.00)	0.486

Notes: P/T: Positive people/Total Number of People; *: Compared with GLM Patients and Healthy Controls, P*<0.05.

Table 4 CK Comparison Between GLM Patients and Healthy Controls

Variables	GLM Patients Q2 (Q1, Q3)	Healthy Examiners Q2 (Q1, Q3)	P-value
IL-1β	3.00 (3.00,3.53)	3.00 (3.00,3.30)	0.537
IL-2	4.00 (4.00,4.00)	4.00 (4.00,4.00)	0.157
IL-4	3.00 (3.00,3.25)	4.60 (3.10,5.20)	0.211
IL-5	3.00 (3.00,3.30)	3.90 (3.00,5.50)	0.077
IL-6	3.40 (2.40,5.25)	2.20 (2.00,2.80)	0.003*
IL-8	3.00 (3.00,16.2)	3.60 (3.00,10.20)	0.548
IL-10	3.00 (3.00,3.30)	3.00 (3.00,3.30)	0.037*
IL-12p70	4.00 (4.00,4.00)	4.00 (4.00,4.90)	0.170
IL-17	5.00 (5.00,6.10)	5.00 (5.00,7.20)	0.379
INF-α	5.00 (5.00,5.00)	5.00 (5.00,5.00)	0.157
INF-γ	5.00 (5.00,5.00)	5.00 (5.00,5.00)	0.157
TNF-α	4.00 (4.00,4.00)	4.00 (4.00,5.10)	0.006*

Notes: *: Compared with GLM Patients and Healthy Controls P*<0.05.
Abbreviation: CK, Cytokine.

Table 5 IM and CK Comparison Between GLMSL and GLMBL Patients

	Variables	GLMSL Patients (n=15) Q2 (Q1, Q3)	GLMBL Patients (n=15) Q2 (Q1, Q3)	P-value
IM	CRP	5.40 (1.45,16.20)	20.60 (6.85,46.50)	0.028*
	C3	1.30 (1.14,1.47)	1.65 (1.47,2.01)	0.007*
	C4	0.26 (0.22,0.33)	0.28 (0.25,0.30)	0.438
	C1q	238.15±52.75	289.05±52.63	0.013*
	IgA	2.35±0.74	2.75±1.03	0.232
	AGP	102.11±28.76	172.68±48.22	0.000*
	AAT	139.81±21.78	181.82±34.39	0.000*
	Anti-dsDNA	10.00 (10.00,10.00)	10.00 (10.00,16.90)	0.133
	Anti-HIS	0.00 (0.00,1.00)	0.00 (0.00,2.00)	0.569
	Anti-Ro52	2.00 (2.00,2.00)	2.00 (1.00,4.00)	0.825
CK	IL-6	2.50 (2.40,5.70)	4.00 (2.90,5.10)	0.123
	IL-10	3.00 (3.00,3.30)	3.00 (3.00,3.30)	0.309
	TNF-α	4.00 (4.00,4.00)	4.00 (4.00,4.00)	0.382

Notes:*: Compared between GLMSL and GLMBL Patients, P<0.05.
Abbreviations: IM, Immune Markers; CK, Cytokine; GLMSL, Granulomatous Lobular Mastitis Small Lump; GLMBL, Granulomatous Lobular Mastitis Big Lump.

Discussion

Recent Research on GLM Immune Markers and Cytokines

Granulomatous Lobular Mastitis (GLM) is widely recognized as an inflammatory disorder with significant immune involvement. However, studies specifically focusing on immune markers and cytokines in GLM remain limited. Our literature review identified only 10 clinical studies that examined immune markers and cytokines in the peripheral serum of GLM patients (Table 9). Among these, five were retrospective studies, which are susceptible to information bias. The other five prospective studies analyzed fewer than 15 immune markers or cytokines per study, potentially leading to an incomplete representation of GLM-related biomarkers. Notably, none of the prospective studies conducted subgroup analyses.

This study introduces three key innovations: 1. Prospective Case-Control Study Design: By selecting GLM patients in acute phases and comparing them to healthy controls, we minimized limitations such as missing data and less rigorous

Table 6 IM and CK Comparison Between GLMWOEN and GLMWEN Patients

	Variables	GLMWOEN Patients (n=26) Q2 (Q1, Q3)	GLMWEN Patients (n=4) Q2 (Q1, Q3)	P-value
IM	CRP	7.95 (2.55,23.00)	39.40 (18.48,59.80)	0.024*
	C3	1.36 (1.21,1.76)	1.79 (1.59,1.99)	0.106
	C4	0.26 (0.24,0.30)	0.30 (0.26,0.34)	0.343
	C1q	238.15±52.75	289.05±52.63	0.013*
	IgA	2.35±0.74	2.75±1.03	0.232
	AGP	102.11±28.76	172.68±48.22	0.007*
	AAT	139.81±21.78	181.82±34.39	0.002*
	Anti-dsDNA	10.00 (10.00,10.00)	24.04 (13.22,28.93)	0.000*
	Anti-HIS	0.00 (0.00,1.25)	1.00 (0.00,2.00)	0.485
	Anti-Ro52	1.00 (2.00,2.25)	3.00 (1.25,52.00)	0.315
CK	IL-6	3.60 (2.40,5.90)	2.960 (2.53,4.63)	0.830
	IL-10	3.00 (3.00,3.30)	3.00 (3.00,3.30)	0.409
	TNF-α	4.00 (4.00,4.00)	4.00 (4.00,4.00)	0.695

Notes: *: Compared between GLMWOEN and GLMWEN Patients, P<0.05.FN.
Abbreviations: IM, Immune Markers; CK, Cytokine; GLMWOEN, Granulomatous Lobular Mastitis without Erythema Nodosum; GLMWEN, Granulomatous Lobular Mastitis with Erythema Nodosum.

Table 7 IM and CK Comparison Between GLMLC>1M and GLMSC≤1M Patients

	Variables	GLMLC>1M Patients (n=14) Q2 (Q1, Q3)	GLMSC≤1M Patients (n=16) Q2 (Q1, Q3)	P-value
IM	CRP	8.45 (2.48,21.78)	11.70 (4.70,47.95)	0.280
	C3	1.38 (1.26,1.61)	1.54 (1.18,2.02)	0.371
	C4	0.26±0.05	0.29±0.09	0.414
	C1q	255.46±49.59	270.72±65.04	0.481
	IgA	2.44±0.79	3.26±1.35	0.493
	AGP	122.08±50.75	150.80±52.81	0.141
	AAT	150.85 (133.45,163.55)	154.30 (141.52,202.03)	0.228
	Anti-dsDNA	10.00 (10.00,12.08)	10.00 (10.00,10.00)	0.481
	Anti-HIS	0.00 (0.50,2.00)	0.00 (0.00,1.00)	0.274
	Anti-Ro52	2.00 (1.00,2.00)	2.00 (1.25,3.75)	0.250
CK	IL-6	3.10 (2.55,7.78)	3.65 (2.40,4.90)	0.545
	IL-10	3.00 (3.00,3.00)	3.00 (3.00,3.30)	0.888
	TNF-α	4.00 (4.00,4.00)	4.00 (4.00,4.00)	0.285

Abbreviations: IM, Immune Markers; CK, Cytokine; GLMLC >1M, Granulomatous Lobular Mastitis Long Course >1 Month; GLMSC ≤1M, Granulomatous Lobular Mastitis Short Course ≤1 Month.

case selection often encountered in retrospective studies. 2. Comprehensive Detection of Immune Markers: This study includes a broader array of immune markers and cytokines than any previous study on GLM, making this the most extensive serological analysis to date. This comprehensive approach significantly enriches our understanding of GLM's immune and cytokine profiles and provides a stronger foundation for future research into its pathophysiology. 3. Subgroup Analysis: Building on positive results from the healthy female control group, we further enriched the study by conducting subgroup analyses of GLM patients to explore the relationship between immune markers, cytokines, and the severity of the disease.

Clinical Manifestations of the GLM

The clinical characteristics observed in this study, such as breast injury history (8/30, 26.67%), nipple inversion (5/30, 16.67%), breastfeeding disorders (2/30, 6.67%), and nipple discharge (5/30, 16,67%), were consistent with the risk

Table 8 IM and CK Comparison Between PGLM and RGLM Patients

	Variables	PGLM Patients (n=25) Q2 (Q1, Q3)	RGLM Patients (n=5) Q2 (Q1, Q3)	P-value
IM	CRP	13.70 (3.00,30.95)	7.20 (2.60,32.45)	0.676
	C3	1.54±0.36	1.44±0.35	0.591
	C4	0.26 (0.24,0.30)	0.28 (0.22,0.39)	0.503
	C1q	263.53±48.94	263.96±99.60	0.993
	IgA	2.53±0.93	2.62±0.84	0.852
	AGP	143.03±56.16	109.22±16.11	0.198
	AAT	163.96±37.44	145.10±18.08	0.285
	Anti-dsDNA	10.00 (10.00,10.00)	10.00 (10.00,10.00)	0.284
	Anti-HIS	0.00 (0.00,2.00)	1.00 (0.00,1.50)	0.657
	Anti-Ro52	2.00 (1.00,2.50)	2.00 (2.00,3.00)	0.314
CK	IL-6	3.60 (2.50,5.80)	2.40 (2.20,4.85)	0.229
	IL-10	3.00 (3.00,3.30)	3.00 (3.00,3.05)	0.742
	TNF-α	4.00 (4.00,4.00)	4.00 (4.00,4.00)	0.665

Abbreviations: IM, Immune Markers; CK, Cytokine; PGLM, Primary Granulomatous Lobular Mastitis; RGLM, Recurrent Granulomatous Lobular Mastitis.

Table 9 Previous Peripheral Cytokine or Immune Markers Studies of GLM

Research	Research Type	Experimental Group	Control Group	Sample Size	Laboratory Indicators	Positive Indicators (P<0.05)
1	RS	GLMA	GLMNA	85/41	WBC, CRP, ESR, NEU%, IL-4, IL-6, IL-10, IL-17, INF-α	WBC, NEU%, CRP, ESR, IL-10, IL-6, IL-4, IL-17, INF-α
2	RS	GLM	BBT	73/60	NEU, NEU%, WBC, CRP, IL-6, IL-10, IL-17, INF-α, IL-β1, IFN-γ	WBC, NEU, CRP, IL-6, TNF-α
3	RS	GLM	HC	11//7	TNF-α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, IL-22, IL-23, GM-CSF, CRP	TNF-α, IL-1β, IL-2, IL-6, IL-12p70, IL-17A, CRP
4	RS	RGLM	PGLM	32/48	CRP, ESR, NLR, PLR, WBC	NLR, CRR
5	RS	GLM/PCM	BF	40/40/40	C3, C5, C3a, C5a	C3a, C5a
6	PS	GLM (A/R)	HC	32(19/13)/18	IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23, IL-33	IL-1β, TNF-α, IL-10, IL-18
7	PS	GLM (A/R)	HC	21(11/10)/12	TT, HT, CyT, NK, RT, MS, WBC, Lym, Eos, NEU, NLR, NCM	NEU, Eos, NLR, CD4 Bregs:, CD25 Bregs:, CD127 Bregs:, MS
8	PS	GLM	HC	26/15	IL-17, IL-22, IL-23	IL-22, IL-23
9	PS	GLM (A/R)	HC	47(21/26)/30	IL-4, IL −8, IL −10, IL −17, TNF-α	IL-8, IL-10, IL-17
10	PS	GLM (A/R)	HC	47(21/26)/26	CD3CD4CD45RAFoxp3++-high aTregs, CD3CD4CD45RAFoxp3 ++-low non-suppressive Tregs, CD3CD4CD45RAFoxp3+++low rTregsC,CD3CD4CD25Foxp3 Teff, Tregs, Bregs	CD3CD4CD45RAFoxp3++-lownon-suppressive Tregs, aTregs

Note: Research details can be found in Supplementary Material 2.

Abbreviations: GLMA, GLM abscess; GLMNA, GLM non-abscess; A, active; R, remission; PS, Prospective study; RS, Retrospective study; HC, Healthy Controls; PCM, Plasma cell mastitis; BBT, Benign breast tumor; BF, Breast fibroadenoma; ESR, Erythrocyte sedimentation rate; WBC, White blood cell; NEU, Neutrophil; GM-CSF, Granulocyte–macrophage colony-stimulating factor; MCP-1, Monocyte chemotactic protein −1; NLR, Neutrophil–lymphocyte ratio; PLR, Platelet-lymphocyte ratio; Eos, Eosinophil; NCM, Non-classical monocyte; Lym, Lymphocyte; TT, Total T cells; HT, Helper T cells; CT, Cytotoxic T cells; NK, Natural killer cells; Tregs, Regulatory T cells; MS, Monocyte subtypes; CD, Cluster of differentiation; Tregs, Regulatory T cells; aTregs, Activated Tregs; rTregs, Resting Tregs; Bregs, Regulatory B cells; Teff, Effector T cells.

factors for GLM reported in the previous literature. These risk factors include the gestational period, breastfeeding disorders, non-medical massage, blunt trauma, nipple discharge, and nipple inversion.[3]

The clinical manifestations of GLM observed in this study align with those reported in most of the literature.[11,12] All patients in this study presented with breast lumps (100%) and breast pain (100%), and a majority had breast erythema (73.33%). A smaller number of patients exhibited breast abscesses (23.33%), erythema nodosum (13.33%), and breast ulcers (13.33%). Since the selected patients were within three months of disease onset, their clinical manifestations were more severe, which may explain the higher incidence of erythema and erythema nodosum compared to other reports.

GLM Diagnosis, Disease Evaluation, and Prognostic Marker

In our study, we found that IL-6, IL-10, TNF-α, C3, C4, C1q, CRP, IgA, AAT, AGP, Anti-HIS, Anti-Ro52, and Anti-dsDNA levels were significantly higher in patients with GLM compared to healthy controls ($P<0.05$). These biomarkers play a crucial role in diagnosing GLM, assessing disease activity, evaluating prognosis, and monitoring treatment efficacy. In our subgroup analysis of GLM patients, individuals with more severe clinical manifestations—such as larger mass sizes and erythema nodosum—showed significantly higher levels of CRP, AAT, AGP, and C1q compared to those with milder symptoms ($P<0.05$). This elevation likely reflects the intrinsic immune response, where CRP, AAT, and AGP act as sensitive indicators of inflammation. Meanwhile, C1q, a key initiator of the classical complement pathway, indicates heightened immune activation during the early stages of disease onset and progression. These biomarkers, therefore, may serve as valuable indicators of both disease severity and treatment response in GLM.

Elevated levels of immune markers and cytokines in GLM play dual roles: they not only indicate the onset, progression, and severity of the disease but also provide insight into its underlying pathogenesis. The recognition of GLM as an autoimmune inflammatory disorder has gained broader acceptance in recent years.[13] Benson's research group proposed a pathogenic pathway for GLM, suggesting that early degeneration of mammary ducts leads to the accumulation of secretions, resulting in ductal lumen expansion and epithelial damage. Subsequently, This, in turn, results in the extravasation of ductal contents into the surrounding lobular connective tissue, triggering local inflammation. Lymphocytes and macrophages then migrate to the periductal area, initiating a granulomatous response and the formation of non-caseating granulomas.[14] Despite these findings, the specific immunological and cytokine factors driving this process have yet to be fully elucidated.

Human immunity encompasses both innate and adaptive branches, with the latter further divided into cellular and humoral immunity. Our study highlights the significant roles of both innate and adaptive immune responses in GLM. In the innate immune response, CRP (C-reactive protein), AAT (alpha-1-antitrypsin), and AGP (alpha-1-acid glycoprotein) are particularly influential. In the realm of adaptive immunity, humoral factors predominate, including complement components C3, C4, and C1q, as well as autoantibodies such as Anti-HIS, Anti-Ro52, Anti-dsDNA, and the immunoglobulin IgA.

Innate immunity represents the body's initial line of defense. Acute phase proteins like CRP, AAT, and AGP play pivotal roles in this process. CRP recognizes and binds to pathogens and apoptotic cells, facilitating their clearance. AAT contributes to anti-inflammatory and tissue-protective effects by inhibiting proteases and reducing the release of inflammatory mediators.[15] AGP, on the other hand, modulates the immune response through the regulation of immune cell function and cytokine activity. Although these proteins operate through distinct mechanisms, they collectively aim to regulate the immune response, curb excessive inflammation, and protect against tissue damage.

The complement system serves as a critical link between innate and adaptive immunity. In this study, elevated levels of C1q, C3, and C4 in GLM patients indicate the activation of the classical complement pathway, a mechanism implicated in the development and progression of GLM, as demonstrated in previous research.[16,17] The increased levels of these complement components facilitate pathogen recognition and clearance, and they significantly contribute to the regulation of immune homeostasis, including processes such as autophagy.[18]

IgA is a pivotal antibody within the immune system, particularly abundant in exocrine fluids such as breast milk. In the event of milk duct rupture and subsequent secondary immunization, IgA can neutralize pathogens by inhibiting adhesion, enhancing phagocytosis, and acting as an immune exclusion agent through antigen binding. Anti-HIS, Anti-Ro52, and Anti-dsDNA are components of humoral immunity, produced by B cells to recognize and bind specific

antigens: Anti-Histidyl-tRNA Synthetase, Anti-Ro52, and double-stranded DNA, respectively. These antibodies have been linked to conditions like dermatomyositis, Sjögren's syndrome, and systemic lupus erythematosus.[19,20] The presence of elevated antinuclear antibodies in GLM patients represents a novel finding in the international literature, as they have not previously been associated with GLM. The potential for antigen presence in GLM patients to increase related antibodies in serum merits further investigation as a direction for future research.

Cytokines

Cytokines, a group of proteins or small peptides with immunoregulatory and effector functions, play a crucial role in the diagnostics and treatment of inflammatory and autoimmune diseases.[21] Our study indicates a correlation between the cytokines IL-6, IL-10, and TNF-α and the development of GLM. IL-6 and TNF-α are pro-inflammatory cytokines, driving acute inflammatory responses and influencing T cell differentiation, while IL-10 serves as an anti-inflammatory cytokine, suppressing inflammation. IL-6 stimulates B cell antibody production, and TNF-α enhances the maturation and function of antigen-presenting cells. The IL-6/JAK2/STAT3 pathway, which is implicated in promoting epithelial-mesenchymal transition in response to inflammation, has been shown to exert pro-inflammatory effects in non-lactating mastitis.[10] TNF-α is known to induce mastitis via the NF-κB signaling pathway.[22] IL-10 regulates macrophage activity to mitigate inflammatory responses and maintains immune tolerance by suppressing effector T cell functions in adaptive immunity.

Interconnections Between Immune Markers and Cytokines

Beyond their individual roles, immune markers are interconnected, shaping both innate and adaptive immunity as well as the broader immune landscape. CRP, for example, activates the classical complement pathway, enhancing pathogen clearance. IL-6 and TNF-α exhibit mutual reinforcement,[23,24] while IL-10 suppresses their production, modulating immune responses and curbing excessive inflammation.[25] AAT regulates cytokines, including IL-6 and TNF-α,[26] and both TNF-α and IL-6 stimulate AGP production, which can further amplify the inflammatory response.[27] These interactions underscore the complexity of immune regulation in GLM and highlight the need for further research into their clinical significance.

Conclusion

This study identified serum levels of CRP, IgA, AAT, AGP, Anti-HIS, Anti-Ro52, Anti-dsDNA, C3, C4, C1q, IL-6, IL-10, and TNF-α as potential diagnostic, prognostic, and disease assessment markers for GLM. Among them, elevated levels of CRP, AAT, AGP, and C1q were significantly associated with increased disease severity, indicating their utility not only as diagnostic markers but also as potential therapeutic targets. However, the small sample size in certain subgroups represents a limitation of this study, potentially affecting the generalizability of the findings. Therefore, larger cohort studies are urgently needed to validate and extend these results, providing deeper insights into therapeutic strategies for GLM.

Data Sharing Statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics Declarations

The studies involving human participants were approved by the Ethics Committee of Beijing Hospital of Traditional Chinese Medicine. The studies were conducted in accordance with local legislation and institutional requirements. All participants provided written informed consent to participate in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This study was supported by the Young Doctor Scholar Project (2022), Capital Research and Transformation of Clinical Diagnosis and Treatment Technology (Z211100002921020), the Special Project on Traditional Chinese Medicine (TCM) Heritage of Ancient Books, Literature, and Distinctive Techniques (GZY-KJS-2022-035), Beijing municipal hospital research and cultivation program (PZ2022009), Scientific research Training Fund of Capital Medical University (PYZ22162) and Beijing science and technology development fund project of traditional Chinese medicine (BJZYQN-2023-08).

Disclosure

The authors declare no conflicts of interest.

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