Rheumatological manifestations, organ damage and autoimmunity in hereditary C2 deficiency

G. Jönsson¹,³, A. G. Sjöholm³, L. Truedsson³, A. A. Bengtsson², J. H. Braconier¹ and G. Sturfelt²

Objective. To analyse rheumatological manifestations, organ damage and autoimmune responses in a large cohort of patients (n = 45) with homozygous C2 deficiency (C2D) and long-term follow-up.

Methods. Medical records were reviewed and were supplemented with a mailed questionnaire for assessment of cardiovascular disease (CVD) risk factors. Organ damage was evaluated using the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI). Causes for disability pensions were investigated. Autoantibodies were determined with established methods.

Results. Patients with rheumatological diseases had systemic lupus erythematosus (SLE, n = 12), undifferentiated connective tissue disease (n = 5) or vasculitis (n = 3). Judging from annual SLICC/ACR DI, C2D patients with SLE run a similar risk of development of severe disease as other patients with SLE. An increased rate of CVD was observed not explained by Framingham-related risk factors. Disability pensions were mainly related to rheumatological disease. The prevalence of anti-nuclear antibodies in C2D and of anti-SS-A was 25% while anti-RNP was found in 4%. Only one patient showed antibodies to dsDNA. Formation of anti-cardiolipin antibodies (aCL) appeared to be increased in C2D despite the absence of an anti-phospholipid syndrome. The prevalence of antibodies to the collagen-like region of C1q (C1qCLR) was also remarkably high and was not related to rheumatological manifestations.

Conclusions. Severity of SLE in C2D is similar to that of SLE in other patients. Conventional risk factors do not explain the occurrence of CVD in C2D. The high prevalence of aCL and anti-C1qCLR indicates mechanisms through which impaired complement function promotes formation of autoantibodies.

Key words: Antiphospholipid syndrome, Autoantibodies, C2 deficiency, Cardiovascular disease, Complement, SLE.

Introduction

Systemic lupus erythematosus (SLE) is a B-cell-dependent autoimmune disease with strong familial aggregation [1]. A Mendelian mode of inheritance is not seen, but multiple candidate genes of susceptibility have been identified by association studies. These include major histocompatibility complex (MHC) alleles, complement deficiency genes, Fcγ receptor (FcγR) alleles and other genetic markers [2]. In experimental murine models, several genes and pathogenetic pathways have been shown to contribute to development of lupus-like disease [2, 3]. Furthermore, a broad variety of environmental factors have been suggested to be implied in the aetiology of the disease [4, 5].

The concept of complement involvement in the pathogenesis of SLE originates from the findings of hypocomplementaemia and deposition of complement proteins in target organs [6, 7] indicating that complement activation is important in the pathogenesis of SLE. During the 1970s, inherited complement deficiencies were surprisingly found to be associated with development of SLE [8]. This suggests that impaired complement function promotes autoimmune inflammation and does not protect against development of the disease.

C2 deficiency (C2D) has an estimated prevalence of ~1/20 000 persons of European descent [6]. The structural gene for C2 is located in the MHC class III region together with genes for C4 and factor B [6]. Nearly all cases of C2D are caused by a 28-bp deletion in the C2 gene, a mutation associated with the HLA-B*18, S04,DRB1*15 haplotype [9]. Deficiencies of C1q, C1r, C1s and C4 have a more heterogeneous genetic background [6].

C2 supplies the catalytic part of the C3 convertase C4b2a, which can be generated through the classical pathway or the lectin pathway of complement [10]. The classical pathway is initiated by interaction of C1q with IgM and IgG in immune complexes or with other C1q-binding structures [11]. In the lectin pathway, mannann-binding lectin (MBL) and ficolins that form complexes with MBL-associated serine proteases (MASPs), bind to target structures such as microbial carbohydrates [11]. C4b2a is generated through the actions of C1s and MASP-2. Hence, abnormal immune functions in C2D may be ascribed to impaired classical pathway or lectin pathway activity. The alternative activation pathway is usually intact in C2D and the recently reported MBL-dependent activation of C3 and the alternative pathway without involvement of C2 may play a role [12].

Among patients with C2D initially reported in the literature, about one-third showed SLE or SLE-like disease with predominance of cutaneous manifestations [6, 8]. Development of severe SLE with kidney involvement appears to be rare in C2D, but may occur [6, 8]. C2D is also known to be associated with susceptibility to invasive infections and a variety of immunological diseases, but many persons with C2D appear to be completely healthy [13, 14].

We recently described a large cohort (n = 40) of C2-deficient patients emphasizing the high prevalence of invasive infections [14]. The C2D cohort has now been enlarged and in this investigation we have focused on rheumatological and cardiovascular manifestations in C2D. Most of the patients were subject to prolonged observation, which enabled analysis of organ damage and working capacity, issues that have not been previously addressed in patients with complement deficiency.

Patients and methods

Patients

Between 1977 and 2006, 45 Swedish persons from 33 families were identified through screening as a routine part of complement

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analysis at the Clinical Immunology Unit, University Hospital of Lund. During this period, about 46 000 analyses for complement deficiency were performed mainly with haemolytic gel assays [15]. The first collected serum samples were retrieved from hospital departments of Dermatology (1%), Internal Medicine (13%), Infectious Diseases (2%), Otorhinolaryngology (1%), Paediatrics (3%), Rheumatology (10%), General (53%) and Private Practice (7%) and from other departments (11%). More than one-third of the patients were found in southern Sweden, the rest were either sent directly to our laboratory or were referred after initial screening from other Clinical Immunology laboratories in Sweden. Seven non-index persons to a first-degree relative with C2D were identified through family studies in 18 families [14]. Among these non-index patients, one patient (Patient 3) developed SLE later in life and two patients (Patient 21 and 25) were documented for severe infection. To the previously described 40 C2D patients (Patients 1–40) [14], two females (Patients 42 and 45) and three male (Patients 41, 43 and 44) patients were added to the study. Of the 45 patients, 25 were females and 20 were males.

In the previous investigation, which included 40 of the patients, 33 were found to be homozygous for the 28-bp C2 gene deletion, DRB1*15 and C4A*4 B*2 [14]. Three patients (Patients 19, 37 and 38) were heterozygous for the 28-bp deletion and two of them (Patients 37 and 38) had MHC haplotypes not previously described in relation to the C2 null genes. Of the five additional C2D persons (Patients 41–45), four were homozygous for the 28-bp deletion and one heterozygous (Patient 45), but DRB1 and C4 variants were not determined.

The mean age at the time of C2D diagnosis was 32 yrs (median 35, range 1–76). The medical records contained at the time of this review a total of 1772 person-years. The average time of follow-up per person was 39 yrs (range 3–77). A control group consisting of patients with genuine SLE (n = 134) of whom 28 had secondary anti phospholipid syndrome (APS) was also available. Informed written consent was given by the participants and the study was approved by the Research Ethics Committee of the University of Lund and six other centres.

Supplementary data regarding clinical, genetic and autoantibody findings in the 45 C2D persons are available in Supplemental Table S1 published online.

Assessment of working capacity

Data from the Regional Social Insurance Office concerning working incapacity, i.e. temporary or permanent disability pension, were utilized for analysis of C2D-associated morbidity in 26 adult persons. Information concerning the number of inhabitants in the labour force was obtained from the Swedish Statistical Database, Stockholm, Sweden. The observation period was 1981–2003 and patients between 18 and 65 yrs with assessable data were investigated. During this observation period, the median year (1992) was chosen for registration. Data from 1992 regarding disability pension in the Swedish population and in the C2D cohort (n = 19) were used for comparison and determination of the point prevalence on 31 December 1992.

Cardiovascular risk factors

A questionnaire modified from Bengtsson et al. [4] and medical records were used for assessment of traditional risk factors for development of cardiovascular disease (CVD). The following traditional risk factors for CVD were recorded: arterial hypertension (blood pressure $\geq 140/90$ mmHg or treatment with anti-hypertensive drugs), diabetes mellitus (fasting glucose $\geq 7.0$ mmol/l or treatment with insulin or oral hypoglycaemic agents), dyslipidaemia (high-density lipoprotein (HDL) cholesterol $\leq 1.6$ mmol/l, low-density lipoprotein (LDL) cholesterol $\geq 3.4$ mmol/l, or triglycerides $\geq 2.3$ mmol/l or treatment for hyperlipidaemia), post-menopausal status, smoking, obesity [body mass index (BMI) $\geq 30$ kg/m$^2$] and a family history of premature CVD in first-degree relatives. Premature CVD was defined as an acute myocardial infarction or sudden death before the age of 55 yrs in males and 65 yrs in females [16, 17]. The questionnaire was given to 25 patients ($\geq 18$ years) and all 25 patients responded. Ten patients were $< 18$ yrs of age and 10 patients were deceased. At the time when the questionnaire was distributed, only two of the six patients with a record of acute myocardial infarction (AMI) were alive. Blood samples for analysis of cholesterol and triglycerides were obtained in 13 females and 7 males. The CVD risk calculator programme published by Anderson et al. [18], was used to assess the risk of a cardiac event in the C2D patients. The upper limit for intervention is, according to this risk assessment model, $\geq 16\%$.

Laboratory studies

Available serum and EDTA plasma samples were stored in aliquots at $-80^\circ$C. Assessment of anti-nuclear antibodies (ANA) was performed by indirect immunofluorescence with HEP-2 cells (Euroimmun, Lübeck, Germany) at a serum dilution of 1/400 corresponding to ANA at 141 U/ml (WHO reference serum 66/233). Rheumatoid factors (RF) were measured by an enzyme-linked immunosorbent assay (ELISA) [19]. Anti-cardiolipin antibodies (aCL) were determined by ELISA [20], native DNA (dsDNA) antibodies with the Crithidia luciliae test [21] using a commercial kit (Euroimmun, Lübeck, Germany) and antibodies to the collagen-like region of C1q (anti-C1qCLR) as described by Märtensson et al. [22]. Anti-C1qCLR values were given in arbitrary units (AU) with values $< 16$ AU/l defined as negative. The aCL values were defined as negative ($< 20$ lg phospholipid units, GPLU/ml), low (20–40 GPLU/ml), medium (41–80 GPLU/ml) and high ($> 80$ GPLU/ml). For reference, analysis of aCL (n = 100) and anti-C1qCLR (n = 96) was performed in healthy blood donors. Autoantibodies to ribonucleoprotein (RNP), histone, ScI-70, Sm, Sm B subunit, SS-A 52/60, SS-A 52 and SS-A 60 were determined by immunoblot analysis (INNO-LIA ANA, Innogenetics, Gent, Belgium). Indirect immunofluorescence for detection of antineutrophil cytoplasmic autoantibodies (ANCA) was performed with BIOCHIP Mosaic (Euroimmun, Lübeck, Germany). Antibodies against proteinase 3 (PR3) and myeloperoxidase (MPO) were determined by ELISA using commercial antigens provided by Wieslab AB, Lund, Sweden.

Assessment of SLE

SLE disease activity and cumulative organ damage were determined by using the SLE disease activity index (SLEDAI-2K) [23] and the SLICC/ACR DI [17], respectively. In addition, information on glucocorticoid treatment and immunosuppressive drugs was documented during the available observation period. Experienced rheumatology specialists carried out clinical evaluation of the patients. SLEDAI and the SLICC/ACR DI were established by information in the medical records.

Statistics

Differences between groups were analysed with Fisher’s exact test, the $\chi^2$ test and the Mann–Whitney test. The Kruskal–Wallis test was used to make comparisons of the aCL and anti-C1qCLR concentrations between the four patient groups given in Figs 1 and 2. All $P$-values were two-tailed. Standard mortality/morbidity ratio (SMR) was calculated in C2D persons considered at risk for AMI (30–79 yrs of age) during the follow-up period 1940–2005. Twenty-eight C2D persons in the cohort could be observed and attributed to person-time until their first AMI was recorded. The person-time found in the C2D persons was compared with data from the Swedish National Board of Health and Welfare Registries concerning age-related AMI incidences in
FIG. 1. Comparison between aCL concentrations in C2D patients (n = 42) and patients with genuine SLE (n = 134) using the earliest collected available serum samples. The aCL levels were defined as negative (<20 IgG phospholipid units, GPLU/ml), low (20–40 GPLU/ml), medium (41–80 GPLU/ml), and high (>80 GPLU/ml) indicated with broken lines. The C2D patients had higher concentrations of aCL than the SLE patients (P < 0.0001, Kruskal–Wallis test).

FIG. 2. Comparison between anti-C1qCLR concentrations in C2D patients (n = 42) and patients with genuine SLE (n = 134) using the earliest collected available serum samples. The upper limit of anti-C1qCLR levels defined as negative (<16 AU/l) is indicated with a broken line. The C2D patients had higher concentrations of anti-C1qCLR than the SLE patients (P = 0.001, Kruskal–Wallis test).
### Results

#### Clinical manifestations

Among the 45 patients with C2D, 12 patients (8 females and 4 males) had a clinically diagnosed SLE and fulfilled four or more of the 1982 ACR classification criteria [24]. The distribution of ACR criteria for SLE and other clinical manifestations are given in Table 1. The most common ACR criteria were arthritis (83%), malar rash (92%), discoid lesions (67%), photosensitivity (67%) and serositis (42%). The mean age at diagnosis of SLE was 37 yrs (median 39, range 10–57). Seventy-five per cent of the SLE patients were above the age of 30 yrs at the time of their SLE diagnosis. Patient 12 developed a diffuse proliferative glomerulonephritis (WHO class IV) with progression to renal failure in 6 months. Atrioventricular block II-III was found in two patients (Patients 11 and 45) and pericarditis with duration for 6 months was documented in two patients (Patients 3 and 12). Patient 43 had gone through surgery intervention against development of CVD (Patient 16%).

#### Organ damage

SLICC/ACR DI was assessed in the 12 C2D patients with SLE. The mean SLICC/ACR DI score was 3.8 at 10 yrs after diagnosis. A main cause of damage was cardiovascular manifestations (Table 2). Documented cardiovascular manifestations included five AMI in three SLE patients (Patients 1, 3 and 43). Two patients had valvular disease (Patients 11 and 12), aortoventricular block II-III was found in two patients (Patients 11 and 45) and pericarditis with duration for >6 months was documented in two patients (Patients 3 and 12). Patient 43 had gone through surgery with a three-vessel coronary bypass grafting. The autopsy report concerning Patient 1, a 34-yr-old woman, revealed severe atherosclerosis, a cerebrovascular accident, a dissecting aorta aneurysm and two myocardial infarctions.

Patients with documented AMI (Patients 1, 3, 5, 10, 26 and 43) did not have high levels of anti-C1qCRL (median <16 AU/l, range <16–31 AU/l) or aCL (median <20 GPLU/ml, range <20–26 GPLU/ml). The patients had their first AMI at a mean age of 56 yrs (median 56, range 33–77 yrs). In general, the frequency of conventional Framingham risk factors was fairly low in the C2D cohort and the calculated percentage risk of suffering a cardiac event during the following 10 yrs was also found to be low in 20 accessible adult C2D patients (mean 6%, median 5%, range 0–16%). Thus, only one patient reached the limit for intervention against development of CVD (Patient 27, 16%).

During the available observation period, the SLE patients were treated with an average dose of glucocorticoids of 2.5 mg/day (range 0–20) with a maximum dose of 40 mg/day. Three SLE patients (Patients 1, 4 and 12) received periodical treatment with azathioprine and hydroxychloroquine. One SLE patient (Patient 45) was treated with pulses of cyclophosphamide for extensive skin manifestations. A cushingoid appearance developed in four patients (Patients 1, 2, 11 and 12).

Calculations concerning the risk of AMI in the C2D cohort (n = 28) showed a statistically significant increased SMR of 4.1

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**Table 1. Clinical findings in the 20 C2D patients with rheumatological disease**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Rheumatological disease</th>
<th>ACR criteria for SLE</th>
<th>Other clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SLE</td>
<td>1, 2, 3, 5, 6, 8, 11</td>
<td>Alopecia, back pain, myalgia, Raynaud's phenomenon, SCLE, AMI x 2, died of AMI (34 yrs).</td>
</tr>
<tr>
<td>2</td>
<td>SLE</td>
<td>1, 5, 6, 9</td>
<td>Alopecia, fractures, gastrointestinal vasculitis, back pain, pancreatitis, Raynaud's phenomenon, ruptured Achilles’ tendon.</td>
</tr>
<tr>
<td>3</td>
<td>SLE</td>
<td>5, 6, 10, 11</td>
<td>AMI, pernicious anaemia, Sjögren's syndrome.</td>
</tr>
<tr>
<td>4</td>
<td>SLE</td>
<td>1, 2, 3, 8, 10, 11</td>
<td>Osteoporosis, SCLE, died of septicaemia (59 yrs).</td>
</tr>
<tr>
<td>8</td>
<td>SLE</td>
<td>1, 3, 4, 5</td>
<td>Asthma.</td>
</tr>
<tr>
<td>11</td>
<td>SLE</td>
<td>1, 2, 5, 4, 5, 11</td>
<td>Allopecia, arthropalgia, aortoventricular block II, diabetes, fractures, glaucoma, back pain, ruptured Achilles’ tendon, died of pulmonary cancer (76 yrs).</td>
</tr>
<tr>
<td>12</td>
<td>SLE</td>
<td>1, 2, 5, 6, 7, 10, 11</td>
<td>Myalgia, pulmonary fibrosis, venous thrombosis and pulmonary emboli, vasculitis with skin manifestations, died of septicaemia and meningitis (51 yrs).</td>
</tr>
<tr>
<td>19</td>
<td>SLE</td>
<td>1, 2, 5, 10</td>
<td>SCLE, Raynaud’s phenomenon.</td>
</tr>
<tr>
<td>23</td>
<td>SLE</td>
<td>1, 2, 3, 5, 10</td>
<td>Myalgia, Sjögren’s syndrome.</td>
</tr>
<tr>
<td>29</td>
<td>SLE</td>
<td>1, 3, 4, 5, 8</td>
<td>Cholecystitis, back pain, myoma, pancreatitis, Raynaud’s phenomenon.</td>
</tr>
<tr>
<td>43</td>
<td>SLE</td>
<td>1, 2, 3, 5, 6, 9</td>
<td>AMI x 2, prostate cancer, SCLE.</td>
</tr>
<tr>
<td>45</td>
<td>SLE</td>
<td>1, 2, 3, 9, 10, 11</td>
<td>Aortoventricular block II-III, parotid gland tumour, polyneuropathy, septicaemia.</td>
</tr>
<tr>
<td>5</td>
<td>UCTD</td>
<td>1, 2, 5</td>
<td>Allopecia, cholecystitis, osteoporosis, pyoderma gangrenosum, venous thrombosis and pulmonary emboli, died of AMI (75 yrs).</td>
</tr>
<tr>
<td>25</td>
<td>UCTD</td>
<td></td>
<td>Atopic dermatitis, meningitis, Pustulosis palmaris et plantaris, Raynaud’s phenomenon.</td>
</tr>
<tr>
<td>27</td>
<td>UCTD</td>
<td></td>
<td>Arthralgia, myalgia.</td>
</tr>
<tr>
<td>33</td>
<td>UCTD</td>
<td>2, 6</td>
<td>Arthralgia, liver steatosis with fibrosis, alveolitis, pulmonary fibrosis.</td>
</tr>
<tr>
<td>42</td>
<td>UCTD</td>
<td>7</td>
<td>Myalgia.</td>
</tr>
<tr>
<td>24</td>
<td>Vasculitis</td>
<td></td>
<td>Malignant melanoma, venous thrombosis, septicaemia x 2.</td>
</tr>
<tr>
<td>31</td>
<td>Vasculitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>Vasculitis</td>
<td>7</td>
<td></td>
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</tbody>
</table>

AMI, acute myocardial infarction; SLE, systemic lupus erythematosus; SCLE, subacute cutaneous lupus erythematosus; UCTD, undifferentiated connective tissue disease; vasculitis, vasculitis with skin manifestations verified by skin biopsy in two patients; ACR, American College of Rheumatology. ACR criteria for SLE: 1, Malar rash, 2, Discoid rash, 3, Photosensitivity, 4, Oral ulcers, 5, Arthritis, 6, Serositis, 7, Renal disorder, 8, Neurological disorder, 9, Haematological disorder, 10, Immunological disorder, 11, Antinuclear antibody.
(95% CI, 1.5–8.9). Continuous long-term glucocorticoid treatment (>5 yrs) for rheumatological disease was observed in eight patients (Patients 1, 2, 4, 11, 12, 34, 42 and 45). There was no statistically significant increased risk for AMI in this group (SMR 2.3, 95% CI 0.06–13). In the 20 patients with no long-term treatment, the risk of AMI resembled that found in the C2D cohort in general (SMR 4.8, 95% CI 1.6–11). In conclusion, the risk of AMI is increased with four times in C2D as compared with the general Swedish population. Whether glucocorticoid treatment of rheumatological disease reduces the risk of AMI in C2D is more uncertain since our calculations are based on observations in only eight patients.

**Working capacity**

Nine females and one male of 26 investigated patients received disability pension. The median age for receiving disability pension was 43 yrs (range 37–63). Among C2D patients, the underlying cause of the pension was SLE in 7/9 patients and other rheumatological diseases (UCTD and vasculitis) in 2/6 patients. One of the 11 C2D patients without rheumatological disease received a disability pension because of severe chronic obstructive pulmonary disease. Thus, rheumatological disease (SLE, UCTD and vasculitis) was the principal cause of disability in C2D (P = 0.01, RR = 2.4, 95% CI 1.1–4.7, Fisher’s exact test). On 31 December 1992 (point prevalence), about 8% of the labour force received disability pension in Sweden compared with 21% of the investigated patients in the C2D cohort.

**Infections**

Invasive infections were documented in nine of the 20 patients (45%) with rheumatological disease, and three of them died of septicaemia (Patients 4, 12 and 24). Pneumonia was documented in six SLE patients, in three patients with UCTD, and in two patients with vasculitis. About 80% of the C2D patients with recurrent invasive infections were under the age of 18 yrs. Invasive infections among the patients without rheumatological disease (n = 25) were documented in 17 patients (68%).

**Autoantibodies**

Findings with regard to ANA are summarized in Table 3. Only three SLE patients showed a positive immunofluorescence test for ANA with HEp-2 cells. Antibodies to dsDNA were only found in one patient (Patient 45). The rarity of anti-dsDNA antibodies was further verified by analysis of samples obtained during repeated SLE flares (SLEDAI-2K > 4). Single flares were investigated in Patients 1, 2, 8, 19 and 23; two flares were investigated in Patients 1, 2, 8, 19 and 23; two flares were investigated in Patient 12. Anti-dsDNA antibodies were not found in these SLE patients. Antibodies to RNP and histone were each present in about 20% of all patients with C2D. Anti-RNP was more prevalent in SLE (P = 0.02, RR = 8.2, 95% CI 1.1–61.2, Fisher’s exact test) than in the other patient groups. All patients with SCLE (Patients 1, 4, 19 and 45) had antibodies to RNP or SS-A. In the three SLE patients with Raynaud’s phenomenon, anti-RNP antibodies were present in two (Patients 1 and 19), and anti-SS-A in one (Patient 4). We found no anti-SS-A antibodies in the two SLE patients with sicca symptoms (Patients 3 and 23), but both had anti-Sm antibodies.

Three patients with SLE and one patient with vasculitis had increased concentrations of RF (median 38, range 16–761 IU/ml). The prevalence of anti-C1qCLR and aCL was high among the patients with C2D (Figs 1 and 2). In six patients (Patients 11, 12, 23, 24, 26 and 33), a medium or high level of aCL were found (median 47, range 42–107 GPLU/ml). The patients with increased aCL levels were significantly older than the aCL negative patients (P = 0.04, Mann–Whitney test). SLE patients with infection have been reported to have transiently increased aCL levels [25]. However, we found no correlation between aCL and a history of invasive infection in the C2D cohort.

The first available blood sample in the SLE control group was used for analysis of aCL and anti-C1qCLR. Twenty-eight patients in the SLE control group had a clinical verified APS. The results found in the SLE control group were compared with the first available blood sample in the C2D patients. The C2D patients had higher concentrations of aCL and anti-C1qCLR as compared with the patients with genuine SLE (Figs 1 and 2, P < 0.0001, P = 0.001, respectively, Kruskal–Wallis test).

Despite the high frequency of aCL in the C2D group, very few patients had venous thrombosis (Patients 5, 12 and 34). Only one of these (Patient 12) had aCL (44 GPLU/ml). The significance of the aCL in Patient 12 is questionable, since the patient died of septicaemia with disseminated intravascular coagulation and severe uraemia that may have caused the pulmonary embolism that was found at autopsy. Prior to that, the patient had neither documented aCL nor APS-associated manifestations. In the two other patients, aCL was negative (Patient 5) or once weakly positive (25 GPLU/ml, Patient 34). Three patients (Patient 5, 11 and 12) had valvular disease and two (Patient 11 and 12) had aCL at a medium level (42 and 44 GPLU/ml, respectively). A Libman–Sacks endocarditis was not documented in these patients.

Longitudinal analysis (mean 7 yrs, range 1–15 yrs) of 20 serum samples from five C2D patients with SLE (Patients 1, 2, 3, 4 and 19) was performed in order to examine if ANA, anti-dsDNA and anti-C1qCLR varied in accordance with disease activity.

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**Table 2.** Predominant organ damage found in C2D patients with SLE was mainly related to CVD and skin manifestations. For each patient, the SLICC/ACR DI scores are given for each category after 10 yrs of observation.

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<th>Patient no</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>8</th>
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<th>23</th>
<th>29</th>
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<tr>
<td>SLICC/ACR DI categories</td>
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<td></td>
</tr>
<tr>
<td>Skin</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Malignancy</td>
<td>1</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Premature gonadal failure</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Table 3.** Presence of antibodies against nuclear antigens in adult C2D patients in relation to diagnosis given as number and percentage of patients.

<table>
<thead>
<tr>
<th></th>
<th>SLE</th>
<th>UCTD</th>
<th>Vasculitis</th>
<th>No rheumatological disease</th>
<th>All C2D patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>(n = 12)</td>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 11)</td>
<td>(n = 31)</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>3 (25%)</td>
<td></td>
<td></td>
<td></td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Antibodiesleasing to</td>
<td>(n = 11)</td>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 10)</td>
<td>(n = 29)</td>
</tr>
<tr>
<td>Histone</td>
<td>1 (9%)</td>
<td></td>
<td>2 (66%)</td>
<td>2 (20%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>RNP</td>
<td>5 (45%)</td>
<td></td>
<td>1 (10%)</td>
<td>6 (21%)</td>
<td></td>
</tr>
<tr>
<td>Sci-70</td>
<td>1 (33%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm</td>
<td>2 (18%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm B subunit</td>
<td>1 (9%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS-A 52/60</td>
<td>1 (9%)</td>
<td>1 (20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS-A 52</td>
<td>1 (20%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS-A 60</td>
<td>2 (18%)</td>
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</table>
Autoantibody levels were stable over time despite moderate changes of the SLEDAI-2K score (range 0–6).

**Causes of mortality**

Ten patients with C2D died during the observation time. In six patients, death was due to severe infection. Three patients died of AMI and one patient died of breast cancer. Of the four patients with SLE, who died during the observation period, two died of invasive infection, one of lung cancer and one of AMI (Table 1).

**Discussion**

Deficiency states within the classical pathway of complement are the strongest known susceptibility factors for development of SLE [2]. We present here clinical and laboratory data from a large C2D cohort gathered at a single centre.

During recent years, a hierarchy within the classical pathway has been established [1] in that the risk for SLE development and disease severity is high in C1 and C4 deficiency states and more modest in C2 deficiency. SLE is rare in patients with complete C3 deficiency. Furthermore, SLE associated with C2D in early studies was described as a generally mild clinical subset of the disease [26]. Skin and joint disease predominated in the patients, while severe manifestations such as serositis, neuropsychiatric SLE and glomerulonephritis were mostly absent. The results of the present study suggest that C2-deficient patients with SLE run virtually a similar risk of development of severe disease as other patients with SLE. Thus, the mean annual organ damage score during course of disease equalled that found in an epidemiologically recruited cohort of SLE patients in southern Sweden [27]. The female predominance among SLE patients with C2D is well established [26] and resembles the ordinary female/male distribution in the disease [28]. This is in contrast to C1q deficiency where female/male distribution is almost equal.

Regarding complement deficiency states within the classical pathway and development of SLE, several issues could be addressed from an epidemiological point of view. Patients with deficiency of C1q or C4 usually develop SLE early in life, which facilitates the recognition in cross-sectional studies. We would like to stress that the true prevalence of SLE in C2D is not known, but has been estimated to be in the order of 10% [6]. We believe that this estimation is probably too low since SLE in C2D persons may develop later in life and cross-sectional surveys might underestimate frequencies. In our C2-deficient patients, SLE was diagnosed at a median age of 39 yrs, which is comparable to findings in epidemiologically recruited SLE patients [28]. Four of our patients developed SLE at an age above 50 yrs. In the C2-deficient patients, the predominate finding during infancy and childhood was recurrent severe infections [14]. Finally, SLE in complete C3 deficiency is considered to be rare, but this could be subjected to an underestimation due to lack of data. Thus, there is a need for long-term prospective cohort studies to assess the prevalence of SLE in complement deficiency states other than C1q and C4 deficiency.

Among the C2D SLE patients, the high frequency of severe organ damage was mainly due to cardiovascular damage resembling that seen in genuine SLE [27]. In an attempt to clarify this finding, medical records and a questionnaire concerning Framingham risk factors were used. However, assessment of Framingham-related risk factors failed to explain the high cardiovascular damage rate. Thus, the cardiovascular damage is likely to be a more direct consequence of the complement deficiency. In recent studies, MBL deficiency has been associated with coronary artery disease [29, 30]. Furthermore, the vascular damage has been shown to be enhanced in genetically engineered C3-deficient mice [31]. These data indicate that development of cardiovascular damage in C2D might be related to impaired function of the classical and also the lectin pathway.

Analysis of working capacity demonstrated a marked impact of C2D on the general health status. Rheumatological disease was the main cause of chronic illness among the adult patients. Thus, 21% of the cohort received a disability pension, which equals previous investigations (19%) of unselected SLE patients during long-term follow-up [32].

Low or absent ANA titre have been consistent findings in C2-deficient patients with rheumatological manifestations [6, 33]. Furthermore, antibodies to native DNA appear to be rare [6, 26]. These serological features were confirmed in the present study. Furthermore, we found no evidence of fluctuating antibody levels in conjunction with changes in disease activity. As compared with some earlier reports [6], the prevalence of anti-SS-A antibodies was not particularly high.

A novel finding was the high prevalence of aCL and anti-C1qCLR in C2D. The cause of this deviated autoimmune response is not known but might be related to the importance of complement for elimination of autoreactive lymphocytes [34] and elimination of potential autoantigens [6]. Our observation that patients with aCL had a higher frequency of anti-C1qCLR than patients without aCL supported this idea. The majority of C2D persons is homozygous for the HLA-B*18, S042, DHR1∗15 haplotype [35]. This implies that their immune responses governed by MHC genes are expected to show a restriction that might contribute to the antibody profile.

Among anti-phospholipid antibodies, aCL predominate and are strongly associated with the APS and development of thrombotic events [36]. The aCL have also been reported to play a role in development of atherosclerosis [37] and might well have contributed to cardiovascular events in C2D. However, in this study the C2D patients with aCL did not show recurrent thrombosis or other manifestations of APS. Fetal loss induced with anti-phospholipid antibodies in mice is prevented by inhibition of complement activation with heparin [38]. Moreover, complement activation with cleavage of C2 has been reported to be a characteristic finding in patients with an APS [39]. Most likely, C2D protects against some manifestations of the syndrome.

A potentially important immunological mechanism in atherosclerosis involves formation of immune complexes showing strong pro-atherogenic activity in animal models [40]. Interestingly, C1q-containing immune complexes may promote development of atherosclerosis by inhibiting the function of cholesterol 27-hydroxylase in human arterial endothelium and macrophages [41]. Even if we found no correlation between the anti-C1qCLR and cardiovascular damage, these antibodies may be regarded as indicators of in vivo formation of C1q-containing complexes providing a potential link between impaired classical pathway function and development of cardiovascular damage in C2D.

In conclusion, a large cohort of C2-deficient patients with long-term follow-up provided a partly unique basis for evaluation of disease manifestations and mechanisms associated with impaired classical pathway and lectin pathway functions. The severity of SLE in C2D does not differ from disease severity in genuine SLE patients. Novel findings included a high prevalence of aCL and anti-C1qCLR in C2D. The absence of APS manifestations suggests that complement dysfunction might partly prevent biological effects of aCL.

**Acknowledgements**

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References