COMBINED AUTOIMMUNE CYTOPENIAS

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ABSTRACT

Background. Autoimmune neutropenia (AIN) can occur in association with autoimmune hemolytic anemia (AIHA) and/or immune thrombocytopenic purpura (ITP), although these associations have not been studied in detail.

Methods and Results. Twenty cases of AIN were found in a group of 55 adults with unexplained neutropenia over a five-year period. Eight subjects with AIN had an associated AIHA and/or ITP (AIN+ITP, n=2; AIN+AIHA, n=2; AIN+ITP+AIHA, n=4). Thorough investigations identified no underlying disease in four patients, and none has appeared during follow-up. Of the other 4, one was found to have been suffering from systemic lupus erythematosus when the combined immunocytopenia was diagnosed, one patient from idiopathic myelofibrosis, one from a combined variable immunodeficiency and the other from disseminated tuberculosis. These last three conditions, while sometimes associated with autoimmune cytopenias, has not been previously reported together with combined immunocytopenias. All patients responded to immunosuppressors, although severe infectious complications occurred in two, leading to death from Pneumocystis carinii pneumonia and to irreversible neurologic damage from Listeria monocytogenes meningitis, respectively.

Conclusions. We conclude that combined autoimmune cytopenias are frequently observed in patients with AIN, and a thorough search for associated conditions can lead to unsuspected diagnoses.

Key words: autoimmune neutropenia, immune thrombocytopenic purpura, autoimmune hemolytic anemia, combined immune cytopenias

In 1949 Evans and Duane first described the association of acquired autoimmune hemolytic anemia (AIHA) with thrombocytopenia and neutropenia and, in a subsequent report, they focused on the coexistence of AIHA and thrombocytopenic purpura. Since then, however, there have been few well-documented reports of combined immunocytopenias with neutrophil involvement. We report the clinical, serological and immunological characteristics of eight such cases studied in a single institution over a five-year period.

Materials and Methods

Patient characteristics

From 55 cases of idiopathic neutropenia referred to the immunohematology laboratory of the Hospital de Sant Pau (Barcelona) between October 1989 and August 1994, we selected all those patients (pts) found to have autoimmune neutropenia (AIN) associated with AIHA and/or immune thrombocytopenic purpura (ITP). Eight such cases were identified, and their clinical records were reviewed in detail. Patient characteristics are shown in Table 1.

Laboratory studies

Peripheral blood counts were obtained using routine automated counters, and low platelet counts were confirmed by chamber counting. Immunohematological studies performed in all eight cases included:

1. Lymphocytotoxicity test (LCT) to exclude the presence of HLA antibodies using a panel of HLA-typed lymphocytes;
2. granulocyte immunofluorescence test (GIFT)\(^8\) to detect granulocyte-bound antibodies. In addition to a direct GIFT, an eluate was obtained and incubated with allo- geneic granulocytes to confirm the antigranulo- cytic specificity of the antibody (eluate test), and the pts' serum was tested for free autoantibody by incubation with these same granulocytes (indirect GIFT);

3. platelet autoantibodies were studied using the platelet immunofluorescence test (PIFT).\(^9\) Both direct and indirect PIFT were performed for granulocytes. When a positive direct PIFT was found, the antibody was eluted and the eluate was incubated with allogeneic platelets to confirm the platelet-specific nature of the antibody; Sera from a non-transfused group AB male blood donor was used as negative control and an HLA-multispecific antiserum as positive control for both GIFT and PIFT. Results were expressed from – to ++++;

4. patients with Coombs’-positive hemolytic anemia had a standard work-up for the study of AIHA. This included a direct antiglobulin test (DAT) with polyspecific antiglobulin, anti-IgG and anti-C3b/C3d. Elution studies were routinely performed, and the coexistence of red-cell alloantibodies was excluded after adsorbing the sera with autologous red cells. Results were expressed from – to ++++. The presence of AIHA required a positive DAT plus evidence of hemolysis (i.e. reticulocyto- sis, indirect hyperbilirubinemia, elevated lactate dehydrogenase, low haptoglobin, etc.). All patients had a complete immunological evaluation which included: immunoglobulin class quantification, differential lymphocyte counts, T-lymphocyte subsets, antinuclear antibodies (ANA), anti-DNA, anti-mitochondrial antibodies, anti-smooth muscle antibodies, anti-SSa and -SSb, rheumatoid factor, circulating immune complexes, anti-thyroglobulin and anti-parietal cell antibodies. Serological studies for human immunodeficiency virus were available for all, and bone marrow evaluation was performed in most cases. Other laboratory testing was carried out as clinically indicated.
Results

From a total of 55 patients with unexplained neutropenia (absolute neutrophil count (ANC) < 1.5×10⁹/L], 20 (36%) were found to have a positive direct GIFT with a positive eluate and were thus considered as having AIN. Of these 20 we identified eight (40% of all AIN) who had associated AIHA and/or ITP: two ITP, two AIHA and four AIHA+ITP. Patients with AIN and thrombocytopenia with a negative PIFT were not included in the study. Tables 1 and 2 summarize the results of all hematological and immunological studies performed, as well as relevant clinical data. Most patients (6/8) were first admitted to the hospital to study an abrupt-onset bicytopenia or pancytopenia.

Case reports

In four patients no associated disease was found to coexist with the autoimmune blood dyscrasia: IPN (individual patient number) 1, 7, 37 and 49. Two had autoimmune pancytopenia (IPN 1 and 37) and two AIN+ITP. Clinical, radiologic and laboratory evaluations were remarkable for moderate splenomegaly in one case (IPN 7) and for positive organ-specific autoantibodies in two (anti-parial cell antibodies in IPN 1 and anti-thyroglobulin antibodies in IPN 49). Three patients received treatment for their blood dyscrasia and two responded to prednisone (PDN). IPN 37 required no treatment, although she has moderate autoimmune pancytopenia. These three patients were followed for 17, 57 and 14 months (IPN 1, 37 and 49, respectively). The fourth patient (IPN 7) did not respond to PDN and intravenous immunoglobulins, and after six weeks of unsuccessful therapy cyclosporin A was begun (10 mg/kg daily). Although the blood counts rose to near-normal levels, six weeks later he developed *Pneumocystis carinii* pneumonia and died from septic complications.

IPN 20 was a 76-year-old male with no significant medical history who was admitted for pancytopenia. Physical examination revealed moderate splenomegaly (6 cm below the left costal margin). Bone marrow aspiration was unsuccessful, and the biopsy was hypercellular with extensive reticulin fibrosis. Teardrop ery-
thrombocytes were seen in the peripheral blood and a presumptive diagnosis of early-stage idiopathic myelofibrosis was established, associated with AIN and ITP. Treatment with PDN led to normalization of platelet and neutrophil counts within two weeks. Over the following 2.5 years, however, the myelofibrosis progressed, and he is currently pancytopenic with massive splenomegaly and is red cell and platelet transfusion dependent.

IPN 40 was a 61-year-old male who was admitted for pancytopenia and fever of unknown origin. Physical examination and X-ray studies revealed only moderate splenomegaly (8 cm below the left costal margin). He had been well until two months before admission when he developed pneumococcal pneumonia; since then he had experienced a urinary tract infection by Enterobacter cloacae, a polymicrobial perianal infection which required surgical drainage and bilateral pneumonia of unknown etiology, all of which had resolved with antibiotics. Immunologic studies revealed a severe panhypogammaglobulinemia of 1.79 g/L (normal, 8.2–14). Two bone marrow examinations showed a hyperplastic marrow with lymphoid nodules distributed all along the biopsy cylinder. A thoracic and abdominal CT scan showed only homogeneous splenomegaly with no evidence of lymphoma. AIHA and AIN were diagnosed, and a presumptive diagnosis of common variable immunodeficiency was established. Treatment with PDN led to a slow rise in the ANC and Hb to 4.4×10^9/L and 12.0 g/dL, respectively. Intravenous immunoglobulins (400 mg/kg every two to four weeks) have prevented further infections due to the acquired humoral immune deficiency; follow-up has been 16 months.

IPN 42 was a 61-year-old man with a 1½ year history of cutaneous discoid lupus and AIHA. In January 1994 he developed bilateral pneumonia, and during this period of hospitalization AIN was first diagnosed. He now met the criteria for systemic lupus erythematosus. PDN was given to treat the AIHA and AIN, with good response. Six weeks later he was readmitted for meningitis caused by Listeria monocytogenes, and after withdrawal of PDN both cytopenias recurred, but no further treatment has been given due to the patient’s critical condition. He died two months later from the neurologic sequelae of listeriosis.

IPN 54 was an 80-year-old woman who was admitted for bilateral pneumonia and severe pancytopenia. Her medical history was significant mainly for adult-onset insulin-dependent diabetes mellitus. Disseminated infection by Mycobacterium tuberculosis was diagnosed, with marrow involvement in the form of typical tuberculous multinucleated giant-cell granulomas; the rest of the marrow showed hyperplasia of all hematopoietic elements. Immunohematologic studies confirmed the existence of AIN +AIHA+ITP. Treatment with PDN led to a significant rise in blood counts within 10 days, but the patient died within two weeks from uncontrollable pulmonary tuberculosis. Postmortem examination found no underlying disease except for multiorgan involvement by M. tuberculosis.

**Discussion**

The term combined immunocytopenias was first used by Wiesneth et al. to describe the association of AIN, ITP and/or AIHA. Evans and Duane first reported this condition in 1949, although the term Evans’ syndrome usually refers to the association of AIHA and ITP. From a group of 55 patients referred to our laboratory with a diagnosis of idiopathic neutropenia, usually chronic, GIFT showed that 36% suffered from AIN. Detailed analyses of these cases identified eight (40%) with associated AIHA and/or ITP (combined immunocytopenias).

The incidence of ITP and/or AIHA in patients with AIN has not been clearly established. Bux et al. recently reported 143 cases of AIN diagnosed from a total of 1500 idiopathic neutropenias (9.5% incidence); of these 143 AIN, 8.4% had associated ITP, 2.1% ITP+AIHA and 0.7% AIHA only. The clinical characteristics of these pts, however, were not specified. Logue et al. found 44 AIN in 121 patients with idiopathic neutropenia (36%). Although combined hematocytopenias were present in 41% of all neutropenic pts, the incidence in those with confirmed AIN was not specified; moreover, the autoimmune origin of these other cytopenias
was not confirmed, except for the occurrence of 7 AIHA in the 44 cases of AIN (16%). Van der Veen et al.\textsuperscript{12} described 91 cases of AIN (49 primary and 42 secondary) and found that 5.5% were associated with ITP and 5.5% with AIHA+ITP. Again, no details regarding these combined cytopenias were given.

These data indicate that the presence of ITP and/or AIHA in patients with AIN is not a rare phenomenon. Nevertheless, there have been few detailed reports of combined immunocytopenias with a component of AIN.\textsuperscript{3-6}

In our experience combined immunocytopenias with AIN are a relatively frequent finding in patients studied for AIN. Only two of these patients had a history of an immune-mediated disease: IPN 37 (AIHA three years earlier) and IPN 42 (cutaneous discoid lupus and AIHA). Thus, in most cases there was no preceding history of an autoimmune disorder, as frequently occurs in patients with AIN.\textsuperscript{10,12} More important, however, is the fact that a diagnosis of autoimmune bi- or pancytopenia prompted the clinician to search for such an associated condition. This search proved unrevealing in four cases (IPN 1, 7, 37 and 49). One of these patients (IPN 7) was refractory to steroids and intravenous immunoglobulins, and splenectomy was contraindicated since the patient was severely pancytopenic and refused blood transfusions (Jehovah’s witness). We had previously obtained long-term remissions in a refractory AIN\textsuperscript{13} and in two refractory Evans’ syndromes (unpublished data) with cyclosporin A, and thus we decided to try this drug. Although blood counts were almost normal after six weeks of therapy, the severe respiratory complications which developed did not allow sufficient follow-up to evaluate long-term response to this agent. In two other cases significant titers of organ-specific autoantibodies were found (anti-parietal cell antibodies in IPN 1 and anti-thyroglobulin antibodies in IPN 49), although no autoimmune disease was diagnosed. IPN 42 met the criteria for SLE when the immune bicytopenia appeared.

Clinical and laboratory studies led to unexpected underlying diseases in three of our pts. IPN 20 was diagnosed as having idiopathic myelofibrosis. Autoimmune phenomena are frequently found in patients with myelofibrosis, in particular a wide range of autoantibodies\textsuperscript{17} and autoimmune diseases such as SLE.\textsuperscript{15,16} AIHA\textsuperscript{14} and, less frequently, ITP\textsuperscript{17} can occur in subjects with idiopathic myelofibrosis. We are aware of several cases of combined immunocytopenias with AIN in cases of myelofibrosis associated with SLE,\textsuperscript{15,16} but to our knowledge there have been no previous reports of AIN and ITP associated with idiopathic myelofibrosis. In our case, a rise in platelet and neutrophil counts following PDN therapy was consistent with the autoimmune nature of this bicytopenia, but the subsequent course was characteristic of myelofibrosis. IPN 40 displayed clinical and laboratory features characteristic of combined variable immunodeficiency.\textsuperscript{18,19} Approximately 20% of these patients develop one or more autoimmune diseases,\textsuperscript{18,19} mainly autoimmune hemocytopenias. To our knowledge, however, combined immunocytopenias have not been previously described. The nodular lymphoid infiltrates observed in the patient’s marrow biopsy are typically found in this disorder, although they usually occur in lymph nodes and intestinal mucosa.\textsuperscript{18,20} These proliferations are generally benign, although lymphomas can occur.\textsuperscript{20} In this patient, as well as in others, the clinicians specifically searched for an underlying lymphoproliferative disorder, in particular a lymphoma or a large granular lymphocyte proliferation, which are known to frequently produce autoimmune cytopenias.\textsuperscript{21} As specified, no such disorder was diagnosed and none was found at autopsy or at follow-up. This is important since steroid treatment could have initially masked such a disease, which, nevertheless, should have been obvious at follow-up or at autopsy.

In IPN 54 the existence of disseminated tuberculosis with bone marrow involvement suggests a myelophthisic component to the pancytopenia. The presence of pancytopenia in disseminated tuberculosis is not rare and appears to carry a negative prognosis, although its exact mechanism is not known.\textsuperscript{22,23} Surprisingly, bone marrow involvement by tuberculous granulomas is frequently not observed in such patients.\textsuperscript{23} The existence of AIHA, however, was obvious in our
patient, and the immunohematologic findings and the response to steroids were both consistent with ITP and AIN. ITP has been reported to occur in association with tuberculosis, as has T-lymphocyte-mediated pure white cell aplasia. The presence of another predisposing condition in this patient cannot be postulated since a detailed postmortem examination found only signs of multiorgan involvement by this infection.

In conclusion, combined immunocytopenias are frequently found in patients with AIN. Detailed study of such cases may reveal the existence of an undiagnosed underlying disease. Treatment can be difficult, frequently requiring splenectomy and/or potent immunosuppressors.

References