The use of C3d as a means of monitoring clinical activity in systemic lupus erythematosus and rheumatoid arthritis

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SUMMARY Plasma samples from 44 patients with systemic lupus erythematosus (SLE) and 43 with rheumatoid arthritis (RA) were assayed for C3d, a breakdown product of the third component of complement (C3), which was also measured in parallel. Levels of C3d varied in direct proportion with disease activity in RA, whereas C3 showed little change. Although C3d values also increased with worsening clinical condition in SLE, this trend was not considered to be sufficiently clear to be useful and did not provide any advantage over the routinely performed C3 assay.

The problems of assessing clinical activity in systemic lupus erythematosus (SLE) by monitoring immunological parameters have been discussed.¹ In particular the lack of any measurement that would reliably reflect the severity of the disease was noted; thus improvement in objective testing is needed.

Low plasma C3 levels can be used as an approximate indicator of increasing clinical activity in SLE,²⁻⁴ although this trend is not as clear for rheumatoid arthritis (RA).⁵⁻⁶ However, it is possible that variations in C3 levels may occur for reasons other than complement consumption; for example an increased synthetic rate may accompany an accelerated catabolism.⁶ In such instances measured values may bear little relation to the severity of disease. C3d, a fragment generated from activated C3, may be more closely related to an inflammatory process, and initial investigations⁷⁻⁸ suggested that the measurement of this component may provide valuable clinical information. This study attempts to evaluate the usefulness of C3d measurements for the assessment of clinical activity in SLE and rheumatoid arthritis (RA).

Patients, materials, and methods

SLE patients. Each patient had 4 or more of the criteria for the classification of SLE as defined by the American Rheumatism Association.⁹ Patients were grouped according to clinical status (inactive, slightly active, moderately active, severely active) as defined in Table 1. Only one plasma sample was taken from any one patient on one instance in any particular clinical grade.

RA patients. RA patients were either 'classical' or 'definite' according to the American Rheumatism Association's criteria.¹⁰ Patients were classified as 'inactive', 'moderately active', or 'severely active' by the method of McCarthy.¹¹ Plasma samples were taken from each subject on only one instance regardless of clinical grade.

Blood sampling. Blood samples were collected in standard EDTA tubes and the plasma was stored at -20°C and assayed for C3d within 2 weeks. It has been clearly established that C3d levels do not increase after this time in these storage conditions.¹² Plasma C3 levels were measured with a laser nephelometer (Hyland Laboratories).

C3d assay. C3d was assayed according to the technique of Perrin *et al.*⁷ without prior knowledge of the clinical state of the patient. C3d was first separated from the higher molecular weight components of C3 by incubation of 50 µl of plasma with an equal volume of a 22% solution of polyethylene glycol (PEG

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Table 1 Criteria for grading clinical severity of SLE

Clinical feature	Score
Visual analogue, 5 cm or an increase of 2 cm or more compared with previous visit	1
Pyrexia (not due to an infection)	1
Lymphadenopathy (not due to an infection)	1
Arthralgia and/or myalgia	1
Pleuritis and/or pericarditis	2
Vasculitic skin rash UCH grade* 1	0
2 }	1
3 }	1
Raynaud's disease (active)	1
Cerebral: UCH grade* 1	1
2 }	3
3 }	3
Renal proteinuria (+ or more) } but little change proteinuria trace +↑BP } compared with proteinuria trace + } previous visit ↑ urea/creatinine }	1
Increasing proteinuria ± rising BP ± rising urea/creatinine	3
Easy bruising/bleeding	1
Steroids, prednisolone 0-4 mg	0
5-24 mg/day	1
25 mg +	2
Maximum	20
Grade 1, inactive 0/1	
Grade 2, mild 2-4	
Grade 3, moderate 5-7	
Grade 4, severe 8+	

* See reference 1.

6000) in 0.1 M Tris-glycine buffer, pH 7.8 for 4 hours at 4°C. After this time the samples were centrifuged and the concentration of C3d in the supernatant was estimated by radial immunodiffusion¹³ in 1% agarose and with 1% anti-C3d antiserum (Dako).

Diffusion rings were related to a standard curve constructed from dilutions of a pool of normal human serum in which C3d had been generated by incubation with cobra venom (Calbiochem) for 48 hours at 37°C. This sample was considered to be 100% activated.

Controls. Overall, plasma samples from 59 control subjects (38 in Brest, 21 in London) were assayed to

Table 2 Mean C3 and C3d values for SLE patients in each clinical group

	Inactive (1) n=15	Slightly active (2) n=31	Moderately active (3) n=10	Severely active (4) n=3
C3 $\mu\text{g ml}^{-1}$	1195±602	1182±354	1010±616	809±263
C3d % activity	27±9	25±12	40±26	31±4

The only significant differences in C3d levels were between (1 + 2) and (3 + 4), $0.025 < p < 0.05$. Identical differences were noted for C3 levels.

establish a normal range for C3d. These subjects comprised laboratory volunteers and staff attending a fracture rehabilitation clinic. The subjects were matched for age and sex against the patients. All these normal controls had a C3d value below 10% activation (below curve), and thus an arbitrary value of 15% activation was assigned as the upper limit of normal for C3d.

Statistics. Data were analysed either by Student's *t* test or by linear regression, the latter being performed on $\log_{10}$ transformed data.

Results

C3d levels in 63 plasma samples from 44 patients with SLE (Fig. 1) and 43 with RA (Fig. 2) are given for the various clinical grades. Further analysis is made in Tables 2 and 3, where it can be seen that C3d gave no better indication of clinical status than C3 in SLE patients. In contrast C3d distinguished well between the clinical grades in RA, although C3 completely failed to do this. In SLE linear regression analysis performed on C3 and C3d values revealed no correlation, either overall or within clinical groups. However, in RA there was a good overall correlation between these 2 parameters in the whole group of patients ($r = 0.61$, $p < 0.001$) and also

Fig. 1 C3d levels in 59 plasma samples from 44 patients with SLE, grouped according to clinical activity.

Fig. 2 C3d levels in plasma samples from 43 patients with RA, grouped according to clinical activity.

in the moderately active category ($r = 0.57$, $p < 0.05$) and the severely active group ($r = 0.84$, $p < 0.001$). Furthermore C3d was markedly increased in 5 patients with extra-articular disease (mean = 45% activation, standard deviation 7.1%), whereas the C3 value was normal (mean = 1510 $\mu\text{g/ml}$, standard deviation 16 $\mu\text{g/ml}$). There was no clear association between C3d levels and pattern of organ involvement in SLE.

Table 3 Mean C3 and C3d values for RA patients in each clinical group.

	Inactive (1) <i>n</i> =12	Moderately active (2) <i>n</i> =26	Severely active (3) <i>n</i> =5
C3 $\mu\text{g ml}^{-1}$	1300 $\pm$ 122	1388 $\pm$ 33.7	1520 $\pm$ 420
C3d % act.	12.0 $\pm$ 8.3	27.5 $\pm$ 15.7	43.75 $\pm$ 11.7

Groups 1 and 2, $p < 0.0025$. Groups 2 and 3, $0.005 < p < 0.01$. There were no significant differences between C3 levels in any of the clinical groups.

Discussion

In this study we have shown that C3d levels were clearly raised above normal in both SLE and RA patients, presumably affecting the inflammatory processes involved in these diseases.

C3d levels were associated with the severity of disease in RA and proved to be a much better indicator than C3, which showed no correlation. This finding is in agreement with the data of Mallya *et al.*¹⁴ and Nydegger *et al.*,⁸ who showed that plasma C3d levels correlated with clinical activity, and also with the data of Franco and Schur,⁵ who found that C3 was rarely below the normal range. It is also of interest to note that C3d was particularly increased in patients with extra-articular disease: this was also observed by Nydegger *et al.*,⁸ Bourke *et al.*,¹⁵ and Perrin *et al.*,¹⁶ who found C3d levels raised in synovial fluid from RA patients.

As regards SLE, C3d measurements proved to be of no greater value than C3 for determining the clinical status of the patients. While both these parameters tended to change with worsening of disease, this trend, though statistically significant, was not considered to be sufficiently clear to be useful. The latter result is perhaps a little surprising, as these components have been said to show an inverse correlation in SLE.⁷ Perrin *et al.*⁷ showed that C3d was increased in SLE in an inverse relationship with C3 but did not comment on the association between C3d and clinical activity except in 2 selected case studies. It is possible that we did not establish a clear trend because of the diverse nature of the disease in our patients. We could not analyse the data further according to the principal clinical features such as arthralgia, renal, cerebral, and dermatological symptoms owing to the small numbers of subjects in these subgroups. It is quite possible that there was a relationship between C3d and clinical status in those patients with kidney disease, as other investigations would appear to suggest that high levels of C3d are associated with nephritis.^{7, 17} A larger sample might clarify this issue.

In contrast to SLE, the correlation between C3 and C3d was very marked in RA, particularly in the severely active groups, though no explanation can be offered for this. Thus routine C3d measurements would appear to be of value for monitoring the clinical progress of RA patients, though our data indicate that this parameter offers little useful information in the case of the SLE patient.

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