

# Predictive value of complement profiles and anti-dsDNA in systemic lupus erythematosus

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**SUMMARY** In a prospective study of 143 patients with systemic lupus erythematosus (SLE) the relation between clinical exacerbations, anti-dsDNA levels, and serum levels of complement components, C1q, C4, C3, C5, and C9 was investigated. In 33 out of these 143 patients a major clinical exacerbation of the disease developed. Evaluation of anti-dsDNA levels in relation to disease activity confirmed our earlier finding that anti-dsDNA levels rose before a major exacerbation and decreased after it. In the remaining 110 SLE patients a nearly constant anti-dsDNA level was seen, but none of these patients experienced a major exacerbation. In the 21 SLE patients who developed deterioration in renal function a decrease of C4 followed by decreases of C1q and C3 levels was seen first, starting about 25 to 20 weeks before the first signs of renal involvement. In the 12 SLE patients who developed an exacerbation without renal involvement an inconsistent profile of the complement components C4, C1q, and C3 was observed. C5 levels were hardly affected at all, while C9 levels were in general higher than normal during the exacerbation, irrespective of the type of exacerbation. These results show that, by following the complement and anti-dsDNA profiles, not only can exacerbations be predicted but also a pointer can be obtained about the pattern of disease well before the first clinical signs of an exacerbation appear.

Serum complement levels are often used as prognostic indicators and diagnostic aids in patients with systemic lupus erythematosus (SLE). Diminished serum complement activity (CH<sub>50</sub> titre) has been claimed to accompany or precede the onset of nephritis.<sup>1</sup> Exacerbations of nephritis have also been described without a fall in the serum complement level.<sup>2</sup> Specifically, the presence of low levels of C1q or C4 has been claimed to be a sign of the presence of renal disease or the tendency to develop it.<sup>3,4</sup> In later studies lower mean levels of C1q, C4, and C3, but not of C5 and C9, were observed in SLE patients with renal involvement.<sup>5,6</sup> In two prospective follow up studies normalisation of serum CH<sub>50</sub> or C1q and C3 levels was associated with improvement in renal function.<sup>7,8</sup> Up till now there have been no available serial data, gathered in

a long term, prospective manner, involving a large group of patients with frequent serum sampling, on the profiles of the complement components C1q, C4, C3, C5, and C9 in the periods preceding, during, and after disease exacerbations (renal and non-renal). In the present study the relation between the clinical course, the anti-dsDNA level, and the profile of the various complement components is described, with special regard to the period preceding exacerbations. An attempt has been made to answer the question whether or not the complement profile in combination with anti-dsDNA can predict an exacerbation and also its nature (renal versus non-renal) at a stage well before the first clinical signs of the exacerbation appear.

## Patients and methods

### PATIENTS AND SERA

Patients were followed up clinically (by AJGS and WB) in three clinical centres in the Netherlands: the

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Department of Internal Medicine of the Slotervaart Hospital (Amsterdam), the Department of Internal Medicine of the Free University Hospital (Amsterdam), and the Department of Rheumatic Diseases of the Dr Daniël den Hoed Hospital (Rotterdam). Venous blood was collected from 143 patients meeting the preliminary criteria for the diagnosis of SLE.<sup>9</sup> Samples from all these patients were obtained at four to six week intervals. After coagulation and centrifugation the sera were stored at  $-20^{\circ}\text{C}$ .

When the patients developed additional complaints or manifestations of their disease the frequency of clinical and serological investigations was increased, if possible, to one to three week intervals. Disease activity was recorded without knowledge of the serological parameters, and treatment of the patients was not influenced by serological data. Signs of disease activity, medication, indications for dosage alterations, and routine clinical laboratory results were recorded on standard clinical record sheets. Exacerbations were defined by the occurrence of major disease features.<sup>8, 10</sup> Minor symptoms were mainly restricted to the musculoskeletal system and the skin, which could be controlled by an increase in prednisolone dosage of 5–15 mg/day without the need for admission to hospital. Major features were characterised by at least one of the following: renal impairment, serositis, anaemia ( $\text{Hb} < 7 \text{ g/dl}$  ( $70 \text{ g/l}$ ) and/or leucopenia (white cell count  $< 4 \times 10^9/\text{l}$ ) and/or thrombocytopenia (platelet count  $< 50 \times 10^9/\text{l}$ ), neurological or psychiatric symptoms. These features always led to hospitalisation, and the dosage of prednisolone was increased to more than 20 mg/day. Episodes in which the features were explained by causes other than SLE were excluded.

Patients with pre-existing renal involvement were considered to be inactive when no significant alterations took place in the creatinine values, proteinuria, and urine sediment. An increase in proteinuria or other evidence of further deterioration of renal function, in the absence of other causes (such as pyelonephritis not related to SLE), were recorded as exacerbations of renal disease.

Renal function was assumed to have deteriorated when urinary protein increased to at least 1 g/24 h, or when there was an increase in red blood cell (RBC) casts in the urinary sediment, or a decrease in glomerular filtration rate (GFR) of more than 20%. Increase of urinary protein, sediment abnormalities, or decrease in GFR were considered significant only if observed on at least three consecutive days.

#### SERUM COMPLEMENT

C1q, C3, C4, C5, and C9 were determined by radial immunodiffusion with monospecific antisera against

C1q, C3c, C4, C5, and C9 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, lot Nos aC1q: KH43-02-PO2, aC3c: KH41-15-POI, aC4: KH12-09-PO2, aC5: KH44-03-PO3, and aC9: KH50-02-PO3). The values were expressed as percentages of a standard pooled donor serum. Results obtained from 200 healthy blood donors yielded normal values (mean  $\pm$  2 SD) of 69–128% for C1q, 37–144% for C4, 66–124% for C3, 73–135% for C5, and 68–133% for C9. For C4, C5, and C9 these normal levels were used. Because earlier studies had indicated that a level of C3 or C1q levels below 40% was always associated with renal involvement,<sup>8, 11</sup> this level was chosen to differentiate very low from decreased levels.

#### DETERMINATION OF ANTI-dsDNA

Anti-dsDNA was determined by the Farr assay, as modified by Aarden *et al.*<sup>12</sup>  $^3\text{H}$ -Labelled circular bacteriophage, PM<sub>2</sub>DNA, with a molecular weight of  $5.9 \times 10^6$ , was used as antigen. This  $^3\text{H}$ -labelled PM<sub>2</sub>DNA was purified according to the method of Espejo and Canelo<sup>13</sup> and found to be free of ssDNA contamination. Up to 50  $\mu\text{l}$  serum was incubated with 100 ng  $^3\text{H}$ -labelled dsPM<sub>2</sub>DNA. The total amount of antibody was expressed in units/ml. One unit is defined as the amount of anti-dsDNA that binds 30% of 100 ng PM<sub>2</sub>DNA under standard conditions.

#### RELATION BETWEEN ANTI-dsDNA LEVELS, COMPLEMENT LEVELS, AND DISEASE STATE (EXACERBATION)

The patients who experienced exacerbations were divided into two groups according to the presence or absence of renal involvement during the exacerbation. The moment of the first evidence of exacerbation was called time zero ( $t=0$ ), corresponding mostly to the highest dsDNA level. From this point the period before and after the exacerbation was divided into four week intervals. For each patient clinical and serological data were evaluated at the end of this investigation, from 24 weeks before to 24 weeks after the exacerbation.

Complement levels were classified according to the normal values (low or normal), at four week intervals. Results were then expressed as percentages of the patients with normal or low levels at the defined periods before and after the exacerbation.

#### Results

ANALYSIS OF THE 143 PATIENTS STUDIED  
Over a period of six years we recorded 33 exacerbations in 143 patients.<sup>11, 14</sup> Of these 33, 21 patients developed a deterioration of the renal function in association with other symptoms, whereas 12

Table 1 Frequency and type of 33 exacerbations in 143 SLE patients

Clinical features	Group I*		Group II*	
	No	%	No	%
Renal disease	21	100	0	0
Haematological disease	9	43	8	67
Pleuritis or pericarditis, or both	7	33	6	50
Neurological or psychiatric, or both	2	10	3	25

\*Group I=21 patients who developed a deterioration of the renal function in association with other symptoms; group II=12 patients who showed exacerbations without signs of renal involvement.

patients showed exacerbations without signs of renal involvement. Table 1 shows the frequency of major symptoms in both groups of patients. Complement profiles were systematically studied only in these 33 patients, whereas anti-dsDNA levels were studied in all 143 patients. During the period of observation the anti-dsDNA levels in the 110 patients that did not develop a major exacerbation remained nearly constant, as reported in our previous study.<sup>14</sup> Results obtained with the other 33 patients confirmed our previous finding that periods of major exacerbations were heralded by increasing anti-dsDNA levels, whereas concomitant with the actual development of these flares anti-dsDNA levels decreased rapidly (Figs 1 and 5). During this follow up study four patients developed a second exacerbation. Only the first was used in the analysis, lest these patients might influence the final results too strongly. Results obtained in those four exacerbations, however, were in agreement with the general results.

COMPLEMENT PROFILES OF 21 PATIENTS WITH RENAL INVOLVEMENT

Fig. 1 shows an example of the changes in the levels of the complement components seen in the patients who developed a deterioration in renal function. First there was a decrease of C1q and C4, later a decrease in C3. A relative decline in the C5 levels was also noted, though the levels remained within normal units. No change was observed in the C9 levels (data not shown).

Follow up data (profiles of the complement components) of all 21 patients, gathered as described in 'Patients and methods', have been combined in Fig. 2. This figure illustrates that for most of the 21 patients who developed renal disease the period preceding the exacerbation was characterised by decreasing C1q and C3 levels. In nearly 80% of the patients C4 was already reduced 16 weeks before the onset of the exacerbation. At that point C1q and

C3 were still 'normal' in 50% of the patients. At time zero C1q, C4, and C3 were decreased in 90% of the patients (C1q  $\leq$ 40%, C4  $\leq$ 37%, C3  $\leq$ 40%). In four of the 21 patients with exacerbations a discrepancy between the C1q and C3 profile was found: at t=0 two patients had normal C1q levels,

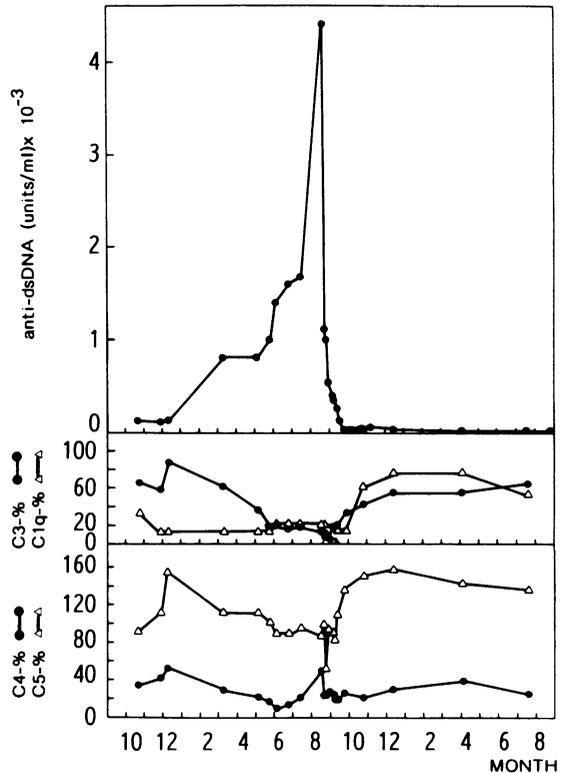


Fig. 1 Correlation between profiles of the complement components, anti-dsDNA, and course of the disease. A 24 year old female (patient 1) was admitted to hospital on 17 August 1981. For four years she had complained about her joints. Routine examination showed a positive LE phenomenon and antinuclear antibodies. In May a butterfly rash developed as well as leucocytopenia ( $2.3 \times 10^9/l$ ) and thrombocytopenia ( $80 \times 10^9/l$ ). At this stage anti-dsDNA levels were increasing. After a holiday (in Italy) the features of discoid lupus erythematosus developed. The serum creatinine levels remained nearly constant ( $73-68 \mu\text{mol/l}$ ) until 16 August 1981 (month 8, at which time the peak in anti-dsDNA level occurred); these increased to  $100 \mu\text{mol/l}$  on 16 August, and a few days later to  $124 \mu\text{mol/l}$  on 20 August and to  $123 \mu\text{mol/l}$  on 23 August. Nephritis was confirmed by the presence of hyaline casts in her urine. Renal biopsies showed diffuse proliferative glomerulonephritis. Prednisolone therapy was started on 16 August 1981 with 40 mg daily; later, the dose was increased to 100 mg daily. On this therapy creatinine levels decreased to  $95-100 \mu\text{mol/l}$ .

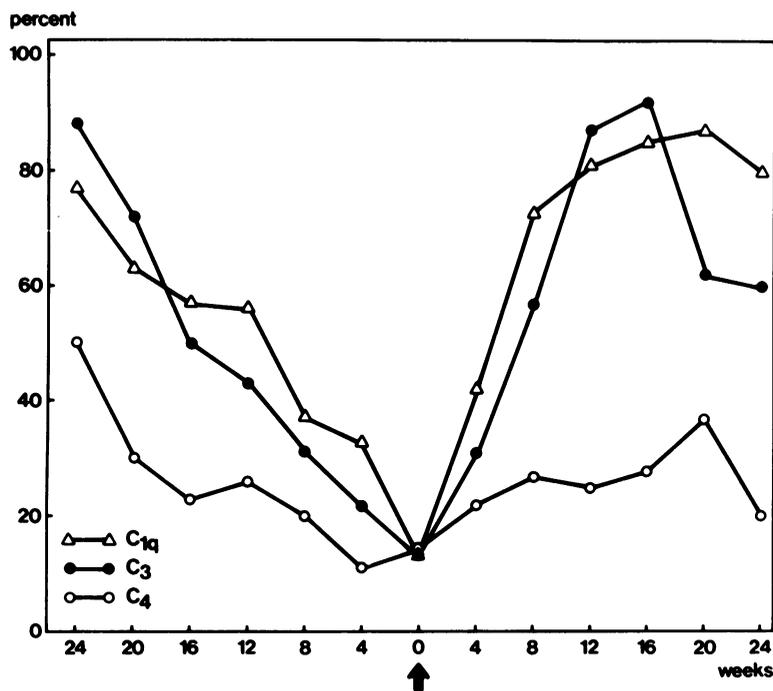


Fig. 2 The course of C1q, C3, and C4 levels in the 21 patients who developed an exacerbation of their disease with renal involvement. Each point in the figure represents the percentage of the 21 patients with levels above the 'normal' value. Note that the slope of the fall is less steep than the rise after  $t=0$  (start of the exacerbation). C4 was already slightly decreased in nearly 50% of the patients 24 weeks before 'the starting point' of the exacerbation.

with decreased C3 and C4 levels, and two patients had normal C3 levels, with decreased C1q and C4 levels. Both Figs 1 and 2 show that in the period during and after the start of the exacerbation C1q, C4, and C3 levels tended to become normal rapidly. The increase seemed to occur faster than the decrease.

Fig. 3 shows results for C5 and C9 data; only in 40% of the patients was C5 decreased at  $t=0$ . The C9 levels were slightly decreased in only one patient; in other patients all levels were normal during the follow up.

COMPLEMENT PROFILES OF 12 PATIENTS WITHOUT RENAL INVOLVEMENT

Complement profiles of the other 12 patients who developed an exacerbation are summarised in Fig. 4. In the period preceding the exacerbation there was a decrease in the C4 levels only. At time zero 60% of the patients had C4 levels lower than 37% of



Fig. 3 The course of C5 and C9 in the 21 patients who developed a deterioration of the renal function. During the whole follow up period values of C9 hardly decreased. At  $t=0$  57% of the patients had normal C5 levels. C5 levels were falling at 20 weeks before the onset of the exacerbation ( $t=20$ ), and after  $t=0$  they increased to normal.

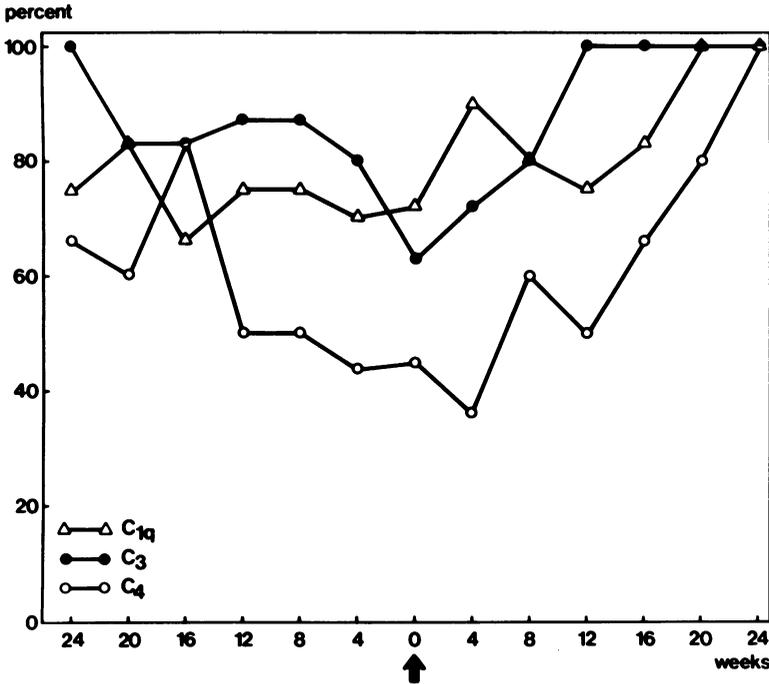


Fig. 4 The course of C1q, C3, and C4 levels in the 12 patients who developed an exacerbation of their disease but without signs of deterioration in renal function. There was a general decrease of C4. At t=0 nearly 60% of the patients showed decreased C4 levels (<37%). A decrease of the C4 level was already evident in 40% of the patients at 24 weeks before the onset of the exacerbation (t=24). C3 and C1q levels fell in 30% of the patients.

the normal value. Of these patients, two had a decreased C1q level at t=0, with normal C4 and C3 levels, three had a decreased C3, with normal C1q and C4 levels and one had decreased C1q and C4 levels (Fig. 5). In the patients with decreasing C1q levels or C3 levels, or both, no clear relation was found between the course of these levels and the developing exacerbation. During a relatively long time period preceding the exacerbation steadily decreasingly C1q or C3 levels, or both, were found. Only the C4 levels showed the clear relation between fall and approaching exacerbation. In the C5 and C9 profiles (Fig. 6) no 'abnormalities' were detected: both complement components were always either within the normal range or increased.

ANTI-dsDNA PROFILES IN THE 33 PATIENTS WHO DEVELOPED AN EXACERBATION

In the 24 weeks preceding exacerbations a continuous increase in the anti-dsDNA was found for all patients with a doubling time (t<sub>2</sub>) of less than 10 weeks. In 28 patients the t<sub>2</sub> was even shorter than six weeks. The actual exacerbations were characterised by rapidly decreasing levels of anti-dsDNA: 29 patients had a t<sub>1/2</sub> shorter than three weeks; the other four patients showed a t<sub>1/2</sub> shorter than six weeks.

PREDICTIVE VALUE OF THE LEVELS OF COMPLEMENT COMPONENTS

Two patients in whom a deterioration of the renal function developed showed first a decrease in the C4 level, followed later by falls in C1q, then C3 (see Fig. 1). In the non-renal patients, however, decreased complement levels were also seen. To evaluate which patients were prone to develop renal involvement at t=0 all data of the 33 exacerbations were taken together. Firstly, the separate complement components were considered in relation to the development of renal involvement. Secondly, to increase the specificity, combinations of the complement components were examined (Table 2). For example, at t24 eight out of the 33 (24%) patients had a decreased C1q level. In five of these 8 patients (63%) renal involvement developed at t=0. At t=8 nearly half of the 33 patients had decreased C1q and/or C3 or C4. Of these patients, 75-90% developed renal involvement at t=0. Since all patients in the period preceding the exacerbation showed increasing anti-dsDNA levels, these values meant that a patient who also developed decreasing levels of C1q or C3 and/or C4 levels lower than 40% was prone to develop renal involvement. When decreasing levels of C1q and C3 occurred at the same time as increasing anti-dsDNA levels then a

deterioration in the renal function could be predicted in nearly all cases. The specificity was hardly increased by addition of the C4 data. For the remaining 110 SLE patients, who did not have clinical signs of an exacerbation during the period of

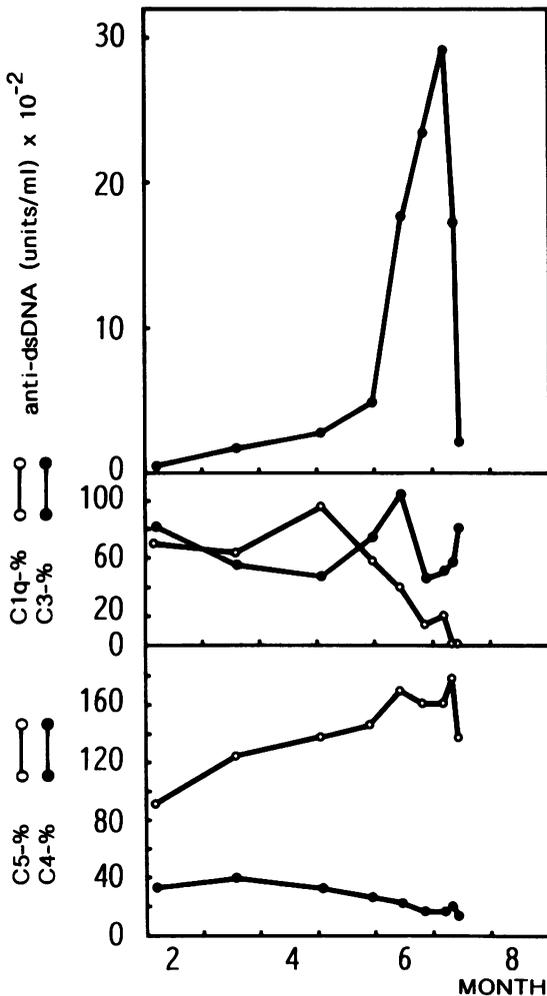


Fig. 5 Correlation between the profiles of complement components, anti-dsDNA, and course of the disease. Patient 2 was a 47 year old male, whose diagnosis of SLE was made in 1974. At that time he was admitted to hospital with pleuritic pain. Serological examination showed anaemia and leucocytopenia. Periods of polyarthritis followed. On 4 July 1982 (month 6) he entered hospital with fever (40.5°C); pleuropericarditis was then diagnosed. The anti-dsDNA on 5 July was 2960 units/ml, on 8 July 1720 units/ml, and on 11 July 216 units/ml. Prednisolone therapy (100 mg/day) was started on 8 July. On 14 July he died due to irreversible forward cardiac failure. (Histopathological diagnosis: pericarditis, serious myocarditis.)

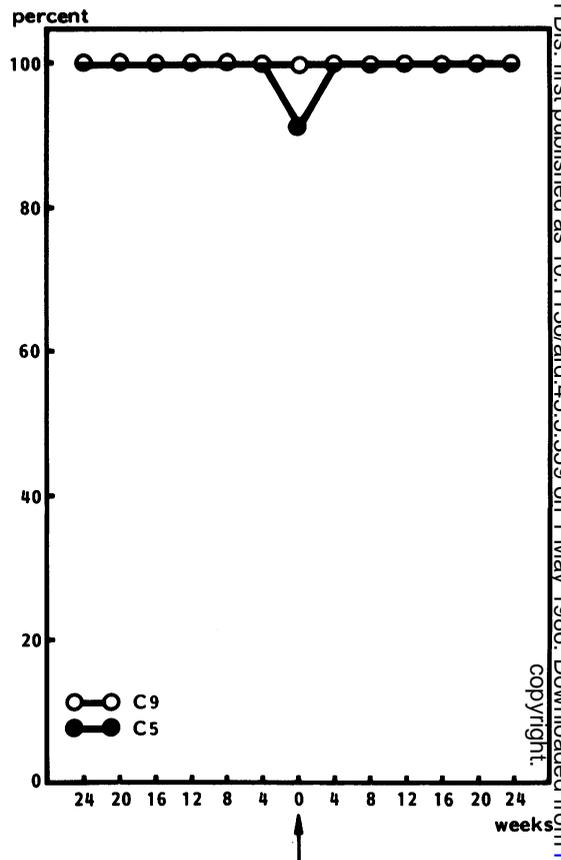


Fig. 6 Levels of C5 and C9 in 12 patients during periods of exacerbation without evidence of renal involvement. Decreased C5 or C9 levels were detected in hardly any of these patients.

this study, increasing levels of anti-dsDNA combined with decreasing complement levels were never observed.

### Discussion

The complement system is thought to be involved in the immune complex mediated lesions of SLE with deposition of complement in renal and other lesions. The level of serum complement, therefore, may represent an indicator of disease activity.

In our retrospective study<sup>8</sup> evidence of renal involvement was observed when the decrease of high anti-dsDNA was accompanied by very low ( $\leq 40\%$ ) serum levels of the complement factors C1q and C3. In that study two out of 43 patients were observed with low C1q and normal C3, but neither of these two patients showed symptoms of

Table 2 Predictive value of the levels of the complement components in the 33 SLE patients

Time*	C1q<40%†	RI‡	C3<40%†	RI‡	C4<37%†	RI‡	C1q+C3<40%†	RI‡	C1q+C3≤40%† +C4≤37%	RI‡
24	24	63	7	100	50	83	0	0	0	0
20	29	80	23	75	62	80	6	100	6	100
16	40	75	40	88	60	90	15	100	15	100
12	37	77	41	90	66	75	13	100	13	100
8	50	83	50	90	70	75	29	100	29	100
4	53	80	59	87	74	70	39	100	32	100
0	66	86	69	82	75	72	54	94	54	94

\*Weeks preceding the onset of the exacerbation (t=0).

†The percentage of all 33 patients with decreased complement components at the defined time interval in the period preceding the exacerbation.

‡The percentage of the patients with decreased complement components who will develop renal involvement (RI) at time 0.

renal impairment. Only one patient showed a deterioration in renal function with low C1q and subnormal C3 (43%) levels.

Until now the most consistent finding in patients with active SLE has been a low C4 level.<sup>15</sup> Other authors regarded the presence of low levels of C1q or C4 as special features of patients with renal involvement.<sup>3,4</sup> Moreover, it was claimed that patients with renal disease tended to have lower levels of C1q, C4, and C3 than those without renal disease.<sup>16-19</sup> Very low levels of C1q, C3, and C4 have often been reported for patients with renal disease but rarely in its absence. All the authors cited described their results from the start of the exacerbation of the disease. Ours is the first study to cover the period before the start of a flare. Only in one study on the relation between complement profiles and disease activity were results described in a prospective manner<sup>1</sup>; in that report a decrease in the CH<sub>50</sub> titre and an increase in antinuclear antibodies were specifically noted before clinical features of exacerbations were found. Each SLE exacerbation was characterised by steadily increasing anti-dsDNA levels; overall, at the first sign of exacerbation (t=0) the highest anti-dsDNA levels were reached.

The complement profiles show that if the anti-dsDNA increases and the various complement components (C1q, C3, and C4) fall the patient is prone to develop an exacerbation with signs of renal involvement. Of the 33 observed exacerbations, there was only one exception to this. For a long time C4 levels have been considered to be the best guide for the follow up of SLE patients. One should be aware that low C4 levels can be observed in other diseases, for example, in viral hepatitis complicated by arthritis.<sup>20</sup> Secondly, this study and others show that C4 may remain depressed over a long period of time, even in the face of an otherwise normal

complement profile in the period before or after an exacerbation.<sup>6</sup> Clear differences in C9 values in SLE patients, with or without renal involvement, have not been described.<sup>5</sup> Moreover, in patients with, for example, membranoproliferative glomerulonephritis, normal or increased C9 levels (our findings) are found.<sup>21</sup>

Levels of C5 in patients with renal involvement tended to be lower than in patients without renal involvement. In this prospective study we confirmed in a larger group of patients our earlier findings of the relation between the course of the disease and the anti-dsDNA profiles. All observed periods of major flares were heralded by a period of increasing levels of anti-dsDNA. Then, once the exacerbation started, the anti-dsDNA levels decreased rapidly. Thirty three such exacerbations were observed in 143 patients. In the remaining 110 patients (none of whom developed a major exacerbation) anti-dsDNA levels remained constant during the time they were studied. This study was specifically focused on those patients with changing anti-dsDNA levels, and it became clear that changes in anti-dsDNA content associated with the three different complement components (C1q, C4, and C3) had the most prognostic significance, as follows: (a) The 21 patients who were about to develop renal involvement were characterised by an initial decrease of C4, followed by C1q and C3. During the phase of exacerbation C1q and C3 increased first, followed later by C4 (Fig. 2). If during the follow up study of an SLE patient a continuous increase of the anti-dsDNA level (with a doubling time of four to six weeks) accompanies decreasing complement components C1q, C3, and C4, not only can an exacerbation be predicted, but also a deterioration in renal function.

(b) In nearly 10% of the SLE patients C1q or C3 were normal at the start of the renal involvement.

These levels dropped soon after the first changes were observed.

(c) In the 12 patients who developed an exacerbation without signs of renal involvement different complement components were variably lowered. The complement component most often decreased was C4.

(d) C5 levels in patients with renal involvement tended to be lower than in patients without renal involvement.

(e) In both groups of patients increased levels of C9 were detected, with no clear relation with anti-dsDNA levels or with the course of the other complement components. Determination of C9 is thus not useful in the management of SLE.

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