

## USEFULNESS OF COMPLEMENT SPLIT PRODUCT, Bb, AS A CLINICAL MARKER FOR DISEASE ACTIVITY OF LUPUS NEPHRITIS

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**Abstract :** To evaluate the usefulness of Bb, a split product of complement factor B, as a clinical marker for disease activity of lupus nephritis, we measured the Bb concentration of sera from 42 patients with lupus nephritis. Serum Bb levels were significantly higher in patients with active nephritis (active nephritis group,  $n=30$ ) than in patients with nephritis in remission (remission group,  $n=12$ ) ( $14.3 \pm 8.3$  versus  $7.4 \pm 5.9$   $\mu\text{g/ml}$ ;  $p=0.012$ ). In contrast, there was no significant difference in serum C3 levels between active nephritis group and remission group ( $42.5 \pm 20.9$  versus  $44.7 \pm 15.9$   $\text{mg/dl}$ ;  $p=0.77$ ). In the comparison of Bb levels between active nephritis group and remission group, the sensitivity was 66.6%, specificity was 83.3%, and the positive and negative likelihood ratios were 3.95% and 0.41%, respectively. The present results suggest that serum Bb level is a useful clinical marker for disease activity in lupus nephritis.

**Key words :** complement, systemic lupus erythematosus

### INTRODUCTION

Activation of the complement system is an important factor in the pathogenesis of systemic lupus erythematosus (SLE). Conventionally, serum C3 and C4 levels have been used as indicators of SLE disease activity. However, in some cases, decreased serum C3 and C4 levels do not always reflect activation of the complement system. Furthermore, because C3 is an acute-phase protein, increased production of C3 may mask C3 consumption in SLE patients with inflammation<sup>1,2)</sup>.

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Complement factor B (fB) is one of the proteins required for activation of the alternative pathway. During activation of the alternative pathway, fB binds to C3b on the surface of a pathogen, and is cleaved into Ba and Bb by factor D, creating the C3bBb complex. Then, properdin binds to the C3bBb complex and stabilizes it. The properdin-stabilized C3bBb complex is a C3 convertase; i.e., it cleaves C3 into C3a and C3b. Thus, more C3b is produced, resulting in additional activation of fB and setting up an amplification loop for activation of C3. C3b binds to the properdin-stabilized C3bBb complex to form the C3bBbC3b complex, which is a C5 convertase. Formation of this C5 convertase leads to activation of the terminal complement components on the pathogen surface, resulting in opsonization and direct damage to the pathogen<sup>3-5</sup>.

The presence of complement split products is clear evidence of complement activation. Recent studies suggest that complement split products are sensitive markers for SLE disease activity<sup>6-15</sup>. In the present study, we measured serum Bb concentration in patients with lupus nephritis, including patients with active nephritis and patients with nephritis in remission. We evaluated the usefulness of serum Bb level as a clinical marker for disease activity of lupus nephritis.

#### MATERIALS AND METHODS

##### *Subjects*

All patients in this study fulfilled the American College of Rheumatology diagnostic criteria for SLE<sup>16</sup>. The subjects were 42 SLE patients (11 men and 31 women) with lupus nephritis. This study was approved by the ethics committee of Fukushima Medical University, Japan.

##### *Renal biopsy*

Renal biopsy was performed on 35 of the 42 patients, and the biopsy specimens were evaluated according to the World Health Organization classification of lupus nephritis<sup>17</sup> in a blinded manner.

##### *Measurement of Bb and C3 in sera from patients with lupus nephritis*

The patients were divided into 2 groups: active nephritis group, patients with urine protein of more than 1.0 g/day; remission group, patients with urine protein of less than 1.0 g/day. Serum Bb levels and serum C3 levels were measured for each group. Bb concentrations in sera were measured using the Bb Fragment Enzyme Immunoassay Kit (Quidel, San Diego, CA) according to the manufacturer's recommendations. C3 levels in sera were measured by rate nephelometry in routine laboratory tests at Fukushima Medical University.

##### *Statistic analysis*

Statistical analyses were performed using the Mann-Whitney two-tailed U test

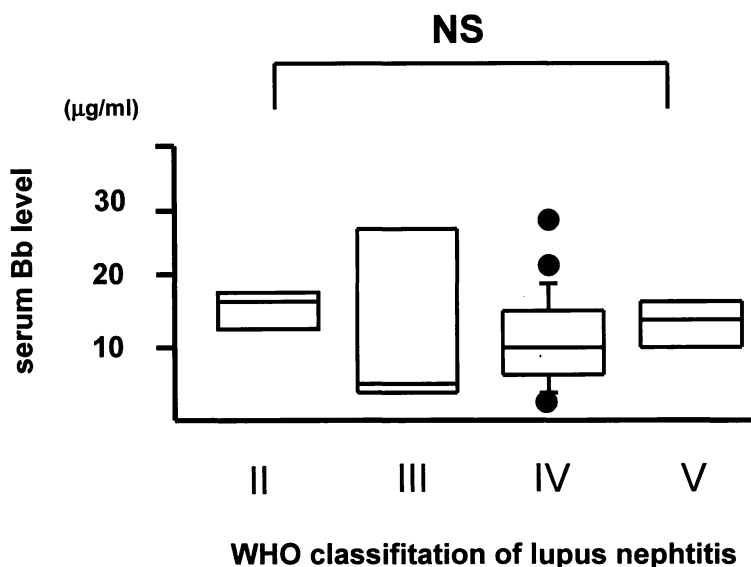


Fig. 1. Relationship between serum Bb level and WHO classification of histologic type of lupus nephritis. The vertical bars indicate the range, and the horizontal boundaries of the boxes represent the first and third quartiles. Lines outside the boxes represent the 10th and 90th percentiles. Outliers are indicated by the closed circles. There were no significant differences in serum Bb levels among the WHO classifications.

and Stat View software (Cary, NC, USA). A probability value of  $p < 0.05$  was considered to indicate significance.

## RESULTS

### *Serum Bb level and WHO classification of the histologic type of lupus nephritis*

The 36 patients who underwent renal biopsy were divided into 4 groups according to the WHO renal histopathology classification: class II lupus nephritis, 5 patients; class III, 3 patients; class IV, 23 patients; class V, 5 patients. To analyze the association of serum Bb level with renal histopathology in patients with lupus nephritis, serum Bb levels were compared among these 4 groups. There were no significant differences in serum Bb levels among the groups (Fig. 1).

### *Serum Bb level and disease activity of lupus nephritis*

To determine whether serum Bb level reflects disease activity of lupus nephritis, serum Bb levels were compared between the active nephritis group ( $n=30$ ) and the remission group ( $n=12$ ). Serum Bb levels were significantly higher in the active nephritis group than in the remission group ( $14.3 \pm 8.3$  versus  $7.4 \pm 5.9$  µg/ml;  $p = 0.012$ ) (normal 1.0–7.3 µg/ml) (Fig. 2). In the comparison of serum Bb levels between the active nephritis group and the remission group, the sensitivity was

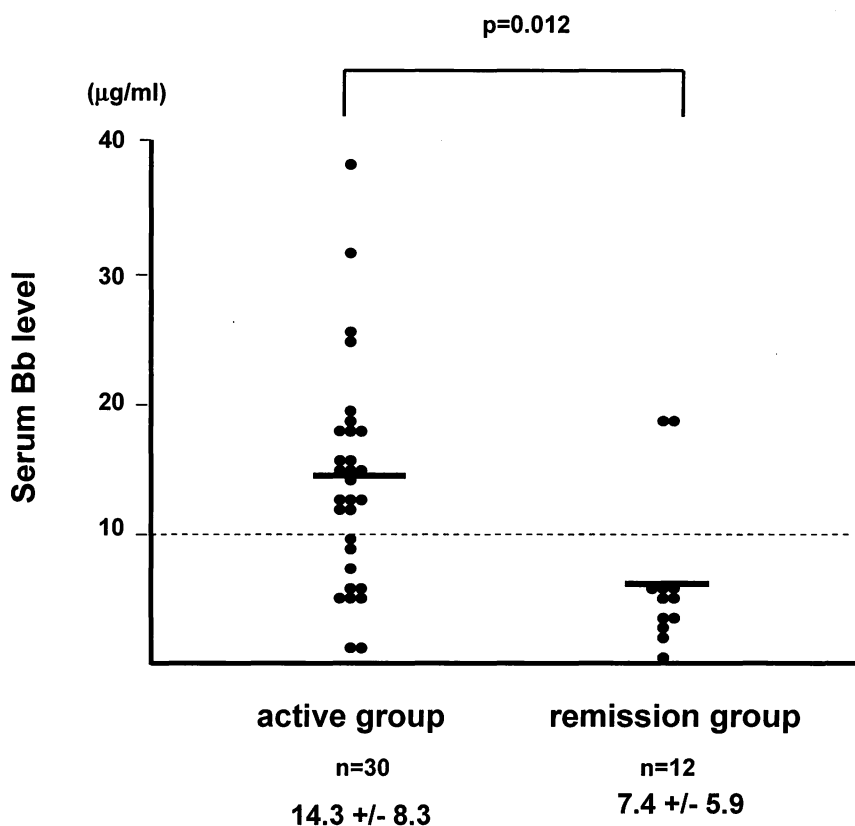


Fig. 2. Comparison of serum Bb levels between active nephritis group and remission group. Serum Bb levels were significantly higher in active nephritis group than in remission group ( $14.3 \pm 8.3$  versus  $7.4 \pm 5.9$  µg/ml;  $p=0.012$ ).

66.6%, specificity was 83.3%, and the positive and negative likelihood ratios were 3.95% and 0.41%, respectively. In contrast, there was no significant difference in serum C3 levels between these 2 groups ( $42.5 \pm 20.9$  versus  $44.7 \pm 15.9$  mg/dl;  $p=0.77$ ) (normal 69–128 mg/dl) (Fig. 3).

#### DISCUSSION

In SLE, the renal deposition of complement-containing immune complexes initiates an inflammatory cascade resulting in glomerulonephritis. Activation of the classical complement pathway with deposition of C3 is pathogenic in lupus nephritis. However, the role of C3 in disease pathogenesis is unknown although the alternative complement pathway is activated in lupus nephritis.

A previous study indicates that complement factor B knock-out MRL/MpJ-Fas<sup>lpr</sup> (MRL/*lpr*) mice, which spontaneously develop severe autoimmune disease similar to human lupus, exhibit less glomerulonephritis than nonB-knock-out MRL/

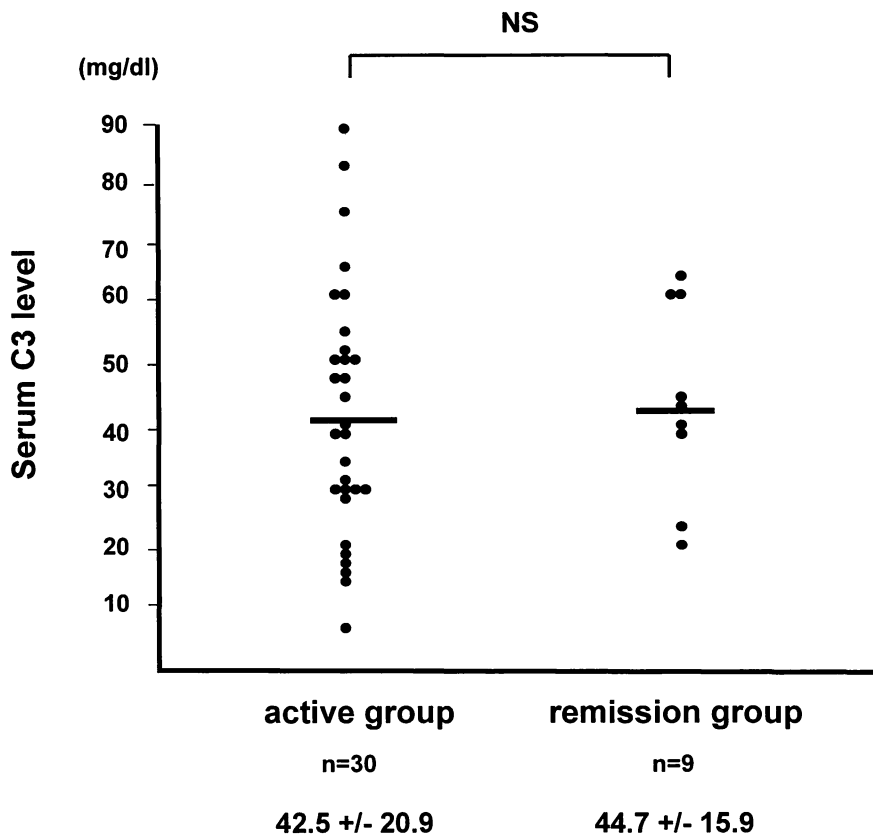


Fig. 3. Comparison of serum C3 levels between active nephritis group and remission group. There was no significant difference between the groups ( $42.5 \pm 20.9$  versus  $44.7 \pm 15.9$   $\mu\text{g/dl}$ ;  $p=0.77$ ). Shaded areas represent normal range of serum C3 level.

*lpr* mice, suggesting that factor B, a key protein of the alternative complement pathway, plays an important role in the pathogenesis of glomerulonephritis in MRL/*lpr* mice<sup>18</sup>. This finding suggests that activation of the alternative complement pathway plays a more important role in the pathogenesis of lupus nephritis than the classical complement pathway which was considered to be important previously.

Recent reports indicate that complement split products are useful indicators of SLE disease activity. Buyon *et al.* reported that complement split products (Ba, Bb, C5b-9, C4d) were as useful for assessing lupus disease activity as C3 and C4<sup>12</sup>. Belmont *et al.* reported that C5a levels were elevated in severe lupus patients with acute central nervous system involvement<sup>7</sup>. Manzi *et al.* showed that C4d and Bb are more sensitive markers for lupus disease activity than are C3 and C4<sup>15</sup>. Other studies indicate that C5b-9 levels in serum are associated with lupus disease activity<sup>6,11</sup>. Furthermore, Manzi *et al.* have reported that C3d is present in urine from patients with active lupus nephritis<sup>15</sup>.

Previously, we observed elevated serum Bb levels in SLE patients with nephritis, compared with SLE patients with other manifestations (data not shown). In the present study, serum Bb levels were significantly higher in SLE patients with active nephritis than in SLE patients with nephritis in remission. Furthermore, in the present case study, serum Bb concentration reflected renal disease activity in a lupus nephritis patient whose serum C3 level remained normal despite clinical evidence of disease activity.

In the present study, we found no significant difference in serum C3 levels between the active nephritis group and the remission group. However, this does not mean that C3 is useless as a marker for SLE disease activity. Previously, we observed a highly significant negative correlation between serum C3 levels and disease activity index of SLE patients (SLEDAI) (data not shown), suggesting that serum C3 concentration reflects activity of nephritis as well as activity of other manifestations. Therefore, in patients with SLE, even if nephritis is in remission, if the activity of other manifestations are still high, serum C3 level may not reflect the disease activity of nephritis.

In conclusion, the present findings suggest that serum Bb level is a useful marker of lupus nephritis activity, and is more sensitive than serum C3 level.

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