CONCISE REPORT

Primary Sjögren's syndrome associated agranulocytosis: a benign disorder?

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Objective: To report on an uncommon association of agranulocytosis in primary Sjögren's syndrome (SS).
Methods: The clinical, haematological, and immunological features of seven patients with primary SS associated with a chronic (>6 months) agranulocytosis, and the outcome of the patients, were analysed.
Results: Patients were white women with an unexplained agranulocytosis. They all had non-erosive arthritis and three had a thrombocytopenia or Evan's syndrome. In three patients, transient or durable expansion of T lymphocytes was present in the peripheral blood or in the bone marrow, but evolved independently from neutrophil counts. There was no paroxysmal nocturnal haemoglobinuria clone or antibodies to neutrophil surface antigens. In vitro bone marrow culture was normal (four patients) or showed a decrease in colony forming unit-granulocyte monocyte (CFU-GM) and colony forming unit-erythroblast (CFU-E) (one patient). Serum levels of soluble Fas ligand (sFasL) were normal, and granulocyte-colony stimulating factor (G-CSF) concentrations were either normal or raised. One patient was treated with steroids associated with intravenous immunoglobulins and achieved a lasting response. Two other patients were treated with steroids and methotrexate, with poor efficacy. Short courses of subcutaneous G-CSF produced a transient and mild response in all three patients. Complete recovery of the neutrophils occurred temporarily during pregnancy in two patients. After a mean follow up of 34.8 months (range 6–139) all patients were alive and none developed serious infections.
Conclusion: A subset of patients with primary SS and non-destructive arthritis may develop a chronic but well tolerated agranulocytosis that is usually poorly responsive to steroids and oral methotrexate.

Sjögren's syndrome (SS) is an autoimmune disease characterised by a lymphocytic infiltration of salivary and lachrymal glands leading to a progressive destruction of these glands, and by production of a large variety of autoantibodies.[1] This disorder is either isolated (primary SS) or associated with other systemic diseases (secondary SS).

Haematological manifestations of SS usually consist of mild anaemia and thrombocytopenia, as well as leucopenia.[1 2] However, severe neutropenia is very unusual.[3 4] This study aimed at describing the clinical and biological features, and the outcome of seven patients with persistent agranulocytosis associated with primary SS.

PATIENTS AND METHODS

Seven patients with severe and durable neutropenia ($<0.5\times10^9$/l, >6 months) associated with primary SS were included in the study. All patients fulfilled the revised version of the European criteria for primary SS.[5]

Patients were evaluated by routine laboratory tests, bone marrow (BM) aspirate, and/or biopsy. All patients had extensive immunological tests, peripheral blood lymphocyte immunophenotyping, and a search for a paroxysmal nocturnal haemoglobinuria (PNH) clone. In vitro BM culture analysis was performed in five patients.

Soluble Fas ligand (sFasL) (Medical and Biological Laboratories Co, Ltd) and granulocyte-colony stimulating factor (G-CSF) (R&D System, Quantikine) serum concentrations were assessed by enzyme linked immunosorbent assay (ELISA; normal values <0.1 ng/ml and <40 ng/l, respectively). Serum samples from eight patients with SS with a normal neutrophil count were used as controls.

RESULTS

Clinical features

All patients were white women who presented with xerostomia, and all of them except patient 6 had keratoconjunctivitis sicca. Minor labial salivary gland biopsy disclosed a Chisholm stage III/IV in all cases. All patients presented with non-destructive peripheral arthritis affecting just small or both small and large joints. None of them fulfilled sufficient criteria for the diagnosis of rheumatoid arthritis[6] or systemic lupus erythematosus.[7] No patient had an organomegaly. No drug intake or exposure to toxic compounds that might induce a neutropenia was recorded. In patients 1, 3, and 7, SS was diagnosed 2, 15, and 10 years before neutropenia, respectively, whereas in patient 5, neutropenia was diagnosed 9 years before SS. In the three remaining patients, neutropenia and SS were diagnosed simultaneously. The mean follow up of SS and neutropenia was 64 months (range 6–192) and 34.8 months (range 6–139), respectively.

Haematological findings

All patients had a severe and chronic non-cyclic neutropenia that was either isolated (four patients) or associated with an immune thrombocytopenia or Evan's syndrome (three patients).

A blood smear disclosed large granular lymphocyte (LGL) expansion in patient 1, accounting for 20% of the lymphocytes, which were polyclonal CD3+, CD8+ TCR $\alpha\beta$+ lymphocytes. No patient had a PNH clone.

Table 1 gives the results of BM analysis. A clonal expansion of CD4–, CD8–, CD3+, TCR $\gamma\delta$+ small lymphocytes was present in patient 2, confined to BM and accounting for 40% of the lymphocytes. In this patient, the karyotype was normal and there was no cytological evidence of a malignancy. Patient

Abbreviations: BM, bone marrow; CFU-E, colony forming unit-erythroblast; CFU-GM, colony forming unit-granulocyte monocyte; G-CSF, granulocyte-colony stimulating factor; IVIg, intravenous immunoglobulins; LGL, large granular lymphocytes; PNH, paroxysmal nocturnal haemoglobinuria; sFasL, soluble Fas ligand; SS, Sjogren's syndrome

Table 1 Haematological findings

Patients	Sex/age (years)	Neutropenia ($\times 10^9$/l)*	Associated cytopenia(s)	BM analysis	In vitro BM culture	T cell clone
1	F/63	0.24	–	MMA	N	–
2	F/30	0.25	Thrombocytopenia	MMA CD4–, CD8– γδ T cell expansion	N	+
3	F/15	0.2	–	N	ND	ND
4	F/74	0.5	Thrombocytopenia	MMA	↓ CFU-GM ↓ CFU-E	–
5	F/63	0.17	Haemolytic anaemia Thrombocytopenia	N	ND	–
6	F/56	0.25	–	Lymphocyte excess	N	–
7	F/42	0.34	–	MMA	N	–

F, female; BM, bone marrow; MMA, myeloid maturation arrest; N, normal; ND, not done; CFU-GM, colony forming unit-granulocyte monocyte; CFU-E, colony forming unit-erythroblast.
*At diagnosis.

6 had a mild BM infiltration with polyclonal T lymphocytes. None of the five patients who were tested, except patient 2, had a clonal expansion of BM (or peripheral blood) T lymphocytes. No patient had blast excess or features of myelodysplasia.

In vitro BM culture studies performed in five patients showed a decrease in colony forming unit-granulocyte monocyte (CFU-GM) and in colony forming unit-erythroblast (CFU-E) in patient 4, whereas BM progenitors were normal in the remainder. Addition of a patient's serum did not alter the growth of haematopoietic progenitors.

Immunological investigations

Antinuclear antibodies were positive in all patients. No patient had anti-dsDNA, anti-Sm, antikeratin, anti-citrullinated filaggrin, antiphospholipid antibodies, or lupus anticoagulant. Antineutrophil cytoplasmic antibodies were present in patients 1 and 6. Antineutrophil antibodies were negative in the five patients tested. A rheumatoid factor was present in patients 1, 2, and 5. Patient 5 had a positive Coombs test and patient 6 had an IgG3 subclass deficiency. Serum levels of sFasL were similar to those of controls (data not shown) in all patients tested (patients 1, 2, 5, 6, and 7), whereas G-CSF levels were either raised or normal (table 2). Table 2 shows the HLA genotyping.

Renal function, urine tests, and chest and abdominal computed tomography were normal in all patients.

Treatment and outcome

Oral steroids were given to patients 1 (0.1 mg/kg/day) and 6 (1 mg/kg/day) for two and one month, respectively, with no improvement of the neutrophil counts. However, steroids induced a lasting decrease in LGL (<9%) in patient 1. Oral methotrexate (up to 15 mg/week) was subsequently given to both patients, resulting in a transient increase of neutrophils (0.7×10^9/l and 1.0×10^9/l, respectively). Subcutaneous G-CSF was then given and again resulted in a transient neutrophil count recovery in both cases. Remarkably, in patient 1, the neutrophils spontaneously rose to 3.2×10^9/l for 10 days during a flu episode then quickly decreased to reach previous counts. A slight and transient increase in serum sFasL (0.12 ng/ml) was detected during this episode. The percentage of LGL remained remarkably stable (<9%) when the neutrophil counts recovered with G-CSF treatment, as well as during the flu episode, and at relapse.

In patients 2 and 3, neutropenia resolved spontaneously by one month and four months of pregnancy, respectively, and relapsed one month after delivery in both cases. Interestingly, the TCR γδ T cell expansion remained unchanged at delivery in patient 2, while the BM myeloid arrest and the neutropenia completely resolved.

In patient 5, oral steroids (2 mg/kg/day) were also ineffective. Intravenous immunoglobulins (IVIg) were subsequently added on day 15 (0.5 g/day for five days), and resulted in a rapid (five days) and persistent neutrophil count recovery (1.8×10^9/l). Steroids were then tapered to 10 mg/day without further relapse of the neutropenia.

Patient 7 had an episode of urinary tract infection due to *Escherichia coli*. Subcutaneous injections of G-CSF (300 µg/day for five days) induced a slight (0.8×10^9/l) and temporary increase in the neutrophil counts.

Patients 3 and 4 were not treated.

After a mean follow up of 34.8 months (range 6–139), no patient experienced serious infectious complications or developed a malignant lymphoid disorder.

DISCUSSION

Haematological manifestations of primary SS essentially include autoimmune thrombocytopenia, and moderate

Table 2 Immunological findings, treatment, and outcome

Patients	ANA titre	Specificity	G-CSF level (ng/l)	HLA-DR	Treatment	Outcome (neutrophil count)
1	80	NI	120	4-16/1-5	Steroids Steroids+MTX	No response Transient recovery
2	80	Ro/SSA	56	4-13/52-53	–	Recovery during pregnancy, then relapse
3	80	NI	ND	15-13	–	Recovery during pregnancy, then relapse
4	160	NI	ND	–	–	Persistent severe neutropenia
5	640	Ro/SSA	ND	3-3	Steroids Steroids+ IVIg	No response Durable recovery
6	320	NI	23	–	Steroids Steroids+MTX	No response Partial recovery
7	1280	Ro/SSA La/SSB	12	3-4/52-53	–	Persistent severe neutropenia

ANA, antinuclear antibodies; NI, not identified; ND, not done; G-CSF, granulocyte-colony stimulating factor; MTX, methotrexate; IVIg, intravenous immunoglobulins.

neutropenia and lymphopenia. Less commonly, patients with SS may develop haemolytic anaemia, agranulocytosis, pure red cell aplasia, aplastic anaemia, and myelodysplastic syndromes.[3 8–10] An increased risk of non-Hodgkin lymphomas has also been reported.[11]

We report herein seven female patients who developed a chronic unexplained agranulocytosis associated with a primary SS. Agranulocytosis was isolated in four patients and associated with haemolytic anaemia or thrombocytopenia, or both, in three others.

The presence of polyarthritis in all patients and expression of HLA-DR4 in 3/5 patients were reminiscent of a Felty's syndrome. However, no patients satisfied the criteria for the diagnosis of rheumatoid arthritis or had a splenomegaly. One patient had an expansion of polyclonal LGL and another had a BM infiltration with monoclonal TCR γδ+ T lymphocytes with no further evidence of malignancy. Remarkably, in the latter, the neutropenia spontaneously resolved during pregnancy and the neutrophil recovery persisted for one month after delivery, although the γδ T cell clone remained unchanged in the BM, indicating that the pathogenic role of the expanded T cell population is questionable. A similar recovery was seen during pregnancy in another patient without any detectable BM or blood T lymphocyte expansion. In a third patient, neutropenia was associated with IgG3 subclass deficiency and mild BM infiltration with polyclonal T lymphocytes, which is similar to our previous report of a subset of patients with IgG2 deficiency and neutropenia possibly due to an autoimmune mechanism.[12]

We failed to detect autoantibodies to surface neutrophil antigens in the patients' sera. BM features and the lack of influence of the patients' sera on granulocyte growth in an in vitro BM cultures system argue against the presence of autoantibodies directed to granulocyte growth factors or to their receptors. However, association of neutropenia with other autoimmune cytopenias in three patients and the presence of a large variety of autoantibodies in all seven patients point to an autoimmune process. The lack of recurrent or severe infections and the normal serum levels of sFasL are similar to reports in children with autoimmune neutropenia. As in our patients, the serum G-CSF levels and the BM features in such children are variable.[13] However, in contrast with our patients, paediatric autoimmune neutropenias are typically isolated and usually recover spontaneously within two years.

Unexplained agranulocytosis has been rarely reported in patients with primary SS.[3 8 9] It is usually associated with antineutrophil antibodies and responds to steroids,[3 9 14 15] used alone or in association with immunosuppressive drugs.[4–5] By contrast, in our series, steroids alone were ineffective in all three treated patients and association with methotrexate in two of them resulted in only a partial and transient response. Combination of steroids and IVIg in one patient induced a complete response. Remarkably, none of our patients had severe or recurrent infections, raising the issue of whether it is reasonable to propose other immunosuppressive treatments or long term subcutaneous injections of G-CSF.

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