

A Case of Autoimmune Neutropenia That Responded to Rituximab

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Abstract

Autoimmune neutropenia (AIN) refers to the immune-mediated destruction of neutrophils. It is a rare condition with an estimated prevalence of less than 1 case per 100,000 per year. Typical treatment involves supportive care with granulocyte colony-stimulating factor (G-CSF) and management of secondary infections with antibiotics. Other therapies targeted at the immune system such as steroids, intravenous immunoglobulin (IVIG), and rituximab have not been thoroughly evaluated, but recently rituximab has shown promising results in one case series. We present a 76-year-old man with the diagnosis of antineutrophil antibody-negative AIN and concurrent immune thrombocytopenic purpura (ITP), whose AIN was treated initially with G-CSF which had a short-lived effect, then treated with rituximab which induced a lasting remission. We then review this case in context of other cases described in the literature, given the paucity of available publications.

Keywords: Autoimmune neutropenia; Antibody-negative; Rituximab; Sustained remission; Concomitant immune thrombocytopenic purpura

Introduction

Neutropenia is associated with increased risk of infection, particularly if the absolute neutrophil count (ANC) falls below 500/ μ L, which may be severe and life-threatening [1]. According to the 2023 European guidelines on diagnosis and management of neutropenia, neutropenia is classified into congenital and acquired; acquired neutropenia is further categorized as primary and secondary [2]. Autoimmune neutropenia (AIN) is a general term referring to a set of conditions in which there is

immune-mediated destruction of neutrophils. It is more prevalent in children than in adults, predominantly affects women over men, and is most often associated with underlying autoimmune disease [3]. Antibody-mediated forms of AIN are characterized by antineutrophil autoantibodies against human neutrophil antigens (HNAs). Within primary neutropenia, there are antibody-mediated (such as primary AIN) and non-antibody mediated (such as chronic idiopathic neutropenia (CIN)) disease classifications. Even in CIN, an autoimmune etiology is thought to be the cause, and various antibody-independent mechanisms of immune destruction have been proposed such as agglutination and complement activation, neutrophil phagocytosis by the reticuloendothelial system, or lymphocyte-mediated apoptosis through the Fas pathway. Secondary neutropenia may also be related to autoimmune diseases, such as systemic lupus erythematosus and Felty's syndrome and is usually associated with antineutrophil antibodies [1, 4-7].

Antineutrophil antibodies, as a hallmark of the disease, are often present at low titers and bind to their targets with low avidity, necessitating multiple tests before concluding whether antibodies are present or not, as no single test can reliably detect antineutrophil antibodies [5]. Patients with antibody-mediated AIN may have antineutrophil antibodies that are undetectable due to limitations in the sensitivity of currently available testing, which includes immunofluorescence to detect autoantibodies towards HNAs on neutrophil surface glycoproteins, either directly on neutrophils or within serum via granulocyte immunofluorescence test (GIFT), granulocyte agglutination test (GAT), or monoclonal antibody immobilization of granulocyte-specific antigen enzyme-linked immunosorbent assay (ELISA) (MAIGA assay) [1]. In these cases, the diagnosis may be made either entirely clinically or supported by detection of antineutrophil cytoplasmic antibodies (ANCA), which are often associated with AIN [1, 4, 5, 8]. In cases where repeat antineutrophil antibody testing is negative, bone marrow biopsy may be performed to support the diagnosis, which is usually heterogeneous, and a characteristic finding is hypercellularity with a distinct reduction in mature cells resembling maturation arrest [5, 9-12]. ANCA testing is also often performed due to its presence in some forms of AIN [4, 10]. The diagnosis of AIN should be suspected in patients with a steady decline in ANC and in whom other causes of neutropenia (infections, drugs, toxins, and hematologic malignancies) have been ruled out, and the diagnosis of AIN is mainly clinical, with antineutrophil antibody testing playing an adjunctive role [4].

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Therapy for AIN has historically included supportive care for associated infections, glucocorticoids, and splenectomy [12]. Corticosteroids and splenectomy have fallen out of favor owing to unpredictable outcomes with the former and risk of severe encapsulated bacterial infections with the latter and have been supplanted by granulocyte- or granulocyte-macrophage-colony-stimulating factor (G-CSF/GM-CSF). G-CSFs have been demonstrated to be effective but are limited by both frequent relapse once treatment is discontinued [10], as well as risk of autoimmune flare particularly in those with secondary AIN [7]. Primary AIN is a chronic condition that does not always require therapy unless presenting with infection, while secondary AIN oftentimes needs to be treated aggressively, and the underlying condition also should be treated. Alemtuzumab and rituximab are biological agents that have been investigated as potential treatments [1, 4, 6].

Rituximab is an anti-CD20 agent that targets B lymphocytes, which express this surface marker. It induces apoptosis and disrupts the normal functioning of B cells in the immune system and may inhibit the maturation to plasma cells and thus antibody production [13]. Rituximab has been viewed unfavorably or equivocally by some [1, 4, 14] but has been recently shown to induce good ANC responses in patients who were both ANCA-positive and negative in a case series by Bigot et al [10]. Regardless of ANCA status, seven of eight patients in the above case series had a complete or partial hematologic response to rituximab, and of those that had a relapse, all had a complete response to a second course.

Here we describe a case of dual AIN and immune thrombocytopenic purpura (ITP) with undetectable antineutrophil antibodies initially requiring frequent G-CSF infusions, which incidentally responded well to induction rituximab for refractory ITP. Though there are few studies demonstrating the benefit of using rituximab, this patient achieved long remissions in neutrophil count after each course.

Case Report

Investigations

A 76-year-old man with remote history of indeterminate colitis, obstructive sleep apnea, chronic kidney disease (CKD) stage 3, diabetes, and chronic obstructive pulmonary disease (COPD) presented with pancytopenia. His physical exam was unremarkable without palpable lymphadenopathy or skin changes.

His hemoglobin (Hb) began trending down in February 2018 to 12.2 (range 13 - 17.1 g/dL) with normal mean corpuscular volume (MCV, range 81 - 100). After total knee replacement in March 2019, white blood cell (WBC) count dropped to 2 (range $3.4 - 11.0 \times 10^3/\mu\text{L}$) with ANC 0.02 (range $1.5 - 7.4 \times 10^3/\mu\text{L}$) and Hb to 9 with MCV 87. He presented to hematology clinic in April 2019 with dizziness and weakness and was hospitalized for neutropenic fever secondary to septic arthritis of his thumb. He responded well to granulocyte stimulating agents. His fluctuating thrombocytopenia, which had previously ranged between 100 and 150 (range $150 - 425 \times 10^3/\mu\text{L}$), began to downtrend significantly after July 2019, reaching 20

- 30 in March 2020.

In July 2020, he was admitted for *Pseudomonas* bacteremia of unclear source in the setting of ANC of 0.17 and again treated successfully with G-CSF and antibiotics. He also received intravenous immunoglobulin (IVIG) for thrombocytopenia of 11. During this admission, steroids were avoided in the setting of sepsis.

The patient's neutropenia transiently responded to G-CSF but required frequent re-dosing to maintain normal neutrophil counts. His thrombocytopenia likewise demonstrated an efficacious but short-lived response to bursts of high-dose dexamethasone and an instance of IVIG.

Diagnosis

A review of the patient's medications was conducted, and no clear inciting medication was identified. Notable initial laboratory workup included C-reactive protein (CRP) of 249 (range $< 10 \text{ mg/L}$), erythrocyte sedimentation rate (ESR) 129 (range $< 20 \text{ mm/h}$). Antinuclear antibody (ANA) was positive in 2014 with low titer (1:20) and negative on repeat in 2019. Antineutrophil antibodies were negative in 2019, and ANCA was negative in 2022.

Bone marrow biopsy in April 2019 showed hypercellular marrow (75%) with left-shifted granulocytic hyperplasia, and less than 3% blasts. There were no clonal abnormalities on fluorescence *in situ* hybridization (FISH), normal karyotype, and normal flow cytometry. Repeat bone marrow biopsy of the contralateral iliac crest in June 2019 showed similar findings.

In May 2019, a computed tomography (CT) of the chest, abdomen, and pelvis showed diffuse lymphadenopathy. Core needle biopsy and excisional biopsy in July 2019 was consistent with benign reactive lymph nodes without evidence of Castleman disease, granulomatous inflammation, lymphoma, Hodgkin disease, or metastatic malignancy. Of note, biopsy showed prominent interfollicular vessels with activated endothelial nuclei but no further evidence of leukocytoclastic vasculitis. In July 2020, a repeat CT revealed spleen size had increased from 15 to 18 cm.

In September 2020 he was evaluated by a second hematologist at an outside institution who concurred that the hypercellularity of marrow without clonal lesions or peripheral cytopenias in the setting of negative infectious or rheumatologic driver was suggestive of an autoimmune etiology.

An extensive rheumatologic, hematologic, malignant, and infectious workup was negative for alternative etiologies for the patient's pancytopenia and imaging findings. Results are listed below: 1) The patient was Epstein-Barr virus (EBV) immunoglobulin (Ig)G positive and IgM negative, and cytomegalovirus (CMV) IgG and IgM negative. Human immunodeficiency virus (HIV) antigen/antibodies were not present. Syphilis treponemal antibody was negative. 2) The following antibodies were negative: anti-Sjogren's syndrome (SSA) antibody, anti-Sjogren's syndrome type B (SSB) antibody, anti-Smith antibody, anti-ribonucleoprotein (RNP) antibody, C3 complement, C4 complement, and anti-double stranded DNA. 3) Serum protein electrophoresis (SPEP), paroxysmal noctur-

nal hemoglobinuria (PNH) studies, and quantitative immunoglobulins were unremarkable. The hematology molecular profile did not note any evidence of mutations in *JAK2*, *MPL*, *CALR*, *FLT3*, *NPM1*, *IDH1* and *IDH2*. Peripheral smear revealed neutrophils decreased in number with occasional binucleated forms and occasional myelocytes and metamyelocytes. Copper and ferritin were within normal ranges, and interleukin (IL)-6 was undetectable.

Treatment

The patient's neutropenia was initially treated with G-CSF (filgrastim) injections which induced a transient normalization in neutrophil count > 1.5 generally for about 3 to 6 weeks.

His thrombocytopenia was treated with dexamethasone burst of 40 mg daily for 4 days in May 2020 with only modest response (8 to 51). His most significant improvement in thrombocytopenia occurred with IVIG in July 2020 (11 to 147). Dexamethasone burst was repeated following this. He was not able to achieve lasting remission with any of the above therapies, and eltrombopag daily was started. A splenectomy was considered and discussed with the patient but was deferred in favor of a trial of rituximab which was felt to be a safer option with the potential for lasting remission.

Starting in December 2020, he received four cycles of weekly rituximab for presumed refractory ITP, which induced a remission in his thrombocytopenia. It was not until the patient was treated with rituximab that he attained long-lasting remissions in both neutrophil and platelet counts.

Follow-up and outcomes

Incidentally, within 3 months of completing rituximab, the patient's G-CSF requirements began to decrease significantly. Eventually, the patient went for 1 year without needing any G-CSF as his neutropenia resolved. From June to August 2022, he had a relapse in his neutropenia (ANC *nadir* of 0.09) but responded rapidly to a dose of G-CSF. His platelets, which had generally sustained > 100 since the rituximab, also dipped below < 100 at this time. In February 2023, the patient received a second round of rituximab, resulting in sustained remission of neutrophil and platelet counts.

Since then, the patient has required G-CSF only once for ANC < 1.5 . For ITP, he continues eltrombopag three times per week. He has maintained a normal ANC and near normal platelet count since this second dose of rituximab and has not had clinically significant infection or bleeding events since then. The patient's blood counts were monitored with labs at least monthly since May of 2019. The patient tolerated all treatments well; no clinically significant adverse effects were noted at the patient's routine follow-up treatments over this period.

Discussion

We present a case of antineutrophil antibody-negative immune

neutropenia and immune thrombocytopenia. After an extensive workup for alternative etiologies which was negative, AIN was favored as the diagnosis, and this was supported by a robust response in neutrophil count to rituximab. Similarly, a diagnosis of concomitant ITP was made given the patient's excellent response to steroids, IVIG, and rituximab. Although he was initially supported with repeated doses of G-CSF every few weeks, the patient achieved long remissions in neutropenia after each course of rituximab. Combined AIN and ITP is a reportedly rare condition, and as such, there are limited data available on treatment options. A prior case report indicated that methotrexate has been used successfully [15].

Alternative diagnoses were considered. Given that his SPEP was negative for significantly elevated polyclonal immunoglobulins, IL-6 was undetectable, and crucially an excisional lymph node biopsy was negative, Castleman disease was ruled out. Contralateral bone marrow biopsy was performed and also negative for abnormality other than regenerative hematopoiesis, with no evidence for myelodysplastic syndrome, leukemia, lymphoma, or other infiltrative marrow processes. Although he developed splenomegaly, after discussion with pathology and hematology tumor board, it was felt that there was a very low likelihood of a malignant process in the spleen that was causing this, given the absence of evidence for such a process elsewhere, including in the peripheral blood, lymph nodes, or bone marrow. The decision was made to defer resection due to the high potential morbidity associated with encapsulated organism sepsis in the setting of chronic neutropenia.

His AIN is of interest in the setting of an undiagnosed chronic diarrhea. Although he was initially suspected to have ulcerative colitis vs. Crohn's disease a decade prior to his pancytopenia, the diagnosis was not consistent with colonoscopy findings or biopsy results. In 2003, his diarrhea went into remission after a course of mesalamine. Biopsy during colonoscopy in 2014 showed normal colonic mucosa off treatment. Despite his long remission, the patient's diarrhea recurred in November 2019, around the same time as the onset of his AIN. Fecal calprotectin in 2021 was elevated at 129. The question remains whether this patient's AIN was directly connected to his gastrointestinal symptoms.

Testing for antineutrophil antibodies was performed only once. Due to the limitations of single tests in reliably detecting antineutrophil antibodies, it cannot be definitively concluded whether they were present or not in this patient. The patient's bone marrow was consistent with AIN, and ANCA antibodies were negative. Inflammatory markers were elevated, and he had other autoimmune conditions. He additionally responded well to the standard treatment of G-CSF, and interestingly, rituximab. In this case, detection of antineutrophil antibodies was not felt to be required to make the diagnosis of AIN, and a clinical diagnosis was made instead given the high degree of suspicion and workup done above. In conclusion, this patient was diagnosed with AIN, though whether it was antibody-associated likely secondary AIN or a non-antibody-mediated condition such as CIN was unable to be determined.

Although prior studies have shown mixed results using

rituximab in AIN [10, 14], a recent case series published by Bigot et al [10] has had success with this treatment in both ANCA-positive and negative AIN. Interestingly, in this case, a long-lasting remission to rituximab was also observed, which supports a potential role of rituximab in the treatment of this rare condition in select patients. Further studies are warranted to identify the patient population most likely to respond to rituximab, such as potentially those with concomitant autoimmune disorders, as well as further characterize the safety and efficacy of this novel and promising treatment.

Learning points

In this case of antibody-negative AIN, G-CSF was an effective therapy but only resulted in transient response and had to be frequently re-dosed. Rituximab was used to treat refractory ITP but incidentally induced long-lasting remissions twice in the patient's AIN. Based on these findings and recent literature, there appears to be a potential role for rituximab in treatment of this rare condition that may have been previously overlooked. More data are needed to further evaluate the efficacy and potential risks of this therapy.

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Conflict of Interest

The authors declare they have no competing interest.

Informed Consent

The patient provided informed consent to the writing and publication of deidentified medical information for this case report.

Author Contributions

JW provided initial literature review, data interpretation, and drafted and reviewed all versions of the manuscript. DB provided clinical review, data interpretation, and drafted and reviewed all versions of the manuscript. EN conceptualized the study, provided data interpretation, editing, oversight, and reviewed all versions of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data Availability

All data were accessed through the Scripps Health System HER (last accessed on May 13, 2024).

Abbreviations

AIN: autoimmune neutropenia; ANA: antinuclear antibody; ANC: absolute neutrophil count; ANCAs: antineutrophil cytoplasmic antibodies; CRP: C-reactive protein; CKD: chronic kidney disease; CMV: cytomegalovirus; COPD: chronic obstructive pulmonary disease; EBV: Epstein-Barr virus; ESR: erythrocyte sedimentation rate; G-CSF: granulocyte colony-stimulating factor; GAT: granulocyte agglutination test; GIFT: granulocyte immunofluorescence test; Hb: hemoglobin; HIV: human immunodeficiency virus; HNA: human neutrophil antigen; Ig: immunoglobulin; ITP: immune thrombocytopenic purpura; IVIG: intravenous immunoglobulin; MAIGA: monoclonal antibody immobilization of granulocyte-specific antigens; MCV: mean corpuscular volume; PNH: paroxysmal nocturnal hemoglobinuria; RNP: anti-ribonucleoprotein; SSA: anti-Sjogren's syndrome type A; SSB: anti-Sjogren's syndrome type B; SPEP: serum protein electrophoresis

References

1. Akhtari M, Curtis B, Waller EK. Autoimmune neutropenia in adults. *Autoimmun Rev*. 2009;9(1):62-66. [doi pubmed](#)
2. Fioredda F, Skokowa J, Tamary H, Spanoudakis M, Farruggia P, Almeida A, Guardo D, et al. The European guidelines on diagnosis and management of neutropenia in adults and children: a consensus between the European Hematology Association and the EuNet-INNOCHRON COST Action. *Hemasphere*. 2023;7(4):e872. [doi pubmed](#)
3. Newburger PE. Autoimmune and other acquired neutropenias. *Hematology Am Soc Hematol Educ Program*. 2016;2016(1):38-42. [doi pubmed](#)
4. Afzal W, Owlia MB, Hasni S, Newman KA. Autoimmune neutropenia updates: etiology, pathology, and treatment. *South Med J*. 2017;110(4):300-307. [doi pubmed](#)
5. Capsoni F, Sarzi-Puttini P, Zanella A. Primary and secondary autoimmune neutropenia. *Arthritis Res Ther*. 2005;7(5):208-214. [doi pubmed](#)
6. Kumakura S, Kobayashi S. Autoimmune neutropenia. *Intern Med*. 2003;42(2):133-134. [doi pubmed](#)
7. Starkebaum G. Chronic neutropenia associated with autoimmune disease. *Semin Hematol*. 2002;39(2):121-127. [doi pubmed](#)
8. Shastri KA, Logue GL. Autoimmune neutropenia. *Blood*. 1993;81(8):1984-1995. [pubmed](#)
9. Mariotti J, Caberlon S, Bertinato E, Podda G, Pugliano MT, Cattaneo M. Primary autoimmune neutropenia in adults: case report and review of the literature. *Transfusion*. 2014;54(11):2906-2910. [doi pubmed](#)

10. Bigot W, Viallard JF, Sellam J, Dellal A, Retbi A, Gobert D, Malard F, et al. Efficacy and safety of rituximab for autoimmune neutropenia: a French case series and literature review. *Rheumatology (Oxford)*. 2020;59(9):2637-2640. [doi](#) [pubmed](#)
11. Bux J, Behrens G, Jaeger G, Welte K. Diagnosis and clinical course of autoimmune neutropenia in infancy: analysis of 240 cases. *Blood*. 1998;91(1):181-186. [pubmed](#)
12. Boxer LA, Greenberg MS, Boxer GJ, Stossel TP. Autoimmune neutropenia. *N Engl J Med*. 1975;293(15):748-753. [doi](#) [pubmed](#)
13. Hanif N, Anwer F. Rituximab. In: StatPearls. Treasure Island (FL). 2024. <http://www.ncbi.nlm.nih.gov/books/NBK564374/>. [pubmed](#)
14. Dungarwalla M, Marsh JC, Tooze JA, Lucas G, Ouwehand W, Pettengell R, Dearden CE, et al. Lack of clinical efficacy of rituximab in the treatment of autoimmune neutropenia and pure red cell aplasia: implications for their pathophysiology. *Ann Hematol*. 2007;86(3):191-197. [doi](#) [pubmed](#)
15. Mitsogianni M, Mitsimponas N, Haase S, Giagounidis A. Treatment of combined autoimmune neutropenia and immune thrombocytopenia with methotrexate. *Acta Haematol*. 2020;143(1):89-90. [doi](#) [pubmed](#)