CASE REPORT

Granulocyte-colony-stimulating Factor-resistant Neutropenia and Polyneuropathy Presenting as Severe Complications of Sjögren’s Syndrome

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Abstract:
Primary Sjögren’s syndrome (pSS) has multi-dimensional manifestations, including neutropenia and polyneuropathy. We herein report a 76-year-old woman with pSS initially presenting as severe granulocyte-colony-stimulating factor (G-CSF)-refractory neutropenia and axonal sensorimotor polyneuropathies (SMP). Systemic glucocorticoid administration had reduced neutrophil-associated immunoglobulin G (NAIgG) on the neutrophil surface as detected using flow cytometry, resulting in the development of neutropenia. A patient with pSS concomitant with axonal SMP might show severe neutropenia as aggressive autoimmune disease. Neutropenia can be treated with systemic glucocorticoids based on the assessment of NAIgG on the neutrophil surface.

Key words: neutropenia, flow cytometry, glucocorticoid, Sjögren’s syndrome

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Introduction

Sjögren’s syndrome has been described as a chronic inflammatory disease of the salivary and lacrimal glands characterized by typical sicca symptoms of dry mouth and eyes and lymphocytic infiltration of glandular tissues (1). However, extraglandular involvement is common and can cause highly varied manifestations (2), which may precede or overshadow sicca symptoms and cause a significant diagnostic delay. We encountered a patient with primary Sjögren’s syndrome (pSS) with concomitant severe neutropenia and polyneuropathy. Patients with pSS develop leukopenia as an initial manifestation in 14-42% of cases (2, 3). This is usually mild, not requiring any therapy. However, neuropathy also often precedes the development of sicca syndrome (4), rendering the recognition of pSS during the early stages of the disease course nearly impossible.

Neutrophil-associated immunoglobulins (NAIgs) include any antibodies that bind to and destroy neutrophils, regardless of any antigenic specificity toward neutrophils in systemic lupus erythematosus (SLE), as secondary autoimmune neutropenia (5, 6). NAIgs bound to neutrophils can be detected using flow cytometry (7).

We herein report a case of pSS that had remained undiagnosed for seven years. The patient initially presented with severe neutropenia and axonal sensorimotor polynueuropathies. In this case, we conducted a repeated flow cytometry analysis to follow-up NAIgG bound to neutrophils as a useful tool for managing treatment with systemic glucocorticoids.

Case Report

An apparently healthy 76-year-old woman had a history of transient, asymptomatic fluctuating neutropenia (neutro-
The neutrophil count fluctuated between 1,030 and 3,100/μL. A physical examination revealed no abnormalities. She was admitted to our hospital due to a two-month history of persistent fatigue. On admission, blood tests showed the following: hemoglobin (Hb), 8.2 g/dL (hypocytic); white blood cell (WBC) count, 1.5×10^9/μL; neutrophil count, 372×10^9/μL. A peripheral blood sample from our patient was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. The sample was washed with 2 mL of phosphate-buffered saline (PBS) (pH 7.4; Gibco, Grand Island, USA). Fluorescein isothiocyanate (FITC)-conjugated F(ab)2 fragments of rabbit anti-human IgG (Dako, Glostrup, Denmark) were used to detect NAiG. Aliquots of blood (100 μL) were mixed with 10 μL of allopurinol (APC)-conjugated anti-CD16 monoclonal antibody (clone 3G8), which detects the low-affinity Fc receptor for IgG (FcγRII), and 20 μL of FITC-conjugated anti-human IgG, and incubated at 4°C for 15 min. Isotype controls of FITC- or APC-conjugated mouse monoclonal antibody were used to establish nonspecific fluorescence. Erythrocytes were lysed with 500 μL of OptiLyse C lysis solution (Beckman Coulter, High Wycombe, UK). Flow cytometry was performed using a Navios flow cytometer (Beckman Coulter). Data collection and analysis employed Navios System software (Beckman Coulter Life Sciences, Indianapolis, USA). Neutrophil populations were gated, and CD16-positive neutrophils were selected to measure levels of uncontaminated viable mature cells (Fig. 3A). Finally, the proportions of NAiG-bound neutrophils were assessed on plots and histograms. In the negative controls (Fig. 3B) and healthy controls (Fig. 3C), neutrophils expressed no signals on the surface. On day 26, the patient responded to treatment and NAIgG had disappeared from the neutrophil surface (8.7%). One month after treatment, NAIgG had disappeared from the neutrophil surface (8.7%). One month after beginning systemic glucocorticoid treatment, the amplitude on left tibia nerve stimulation had recovered (Fig. 2B).

The amplitude on nerve conduction study recovered in accordance with the reduction in the patient's numbness and pain at 0.1 mL/min. Labial salivary gland biopsy demonstrated focal lymphocytic sialadenitis, which describes the presence of dense aggregates of ≥50 mononuclear cells, with periductal localization (Fig. 1). She had no history of radiation exposure to the head or neck, active hepatitis C infection, acquired immunodeficiency syndrome, sarcoidosis, amyloidosis, graft-versus-host disease, or IgG4-related disease.

Around the same time as the transient neutropenia occurred, she had complained of sensory symptoms with symmetrical stocking- and glove-like numbness and pain. After admission, nerve conduction studies confirmed a diagnosis of axonal polyneuropathy, presenting as a reduction in amplitude (Fig. 2A). Negative results were obtained for both serum antibodies to anti-ganglioside GM1 antibody (IgG) and anti-ganglioside GQ1b antibody (IgG).

The second day after hospital admission, the neutrophil count remained low, at 400/μL, so lenograstim was administered. The neutrophil count rose progressively to 3,510/μL. On day 6, the neutrophil count decreased again to 280/μL. Lenograstim was required every 4-5 days, with the neutrophil count fluctuating between 5,320/μL and 260/μL.

After admission, NAiG was detected using flow cytometry (7). A peripheral blood sample from our patient was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. The sample was washed with 2 mL of phosphate-buffered saline (PBS) (pH 7.4; Gibco, Grand Island, USA). Fluorescein isothiocyanate (FITC)-conjugated F(ab)2 fragments of rabbit anti-human IgG (Dako, Glostrup, Denmark) were used to detect NAiG. Aliquots of blood (100 μL) were mixed with 10 μL of allopurinol (APC)-conjugated anti-CD16 monoclonal antibody (clone 3G8), which detects the low-affinity Fc receptor for IgG (FcγRII), and 20 μL of FITC-conjugated anti-human IgG, and incubated at 4°C for 15 min. Isotype controls of FITC- or APC-conjugated mouse monoclonal antibody were used to establish nonspecific fluorescence. Erythrocytes were lysed with 500 μL of OptiLyse C lysis solution (Beckman Coulter, Marseille, France) and incubated for 10 min at 4°C in the dark, adding 500 μL of PBS. The sample was washed with 3 mL of PBS.

Flow cytometry was performed using a Navios flow cytometer (Beckman Coulter). Data collection and analysis employed Navios System software (Beckman Coulter Life Sciences, Indianapolis, USA). Neutrophil populations were gated, and CD16-positive neutrophils were selected to measure levels of uncontaminated viable mature cells (Fig. 3A). Finally, the proportions of NAiG-bound neutrophils were assessed on plots and histograms. In the negative controls (Fig. 3B) and healthy controls (Fig. 3C), neutrophils expressed no signals on the surface. On day 26, the patient was administered systemic glucocorticoid pulse therapy followed by 0.5 mg/kg of prednisolone. Before treatment, some neutrophils had expressed NAiG on the surface (8.7%) (Fig. 3D). One week after treatment, NAiG had disappeared from the neutrophil surface (Fig. 3E). One month after beginning systemic glucocorticoid treatment, the amplitude on left tibia nerve stimulation had recovered (Fig. 2B).

The amplitude on nerve conduction study recovered in accordance with the reduction in the patient’s numbness and
We presented a case of pSS with severe granulocyte-colony-stimulating (G-CSF)-refractory neutropenia and axonal sensorimotor polyneuropathies. Neutrophils showed NAIgG on their cell surfaces as detected by flow cytometry. Systemic glucocorticoids improved neutropenia alone with the decreased expression of NAIgG on the neutrophil surface. Complaints suggestive of pSS were not volunteered by the patient until specifically questioned, following which the diagnosis of pSS was readily established. Such atypical presentations, including neutropenia and polyneuropathy, may be responsible in part for long diagnostic delays, which average 3.1 years from patient presentation (9); indeed, in our case, the patient remained undiagnosed for 7 years. The essentially asymptomatic course and fluctuating nature of the neutropenia over many years appeared characteristic of the systemic autoimmune neutropenia of pSS (10).

In our case, NAIgG was detected on the neutrophil surface using flow cytometry. Lamour et al. reported that 14-45% of pSS patients showed anti-neutrophil antibodies (11). Secondary autoimmune neutropenia can occur in association with systemic autoimmune diseases, such as rheumatoid arthritis or SLE (12). Our result supported the findings of Riera et al. that the percentage of neutrophils bearing in vivo bound IgG in patients with secondary autoimmune neutropenia was <10% (13). However, the mechanisms of neutropenia remain unclear.

Chronic neutropenia is caused by autoantibodies directed at specific neutrophil antigens (14). Most such antigens have been reported as surface glycoproteins, including FcγIIb, CD177, CD11a, CD11b (15), and CD4 (11, 16). We did not assess circulating neutrophil antibody binding to these surface neutrophil antigens.

Our patient showed a high titer of anti-Ro/La antibody. The prevalence of neutropenia concomitant with pSS might be elevated due to close associations with anti-Ro/La antibodies, rheumatoid factor, and low C4 levels (17). In cases of neutropenia of unknown etiology, high titers of these factors might offer clues to the diagnosis. Inflammation of the peripheral nervous system (PNS) rep-
Figure 3. Flow cytometric detection of neutrophil-associated immunoglobulin G (NAIgG) in a healthy individual and in our case. (A) Distribution of CD16-positive neutrophils (P1). (B) No isocontrol IgG bound to CD16-positive neutrophils in our case. (C) NAIgG-negative findings on plots in the healthy control. (D) NAIgG-positive findings on plots in our case before treatment with systemic glucocorticoids. (E) Disappearance of the NAIgG finding on plots in our case when neutropenia recovery occurred one month after treatment.

represents another complication of pSS. PNS inflammation is common, reported in 89% of pSS patients, and the prevalence of PNS inflammation associated with pSS increases with age (18). Sensory symptoms occurred symmetrically and showed a stocking- and/or glove-like distribution in 87% of those patients (19). PNS involvement in pSS had two different features: pure sensory neuropathy and axonal sensorimotor polyneuropathy (SMP). Pure sensory neuropathy is reported as a mild phenotype, whereas patients with SMP involvement exhibit a phenotype of more aggressive autoimmune disease and comorbidity with neutropenia, as in our case (20).

Steroids have been shown to be effective and safe in patients with immune-mediated neutropenia who do not tolerate G-CSF therapy (10, 21-23). The main impact of glucocorticoid treatment on the neutrophil function is impairment of migration to sites of inflammation or infection. Neutrophil migration through the vasculature to sites of inflammation is severely impaired. This, combined with enhanced release of cells from the bone marrow and inhibition of neutrophil apoptosis, results in increased numbers of circulating neutrophils (24-26). We also suggest a hypothetical mechanism by which glucocorticoid inhibits the presentation of NAIgG and antigens on the neutrophil surface through signal transduction passage, as evidenced by the finding that glucocorticoid inhibited immunoglobulins on B cells (27).

We reported the case of a 76-year-old woman who presented with pSS concomitant with severe neutropenia and polyneuropathy. Systemic glucocorticoid administration may achieve release from G-CSF-refractory neutropenia and axonal polyneuropathy with the disappearance of NAIgG on the neutrophil surface.

The authors state that they have no Conflict of Interest (COI).

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