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ORIGINAL RESEARCH

Serum IFN-γ Predicts the Therapeutic Effect of Belimumab in Refractory Lupus Nephritis Patients

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Objective: To evaluate belimumabf's efficacy in refractory lupus nephritis (LN) patients and identify predictive serum biomarkers for treatment response.

Methods: In this single-arm retrospective study, we assessed clinical responses in LN patients at baseline and six months after initiating belimumab. Serum cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ) were quantified using multiplex magnetic bead flow immunoassay before and after treatment.

Results: Fourteen patients with various subtypes of refractory LN participated in the study: seven with class III and V LN, three with type V alone, two with class III, and two with class IV+V and V LN. Post six months of belimumab therapy, all participants exhibited a reduction in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)-2K scores from their respective baseline values. Notably, most patients showed a decrease in the dosage of prednisone, levels of 24-hour urinary protein, immunoglobulins, erythrocyte sedimentation rate (ESR), and anti-double-stranded DNA antibody IgM, along with serum levels of IL-4, IL-6, IL-10, and IFN-γ. Meanwhile, levels of C3, C4, IL-2, and TNF-α were observed to increase. Of the participants, nine (64.29%) achieved a complete renal response, one (7.14%) showed a partial response, and four (28.57%) exhibited no response. Significantly, higher baseline serum IFN-γ levels were found in patients who did not achieve complete renal response (CR) compared to those who did ($p = 0.009$). Receiver operating characteristic (ROC) curve analysis demonstrated that baseline IFN-γ levels had an area under curve (AUC) of 0.96 (0.70–1.00), with a sensitivity of 0.89 and a specificity of 1.00 ($p < 0.001$).

Conclusion: Belimumab shows potential efficacy in treating refractory LN. Baseline serum IFN-γ levels may predict response to belimumab therapy, potentially enabling more targeted treatment approaches for this challenging condition.

Keywords: refractory lupus nephritis, belimumab, treatment, biomarker, IFN-γ

Introduction

Renal involvement ranks among the most significant complications in patients with systemic lupus erythematosus (SLE), with lupus nephritis (LN) being its most prevalent renal manifestation and a primary contributor to mortality within this patient group. Within the initial five years post-diagnosis, the majority of SLE patients develop LN, which also presents as the initial diagnostic indicator in 25–50% of these cases.[1,2] The pathogenesis of LN encompasses multiple pathways, such as abnormal cellular apoptosis, the production of autoantibodies, deposition of immune complexes, and activation of the complement system.[3] Despite the application of prompt diagnostic measures and aggressive immunosuppressive treatments, a substantial proportion of LN patients-between 14% and 33%-fail to respond to these therapeutic interventions.[4] Currently, the management of LN primarily involves the use of hormonal therapies and immunosuppressive medications.[5] However, these treatments are often associated with severe side effects, including infections, premature gonadal dysfunction, and an increased risk of malignancies. Given these challenges, there is a pressing need to explore and develop new targeted therapeutic options, such as biological therapies, which could potentially enhance the prognosis for LN patients. Additionally, investigating the specific mechanisms underlying LN pathogenesis could provide further insights into more effective and targeted treatment strategies.

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The complement system plays a crucial role in the pathogenesis of lupus nephritis. In SLE, excessive activation of the complement cascade, particularly the classical pathway, leads to the deposition of complement components in the kidneys. This process is initiated by autoantibodies forming immune complexes, which activate C1q, leading to the sequential activation of C4 and C2 to form C3 convertase. The alternative and lectin pathways can also contribute to complement activation in lupus nephritis. Activation of these pathways results in the generation of inflammatory mediators, recruitment of inflammatory cells, and direct tissue damage. Paradoxically, deficiencies in early components of the classical pathway (C1q, C4, C2) are associated with an increased risk of SLE, highlighting the complex role of complement in this disease. Monitoring complement levels, particularly C3 and C4, is an important part of assessing disease activity in LN.[6]

Belimumab, a recombinant IgG-1λ monoclonal antibody, acts by inhibiting B cell activation and is approved for use in SLE patients aged five years and older exhibiting autoantibody activity.[7] It specifically targets the soluble B cell activating factor (BAFF, also known as BLyS), potentially preventing the proliferation of self-reactive B cells. As an emerging therapeutic option, belimumab has demonstrated increased response rates in LN patients when combined with standard treatments compared to those receiving only standard therapies.[8] Currently, comprehensive studies assessing the effectiveness of belimumab alongside standard treatment regimens for refractory lupus nephritis are lacking, with available data primarily derived from case studies.[9,10] In this preliminary investigation, we assess both the efficacy and safety of belimumab in patients with refractory LN. Additionally, we aim to identify potential predictors of response to belimumab in this challenging patient cohort.

Methods

Patient Selection and Experimental Design

This investigation focused on patients diagnosed with refractory LN. The criteria for inclusion were set as follows: (i) patients must have a diagnosis that aligns with the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) criteria for SLE;[11] (ii) confirmation of LN through renal biopsy; and (iii) patients must fit the definition of refractory LN, characterized by persistent proteinuria and/or a failure to improve or a decline in the estimated glomerular filtration rate (eGFR) despite adhering to two different standard induction therapies over a period of 4–6 months.[12] Exclusion criteria were rigorously defined to maintain the integrity of the study. These included: any patient whose condition did not meet the 2019 EULAR/ACR SLE classification criteria, those with baseline proteinuria less than 500 mg per day, renal biopsy findings inconsistent with LN, presence of another connective tissue disease, concurrent hepatitis B or C infection, and insufficient follow-up data. These criteria ensured the selection of a homogeneous study population specifically affected by refractory LN.

This study was conducted as a retrospective single-arm cohort study. Patients received belimumab via intravenous infusion at a dosage of 10 mg/kg over eight cycles, with the first three doses administered biweekly and subsequent doses given every four weeks. The dosage of prednisolone was tapered according to a standardized schedule. Following the administration of belimumab, patients continued on a stable dose of other immunosuppressive or immunomodulatory agents, including hydroxychloroquine sulfate, mycophenolate mofetil, tacrolimus, and cyclosporine.

Data were extracted from the electronic medical records of the patients, including demographic information such as gender, age at onset, ethnicity, height, and weight; clinical manifestations and laboratory characteristics, which included renal biopsy pathology results, 24-hour urinary protein quantification, complete blood count, urinalysis, creatinine, urea nitrogen, uric acid, complement levels, immunoglobulins (Ig), erythrocyte sedimentation rate (ESR), antinuclear antibodies (ANA), anti-ds-DNA antibody IgM, and cytokine levels such as IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ. Additionally, data related to the treatment, including the drugs and dosages used in the induction therapy for LN and prednisone dosages, were collected. For each patient, the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was calculated at baseline and after six months, along with the estimated Glomerular Filtration Rate (eGFR) at these time points.

Outcome Assessment and Response Criteria

The efficacy of the treatment was assessed using specific criteria for renal response at the six-month mark. A complete renal response (CR) was defined by serum creatinine levels below 115 μmol/L, normal urinalysis results, and estimated 24-hour urine protein excretion below 500 mg/d. A partial renal response (PR) was characterized by serum creatinine remaining under 115 μmol/L, normal urinalysis, and a reduction of more than 50% in proteinuria, with 24-hour urinary protein levels being at least 500 mg/d but less than 2000 mg/d. A classification of No Response (NR) was applied when neither CR nor PR criteria were met within six months.[13] The broader category of Renal Response (RR) was assigned to patients who achieved at least a PR within this timeframe.

Throughout the duration of treatment, both adverse events (AEs) and serious adverse events (SAEs) were meticulously monitored to assess the overall safety and tolerability of belimumab. This comprehensive monitoring was critical in evaluating the risk-benefit profile of the therapy.

This study was designed as a retrospective cohort study. Although we collected data at baseline and after 6 months of treatment, all data were obtained from existing medical records. Patients were identified retrospectively based on their treatment with belimumab for refractory lupus nephritis between start date and end date. We collected data on patients' clinical characteristics, laboratory findings, and treatment outcomes at two time points: before the initiation of belimumab treatment (baseline) and 6 months after starting belimumab therapy. This approach allowed us to analyze the changes in various parameters over time while maintaining the retrospective nature of the study.

Ethical Statement

This study was approved by the Ethics Committee of the affiliated Huaian No.1 People's Hospital of Nanjing Medical University, and carried out in accordance with the Helsinki Declaration.

Statistical Analysis

The data for this study were analyzed using R software, version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). To assess the distribution of the data, the Shapiro–Wilk test was utilized for evaluating normality. Differences in outcomes between CR and PR, as well as between RR and NR, were examined using the Mann–Whitney *U*-test and the chi-square test. For the analysis of pre-treatment and post-treatment data concerning belimumab, a paired-samples *t*-test was conducted. Furthermore, Spearman's rank correlation coefficient was employed to explore the relationships between clinical features and laboratory findings. A p-value of less than 0.05 was designated as the threshold for statistical significance.

Results

Baseline Characteristics of Patients

A total of 14 patients were included in this study. The mean age of onset was 31 years (range 16–60 years), and all were female. Renal biopsy showed that 7 cases were class III+V LN, 3 cases were class V LN, 2 cases were class III LN, 2 cases were class IV+V LN. All patients were treated with prednisone (or methylprednisolone) at a dose of 27.86 ± 13.97 mg/d. Twelve patients (85.71%) were treated with concomitant hydroxychloroquine, and all patients were treated with at least one immunosuppressive agent, including mycophenolate, tacrolimus, and cyclosporine (Table 1).

Clinical Response to Belimumab for Refractory LN

After 6 months of treatment with belimumab, the SLE-2K scores of all patients were lower than the baseline levels (Figure 1). In most patients, the dose of prednisone, 24-hour urine protein quantification, immunoglobulin, ESR, anti-ds-DNA antibody IgM, IL-4, IL-6, IL-10 and IFN-γ decreased, while the level of C3, C4, IL-2 and TNF-α increased (Figure 1). Nine patients (64.29%) and one patient (7.14%) obtained CR and PR after 6 months of treatment with belimumab, while four patients (28.57%) did not respond.

Table 1 Baseline Demographics and Clinical Characteristics of Patients with Refractory LN

Variables	Refractory LN Patients (n = 14)
Age (years)	31.43 ± 12.07
Gender (F/M)	14/0
SLEDAI-2K	10.93 ± 2.92
WBC count ($10^{9/}$ L)	6.59 ± 2.98
Platelet count ($10^{9/}$ L)	239.5 ± 80.77
24-hour urinary protein quantitative (g/d)	1.28 (0.86–2.59)
BUN (μmol/l)	7.51 ± 3.63
Creatinine (μmol/l)	54.90 (44.95–93.65)
eGFR (mL/min)	111.31 (85.72–162.98)
UA (μmol/l)	378.4 (325.75–417)
C3 (g/L)	0.63 ± 0.31
C4 (g/L)	0.07 (0.07–0.18)
IgG (g/L)	9.86 (9.26–14.9)
IgA (g/L)	1.97 ± 0.63
IgM (g/L)	0.84 ± 0.46
ESR (mm/h)	44.14 ± 30.81
Anti C1q antibody positive, n (%)	1 (7.14%)
Anti-ds-DNA antibody IgM positive, n (%)	4 (28.57%)
ANA positive, n (%)	11 (78.57%)
Anti-nRNP antibody positive, n (%)	7 (50%)
Anti-nucleosome antibody positive, n (%)	8 (57.14%)
Anti-AHA antibody positive, n (%)	8 (57.14%)
Anti-ribosomal P protein antibody positive, n (%)	8 (57.14%)
Anti-AMA antibody positive, n (%)	1 (7.14%)
Anti-SSA antibody positive, n (%)	4 (28.57%)
Anti-Ro52 antibody positive, n (%)	3 (21.43%)
Anti-SM antibody positive, n (%)	7 (50%)
IL-2 (pg/mL)	0.77 ± 0.37
IL-4 (pg/mL)	1.7 (1.67–1.71)
IL-6 (pg/mL)	37.63 (31.91–51.93)
IL-10 (pg/mL)	5.35 ± 1.34
TNF-α (pg/mL)	1.52 (1.43–1.65)
IFN-γ (pg/mL)	2.37 ± 0.81
Mean prednisone dose (mg/d)	27.86 ± 13.97
Tacrolimus, n (%)	9 (64.29%)
HCQ, n (%)	12 (85.71%)
Cyclosporine, n (%)	2 (7.14%)
MMF, n (%)	3 (14.29%)

Abbreviations: SLEDAI, systemic lupus erythematosus disease activity score; WBC, white blood cell; BUN, urea nitrogen in serum; eGFR, estimated glomerular filtration rate; UA, uric acid; ESR, erythrocyte sedimentation rate; ANA, antinuclear antibody; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil.

Biomarkers to Predict the Effectiveness of Belimumab for LN

The level of serum IFN-γ in patients with no-CR was significantly higher than that in patients with CR ($p = 0.009$; Figure 2A, Table 2). Receiver operating characteristic (ROC) analysis showed that the area under curve (AUC) value of serum IFN-γ level at baseline was 0.96 (0.70–1.00), the sensitivity was 0.89, and specificity was 1.00 ($p < 0.001$; Figure 2C). The remaining laboratory indices at baseline were not significantly different between patients with CR and patients with no-CR (Table 2). After 6 months of treatment with belimumab, there was no significant difference in serum IFN-γ levels between patients with

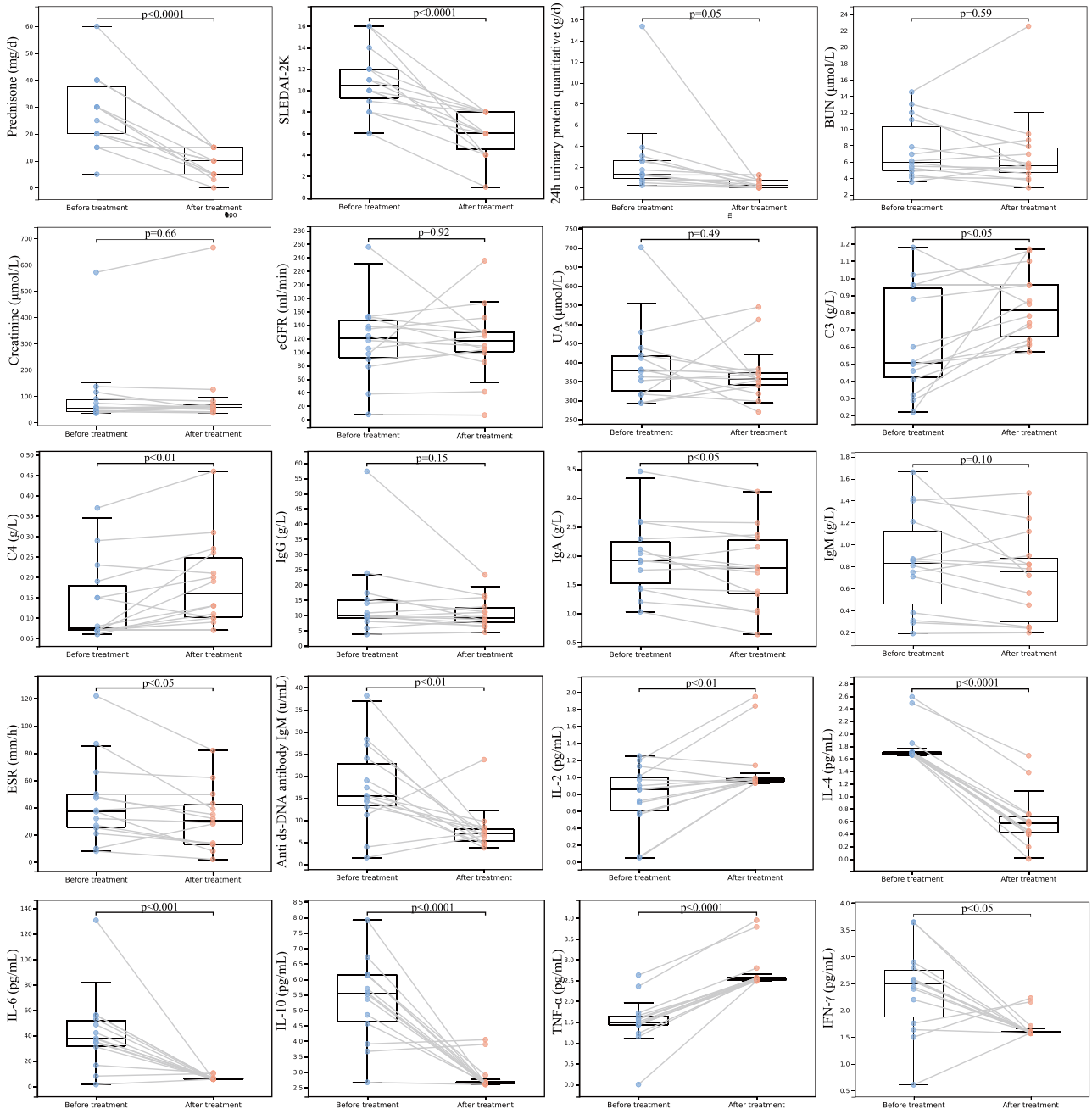

Figure 1 Comparison of clinical features and laboratory indices of patients with refractory LN at baseline examination and after 6 months of treatment with belimumab.
Abbreviations: SLEDAI, systemic lupus erythematosus disease activity score; BUN, urea nitrogen in serum; eGFR, estimated glomerular filtration rate; UA, uric acid; ESR, erythrocyte sedimentation rate; ANA, antinuclear antibody; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil.

CR and patients with no-CR (p = 0.42; Figure 2B). There were no significant differences between patients with RR and patients with NR in any laboratory indexes at baseline (Supplementary Table 1).

Correlation Between Clinical and Laboratory Results

As shown in Figure 3A, at baseline, prednisone dose was positively correlated with IgG (r = 0.666, p = 0.009), creatinine was positively correlated with ESR (r = 0.698, p = 0.006), TNF-α was positively correlated with IL-2 (r = 0.718, p = 0.004) and IL-4 (r = 0.726, p = 0.003), respectively, and IL- 4 and IgA were negatively correlated (r = −0.671, p = 0.009).

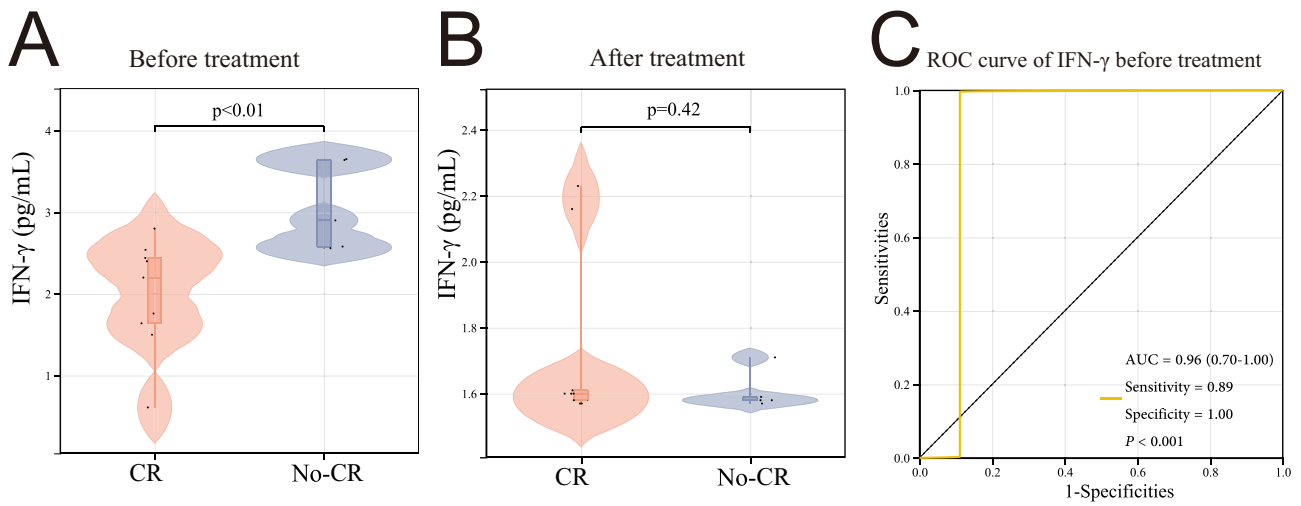

Figure 2 Serum levels of serum IFN-γ in patients with refractory LN before and after treatment with belimumab. (**A**) Serum IFN-γ levels between CR and No-CR at baseline examination. (**B**) Serum IFN-γ levels between CR and No-CR in patients after 6 months of belimumab treatment. (**C**) ROC analysis of serum IFN-γ in patients at the baseline examination.

Abbreviations: CR, complete renal response; IFN, interferon; ROC, receiver operating characteristic; AUC, area under curve.

As shown in Figure 3B, there was a strong positive correlation between all cytokines after 6 months of treatment, except for ESR, and ESR was positively correlated with the dose of prednisone ($r = 0.704$, $p = 0.005$).

Safety Data

Of the 14 patients with refractory LN included in this study, we observed that 4 patients had 6 AEs during treatment with belimumab, including respiratory tract infections (n = 3), allergic skin rash (n = 2) and leukopenia (n = 1). All these adverse events were mild, and there was no AE that resulted in dose reduction or discontinuation of belimumab. No SAEs, such as death, severe infections, severe infusion or allergic reactions, abnormal liver function, tuberculosis, tumor or bone marrow suppression, was observed.

Table 2 Comparison of Clinical Characteristics and Laboratory Indices of CR and No-CR Patients at Baseline Examination

Variables	CR (n = 9)	No-CR (n = 5)	*p*
SLEDAI-2K	11.11 ± 3.62	10.6 ± 1.14	0.704
WBC Count ($10^{9/}$ L)	6.74 ± 3.07	6.31 ± 3.16	0.808
Platelet Count ($10^{9/}$ L)	212.22 ± 74.36	288.6 ± 73.88	0.09
24-hour urinary protein quantitative (g/d)	2.92 ± 4.76	2 ± 1.21	0.687
BUN (μmol/l)	7.02 ± 3.19	8.39 ± 4.58	0.521
Creatinine (μmol/l)	65.98 ± 31.61	164.96 ± 229.66	0.391
eGFR (mL/min)	124.67 ± 61.31	103.97 ± 57.56	0.548
UA (μmol/l)	374.77 ± 61.06	429.22 ± 158.8	0.368
C3 (g/L)	0.63 ± 0.34	0.63 ± 0.28	0.968
C4 (g/L)	0.15 ± 0.12	0.12 ± 0.06	0.696
IgG (g/L)	14.94 ± 16.29	13.96 ± 6.52	0.901
IgA (g/L)	2.07 ± 0.72	1.79 ± 0.46	0.447
IgM (g/L)	0.76 ± 0.46	0.97 ± 0.47	0.451
ESR (mm/h)	40.33 ± 23.89	51 ± 43.03	0.556
Anti ds-DNA antibody IgM (u/mL)	15.21 ± 9.12	21.19 ± 10.4	0.285
ANA positive, n (%)	88.89	60	0.207

(*Continued*)

Table 2 (Continued).

Variables	CR (n = 9)	No-CR (n = 5)	*p*
Anti-C1q antibody positive, n (%)	11.11	0	0.439
Anti-nRNP antibody positive, n (%)	55.56	40	0.577
Anti-nucleosome antibody positive, n (%)	66.67	40	0.334
Anti-AHA antibody positive, n (%)	55.56	40	0.577
Anti-ribosomal P protein antibody positive, n (%)	55.56	40	0.577
Anti-AMA antibody positive, n (%)	0	20	0.164
Anti-SSA antibody positive, n (%)	22.22	40	0.481
Anti-Ro52 antibody positive, n (%)	11.11	40	0.207
Anti-SM antibody positive, n (%)	55.56	40	0.577
IL-2 (pg/mL)	0.67 ± 0.4	0.95 ± 0.24	0.186
IL-4 (pg/mL)	1.88 ± 0.38	1.71 ± 0.08	0.235
IL-6 (pg/mL)	41.48 ± 38.51	43.1 ± 7.74	0.929
IL-10 (pg/mL)	5.12 ± 1.59	5.76 ± 0.7	0.419
TNF-α (pg/mL)	1.49 ± 0.75	1.55 ± 0.13	0.865
IFN-γ (pg/mL)	1.99 ± 0.68	3.07 ± 0.55	0.009

Abbreviations: CR, complete renal response; SLEDAI, systemic lupus erythematosus disease activity score; WBC, white blood cell; BUN, urea nitrogen in serum; eGFR, estimated glomerular filtration rate; UA, uric acid; ESR, erythrocyte sedimentation rate; ANA, antinuclear antibody; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil.

Discussion

Comprising 285 amino acids, BAFF is a critical transmembrane protein essential for B-cell maturation, survival, proliferation, the enhancement of antibody production, and the mediation of immunoglobulin isotype conversion.[14] A significant reduction in mature B cells was observed in BAFF-deficient mice, which led to a marked decrease in serum levels of IgM, IgG, and IgA.[15] In contrast, BAFF overexpression in mice resulted in significantly elevated levels of these serum immunoglobulins.[16] Further research has indicated a substantial rise in BAFF levels in the circulation of patients with SLE, correlating this increase with elevated levels of serum IgG and anti-dsDNA antibodies. This elevation in BAFF is believed to contribute significantly to polyclonal hypergammaglobulinemia and the escalation of autoantibody titers in SLE patients, thus laying a theoretical foundation for the development of BAFF antagonists.[17,18]

A landmark in the management of SLE occurred in 2011 with the global approval of belimumab, the first new SLE treatment approved since hydroxychloroquine in 1955. This approval marked a significant milestone in the history of SLE treatment. Belimumab's increasing use in clinical practice has been underpinned by numerous studies demonstrating

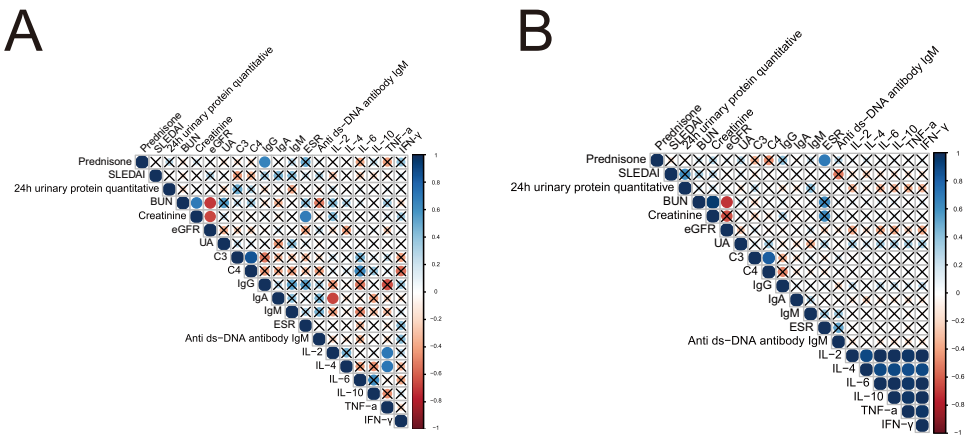

Figure 3 Correlation between clinical and laboratory findings. (**A**) Correlation between clinical and laboratory results at baseline examination. (**B**) Correlation between clinical and laboratory results after 6 months of belimumab treatment. Blue indicates $r > 0$, red indicates $r < 0$, and × indicates $p > 0.05$.

its capacity to improve disease activity indices and reduce glucocorticoid dosage in SLE patients.[19] Baseline levels of anti-ds-DNA antibody, anti-SM antibody, complement, and BAFF in the serum are established as predictors of response to belimumab therapy in SLE patients.[20–22] However, factors such as smoking and pre-existing organ damage have been shown to diminish the therapeutic efficacy of belimumab.[23,24]

Recently, the Food and Drug Administration endorsed belimumab as the inaugural therapy for active LN in adults, marking a significant breakthrough in overcoming the previous therapeutic barriers and introducing a novel biologically targeted therapy for patients with LN. Recent investigations have confirmed that belimumab substantially diminishes the risk of exacerbation in LN and decreases the decline in eGFR, while also facilitating the discontinuation of glucocorticoids.[25,26] Studies comparing belimumab to control treatments indicate that patients receiving belimumab are 1.71 times more likely to achieve complete renal remission and 34% less likely to exhibit non-response.[27] Refractory LN, characterized by an inadequate response to standard therapies, often leads to sustained disease activity or progression despite treatment efforts. Research exploring the efficacy of a combination therapy of rituximab and belimumab for treating refractory LN demonstrated that this regimen, even when combined with cyclophosphamide, does not enhance clinical outcomes compared to rituximab alone.[28] Currently, the literature lacks comprehensive studies on the impact of integrating belimumab with standard therapies for refractory LN, with only individual case reports shedding light on this approach. Our study revealed promising results: 10 out of 14 patients (71.43%) responded positively to belimumab, with 9 achieving CR, accounting for 64.29% of the participants. This outcome confirms belimumab's potential efficacy in managing patients with refractory LN.

Prior research has established the influence of belimumab on serum levels of anti-ds-DNA antibodies and immunoglobulins IgM, IgG, and IgA.[29] Increasing evidence suggests a dysregulation of Th1/Th2 cytokine levels in patients with SLE compared to healthy individuals.[30,31] This study aims to examine the effect of belimumab on Th1/Th2 cytokines, immunoglobulin, and anti-ds-DNA antibody IgM levels in patients with refractory LN post-treatment. Following six months of treatment with belimumab, most patients exhibited decreased levels of prednisone dosage, 24-hour urinary protein quantitation, immunoglobulins, ESR, anti-ds-DNA antibody IgM, IL-4, IL-6, IL-10, and IFN-γ. Conversely, increases were noted in C3, C4, IL-2, and TNF-α levels. The study also analyzed the predictive value of baseline cytokine levels and autoantibody positivity for refractory LN response to belimumab treatment. Notably, while all cytokines showed significant changes post-treatment, only baseline IFN-γ levels were markedly different, with CR patients exhibiting significantly lower serum IFN-γ levels compared to those without CR, indicating that baseline serum IFN-γ levels might serve as a predictive biomarker for belimumab efficacy in refractory LN. Furthermore, serum and urine IFN-γ levels in patients with SLE were significantly elevated compared to healthy controls, with even higher levels observed in patients with LN versus those without LN. The expression of IFN-γ in renal tissues of LN patients was significantly increased and correlated with the activity of pathological lesions,[32] highlighting its potential as a biomarker for SLE disease activity and renal damage. Additionally, baseline IFN-γ levels have been shown to predict the clinical response of active SLE patients to mesenchymal stem cell transplantation, where higher baseline levels indicate a better response.[33] These findings suggest that lower baseline IFN-γ levels might predict a favorable response to belimumab in patients with refractory LN. Future research will explore the relationship between serum IFN-γ levels and belimumab efficacy in larger and paired sample studies, aiming to further elucidate the role of IFN-γ in refractory LN.

Our study revealed that baseline serum IFN-γ levels were significantly lower in patients who achieved CR compared to those who did not. This finding suggests that baseline IFN-γ levels could serve as a potential biomarker for predicting response to belimumab in refractory LN patients. Interestingly, after 6 months of treatment, the difference in IFN-γ levels between CR and non-CR patients was no longer significant. This change might indicate that belimumab treatment influences IFN-γ production or signaling pathways, potentially contributing to its therapeutic effect. Further research is needed to elucidate the exact mechanism by which belimumab affects IFN-γ levels and how this relates to treatment response in LN patients.

Limitations and Future Directions

This study has several limitations that should be acknowledged. Firstly, the small sample size (n=14) limits the generalizability of our findings and may have affected the statistical power of our analyses. Secondly, the retrospective

nature of the study introduces potential biases in patient selection and data collection. Thirdly, the follow-up period of six months may not be sufficient to fully assess the long-term efficacy and safety of belimumab in refractory lupus nephritis patients. Additionally, the lack of a control group makes it difficult to definitively attribute the observed improvements to belimumab treatment alone.

Future studies should address these limitations by conducting larger, prospective, randomized controlled trials with longer follow-up periods. Such studies could provide more robust evidence for the efficacy of belimumab in refractory lupus nephritis and help identify additional predictive biomarkers. Furthermore, investigating the mechanisms by which belimumab affects IFN-γ levels and other cytokines could provide valuable insights into its mode of action and potentially guide more personalized treatment approaches.

Data Sharing Statement

The data sets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the affiliated Huaian No.1 People's Hospital of Nanjing Medical University. The patients/participants provided their 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.

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Disclosure

The authors report no conflicts of interest in this work.

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