Antiphospholipid antibodies and rheumatoid arthritis

We read with interest the letter entitled “Antiphospholipid antibodies and RA: presence of β2GPI independent aCL” by Bonnet et al published in the *Annals* in March 2001.1 We believe that the letter needs additional clarification owing to inconsistencies in the terminology, methodology of antiphospholipid antibody (aPL) detection, and determination of positive values.

The term “anti-cardiolipin antibodies” was somewhat misleading. The use of the term “anticardiolipin antibodies” was introduced and abbreviated as “aCL”, a term not completely appropriate. The term aCL was widely accepted, but the group of antibodies detected in many conditions was not defined even though the authors focused on β2GPI independent aCL. It is generally agreed that the term aCL, if not stated otherwise, defines the antigen (aPL) detection, and determination of positive values.

There were some potential methodological errors in determining β2GPI independent aCL. It was shown that antibodies against β2GPI (anti-β2GPI) from patients with the antiphospholipid syndrome (APS) have the ability to bind to β2GPI in complexes with cardiolipin only if the β2GPI concentration in solution is high enough. The threshold concentration of β2GPI was found to be just about 2 µg/ml, because no binding of anti-β2GPI was seen when serum samples were diluted 1:200 or more.2 As the physiological concentration of β2GPI in human serum is approximately 200 µg/ml, the threshold binding concentration is reached at a serum dilution of 1:100. In the presence of a relatively high concentration of endogenous β2GPI, the statement that antibodies detected by this method are exclusively β2GPI independent is unjustified, as the sera containing high titres of anti-β2GPI might have yielded positive results by the method described in the letter.

The definition of antibody units in the letter is not clear and using Harris’s standards for β2GPI independent aCL is not appropriate. With the use of Harris’s standards,3 the units should be abbreviated as GPL (for IgG) and MPL (for IgM) as previously defined.4 However, Harris’s standards were designed for use in the classical aCL ELISA and were prepared by pooling serum samples from patients with APS. Therefore, they contain mainly, or predominantly, monoclonal aCL. β2GPI independent aCL were not defined in those standards and they were not meant as standards for β2GPI independent assays.

The interpretation for both aCL ELISAs as a method to detect β2GPI dependent aCL may not be valid in all cases. It was shown that not all anti-β2GPI binding β2GPI adsorbed on polystyrene high binding plates also recognise β2GPI associated with cardiolipin. We reported this binding pattern for anti-β2GPI in children with atopic dermatitis,5 and the same was shown also for some patients with autoimmune diseases, including APS.6

The method for purification of β2GPI was not described. Because the authors focused on patients with rheumatoid arthritis (RA), it should be ensured that immunoglobulins were specifically removed from the β2GPI preparation. If this purification step was not carried out, traces of immunoglobulins in the β2GPI preparation might have yielded positive results for sera containing high titres of rheumatoid factor (RF). In fact, all sera containing IgM anti-β2GPI also had RF and the authors already suspected that this might be due to non-specific binding involving RF.

The method for determining cut off values was not explained and the number of normal human sera (NHS) included in the study as negative controls was not given. From the data presented in the letter, one may conclude that the cut off values were arbitrarily set at 20 units for both IgG and IgM isotypes of β2GPI independent aCL and for anti-β2GPI. We recently compared the sensitivity of anti-β2GPI ELISA and classical aCL ELISA. The results showed great differences between their sensitivities and therefore also between the cut off values calibrated by the same standards.7 In addition, the authors did not report the proportion of NHS positive for each assay and the values of positive samples compared with patients with RA. Instead, they just referred to one study, which is only one of the several published estimations of aPL in healthy subjects.

We would like to support our criticism by adding some data about aPL in our patients with RA. We randomly selected 53 serum samples from patients fulfilling the ARA criteria for RA and 53 NHS as negative controls. The samples were tested for anti-β2GPI, β2GPI dependent aCL, and β2GPI independent aCL. The cut off values for anti-β2GPI were set as described8 by calculating the mean ± 2 SD of logarithms of absorbance values for NHS and the 95th centile value of 32 NHS sera for both β2GPI dependent and β2GPI independent aCL. For the anti-β2GPI determination, we used affinity purified β2GPI adsorbed on Costar high binding plates as previously described.9 The β2GPI preparation did not contain any immunoglobulins. β2GPI independent aCL were tested as described in the letter, but the sera were diluted 1:200. Serum samples were tested simultaneously for both β2GPI dependent aCL on the same plate by adding β2GPI in parallel duplicate wells. The final concentration of β2GPI was 10 µg/ml. This experimental design enabled direct comparison of binding to cardiolipin coated wells in the presence and absence of β2GPI. For the final determination of β2GPI dependent binding, the values obtained in wells without β2GPI were subtracted from the values measured in wells with added β2GPI. The patients’ histories were evaluated for the occurrence of arterial or venous thrombosis and recurrent fetal loss. Statistical analysis was performed with the χ2 test where appropriate.

Table 1 presents the frequency of positive sera in each group (NHS, RA, RA-RF positive, and RA-RF negative). The frequency of increased anti-β2GPI, β2GPI dependent aCL, and β2GPI independent aCL was higher in patients with RA than in controls, but the difference was significant only for anti-β2GPI. There were no differences in the frequency of...
any type of antibodies between the RF positive and negative patients. One patient (a male, 66 years old) had a history of deep venous thrombosis and pulmonary embolism together with positive anti-β2GP1 and β2GP1 dependent aCL of IgA isotype. Interestingly, 3/11 RA sera which showed binding to β2GP1 adsorbed on a high binding plate did not recognise β2GP1 associated with cardiolipin, as already reported. In contrast, 3/9 RA sera binding β2GP1 complexed with cardiolipin did not recognise β2GP1 adsorbed on the surface of high binding plates. This phenomenon probably reflects the heterogeneous nature of anti-β2GP1 in RA, which may differ in fine specificity to anti-β2GP1 in APS.

The sera from our patients with RA exhibited an even higher frequency of β2GP1 independent aCL than that reported in the letter. As expected from reported data, the presence of β2GP1 independent aCL was not associated with signs of APS in our patients. We also found that the addition of β2GP1 (10 µg/ml) lowered the binding of β2GP1 independent aCL by about 50%, most probably owing to the competition between β2GP1 independent aCL and β2GP1 for the same binding sites on cardiolipin. In conclusion, patients with RA may have anti-β2GP1 and β2GP1 dependent aCL, which might be associated with the signs of APS. The importance of distinguishing β2GP1 independent aCL has not been fully clarified. It seems likely that β2GP1 independent aCL do not confer an increased risk for APS in RA.

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References

Authors' reply
In response to the comments of Ambrozic et al we would like to comment on the data published earlier in the Annals. The term “anticardiolipin antibodies” (aCL) is classically used to designate antibodies directed against the cardiolipin antigen and detected in sera of patients suffering from the dependence of aCL on β2-glycoprotein I (β2GP1) as is assessed by an enzyme linked immunosorbent assay (ELISA) test using exogenous β2GP1 in block buffer (containing fetal calf sera or ovine serum). In our previous study the solution did not contain bovine or calf sera but only purified bovine serum albumin. So, this method was adapted to detect antibodies directed against cardiolipin antigen alone and not against the complexes of cardiolipin bound to exogenous β2GP1. This method justified the terminology of β2GP1 independent aCL for sera containing aCL without anti-β2GP1 antibodies. The absence of anti-β2GP1 antibodies was shown by another ELISA test specific for the detection of these antibodies. Both ELISAs were used to screen all sera.

The concentration of exogenous β2GP1 contained in human serum is not significant at a 1:100 dilution (the dilution employed to screen our sera), in comparison with the 10% of calf sera added to the test as source of exogenous β2GP1 in the assays used for the detection of β2GP1 dependent aCL. In addition, the sera containing aCL (detected by an ELISA without addition of exogenous β2GP1) did not react with β2GP1 in the other ELISA test specifically designed to detect anti-β2GP1 autoantibodies, and therefore which could detect hypothetically high titer of anti-β2GP1 antibodies contained in these sera.

Harris's standards were used after calibration of our positive control sera from patients with proven antiphospholipid syndrome (APS), which were obtained for each positive control (APS). We used these for the detection of aCL in our previous studies. In contrast with the report of Ambrozic et al we did not find raised levels of aCL or anti-β2GP1 antibodies in normal sera. The percentage of positive normal serum samples was <3%. These differences between our results and those of Ambrozic et al are probably associated with a differing sensitivity and specificity of the methods between the two laboratories.

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References

Methotrexate and postoperative complications
Grennan et al report the safety of continued methotrexate in the perioperative period. Previous investigators have despaired of answering this question definitively owing to the difficulty in recruiting subjects. It is reassuring to see that methotrexate use throughout the postoperative period does not interfere with wound healing or increase the incidence of postoperative complications.

Despite this important finding, we believe that the results of this study should be regarded with some reservation: continuation of methotrexate throughout the perioperative period should be accompanied by significant caution. The elderly and those with renal impairment are at increased risk of methotrexate-related pancytopenia. Indeed, in a community based, observational study of methotrexate use in 460 patients we found the...
periprosthetic period to be especially hazardous for patients with renal impairment and sepsis. Two subjects developed pancytopenia under these conditions, one of whom died. Although all consecutive patients were included in the study by Grennan et al, it is unclear whether Wrightington Hospital is a tertiary referral centre. Renal impairment is an important comorbidity, although no comment is made about the prevalence of this in the study group. It is important to note that this is a study of methotrexate use in elective surgery. We suggest caution should be taken in patients with renal impairment (best assessed by creatinine clearance) and in the elderly patients with renal impairment (best assessed by creatinine clearance) and in the elderly.

**Letters**

Proximal myopathy and bone pain as the presenting features of coeliac disease

It is rare for coeliac disease to present only with symptoms of osteomalacia, without the classic symptoms of diarrhoea, steatorrhoea, and abdominal discomfort.1-4 A 22 year old woman presented with 18 months of a waddling gait disturbance. Hip and back x rays were normal. She experienced bone pain when being hugged, when laughing, or coughing, and had difficulty standing up from a low chair and holding her arms up to blow-dry her hair. She had extreme tiredness and thought she might have lost some weight, but there were no gastrointestinal symptoms.

On examination, she was pale and had difficulty squatting and holding her arms above her head.

Investigations showed a mild anaemia secondary to β thalassaemia minor and iron deficiency. Other investigations disclosed a raised alkaline phosphate of 1375 U/L (normal 30–120 U/L), reduced red blood cell folate level of 290 nmol/l (>300 nmol/l), corrected calcium of 1.75 mmol/l (2.15–2.65 mmol/l), phosphate 1.0 mmol/l (0.8–1.4 mmol/l), 25-hydroxy vitamin D <5 nmol/l (15–110 nmol/l), and raised parathyroid hormone 53.1 pmol/l (1.0–6.5 pmol/l).

Investigations were carried out for a malabsorption syndrome. Antigliadin, antientomysial, and antigluten antibodies were strongly positive, and a small bowel biopsy showed almost total villous atrophy, confirming the diagnosis of coeliac disease.

A bone scan demonstrated increased activity throughout the skeleton, consistent with secondary hyperparathyroidism. Osteopenia was demonstrated by dual emission x ray absorptionmetry estimation of bone mineral density, with the lumbar spine measuring 0.882 g/cm² (2.65 SD below the young adult female mean) and the neck of the femur 0.633 g/cm² (2.9 SD below the mean).

Treatment involved a gluten free diet, ergocalciferol 3000 IU daily, calcium carbonate 600 mg twice a day, slow release ferrous sulphate 350 mg daily, and folic acid 5 mg daily.

Within two months her bone pain and tiredness resolved and her strength had returned to normal. Calcium was within the normal range, and alkaline phosphate reduced to 374 U/L. Bone mineral density had increased markedly after 12 months of treatment, with the lumbar spine increasing by 37% to 1.204 g/cm² (mean level for young adult women), and the neck of the femur by 39% to 0.878 g/cm² (0.8 SD below the mean). She had also gained more than 7 kg in weight, and repeat gastroscopy and duodenal biopsy were normal.

Osteomalacia is now an uncommon disease, and even more uncommon is the presenting symptom of coeliac disease. Since its first description in 1965, there have been several more case reports of coeliac disease presenting with bone pain, proximal myopathy, radiographic findings of pseudo fractures and Looser’s zones, or secondary hyperparathyroidism evident on bone scan.5-9 Most patients were middle aged and responded within six months to treatment with a gluten-free diet, supplemental calcium, and vitamin D, and in some cases with the addition of bisphosphonates.1 A recent case finding study of coeliac disease showed that many patients in fact present with non-gastrointestinal symptoms, of which anaemia is the most common.

Hypocalcaemia in coeliac disease is caused by reduced gut absorption of calcium as a consequence of reduced levels of the fat soluble vitamin D. It is also due to reduced absorptive surface area, secondary hyperparathyroidism, and calcium lost in the stools by binding to unabsorbed fatty acids to form insoluble calcium soaps.10

Secondary hyperparathyroidism can develop if this did in this case, causing increased bone turnover. Low bone mineral density is probably due to a combination of hypocalcaemia, impaired bone mineralisation, and reduced exercise because of skeletal pain and proximal weakness.11

Early diagnosis of coeliac disease is important because untreated patients have an increased risk of gastrointestinal lymphomas. Useful screening blood tests include determination of anti-transglutaminase and antidentomyosial antibodies. They have a high sensitivity and specificity, with a negative predictive value of around 95%.12 There is a genetic influence on the susceptibility to coeliac disease, with a 10% prevalence rate among first degree relatives. On screening our patient’s relatives, one of two siblings was also found to have coeliac disease. A strong association has been found with HLA-DR3 and DR5/DR7.13

Treatment with a gluten-free diet with subsequent villous restitution on repeat biopsy has been associated with rapid gains and even normalisation of bone mineral density: the greater the degree of osteopenia, the more rapid the gain.14 The change is due to improvement of calcium and vitamin D status, leading to remineralisation of the large volume of unmineralised osteoid matrix.15 Introduction of hormone replacement therapy in women approaching the menopause, and bisphosphonates in patients with osteoporotic fractures, should also be considered.14

Osteomalacia presenting with muscle weakness and aches may be the only presenting features of coeliac disease. Prompt treatment and diagnosis is important because treatment with a gluten-free diet and medical treatment including vitamin D may lead to rapid and effective recovery.16

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References


Authors’ reply

Dr Wluka draws attention to the potential hazard of methotrexate prescribing in sub-

Plasma and peripheral blood mononuclear cells levels of Zn and Cu among Indian patients with RA

Plasma and serum levels of zinc (Zn) and copper (Cu) have been reported to be altered in patients with rheumatoid arthritis (RA). Few studies have measured these levels in tissues, particularly peripheral blood mononuclear cells (PBMCs), the site for a host of immunological aberrations. In a previous study we measured levels of Zn and Cu in plasma and PBMCs to see if they correlated with disease activity and reported reduced levels of Zn in the serum of patients with active RA. Patients attending the rheumatology clinic at our institute and satisfying the American College of Rheumatology (formerly American Rheumatism Association) criteria for the diagnosis of RA were studied. Patients were categorised as either active or inactive RA. All patients classified as active RA had at least three of the following: morning stiffness for more than 45 minutes, five swollen joints, five tender joints, and erythrocyte sedimentation rate (Westergren) more than 45 mm/1st h.

Both plasma and lysed PBMC samples were read on atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT) at a wavelength of 213.8 nm for Zn and 324.7 nm for Cu. The atomic absorption spectrophotometer was calibrated with reference standards obtained from Sigma Chemicals Company (St Louis, MA).

Thirty nine patients (31 women) with RA had a mean (SD) age of 36.2 (8.3) years (range 18–52) and mean disease duration of 55.8 (36.6) months (range 6–168). Twenty patients had inactive and 19 patients active disease, respectively. Twenty two healthy controls (14 women), well matched for age (mean age 34.2 (6.2) years, range 20–56) with the two patient groups, were studied at the same time. Both patients and controls were of middle socioeconomic status. Table 1 shows the plasma and PBMC levels of Zn and Cu. Our results are in agreement with earlier studies which showed that plasma Zn levels are significantly lower and plasma Cu levels significantly higher in patients with active RA. Additionally, it is shown here that PBMC levels of these elements have an inverse relation with plasma levels.

With acute inflammation, the acute phase response may move Zn into the liver and the reduced plasma concentration may not be indicative of overall deficiency. Possibly, also, PBMCs may be an additional site to which Zn is moved during inflammatory states. The average disease duration of patients with active disease was more than 54 months. In such a long process it is unclear whether chronic cytokine release, as is seen in RA, causes a shift of Zn from one compartment to another or if there is a true Zn depletion. Significantly, there was no correlation between age or duration of disease and plasma or PBMC levels of Zn.

The finding of raised Cu levels in the plasma is to be expected because of a concomitant rise of caeruloplasmin, which is an acute phase reactant. The reduced levels in PBMCs may signify a movement of Cu from PBMCs to the liver where it is absorbed and attached to caeruloplasmin. Thus the findings of plasma and PBMC Cu levels may merely be a reflection of an acute phase response, and the alterations may be due to increased hepatic synthesis of caeruloplasmin.

The effect of concomitant drugs also needs to be considered. The number of patients receiving non-steroidal anti-inflammatory and second line drugs was similar. None of the patients received corticosteroids in the preceding eight weeks.

It would be premature to speculate about a possible role for supplementation with Zn and Cu for patients with RA. From the results shown in this study, patients with inactive RA had similar levels of Cu and Zn as controls. If the diet of patients with active RA were deficient in Zn (as shown by plasma levels) it would be unlikely to contain an excess of Cu and vice versa for PBMC levels. The more plausible explanation would be that this represents a redistribution of trace elements between plasma and PBMCs, and a control of inflammation would lead to these levels in controls. Hence, further studies need to be carried out on paired samples in a cohort of patients, once the disease is active and again when it becomes inactive. If plasma Zn levels increase, but PBMC levels decrease with the control of inflammation and attain the levels of controls then there would be no indication for dietary supplementation with these metals.

Table 1 Copper and zinc levels in plasma and PBMCs of patients with RA. Results are given as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Active RA</th>
<th>Inactive RA</th>
<th>Overall RA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Zn (µg/l)*</td>
<td>687 (466)</td>
<td>982 (264)</td>
<td>824 (386)</td>
<td>1024 (428)</td>
</tr>
<tr>
<td>PBMC zinc (µg/10⁶ cells)†</td>
<td>135.2 (28.6)</td>
<td>108.3 (38.4)</td>
<td>121.4 (34.4)</td>
<td>98.4 (16.4)</td>
</tr>
<tr>
<td>Plasma copper (µg/l)‡</td>
<td>1646 (357)</td>
<td>1016 (296)</td>
<td>1426 (324)</td>
<td>946 (446)</td>
</tr>
<tr>
<td>PBMC copper (µg/10⁶ cells)§</td>
<td>58.0 (43.2)</td>
<td>86.4 (33.2)</td>
<td>74.3 (38.2)</td>
<td>104.2 (8.5)</td>
</tr>
</tbody>
</table>

PBMCs, peripheral blood mononuclear cells; there was no correlation between age, duration of disease, rheumatoid factor positivity, or any second line drug with plasma or PBMC levels of Zn and Cu.

*Overall levels were significantly lower among patients than controls (p<0.05) and patients with active RA had lower levels than those with inactive RA (p<0.05).†Overall levels were significantly higher than controls (p<0.05) and patients with active RA as compared with those with inactive RA (p<0.05).‡There was overall a negative correlation between plasma and PBMC copper levels (p<0.05).

References


Essential cryoglobulinaemia (type 1) in three patients characterised by Raynaud’s phenomenon, arthralgia-arthritis, and skin lesions

The relevance of monoclonal gammapathy in relation to rheumatic disorders has recently been reviewed. Monoclonal gammapathy or
paraproteins in adults is about 1%. This incidence is higher in people over 70 and increases with age. When a paraprotein is detected and no underlying disease is present, the condition is referred to as a monoclonal gammopathy of undetermined significance. Owing to their immunological properties, paraproteins can be precipitated by lowering the temperature below 37°C. In this way they form an essential part of the so-called cryoglobulins. When cryoglobulins are detected in the serum of a patient, this finding is usually associated with the coexistence of paraproteins. Recently, three patients with a clinical picture of a necrotising vasculitis associated with an essential cryoglobulinaemia (type 1) were admitted to our department. The causative relationship between the cryoglobulinaemia and the clinical symptoms was reduced by the reduced severity of the clinical signs when paraprotein levels were decreased.

Case reports
Patient A was a 69 year old man who, in May 1999, developed extremely painful purpura of the upper part of the third finger of his left hand. In the following days the upper part of his foot became necrotic. Angiography of his arteries showed normal vessels. Immune electrophoresis showed the presence of 8 g/l of an M component (IgG). An assay for the detection of cryoglobulinaemia was positive. Scintigraphic imaging showed normal plasma viscosity; antinuclear antibodies could not be detected and neither could rheumatoid factor. Complement components showed decreased C4 levels (46 IE/ml (normal 81–128), C3 1.3 g/l (normal 0.9–1.8), and low C4 levels (35 mg/l (normal 150–400)). Virus serology was negative for cytomegalovirus, hepatitis A, B, and C. A skin biopsy of non-affected skin showed no evidence for vasculitis, but thrombosis formation was detected in one artery. A diagnosis of multiple myeloma was ruled out by extensive laboratory examination.

Treatment was started with prednisone 60 mg daily for the first 2 weeks and chlorambucil 8 mg daily until the M component concentration reached a plateau. During this treatment the skin lesions on his foot disappeared and his finger became necrotic. The M component decreased to 5 g/l. After almost a year of follow up he is still free of complaints.

Patient B was a 60 year old man who was admitted to our hospital in January 2000 with arthritis of the small joints and severe Raynaud's phenomenon of his ears, which affected him so severely that he could not leave his house. Furthermore, he felt short of breath when breathing cold air. Physical examination revealed purpura skin lesions on both heels of his cars. A paraprotein was detected with an M component of 4 g/l. The presence of a cryoglobulinemia was shown, which consisted exclusively of the M component and other laboratory examination showed very low levels of the complement component C1q <15 IE/ml (normal 81–128), C3 1.42 g/l (normal 0.9–1.8), and C4 300 mg/l (normal 60–150). Virus serology was positive for cytomegalovirus and negative for hepatitis A, B, and C. Plasma viscosity was normal. No evidence for multiple myeloma or lymphoma was obtained. A skin biopsy of the non-affected skin showed only a slight perivascular infiltrate, and no evidence for a necrotising vasculitis was seen. He was treated with chlorambucil 8 mg daily, which after two weeks was switched to melphanal (6 mg/m²) and prednisone (60 mg/day) for every four weeks for six months. The M component fell to 2 g/l and the severe Raynaud's phenomenon disappeared.

Patient C was a 78 year old woman who was admitted to our hospital in May 2000 with cyanosis in both feet, indicating possible arterial occlusion of the small vessels in both feet were cold and very painful. Angiography showed normal vessels, which strongly suggested vasculitis of the end arterial vessels of her feet.

Laboratory examination showed a paraprotein (8 g/l) consisting exclusively of the cryoglobulinaemia (IgGκ). Other laboratory examinations showed no abnormalities. Virus serology for cytomegalovirus and hepatitis A, B, and C was also negative. She was treated with chlorambucil (8 mg/day) and prednisone (60 mg/day), which improved the necrosis of her legs. The necrosis of her right leg disappeared and on the left foot the necrosis began to demarcate to the upper part of her foot. While waiting for the complete demarcation so that an amputation could be planned, she developed a sepsis and died.

Few patients with essential cryoglobulinaemia type 1 have been reported. Until now a defined clinical syndrome could never be associated with classification of the cryoglobulins. Overall, Raynaud's phenomenon, and necrosis of the skin has been described as in our three patients. None of our three patients showed abnormalities on angiographic examination, which may indicate that only the small vessels are affected in the disease process.

In our patients we were able to show that the cryoglobulins were formed by the monoclonal immunoglobulin. When the serum concentration of the cryoprotein was reduced, the disease symptoms in our patients improved. These cases suggest that a paraprotein found in patients with a rheumatic syndrome is not only indicative of a developing malignancy but other disease may also be interpreted as a causative agent. We conclude that paraproteins seen in rheumatic syndromes have a role in the pathogenesis and should be treated when serious symptoms are present.

References

Cryoglobulinaemic vasculitis as presenting manifestation of infective endocarditis
Seroimmunological alterations, including antibodies and/or cryoglobulins, are common in infective endocarditis (IE), but specific autoimmune disorders, such as cryoglobulinaemic vasculitis (CV) associated with IE have seldom been described. CV is related to the vascular deposition of circulating immune complexes, mainly cryoglobulins, and complement; in 70–90% of patients with CV a triggering role of hepatitis C virus (HCV) has been suggested. We report the case of two patients who showed a typical CV with severe neurological involvement as the presenting manifestation of underlying IE.

Case 1
In November 1994 a 63 year old woman presented with fever, purpura, paraesthesia, and pseudoaxotic gait. Her past clinical history was unremarkable except for a prothetic implant of the left hip four years previously. Table 1 shows the main clinicoserological features. Repeated blood cultures were negative. Neurological examination showed abnormal tactile sensation in the fingers and legs; mild ideomotor slowing down; shaky movements; and unsteady gait. An electroencephalographical study recorded a moderate sensorimotor peripheral neuropathy, while ECG, chest x ray examination, abdominal echography, and echocardiography were normal. Cutaneous purpura biopsy disclosed a leukocytoclastic vasculitis. Truncocerephal magnetic resonance imaging showed a poorly defined high signal intensity, punctiform lesions at the white matter consistent with brain vasculitis. Thus a central and peripheral neuropathy complicating CV was established, and prednisone (50 mg) combined with cyclophosphamide (100 mg) was given daily. However, the patient's clinical status progressively worsened and, finally, she died owing to cardiorespiratory failure two months after the start of treatment. Necropsy disclosed coarse endocardial vegetations on the left side valves infected by *Kingella*.

Case 2
In January 1999 a 75 year old woman with no risk factors for infections presented with fever, purpura, and acropaesthesia. Table 1 shows the main clinical and laboratory features suggestive of CV. Prednisone (25 mg/day) was started, with a rapid clinical improvement. One month later, she had an exacerbation of purpura, arthralgias, acropaesthesias, and impairment of distal muscle strength. An electrophysiological study confirmed a sensorimotor peripheral neuropathy. Thus a higher steroid dose (50 mg/day) was given. A week later fever persisted and the patient complained of precordial pain and cardiac murmurs were found. A chest x ray examination and transoesophageal echocardiography detected cardiomyalg and endocardial vegetations on the tricuspid valve; in addition, *Staphylococcus aureus* infection was shown by repeated blood cultures. Despite appropriate antibiotic treatment, the patient died one month later because of severe, refractory heart failure.

Discussion
Our two patients show some interesting peculiarities: the unusual presentation of IE
as CV with severe neurological involvement; and the difficulty of making a timely diagnosis of IE by routine investigations. In both cases, the thrombocytopenia characteristic of CV, together with their transient favourable response to corticosteroids (case 2), further delayed the detection of IE responsible for the fatal outcome. Previous reports (Medline) show that the association of IE with “asymptomatic” cryoglobulinaemia is not uncommon, but only a few studies report IE clinically presenting as CV. This latter presentation can mean a misleading context, moreover, steroid treatment can contribute to masking and worsening of the underlying infectious disorder.

In the course of CV, we can reasonably exclude the possibility that IE represented a complication of the CV. In over 300 of our patients with CV, bacterial manifestations have rarely been seen, even in subjects undergoing steroid or immunosuppressive treatments, or both. Moreover, the CV seen in our two patients had quite unusual clinical and virological characteristics: absence of HCV or other hepatotropic viruses; the presence of particularly severe skin purpura; and the presence of neuropathy as important organ involvement. The linked immune, peripheral neuropathy, in one case associated with central nervous system vasculitis, was the only prevalent organ manifestation seen in our patients. This is one of the most common clinical manifestations in patients with CV, the aetiopathogenesis of which is still unclear. In a considerable number of patients with IE negative blood cultures have also been unclear. In a considerable number of patients

Table 1  Epidemiological, clinical, and seroimmunological features in two female patients with infective (bacterial) endocarditis, at the first visit

<table>
<thead>
<tr>
<th>Feature</th>
<th>Patient 1</th>
<th>Patient 2</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>63</td>
<td>75</td>
</tr>
<tr>
<td>Disease duration (weeks)</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Purpura</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Weakness</td>
<td>Haemorrhagic papulonodular</td>
<td>Haemorrhagic nodular</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>Recurrent</td>
<td>Constant</td>
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<tr>
<td>Hepatopathy</td>
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</tr>
<tr>
<td>Neurological involvement</td>
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</tr>
<tr>
<td>ESR (mm/1st h)</td>
<td>83</td>
<td>77</td>
</tr>
<tr>
<td>CRP (normal ≤5 mg/l)</td>
<td>53</td>
<td>41</td>
</tr>
<tr>
<td>WBC (normal 3.5–10.5 × 10^9/l)</td>
<td>89 000</td>
<td>83 600</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>87</td>
<td>81</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>γGlobulinemia (g/l)</td>
<td>19.5</td>
<td>21.3</td>
</tr>
<tr>
<td>RF (normal ≤20 IU/ml)</td>
<td>575</td>
<td>137</td>
</tr>
<tr>
<td>C3 (normal 500–1200 mg/l)</td>
<td>930</td>
<td>790</td>
</tr>
<tr>
<td>C4 (normal 200–550 mg/l)</td>
<td>&lt;60</td>
<td>100</td>
</tr>
<tr>
<td>Crystalline, % [cryo-type]</td>
<td>0.5 (IgG-IgM)</td>
<td>2 (IgG-IgM)</td>
</tr>
<tr>
<td>Hepatitis virus markers*</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*HBsAg, anti-HBs, anti-HBeAg, anti-HBc; anti-HCV by ELISA and RIBA; anti-EBV IgM, anti-HIV.

References


ANCA antibodies in Graves’ disease

Several drugs have been associated with antineutrophil cytoplasmic antibodies (ANCA) positivity—namely, hyalurazine, penicillamine, allopurinol, and propylthiouracil.

Although propylthiouracil is often implicated in the induction of ANCA positive vasculitis, other antithyroid drugs, such as carbimazole and thiamazole, have been linked. Furthermore, ANCA positivity has been described in the course of Graves’ disease without vasculitis.

This study aimed at determining the frequency and specificity of ANCA in a series of patients with Graves’ disease.

We retrospectively examined 35 serum samples from patients with Graves’ disease. Diagnosis of the disease was based on typical signs and symptoms of hyperthyroidism, raised serum triiodothyronine and thyroxine, very low or undetectable thyroid stimulating hormone, and increased thyroid radioactive iodine uptake. All patients had been receiving treatment with carbimazole (30–45 mg) for at least two months. None of the patients were treated with propylthiouracil or any drug affecting the immune function. ANCA antibodies were determined by indirect immunofluorescence (IF) on ethanol fixed granulocytes, as described elsewhere.

Staining patterns were described as cANCA, when a diffuse granular cytoplasmic staining with central accentuation was seen, as pANCA, when a perinuclear pattern was observed, and as ANCA when a distinct, homogeneous, non-granular cytoplasmic staining pattern was seen. Autoantibodies against proteinase 3 and myeloperoxidase (MPO) were detected by enzyme linked immunosorbent assay (ELISA; Orgentec) as described elsewhere.

Hospital Universitari Germans Trias i Pujol is a 533 bed hospital situated on the outskirts of Barcelona. It is a referral hospital serving a population of 700 000 inhabitants. The immunology laboratory is a reference centre.

ANCA (IF) were detected in 21 (60%) of the serum samples. The titre ranged from 1/40 to 1/260. The immunofluorescence staining pattern was as follows: nine (26%) pANCA, seven (20%) cANCA, and five (14%) cANCA.

We retrospectively examined 35 serum samples from patients with Graves’ disease.

The IF staining pattern was cANCA in five cases and MPO in one case. Anti-MPO antibodies were detected only in one (5%) of the patients. In our study ANCA were detected in 21 (60%) serum samples. The IF staining patterns were more heterogeneous, but the ELISA results were similar.

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Human MPO and human thyroid peroxidase (TPO) share global similarities which indicate that MPO and TPO are members of the same gene family. Therefore, it seems conceivable that MPO autoantibodies may cross react with TPO. Findings suggesting such a relationship were reported by Haapala et al, who found antibodies against both TPO and MPO in 19 patients, three with vasculitis and 16 with thyroid disorders. Three patients had hyperthyroidism and one with hypothyroidism.

There is a need to determine the autoantigenic role of ANCA antibodies in Graves’ disease, the precise relation between ANCA and antithyroid drugs and, lastly, the antigens which are responsible for the ANCA positivity.

ANCA positivity in Graves’ disease may be attributable to either antithyroid drugs (thiazide and propylthiouracil) or to the disease itself.

**Lupus relapse after prostaglandin E, administration: activation of a cytokine cascade?**

A variety of abnormalities in cytokine production occur in human and murine lupus, but their specific role in lupus pathogenesis is unknown. Recent in vitro studies emphasize the role of prostaglandins in the cytokine induction and modulation of the humoral immune response. We present a patient with systemic lupus erythematosus (SLE) who had a relapse after prostaglandin E (PGE), administration, which to our knowledge has not been previously reported. A 25 year old woman was admitted to hospital to receive treatment with IV PGE, owing to severe Raynaud’s phenomenon. Fifteen years previously SLE had been diagnosed according to American Rheumatism Association (ARA) criteria, with renal biopsy proven diffuse proliferative lupus glomerulonephritis (WHO class IV). A physical examination showed only painful, violaceous, and atrophic finger pads with no signs of systemic inflammatory disease. The chest x ray films were normal and laboratory investigations showed antinuclear antibodies (ANA; titre 1/160) and hypocomplementaemia (C3 0.6 g/l, C4 0.1 g/l), with normal liver, renal, and haematological parameters. Treatment with 40 mg/12 h IV PGE, was started. On the sixth day of treatment the patient began to have chest pain, fever, dyspnoea, and pericardial friction rub. The laboratory showed anaemia, modest thrombocytopenia, and ANA 1/320, with no changes in the rest of the biochemical serum parameters. Echocardiography and chest x ray examination showed moderate pericardial and bilateral pleural effusions, PGE, was withdrawn after 72 hours. Thrombocytopenia, 60 mg/day, was started with prompt improvement in the symptoms.

We investigated the possibility that PGE, mediated cytokine production might be the cause of the relapse of SLE in this patient. Intracellular expression of cytokines in the patient’s T lymphocytes after specific PGE, stimuli (10 ng/ml) was determined by flow cytometry using anti-cytokine conjugates in combination with surface anti-CD3 (Pharmingen, San Diego, CA), as previously described. The test performed eight months after the PGE, treatment showed a dramatic rise in interleukin 4 (IL4) production (table 1).

It has been suggested that cytokines have an important role in the immune dysregulation seen in lupus prone mice and in patients with SLE. Increasing evidence supports a role for T helper cell type 2 (Th2) cytokines, such as IL4, in promoting and perpetuating B cell hyperactivity and autoantibody formation. A change in the proportion of Th2 cytokines might be associated with the polyclonal B cell activation seen in SLE. Restoration of Th1 and Th2 cytokines to levels similar to those seen in healthy mice results in amelioration of the clinical manifestations of an already established experimental SLE.

On the other hand, in vitro studies it has been suggested that PGE, alters the Th1/Th2 balance of T cells to a dominant Th2 response. We suggest that the rise in IL4 production induced by the PGE, as shown in vitro, in this patient, may be a marker of dysregulation of the Th1/Th2 profile and might have been the cause of her lupus relapse.

**References**


**Table 1 Intracellular cytokine production after PGE, stimulation in the patient and in an asymptomatic lupus patient who served as a control. Results are shown as the percentage of T lymphocytes with cytokine synthesis**

<table>
<thead>
<tr>
<th></th>
<th>IL2</th>
<th>INFγ</th>
<th>IL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal PGE</td>
<td>Basal</td>
<td>PGE</td>
<td>Basal</td>
</tr>
<tr>
<td>Control</td>
<td>1.2</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Patient</td>
<td>0.8</td>
<td>1.6</td>
<td>1.8</td>
</tr>
</tbody>
</table>

IL, interleukin; INFγ, interferon γ.

Whole blood was incubated with or without PGE, 10 ng/ml, for six hours in the presence of Brefeldin A. Cells were stained with FITC-anti-CD3 and, after erythrocyte lysis and permeabilisation, with the phycoerythrin conjugated anti-cytokines. Samples were analysed by flow cytometry.
Osteocalcin: a marker of disease activity in ankylosing spondylitis?

In rheumatic diseases the synovial concentration of osteocalcin, which represents osteoblast activity, is inversely correlated with the extent of joint inflammation. Synovial and serum osteocalcin correlate positively. In ankylosing spondylitis (AS) the serum concentration of osteocalcin has been reported to be low or normal. Cross sectional studies have shown no significant correlation between osteocalcin serum concentration and erythrocyte sedimentation rate (ESR) or C reactive protein.

To answer the question whether serum osteocalcin is a useful marker of disease activity in AS, longitudinal studies may be more sensitive and specific. For this reason changes in serum osteocalcin were correlated with changes in ESR, which is probably still the best marker of inflammation in AS.

In 89 patients with ankylosing spondylitis (modified New York criteria; 75 male, 14 female; age 43 (11) years; disease duration 14 (6) years) venous blood was taken at the start and the end of a three week rehabilitation course consisting of physical exercise, physiotherapy, electrotherapy, underwater exercises, and radial treatment as prescribed by the patient's doctor. Patients were advised not to change their drug treatment. The ESR was determined according to a standard procedure. The result at one year was used for calculation. Serum was frozen at −18°C until further analysis. Osteocalcin was measured in one batch with a commercially available test kit (IRMA, Biocis, Vienna; normal range according to the manufacturer 7.5–31.5 ng/ml in men, 3.7–31.7 ng/ml in women). Results are given as median (25th, 75th centile). The Mann-Whitney rank sum test and Spearman rank order correlation test were used to test significance.

Values at the first measurement were ESR 18 (8, 28) mm/1st h, serum osteocalcin 25 (20.5, 52.8) ng/ml. The osteocalcin serum concentration was within the normal range in 66 of the 89 patients, and 23 patients had increased serum concentrations. Values at the end of treatment were ESR 16 (8, 26.5) mm/1st h, osteocalcin 26.1 (18.9, 32.7) ng/ml (no significant changes). The ESR and osteocalcin at the first examination did not correlate significantly ($r_1=0.07; p=0.5$). The changes in ESR (1 to 4, 6 mm/1st h) and in osteocalcin (−0.5 to −2.6, 5.7) ng/ml showed a significant correlation ($r_1=−0.28; p<0.01$).

The results confirm previous findings showing no significant correlation between serum osteocalcin and ESR in cross sectional studies. Changes in osteocalcin after three weeks, however, correlated significantly with changes in ESR, but in view of the weak correlation ($r_1=0.28$) the clinical relevance of serum osteocalcin determination for assessing disease activity seems limited.
CD4+ T lymphocytopenia may cause dysgammaglobulinemia and autoimmune syndromes such as Takayasu arteritis.

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Uludag University Medical Faculty, Department of Paediatrics, Göürükle, Bursa 16059, Turkey

Correspondence to: Dr Sebnem Kilic; sebnemkl@uludag.edu.tr

Figure 1 Magnetic resonance imaging shows dilatation and irregular contour of the descending aorta and narrowing of the abdominal aorta.

Low CD4+ T lymphocyte counts are rare in the absence of immunodeficiency, most commonly infection with HIV. In our patient, major histocompatibility complex class II deficiency was excluded by the expression of HLA-DR on peripheral blood lymphocytes. Serological testing for HIV infection was negative and, additionally, the patient had no risk factor for transmission of HIV infection or recent immunosuppressive treatment. All patients with idiopathic CD4+ T lymphocytopenia need to be observed prospectively and tested after their opportunistic infections, or after their first CD4+ cell count less than 0.4 × 10^9/L, to determine the natural history of their infections and lymphocyteopenia. Two recent preliminary reports suggest an immunological cause and, possibly, an autoimmune process.1

CD4+ T lymphocytopenia is an important and specific marker of HIV infection.1 It occurs in approximately 40% of all patients with AIDS at some time during the course of the disease.2 However, CD4+ T lymphocytopenia has been reported previously.1 In patients with aortic arteritis, immunological investigations have shown a decrease in the titre of complement and phagocytic activity of neutrophil granulocytes, deep depression of T cell immunity, and hypergammaglobulinemia.3 Wiskott-Aldrich and Takayasu arteritis have been reported previously.4 It is rare for patients to have both disorders and with this case report, we draw attention to this association. This case report suggests that low

Recurrent orbital pain and diplopia in a 12 year old boy

A previously healthy 12 year old boy was referred to our unit in May 2000, with a history of persistent ocular pain and recurrent diplopia. The first episode of pain had started three years before, when the patient suddenly presented with diplopia and painful periorbital and eyelid oedema. Limited abduction of the right medial rectus muscle was excluded. Orbital MRI showed significant narrowing of the right rectus muscle. Electromyography showed increased insertion activity, fibrillations, and positive sharp waves. Ocular myositis was diagnosed. The oral prednisone dose was raised to 30 mg/day, and rapidly tapered after improvement of signs and symptoms. In November 2000, ciclosporin (3 mg/kg/day) was introduced; no relapse of the ocular findings has been seen so far, and prednisone has been progressively reduced to the current dose of 5 mg/day.

The group of idiopathic inflammatory myopathies encompasses a variety of common and uncommon syndromes. The uncommon variants of myositis include orbital myositis, a condition that is rare in adults and even rarer in children.5 Orbital muscle inflammation may be seen in association with other autoimmune diseases, such as SLE, Scl, giant cell myocarditis, and Crohn’s disease. Primary conditions that are important to distinguish from orbital myositis include thyroid eye disease; ocular myopathies, such as mitochondrial disorders and ocular dystrophies; and orbital pseudotumours. Cellulitis, neoplasms, arteriovenous malformations, and cavernous sinus thrombosis are also included in the differential diagnosis.

Orbital myositis implies orbital inflammation confined to one or more of the extraocular muscles; orbicularis oculi, extraocular muscles, and the levator palpebrae superioris. In these cases, orbitomaxillary, orbital, and cranial symptoms are usually present.7 Erythema, pain, and periorbital oedema are common.9 Inflammatory myopathy is the pathological basis for most orbital inflammatory conditions. In orbital myositis, the inflammatory process affects the muscle and the periorbital structures. In this case, orbital MRI showed significant narrowing of the right rectus muscle and loss of the normal fatty plane between the muscle and the retrobulbar fat. Orbital MRI can be used to diagnose and manage patients with orbital myositis.10

Figure 1 Orbital MRI (T1 weighted image with contrast) that shows increased signal intensity of the right rectus lateralis muscle.
Hodgkin’s lymphoma is rare. The involvement of the central nervous system is unusual and extended investigation that must include isoniazid myositis in the differential diagnosis.

References

Sciatica or spinal lymphoma

The involvement of the central nervous system and vertebrae by low grade non-Hodgkin’s lymphoma is rare. In a previous “lesson of the month” in this journal, it was implied that there is a good prognosis for patients with spinal lymphoma; however, milder cases may also occur.

A 71 year old man presented to us in January 2000 with a three month history of severe low back pain affecting mainly the left lumbar area and buttock, radiating to the outer aspect of the left thigh and calf. He did not have bladder symptoms or history of recent falls. On examination, he looked well; there was no lymphadenopathy. He had restricted back movement with tenderness of lower lumbar spinal processes. Straight leg raising test was restricted to 45° bilaterally, and produced lumbar pain. Neurological examination of the legs showed normal tone, power, and coordination. Knee jerks were reduced, ankle jerks were absent, both plantars were down going, and there was no sensory deficit.

He had a past history of epilepsy, which was controlled by phenytoin and phenobarbitone. In 1993 he was admitted with abdominal pain, splenomegaly, and pancytopenia; this was diagnosed as low grade B cell lymphoma and hypoplasmenia. Spleenectomy was performed in 1994, his blood count returned to normal, and repeated full blood counts were stable. In 1995 the patient had a fall and severe back pain. A magnetic resonance imaging (MRI) scan showed collapse of T7 and wedging of T4, with evidence of osteoporosis but no infiltration. Treatment was started with etidronate and calcium.

Investigations showed normal serum biochemistry apart from a mild increase of alkaline phosphatase, which was 242 IU/l (normal 40–140). The total white cell count was 24.7×10⁹/l, differential count showed neutrophils 3.5×10⁹/l (14%), lymphocytes 17×10⁹/l (69%), monocytes 4.0×10⁹/l (16.0%), eosinophils 0.2×10⁹/l (1.0%), basophils 0.0×10⁹/l (0.0%), and occasional atypical lymphocytes were seen in blood film. The erythrocyte sedimentation rate was 4 mm/1st h, a myeloma screen was negative, and prostate specific antigen was normal.

A lumbosacral spine x ray examination showed biconcave L5 with diffuse osteopenia. Abdominal ultrasound confirmed splenectomy, but no enlarged lymph nodes were detected. A bone isotopic scan showed increased focal activity in the upper lumbar spine and lumbosacral junction, which was compatible with osteoporosis and degenerative changes.

An MRI scan showed extensive infiltration involving vertebral bodies and appendages throughout the lumbosacral spine, being most intense at the biconcave L5; the appearance was consistent with lymphoma or myeloma (fig 1).

During his stay in hospital, the patient’s pain resolved completely after treatment with non-steroidal anti-inflammatory drugs, analgesics, and physiotherapy. His haematologist started treatment of the patient with chlorambucil 10 mg/day for 10 days to be repeated every three weeks, these cycles to be continued for 12 months.

Eight months after the diagnosis of spinal lymphoma, the patient has remained well and active; his back pain is minimal.

In rheumatology, it is essential to differentiate between malignant disease and the more common causes of back pain. Our patient was in a good physical condition, which is unusual for someone with malignancy, his presentation with low back pain appeared to be a typical case of sciatica, and the pain settled down with conventional treatment. Clinically there was no evidence of recurrence of lymphoma for example, enlarged lymph nodes, weight loss, or fever. However, because of his age at presentation and significant past history, thorough investigations were mandatory.

Unusual complications in the Churg-Strauss syndrome

Although abdominal complications are occasionally reported in the Churg-Strauss syndrome (CSS), bowel perforations, cholecystitis, eosinophilic peritonitis, and oophoritis are very unusual and normally resolve after immunosuppressive treatment. We report the case of a patient with CSS with these complications, which was fatal despite proper treatment.

A 64 year old woman with a 13 year history of urticaria, recurrent rhinitis, and asthma was admitted for abdominal pain. An increasing peripheral eosinophilia rising from 1% to 22% in the past five years was detected. Two years before hospital admission an extensive urticarial erythema developed. An abdominal ultrasonography performed during an asthmatic exacerbation when she had no abdominal pain disclosed a thick-walled gall bladder with no cholegetic contents. An excised nasal polyp showed polyoid hyperplasia with many eosinophils.
Two and six weeks later she was readmitted owing to right upper quadrant pain. The leucocyte count was 1×10^9/l with 34% eosinophils. Abdominal ultrasonography and computed tomography (CT) scan showed acalculous cholecystitis. A laparotomy disclosed a purulent peritoneal collection and enlarged inflamed gall bladder and right ovarium. A cholecystectomy and right oophoritis were performed.

One month after surgery she was readmitted with severe abdominal pain, diarrhoea, and fever. The leucocyte count was 4.89×10^9/l with 22% eosinophils, erythrocyte sedimentation rate (ESR, Westergren) 39 mm/1st h, rheumatoid factor (RF) 765 IU/ml (normal <80), and total IgE 769 IU/ml (normal <100), and serum urea and creatinine, complement C3 and C4, antinuclear antibody and antineutrophil cytoplasmic antibody values were normal or negative. The urine contained 300 mg/l proteins and the sediment 6–8 red cells/low power field, 3–5 leucocytes, and hyaline and hyaline granular casts. An abdomen CT scan showed moderate ascites. The ascitic fluid was serofibrinous with a protein concentration of 55 g/l, a leucocyte count of 1.05×10^9/l with 44% eosinophils, and negative standard and Lowenstein cultures. A diagnosis of CSS was made after reviewing the previous gallbladder and ovariun histopathological specimens (fig 1) and considering the history of asthma, eosinophilia, and nasal polyposis.

Oral methylprednisolone 60 mg/day and cyclophosphamide 100 mg/day were started, with initial clinical improvement. However, the abdominal pain recurred and the patient underwent a second laparotomy after three weeks of treatment. Peripheral blood leucocytes were 18.1×10^9/l with 3% eosinophils. Blood urea, creatinine, and urinary sediment were normal, the ESR fell to 15 mm/1st h and the RF to 435 IU/ml. Purulent fluid in the peritoneal cavity and two perforations in the ileal wall were found. A diagnosis of CSS was made after reviewing the previous gallbladder and ovariun histopathological specimens (fig 1) and considering the history of asthma, eosinophilia, and nasal polyposis.

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22nd European Workshop for Rheumatology Research
28 Feb–3 Mar 2002; Leiden, The Netherlands
Contact: Professor F C Breedveld, Leiden University Medical Centre, Department of Rheumatology, PO Box 9600, 2300 RC Leiden, The Netherlands
Tel: +31 (0)71 526 3598
Fax: +31 (0)71 526 6752
Email: F.C.Breedveld@lumc.nl
Website: www.euror.org

Tenth Intensive Applied Epidemiology Course for Rheumatologists
11–15 Mar 2002; Manchester, UK
No previous experience in epidemiology is needed. The course is residential and limited to 25 places.
Contact: Ms Lisa McClair, ARC Epidemiology Unit, University of Manchester, Oxford Road, Manchester M13 9PT, UK
Tel: +44 (0)161 275 5993
Fax: +44 (0)161 275 5043
Email: Lisa@f1.ser.man.ac.uk

OMERACT VI
11–14 Apr 2002; Bali
Includes two modules: MRI and economics; and four workshops: patients’ perceptions, imaging (healing), progressive systemic sclerosis, minimally important clinical difference and osteoarthritis.
Contact: Conference Organisers Q2Q, 7 Swann Street, Old Iselworth, Middlesex TW7 6RJ, UK; or Peter Brooks, Faculty of Health Sciences, Level 1, Edith Cavell Building, Royal Brisbane Hospital, Herston 4029, Australia
Fax: +44 208569 9553
Email: tony@q2q.co.uk or p.brooks@mailbox.uq.edu.au

British Society for Rheumatology XIth AGM
23–26 Apr 2002; Brighton, UK
Contact: BSR, 41 Eagle Street, London WC1R 4TL, UK
Website: www.rheumatology.org.uk

4th EULAR Sonography Course
25–28 April 2002; Madrid, Spain
The course is entitled “Practical use of musculoskeletal ultrasonography”
Contact: Esperanzo Naredo
Email: enaredo@cremas.com
Website: www.eular.org/courses and www.sameint.it/eular

10th International Vasculitis and ANCA Workshop
25–28 Apr 2002; Cleveland, Ohio, USA
Contact: Debora J Bork, The Cleveland Clinic Foundation, Desk A50, Center for Vasculitis Care and Research, 9500 Euclid Avenue, Cleveland, OH 44195, USA
Tel: 216 445 8533
Fax: 216 445 7569
Email: borkd@ccf.org
Website for registration and abstract submission: www.clevelandclinicmeded.com/courses/Vasculitis2002.asp

International Congress: New Trends in Osteoarthritis
9–11 May 2002; Milan, Italy
Contact: Organising Secretariat, O.I.C. S.R.L., Via Fatebenefratelli 19, 20121 Milan, Italy
Tel: +39 02 65 71 200
Fax: +39 02 65 71 270
Email: osteoarthritis@oic.it

IOF World Congress on Osteoporosis
10–14 May 2002; Lisbon, Portugal
Contact: IOF Secretariat, 71 cours Albert Thomas, F-69003 Lyon, France
Tel: +33 472 91 41 77
Fax: +33 472 36 90 52
Email: info@ioflyon.org
Website: www.osteounited.org

5th European Conference on Systemic Lupus Erythematosus
26–30 May 2002; Athens, Greece
Chairman Professor HM Moutsopoulos
Secretariat: Amphihtion Congress Organising Bureau
Email: hmoutsop@med.uoa.gr
Website: congress@amphitron.gr

Annual European Congress of Rheumatology
12–15 June 2002; Stockholm, Sweden
Contact: Fred Wyss, Executive Secretary EULAR, Viltikerstrasse 15, CH-8032, Zurich, Switzerland
Tel: +41 1 383 9690
Fax: +41 1 383 9810
Email: eulrar@bluewin.ch
Website: www.eular.org

10th International Congress on Behcet’s Disease
27–29 June 2002; Berlin, Germany
Under the auspices of the International Society for Behcet’s Disease.
Up to eight young investigator awards, each of $500, will be awarded on the basis of abstracts submitted.
Contact: Professor Ch C Zoubboulis, Department of Dermatology, University Medical Centre Benjamin Franklin, The Free University of Berlin, Fabecstrasse 60–62, 14195 Berlin, Germany
Fax: 49 30 84456908
Email: zoubboure@zedat.fu-berlin.de
Website: www.userpages.fu-berlin.de/~zoubbouer
ISBD website: www.behcet.ws

29th Scandinavian Congress of Rheumatology
15–18 Aug 2002; Tromso, Norway
Contact: Hans Nossent, Department of Rheumatology, University Hospital Tromso, Norway
Tel: 47 776 27294
Fax: 47 776 27288
Email: 29scrz2002@rit.no or revhan@rit.no

Translational Research in Autoimmunity
21–22 Sep 2002; Pavia, Italy
Contact: Organising secretariat: eventi S.R.L., Corso Cavour, 18/20 - 27100 Pavia, Italy
Email: tra@e20pr.com
Website: www.e20pr.com
Congress website: www.medicine.ucsd.edu/albani/2001meeting

OsteoArthritis Research Society International (OARSI) World Congress
22–25 Sep 2002; Sydney, Australia
Contact: OsteoArthritis Research Society International (OARSI), 2025 M Street, NW, Suite 800, Washington DC 20036, USA
Tel: +1 202 367 1177
Fax: +1 202 367 2177
Email: oarsi@oarsi.org
Website: www.oarsi.org

10th International Congress on Antiphospholipid Antibodies
29 Sep–3 Oct 2002; Sicily, Italy
Deadline for abstracts 1 April 2002.
Contact: Secretariat, 10th International Congress on Antiphospholipid Antibodies, c/o Kunes International, PO Box 50006, Tel Aviv 61500, Israel
Tel: 972 3 5140018
Fax: 972 3 5172484
Email: aps@kunes.com
Website: www.kunes.com/aps

7th International Conference on Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Related Diseases
14–17 Oct 2002; Nashville, Tennessee, USA
Contact: Lawrence J Marnett, Biochemistry Department, Vanderbilt University, School of Medicine, Nashville TN 37232-0146, USA
Fax: (615) 343 7329
Fax: (615) 343 7534
Website: www.eicosanoids.science.eayne.edu

66th American College of Rheumatology AGM
25–29 Oct 2002; New Orleans, USA
Contact: ACR, Ronald F Olejko, Director of Conferences and Meetings, 1800 Century Place, Suite 290, Atlanta, Georgia 30045–4300, USA
Tel: +1 404 633 3777
Fax: +1 404 633 1870
Email: acr@rheumatology.org
Website: www.rheumatology.org

Third International Meeting on Social and Economic Aspects of Osteoporosis and Osteoarthritis
7–9 November 2002; Barcelona, Spain
Contact: Yolande Plichte Communication, Boulevard Kleyer 108, 4000 Liége, Belgium
Tel: 32 4 254 12 25
Fax: 32 4 254 12 90
Email: yp@compuserve.com

Certifying Examination in Pediatric Rheumatology
18 Nov 2002
Contact: American Board of Pediatrics, 111 Silver Cedar Court, Chapel Hill, NC 27514-1513, USA
Tel: 919 929 0461
Fax: (615) 343 7534
Website: www.abp.org

Future EULAR congresses
18–21 June 2003; EULAR 2003 Lisbon, Portugal
9–12 June 2004; EULAR 2004 Berlin, Germany
8–11 June 2005; EULAR 2005 Vienna, Austria
21–24 June 2006; EULAR 2006 Amsterdam, The Netherlands
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