B-cell signatures for disease flare and response to pre-emptive immunosuppressive therapy in patients with lupus nephritis: abridged secondary publication

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KEY MESSAGES

1. Pre-emptive increase in immunosuppression in patients with lupus nephritis experiencing asymptomatic serological reactivation can reduce renal relapse and is well-tolerated.
2. B-cell signatures can be modulated by pre-emptive treatment in patients with lupus nephritis and may serve as biomarkers for treatment response monitoring.

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Introduction

Lupus nephritis (LN) is a serious organ manifestation in patients with systemic lupus erythematosus (SLE) and a robust predictor for adverse clinical outcomes. Clinical relapses are common and associated with poor long-term renal prognosis. In patients with LN, prediction and prevention of clinical relapses are challenging. Serological reactivation often occurs after achieving renal remission and may precede clinical relapses; however, LN may be clinically quiescent in some patients despite experiencing serological reactivation. The use of conventional serological parameters (eg, levels of anti-double-stranded DNA [dsDNA] antibodies and complement) in guiding management decisions remains controversial. Therefore, management of asymptomatic serological reactivation (ASR) is an important clinical consideration.

Changes in B cell-related cytokines such as B-cell activation factor (BAFF), interleukin (IL)-6, IL-10, and IL-21 have been observed in patients with SLE, particularly during active disease.[1] These cytokines have pivotal roles in the differentiation/maturation and survival of various B-cell subsets.[1] Transcription repressors such as BTB domain and CNC homologue (BACH)1, BACH2, and paired box (PAX)5 are key regulators of B cell/plasma cell differentiation and maturation.

In our previous studies, pre-emptive increase in immunosuppression in patients with LN experiencing ASR was associated with a lower risk of renal relapse and a slower decline in renal function; it also was well-tolerated.[2] B-cell signatures including BACH1, BACH2, and PAX5 were associated with disease relapse in patients with LN.[3] We conducted a prospective randomised controlled trial to investigate the efficacy and safety of pre-emptive treatment in patients with LN experiencing ASR, and to determine whether B-cell signatures could be used to guide this management approach.

Methods

Recruited patients were randomly assigned to either the pre-emptive treatment group or the control group. In the pre-emptive treatment group, prednisolone was increased to 0.4 to 0.5 mg/kg/day, tapered by 5 mg every 2 weeks to reach 15 mg/day, then reduced by 2.5 mg every 2 weeks to reach 5 to 7.5 mg/day after 12 weeks. The second agent was adjusted as follows. For patients who received azathioprine <100 mg/day, the dose was increased to 100 mg/day. For patients who received mycophenolate mofetil <1.5 g/day, the dose was increased to 1.5 g/day. The dosages of azathioprine and mycophenolate mofetil remained at 100 mg/day and 1.5 g/day, respectively, for 12 weeks after treatment intensification; the dosages were subsequently tapered according to clinical status. Patients without serological remission after a course of pre-emptive treatment were closely monitored for renal function, urine microscopy, proteinuria, anti-dsDNA antibody level, and C3 level at 4-week intervals for up to 12 months. In the control group, the current immunosuppressive treatment regimen and dosage were not modified until the onset of renal or extra-renal flares.

To quantify the expression of BACH1, BACH2, and PAX5, lymphocytes were isolated by the Ficoll gradient method; B-cell subsets were stained with appropriate monoclonal antibodies (naïve B cells: $CD20^+CD27^-$, memory B cells: $CD20^+CD27^+$) and isolated by cell sorting. mRNA was extracted from naïve and memory B cells, then measured by

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quantitative polymerase chain reaction. To quantify serum cytokines, the levels of BAFF, IL-6, and IL-10 were determined by enzyme-linked immunosorbent assays using standard methods.

Primary outcomes were the incidences of renal and extra-renal flares in patients with LN experiencing ASR who had or had not received pre-emptive treatment, and changes in B-cell subsets and relevant regulatory genes (BACH1, BACH2, PAX5), serum and urine cytokine profiles, and miRNA148a levels in patients who had or had not received pre-emptive treatment. Secondary outcomes were associations of B-cell signatures with subsequent renal or extra-renal flares, and associations of B-cell signatures with clinical parameters (anti-dsDNA antibody level, C3/4 level, renal function, proteinuria, and Systemic Lupus Erythematosus Disease Activity Index) at 12 months.

Results

In total, 43 patients with LN experiencing ASR were randomly assigned to the pre-emptive treatment group (n=20) or the control group (n=23) [Table]. Compared with the control group, the pre-emptive treatment group had lower incidences of clinical flares (10.0% vs 34.8%, P=0.028) and renal flares (0% vs 13.0%, P=0.047) during subsequent follow-up. The incidence of extra-renal flares did not differ between the two groups (P>0.05).

In the pre-emptive treatment group, the anti-dsDNA antibody level began to decrease at 4 weeks and remained stable until 52 weeks. In the control group, the anti-dsDNA antibody level remained elevated until 24 weeks after recruitment, then decreased at 52 weeks. There were no significant differences in anti-dsDNA antibody levels at 4, 12, 24, or 52 weeks between two groups (all P>0.05). Additionally, the two groups did not differ in terms of C3 levels at baseline or at 4, 12, 24, or 52 weeks (all P>0.05).

Infectious complications occurred in two patients in the pre-emptive treatment group (one each had herpes zoster or urinary tract infection) and in four patients in the control group (one each had herpes zoster, pneumonia, upper respiratory tract infection, or urinary tract infection). All infectious episodes were successfully managed with appropriate antimicrobial agents. There was no case of new-onset diabetes mellitus in the pre-emptive treatment group.

The serum BAFF level in the pre-emptive treatment group initially declined at 4 weeks, then gradually increased to a near-baseline level (Fig 1). Compared with the control group, the pre-emptive treatment group had a significantly higher BAFF level at baseline (P=0.02) and 52 weeks (P=0.03), but there were no significant differences at 4, 12, or 24 weeks. There were no significant differences between the two groups in terms of serum IL-6 and IL-10 levels at baseline or at 4, 12, 24, or 52 weeks (all P>0.05).

TABLE. Clinical characteristics of patients with lupus nephritis experiencing asymptomatic serological reactivation who had or had not received pre-emptive treatment

	Pre-emptive treatment (n=20)*	Control (n=23)*	P value
No. of men/women	3/17	2/21	0.520
Age, y	50.7±12.3	47.5±12.1	0.401
Duration of systemic lupus erythematosus, y	23.5±8.7	18.6±9.9	0.097
Class of lupus nephritis			
Class III ± V or IV ± V	16 (80.0)	19 (82.6)	0.494
Class V	4 (20.0)	4 (17.4)	0.826
Maintenance treatment			
Prednisolone alone	6 (30.0)	7 (30.4)	0.975
Prednisolone + mycophenolate mofetil	10 (50.0)	13 (56.5)	0.669
Prednisolone + azathioprine	4 (20.0)	3 (13.0%)	0.538
Serum creatinine, μmol/L	80.3±22.7	69.4±22.9	0.137
Estimated glomerular filtration rate, mL/min/1.73m^2	74.7±15.9	84.0±10.8	0.067
Anti–double-stranded DNA antibodies, IU/mL	159.4±65.0	141.7±65.7	0.397
C3, mg/dL	84.1±27.3	85.2±23.5	0.892
C4, mg/dL	18.5±6.4	16.4±10.4	0.482

* Data are presented as mean ± standard deviation or No. (%) of patients

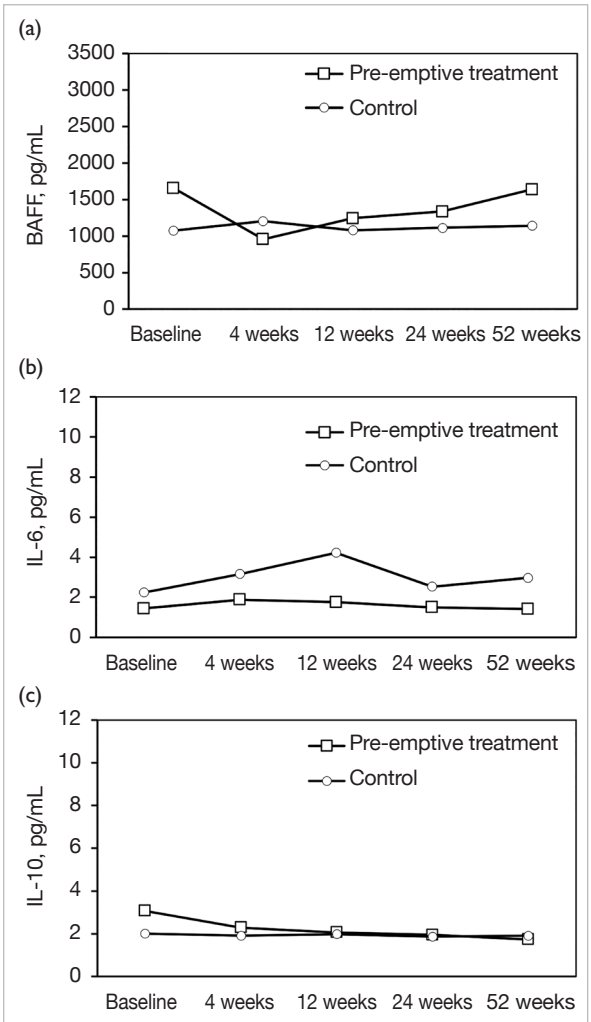

FIG 1. Changes in (a) B-cell activation factor [BAFF], (b) interleukin-6 (IL-6), and (c) interleukin-10 (IL-10) levels in patients with lupus nephritis experiencing asymptomatic serological reactivation who had or had not received pre-emptive treatment

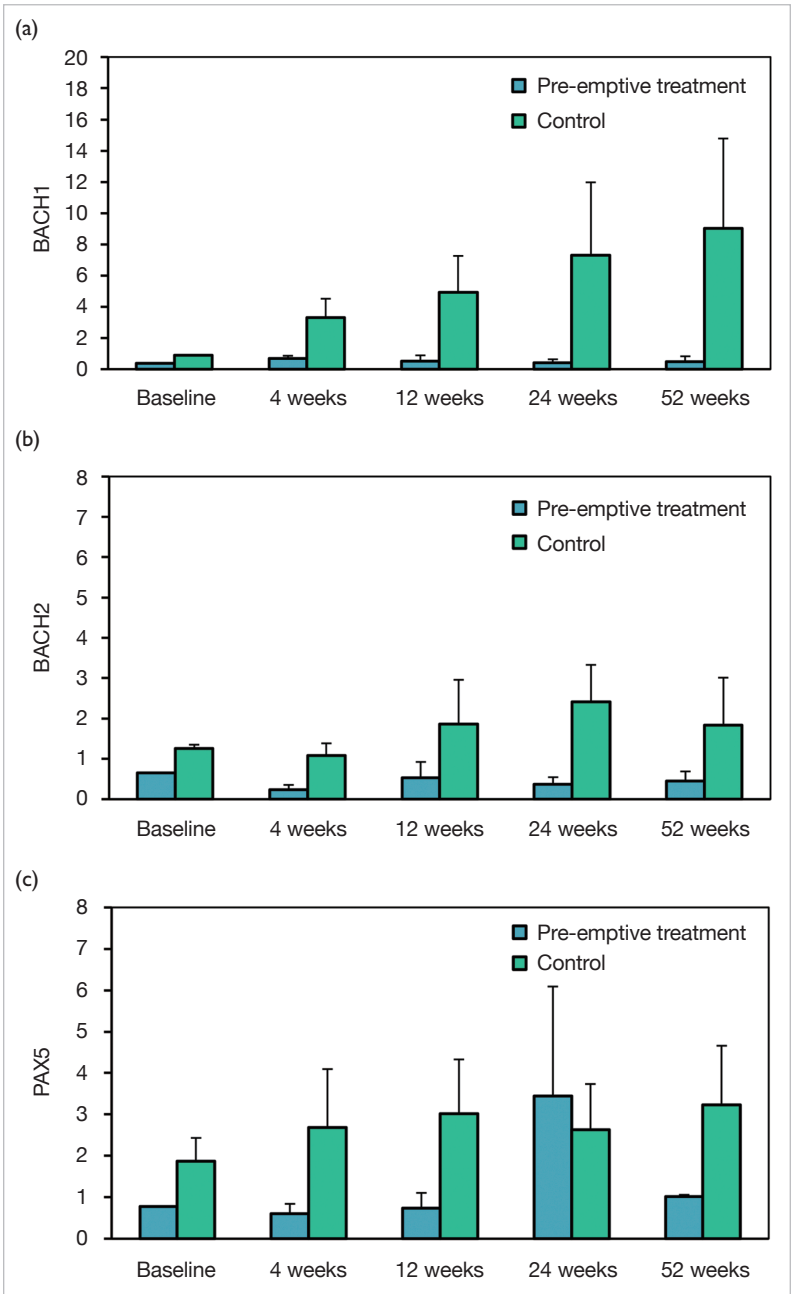

FIG 2. Changes in (a) BACH1, (b) BACH2, and (c) PAX5 expression levels in naive B cells from patients with lupus nephritis who had or had not received pre-emptive treatment

BACH1, BACH2, and PAX5 expression levels in naïve B cells remained stable in the pre-emptive treatment group. However, in the control group, these levels progressively increased over time (Fig 2). BACH1, BACH2, and PAX5 expression levels in naïve B cells were all lower (but not significantly) in the pre-emptive treatment group than in the control group at 4, 12, 24, and 52 weeks). In the pre-emptive treatment group, the BACH1 expression level in memory B cells was similar to the level in naïve B cells; although this level was lower (but not significantly) than that in the control group at 12, 24, and 52 weeks. In the pre-emptive treatment group, the BACH2 expression level in memory B cells increased from 4 weeks to 12 weeks, then decreased to a level similar to the that in the control group (all $P>0.05$). PAX5 expression in memory B cells did not significantly differ between the two groups.

Discussion

Pre-emptive increase in immunosuppression in patients with LN experiencing ASR effectively reduced the risks of clinical and renal relapse. This management approach was not associated with excess adverse effects. Our results were consistent with previous findings that pre-emptive increase in immunosuppression can mitigate impending renal flares in patients with LN experiencing ASR.[2] Nonetheless, our results did not show a benefit of

pre-emptive treatment in terms of preventing extra-renal flares.

The use of biomarkers is important to guide patient selection for pre-emptive treatment and treatment response monitoring. B-cell and B cell-related signatures are promising biomarkers for use in pre-emptive treatment. In the present study, the pre-emptive treatment group displayed a significantly higher BAFF level at baseline. This finding implies that B cells in the pre-emptive treatment group have greater immunological activity at baseline; the lower incidence of clinical flares suggests that this management approach is effective. Notably, there was an initial decline in the BAFF level at 4 weeks in the pre-emptive treatment group, followed by a gradual return to baseline as immunosuppression was tapered. Based on these observations, it remains unclear whether immunosuppression should be reduced more slowly to achieve a greater decrease in overall disease activity. There were no differences in IL-6 and IL-10 levels between the two groups, suggesting that these cytokines are not good biomarkers for patient selection and treatment response monitoring.

To further characterise the mechanistic aspect of pre-emptive treatment, we studied the effects of pre-emptive treatment on key B-cell transcription factors (ie, BACH1, BACH2, and PAX5). We demonstrated that pre-emptive treatment in patients with LN experiencing ASR was associated with increased BACH1, BACH2, and PAX5 expression levels in naïve B cells; it was also associated with increased BACH1 expression in memory B cells. BACH1 has key roles in immune responses and autoimmune conditions; it regulates the expression of core macrophage-associated genes. In a murine osteoarthritis model, BACH1 deficiency is associated with impaired development of antigen-presenting cells and partial protection from experimentally induced autoimmune encephalomyelitis; it was also associated with lower inflammation severity mediated by the upregulation of haem-oxygenase 1. BACH2 has dual effects on B cell homeostasis. In our previous study, BACH1, BACH2, and PAX5 expression levels in B lymphocytes were lower in patients with LN experiencing multiple relapses than in such patients who did not experience relapse.[3] BACH2 overexpression represses 'myeloid genes' in pre- and pro-B cells, hindering their progression to myeloid differentiation; rather, it promotes the commitment of those cells to the lymphoid lineage.[4] Other studies revealed that BACH2 expression was reduced in B cells isolated from patients with SLE; the transfection of BACH2 into B cells from such patients suppressed proliferation and augmented apoptosis. The exact role of BACH1 and BACH2 in SLE and LN remains poorly understood. A major limitation of the present study was the small sample size in each arm, which was related to patient recruitment difficulty during the COVID-19 pandemic. The follow-up duration was relatively short because of funding constraints. There is a need for additional studies regarding the effects of pre-emptive treatment on long-term renal function and disease stability in patients with LN experiencing ASR.

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Disclosure

The results of this research have been previously published in:

1. Yap DYH, Tang CSO, Chung HY, et al. A prospective randomized study on pre-emptive immunosuppressive treatment in lupus nephritis patients with asymptomatic serological reactivation. J Am Soc Nephrol 2020;31(Suppl):552.
2. Yap DYH, Tang CSO, Chung HY, et al. A prospective randomized study on pre-emptive immunosuppressive treatment in lupus nephritis patients with asymptomatic serological reactivation. Nephrol Dial Transplant 2020;35(Suppl 3):iii81-iii83.

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