Hypocomplementaemia as an immunological marker of morbidity and mortality in patients with primary Sjögren’s syndrome


Objective. To analyse the prevalence and clinical significance of hypocomplementaemia in a large series of patients with primary Sjögren’s syndrome (SS), focusing on the association of low complement levels with clinical manifestations, immunological features, lymphoproliferative disorders and mortality.

Methods. Complement determinations (C3 and C4 levels, CH50 activity) were made in 336 consecutive patients with primary SS (313 women and 23 men, mean age 58.5 yr). We also analysed complement levels in 46 patients with SS associated with hepatitis C virus (HCV) infection and 184 with HCV-related cryoglobulinaemia as control groups.

Results. Hypocomplementaemia was detected in 81 (24%) of patients with primary SS, low CH50 being detected in 51 (15%), low C3 values in 42 (12%) and low C4 values in 39 (12%). In the multivariate analysis, patients with low C4 levels showed a higher prevalence of peripheral neuropathy, cutaneous vasculitis, RF, cryoglobulins and lymphoma compared with those with normal C4 levels. The analysis of the 218 SS patients followed prospectively since 1994 showed a lower probability of survival in patients with hypocomplementaemia (with low C3, C4 or CH50 levels) at protocol entry. SS-HCV patients presented a higher frequency of hypocomplementaemia than patients with primary SS (76 vs 24%, P < 0.001); nine (20%) of these patients had persistent, unquantifiable complement levels.

Conclusion. Hypocomplementaemia is closely associated with systemic expression and adverse outcomes (lymphoma development and death) in patients with primary SS. Our results support the inclusion of complement determination at diagnosis as a predictor of the outcome of patients with primary SS and its routine determination in the clinical follow-up.

Key words: Hypocomplementaemia, Complement, Lymphoma, Sjögren’s syndrome, HCV, Cryoglobulinaemia.

Sjögren’s syndrome (SS) is a systemic autoimmune disease that mainly affects the exocrine glands and usually presents as persistent dryness of the mouth and eyes due to functional impairment of the salivary and lacrimal glands [1]. In the absence of an associated systemic autoimmune disease, patients with this condition are classified as having primary SS. The histological hallmark is focal lymphocytic infiltration of the exocrine glands and the spectrum of the disease extends from an organ-specific autoimmune disease (autoimmune exocrinopathy) [2] to a systemic process with diverse extraglandular manifestations [3, 4].

The complement system is a group of individual proteins that act sequentially to form enzyme cascades and is activated by three initiating pathways: the classical, alternative and mannose-binding protein pathways. Complement activation is usually assessed by the determination of levels of individual complement components, such as C3 and C4, and by the quantification of CH50 activity, which reflects the sequential interaction of all the components of the classical and alternative pathways [5]. The routine measurement of the serum complement profile (C3, C4 and CH50) is an important clinical tool in the management of some systemic autoimmune diseases. The best example is systemic lupus erythematosus (SLE), in which hypocomplementaemia is closely correlated with disease activity, especially the development of nephropathy [6]. However, the clinical significance of hypocomplementaemia in systemic autoimmune diseases other than SLE has been little studied. In patients with primary SS, there is growing interest in the clinical significance of low complement levels due to recent studies that have associated low C4 levels with lymphoma development [7] and mortality [8, 9].

In this study, we analysed the prevalence and clinical significance of hypocomplementaemia in a large series of consecutive patients with SS, focusing on the association of low complement levels (C3, C4 and CH50) with clinical manifestations, immunological features, lymphoproliferative disorders and mortality.

Materials and methods

Between 1992 and 2003, complement determinations (C3 and C4 levels, CH50 activity) were made in 336 consecutive patients diagnosed with primary SS (313 women and 23 men, mean age 58.5 yr). All patients fulfilled four or more of the 1993 European Classification Criteria for primary SS [10] (including as the mandatory criterion either positive immunological markers or salivary lip biopsy). Exclusion criteria for the diagnosis of primary SS were considered as the coexistence of other systemic autoimmunological diseases, including the presence of features suggestive of systemic lupus erythematosus, rheumatoid arthritis, Sjögren’s syndrome, systemic vasculitis, systemic autoimmune disease, inflammatory bowel disease, multiple sclerosis, Sjögren’s syndrome with antiphospholipid syndrome, primary biliary cirrhosis, porphyria, mastocytosis, autoimmune thyroid disease and primary hypothyroidism. All cases were reviewed and reclassified by three independent rheumatologists.

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autoimmune diseases, pre-existing haematological diseases and hepatitis B virus, hepatitis C virus (HCV) or HIV infections. Complement determinations were routinely made at the first visit and annually during the follow-up on an out-patient basis. Additional determinations were made in patients with suspected disease activity or during hospitalizations. Clinical and serological characteristics of patients accumulated until the last visit were collected retrospectively using a protocol form that included the main clinical and analytical features defined for each disease, as described previously [3, 4]. In addition to the retrospective analysis, we also investigated the data from 218 patients with primary SS included in the SS mortality project begun in 1994 in our department, which has a single-centre, consecutive, prospective design. The end-point (mortality) was analysed according to the CH50, C3 or C4 levels at the time of protocol inclusion.

We analysed complement determinations in 46 patients with SS associated with HCV infection (35 women and 11 men, mean age 66.1 yr) and in 184 patients with cryoglobulinaemia associated with HCV infection (110 women and 74 men, mean age 56.2 yr). These were the control groups.

**Laboratory studies**

Complement measurement consisted of the determination of C3 and C4 levels by nephelometry (BNII nephelometer; Dade Behring, Marburg, Germany), and CH50 activity measured by Autokit CH50, a commercial liposome immunoassay (LIA) in an automated analyser (Wako Chemicals, Neuss, Germany). Serum levels of C3, C4 and CH50 were determined on serum samples frozen immediately after extraction at −20°C and subsequently stored at −80°C. Normal values are 0.82–1.87 g/l for C3, 0.11–0.45 g/l for C4 and 34–71 U/ml for CH50. The lowest detectable levels were 0.17 g/l for C3, 0.02 g/l for C4 and 9 U/l for CH50. Hypocomplementaemia was defined as a low C3 level (C3 <0.82 g/l), a low C4 level (C4 <0.11 g/l) and/or low CH50 (CH50 <34 U/ml) in at least two different determinations.

Other immunological tests included antinuclear antibodies (ANA; indirect immunofluorescence using mouse liver and Hep-2 cells as substrate) and precipitating antibodies to extractable nuclear antigens (ENA), including Ro/SS-A, La/SS-B, U1-snRNP and Sm (enzyme-linked immunosassay). Rheumatoid factor (RF) was detected by nephelometry. Serum cryoglobulins were determined as described previously [11].

**Statistical analysis**

We used conventional $\chi^2$ and Fisher’s exact tests to analyse qualitative differences. For comparison of quantitative parameters, Student’s t-test was used in large samples of similar variance, and the non-parametric Mann–Whitney U test for small samples. A value of $P < 0.05$ indicated statistical significance. When several independent variables appeared to have statistical significance in the univariate analysis, a logistic regression test was performed for the multivariate analysis. Survival probabilities were calculated according to the Kaplan–Meier lifetime analysis method. The statistical analysis was performed by means of the SPSS program (SPSS, Chicago, IL, USA).

The design of this study conformed to the ethical standards currently applied in Spain. Informed consent was obtained from each participant.

**Results**

**Prevalence of hypocomplementaemia**

Hypocomplementaemia was detected in 81 (24%) of patients with primary SS (Table 1), low CH50 being detected in 51 (15%), low C3 values in 42 (12%) and low C4 values in 39 (12%). A small number of patients with primary SS had persistent, unquantifiable complement levels; two (0.6%) patients presented persistent C4 levels <0.07 g/l, suggesting a possible homozygous C4 deficiency. SS-HCV patients presented a higher frequency of hypocomplementaemia than patients with primary SS (76% vs 24%, $P < 0.001$). Hypocomplementaemia was detected in 35 patients with HCV-related SS: low CH50 in 31 (67%), low C3 values in 16 (35%) and low C4 values in 22 (48%). Nine (20%) of these patients had persistent, unquantifiable complement levels; eight presented a possible homozygous C4 deficiency, with persistent C4 levels <0.07 g/l, while another patient had repeated CH50 measurements of 0 U/ml, suggesting a possible homozygous deficiency of some terminal component (C5–C9).

Hypocomplementaemia was detected in 143 (78%) of the 184 patients with HCV-related cryoglobulinaemia, low CH50 values being detected in 126 (68%) patients, low C3 values in 75 (41%) and low C4 values in 85 (46%). A small number of patients with HCV-related cryoglobulinaemia had persistent, unquantifiable complement levels; two presented a possible homozygous C4 deficiency, with persistent C4 levels <0.07 g/l, while two other patients had repeated CH50 measurements of 0 U/ml, suggesting a possible homozygous deficiency of some terminal component (C5–C9).

**Clinical and immunological associations**

We analysed the correlation of C3, C4 and CH50 levels with clinical and immunological features of primary SS (Table 2). Compared with patients with normal CH50 values, those with CH50 <34 U/ml showed a higher prevalence of parotidomegaly (31 vs 17%, $P = 0.032$), pulmonary involvement (18 vs 8%, $P = 0.04$), cutaneous vasculitis (27 vs 7%, $P < 0.001$), positive ANA (98 vs 81%, $P = 0.001$), cryoglobulins (25 vs 5%, $P = 0.001$) and lymphoma (10 vs 1%, $P = 0.005$) in the univariate analysis, although only cutaneous vasculitis and cryoglobulins were significant independent variables in the multivariate analysis. In comparison with patients with normal C4 levels, those with low C4 levels showed a higher prevalence of lymphadenopathy (18 vs 5%, $P = 0.01$), peripheral neuropathy (20 vs 5%, $P = 0.003$), cutaneous vasculitis (31 vs 8%, $P < 0.001$), positive ANA (97 vs 82%, $P = 0.01$), RF (60 vs 34%, $P = 0.002$), cryoglobulins (35 vs 4%, $P < 0.001$) and lymphoma (10 vs 2%, $P = 0.013$) in the univariate analysis, although only peripheral neuropathy, cutaneous vasculitis, RF, cryoglobulins and lymphoma were significant independent variables in the multivariate analysis.

With respect to C3 levels, patients with low C3 levels showed a higher prevalence of lymphadenopathy (21 vs 5%, $P = 0.001$), pulmonary involvement (17 vs 8%, $P = 0.005$), cutaneous vasculitis (27 vs 7%, $P < 0.001$), positive ANA (98 vs 81%, $P = 0.001$), cryoglobulins (25 vs 5%, $P = 0.001$) and lymphoma (10 vs 1%, $P = 0.005$) in the univariate analysis, although only cutaneous vasculitis and cryoglobulins were significant independent variables in the multivariate analysis.

| Table 1. Epidemiological profile and prevalences of hypocomplementaemia, low C3, low C4 and unquantifiable complement levels in 566 patients |
|-------------------------------|-----------------|-----------------|-----------------|
| Number of patients            | Primary SS      | SS-HCV          | HCV-cryoglobulinaemia |
| Sex (female)                  | 336             | 46              | 184              |
| Mean age (yr)                 | 58.3            | 66.1            | 56.2             |
| Hypocomplementaemia           | 81 (24%)        | 35 (76%)        | 143 (78%)        |
| Low CH50 activity             | 51 (15%)        | 31 (67%)        | 126 (68%)        |
| Low C3 values                 | 42 (12%)        | 16 (35%)        | 75 (41%)         |
| Low C4 values                 | 39 (12%)        | 22 (48%)        | 85 (46%)         |
| Unquantifiable complement levels* | 2/81 (2%)       | 9/35 (26%)      | 4/143 (3%)       |

*Persistent, unquantifiable levels of C4 or absent CH50 activity.
Hypocomplementaemia in primary Sjögren’s syndrome

Table 2. Clinical and immunological manifestations of primary SS in patients with low CH50 activity, low C3 and low C4 levels compared with those with normal complement determinations

<table>
<thead>
<tr>
<th></th>
<th>Normal CH50 (n = 285)</th>
<th>Low CH50 (n = 51)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotidomegaly</td>
<td>49 (17%)</td>
<td>16 (31%)</td>
<td>0.032</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>23 (8%)</td>
<td>9 (18%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>21 (7%)</td>
<td>14 (27%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>ANA</td>
<td>229 (81%)</td>
<td>50 (98%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>11/225 (5%)</td>
<td>10/40 (25%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>4 (1%)</td>
<td>5 (10%)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Normal C3 levels (n = 294)</th>
<th>Low C3 levels (n = 42)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphadenopathy</td>
<td>14 (5%)</td>
<td>9 (21%)</td>
<td>0.001a</td>
</tr>
<tr>
<td>Articular involvement</td>
<td>95 (32%)</td>
<td>25 (59%)</td>
<td>0.001a</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>5 (2%)</td>
<td>4 (10%)</td>
<td>0.017</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Normal C4 levels (n = 297)</th>
<th>Low C4 levels (n = 39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphadenopathy</td>
<td>16 (5%)</td>
<td>7 (18%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Peripheric neuropathy</td>
<td>16 (5%)</td>
<td>8 (20%)</td>
<td>0.003a</td>
</tr>
<tr>
<td>Cutaneous vasculitis</td>
<td>23 (8%)</td>
<td>12 (31%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>ANA</td>
<td>241 (81%)</td>
<td>38 (97%)</td>
<td>0.01</td>
</tr>
<tr>
<td>RF</td>
<td>96/284 (34%)</td>
<td>23/38 (60%)</td>
<td>0.002a</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>10/234 (4%)</td>
<td>11/31 (35%)</td>
<td>&lt;0.001a</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>5 (2%)</td>
<td>4 (10%)</td>
<td>0.013a</td>
</tr>
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</table>

*aIndependent variables in the multivariate analysis.

Prospective analysis of mortality

The analysis of the 218 SS patients followed prospectively since 1994 showed a lower probability of survival in patients with hypocomplementaemia at protocol entry (73.6 vs 92.1%, P = 0.0005) (Fig. 1a). This lower probability of survival was also observed in patients with low C3 values (75 vs 89.5%, log rank = 0.0357), low C4 values (66.7 vs 90.6%, log rank = 0.0002) and low CH50 activity (68.6 vs 91.2%, log rank = 0.0004) (Fig. 1b, c and d respectively).

Discussion

This study analysed the prevalence and clinical significance of routine C3, C4 and CH50 determinations during the follow-up of 382 patients diagnosed with SS in our department. The prevalence of hypocomplementaemia was three times greater in HCV-associated SS than in primary SS. In patients with primary SS, hypocomplementaemia was found in 24% of patients, a similar prevalence to that observed in previous studies [7–9, 12], although the prevalence of low C3 or C4 levels varied according to the study. Skopoulis et al. [7] detected low C3 levels in 4 (2%) and low C4 levels in 44 (17%) of 261 Greek patients, while Ioannidis et al. [8] found low C3 in 17 (3%) and low C4 in 122 (20%) of 601 Greek patients in a multicentre study. Recently, Theander et al. [9] described low C3 levels in 98 (25%) and low C4 levels in 105 (27%) out of 386 Swedish patients. These differences may be related to the different classification criteria used, the cut-off levels of the complement assays used or differences in the study design.

The role of hypocomplementaemia in the clinical expression of primary SS has been little analysed, previous studies describing an association with neurological [13] or renal [14] involvement. In this study, hypocomplementaemia was closely associated with systemic expression of SS and outcomes. We found a significant association between low complement levels and systemic SS features, including both extra-glandular features (fever, articular involvement, cutaneous vasculitis and peripheral neuropathy) and immunological markers (cryoglobulinaemia, RF). These features are typical of the clinical and immunological expression of cryoglobulinaemic vasculitis, suggesting an important role for cryoglobulinaemia in the systemic expression of SS in patients with hypocomplementaemia. Cryoglobulinaemia and hypocomplementaemia are closely related immunological markers, which both suggest systemic involvement in patients with primary SS.

Hypocomplementaemia was also closely associated with the two main adverse outcomes of primary SS (lymphoma development and death). We found that lymphoma was associated with low C3, C4 and CH50 levels in the univariate analysis, although only low C4 levels were an independent significant variable in the multivariate analysis. This close association was also described in a recent multicentre study by Ioannidis et al. [8], who found a higher risk of lymphoproliferation in prevalent cases than in incident cases, and also that low C4 levels were an independent predictor of lymphoproliferation in the multivariate model. In another recent study, Theander et al. [9] found that patients with low C4 levels had an increased cause-specific standardized mortality ratio for lymphoproliferative disease and an increased hazard ratio for death compared with patients with normal C4 levels. This close association between hypocomplementaemia and lymphoma might be directly related to the concomitant presence of cryoglobulinaemia. Tzioufas et al. [15] were the first to demonstrate that mixed cryoglobulinaemia was a predictive laboratory factor for lymphoma development in SS. However, the studies by Ioannidis et al. and Theander et al. on mortality in primary SS patients did not analyse cryoglobulinaemia at protocol entry [8, 9]. Prospective studies including both markers are needed to define their joint or independent value as predictive factors for lymphoma development in patients with primary SS.

In addition, we found that low complement levels are prospectively associated with a higher risk of mortality in patients with primary SS. This confirms the results of Ioannidis et al. [8], who showed that low C4 levels were an independent predictor of mortality, and Theander et al. [9], who described a similar association with low C3 and C4 levels. In our study, we found an additional association between mortality and low CH50 activity and hypocomplementaemia, although the closest statistical association with mortality was found in patients with low C4 levels. Ioannidis et al. [8] proposed two types of SS according to the risk of unfavourable outcome: a low C4 level at diagnosis and palpable purpura were indicative of SS type 1, which was associated with unfavourable outcome, although, as we suggest above, the predictive role of hypocomplementaemia was closely related to the concomitant presence of cryoglobulinaemia. Hypocomplementaemia and cryoglobulinaemia may be the main immunological markers that differentiate type I (high-risk) from type II (low-risk) primary SS [8], type I patients showing a higher frequency of both clinical (purpura, neuropathy, arthritism) and immunological (hypocomplementaemia, RF) cryoglobulinaemia-related features.

Patients with SS associated with HCV infection had a prevalence of hypocomplementaemia three-fold higher than patients with primary SS, although this prevalence was very similar to that found in patients with HCV-related cryoglobulinaemia. This suggests a key role for HCV as an inducer of complement activation in SS-HCV patients. HCV patients (especially those with cryoglobulinaemia) showed a higher frequency of hypocomplementaemia and RF [16], due to the continuous stimulation of the immune system by HCV, which leads to the production of circulating immune complexes with RF activity and complement activation. Recently, we reported the important contribution of...
Fig. 1. Kaplan–Meier plots for the risk of death in 218 patients with primary SS followed prospectively since 1994, according to the following complement values at the beginning of the protocol study. (a) Hypocomplementaemia (yes vs no); (b) C3 values (low vs normal); (c) C4 values (low vs normal); (d) CH50 values (low vs normal).
cryoglobulinaemia to the extraglandular features of SS-HCV [17] in addition to its predominant role in the immunological pattern of these patients, due to its close association with hypocomplementaemia (present in 71% of the SS-HCV-cryoglobulinaemia patients) and RF (present in 68% of these patients). The RF activity due to HCV-related cryoglobulinaemia has additional clinical significance, being a criterion for the fulfilment of the 1993 European criteria for SS diagnosis. Thus, we may conclude that the immunological marker that most strongly defines the pattern of immunological expression of SS associated with HCV is cryoglobulinaemia, and this may explain the presence of RF and low complement levels in the majority of SS-HCV cryoglobulinaemic patients.

Persistent, repeated, unquantifiable complement levels were infrequently observed in patients with primary SS, suggesting that acquired hypocomplementaemia seems to be the main cause of low complement values in the majority of patients. There were two (0.6%) out of 336 patients with a possible homozygous C4 deficiency, with persistent C4 levels < 0.07 g/l. The existence of possible inherited complement deficiencies in primary SS has been very little studied [18], isolated cases also being reported [19, 20]. In patients with HCV infection, there are only two studies describing a familial C4 deficiency in patients with essential cryoglobulinemia [21, 22] before the isolation of HCV. An interesting result was that nearly 20% of our SS-HCV patients had persistent, repeated, unquantifiable complement levels, in contrast to fewer than 1% of patients with HCV-related cryoglobulinaemia. This suggests inherited complement deficiencies in a substantial percentage of patients and might contribute to the development of SS in these HCV patients. A possible role of the complement system in the immune response to HCV infection has recently been suggested by Meyer et al. [23]. Thus, it may be hypothesized that HCV patients carrying an inherited complement deficiency are probably at higher risk of developing autoimmune diseases. Nevertheless, the existence of inherited deficiencies in our hypocomplementaemic patients cannot be confirmed without a genetic study. In addition, the inherited nature of these defects needs to be confirmed by family studies demonstrating either homozygous and/or heterozygous defects.

In conclusion, hypocomplementaemia is closely associated with systemic expression and adverse outcomes (lymphoma development and death) in patients with primary SS. Patients with SS associated with HCV infection had a prevalence of hypocomplementaemia three-fold higher than patients with primary SS and, interestingly, a higher percentage of these patients had persistent, repeated unquantifiable complement levels, suggesting a possible inherited complement deficiency. Our results support the inclusion of complement determination at diagnosis as a predictor of the outcome of patients with primary SS, and its routine determination in the clinical follow-up.

Key messages

- Hypocomplementaemia is associated with systemic expression, lymphoma and death in patients with primary SS.
- The clinical follow-up of primary SS should include routine complement determination.

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References


