Abstract:
Although anti-neutrophil antibodies (ANAs) often exist and immunoreaction may be involved in agranulocytosis, few reports have so far described ANA-positive cases of agranulocytosis with an unknown etiology. We herein describe the case of a 69-year-old woman who presented with ANA-positive agranulocytosis. In this case, both the withdrawal of the drugs that had possibly caused neutropenia and the use of granulocyte-colony stimulating factor (G-CSF) were ineffective treatment measures. Approximately 2 weeks after the discontinuation of the suspected drugs, we initiated corticosteroid pulse therapy; the neutrophil count recovered by day 19 of steroid therapy. High-dose methylprednisolone therapy should thus be considered for patients demonstrating ANA-positive agranulocytosis with an unknown etiology that is refractory to G-CSF treatment.

Key words: agranulocytosis, anti-neutrophil antibody, high-dose methylprednisolone

Introduction
Agranulocytosis is a well-known but rare disease that presents with severe neutropenia mainly due to drug side effects. Two major mechanisms of agranulocytosis are reported: “direct” drug toxicity to myeloid precursors and “indirect” immune- or antibody-mediated responses (1-3). In cases of drug-induced agranulocytosis, the immediate discontinuation of the causative drugs is vital and the use of granulocyte colony stimulating factor (G-CSF) may be effective (4). Several case reports have shown the effectiveness of corticosteroids for drug-induced agranulocytosis (5-7). However, there is a paucity of information focusing on non-drug-induced agranulocytosis cases related to anti-neutrophil antibody. To our knowledge, only one case report of severe antibody-mediated non-drug-induced agranulocytosis that was treated with high-dose steroid treatment has been published to date (8).

We herein describe the case of an elderly patient with anti-neutrophil antibody-positive agranulocytosis with no obvious agranulocytosis etiology that did not respond to G-CSF administration, but ultimately responded to corticosteroid therapy.

Case Report
A 69-year-old woman with progressive leucopenia and persistent fever was admitted to our hospital. Two months prior to the admission, she presented with a skin rash of unknown etiology that was ultimately managed by the intermittent use of bepotastine besilate and very low dose prednisolone (PSL) at a dose of 1 mg/day. She thereafter developed a high fever, sore throat, and general fatigue for 9 days despite antibiotic treatment, and blood tests revealed a remarkably decreased leukocyte count. At admission (Day 1),...
she had a fever (≥38°C) and pharyngeal erythema, but no other signs or symptoms of hematological malignancies or autoimmune disease, such as splenomegaly, skin rash, or joint pain. She had been administered anti-hypertensive drugs (amlodipine besilate), anti-dyslipidemia drugs (fenofibrate), and thrombosis prophylactic aspirin for several years as well as ursodeoxycholic acid for non-viral hepatitis for the previous year.

The laboratory findings are shown in Table. The patient showed a decreased absolute neutrophil count, below the limit of detection, and mild anemia with an increased reticulocyte count. Serology testing revealed the presence of antinuclear and anticientromere antibodies. A bone marrow biopsy revealed severe hypoplasia without any significant dysplasia (Fig. 1). The number of myeloid cells were markedly decreased, and also the number of megakaryocytes were decreased in the nucleated cells, while lymphocytes were predominant. A chromosomal analysis showed a normal karyotype. Abdominal ultrasonography revealed moderate splenomegaly and hepatic steatosis.

Although these findings suggested a diagnosis of aplastic anemia, it was ruled out for the following reasons: the decreased percentage of white blood cells, which was in the normal range. Thus, we made a provisional diagnosis of drug-induced agranulocytosis and discontinued all the drugs (aspirin at Day 7 and the other drugs at Day 1) suspected of causing the agranulocytosis. On Day 4, we initiated G-CSF treatment and conducted laminar air flow isolation. Although the use of broad-spectrum antibiotics resolved the high fever and pharyngalgia, the neutrophil count did not return to normal. She then developed severe pneumonia. Computed tomography (CT) performed on Day 11 revealed an anterior mediastinal tumor (Fig. 2a). A fluorodeoxyglucose positron emission tomography (FDG-PET) examination performed on Day 23 showed an abnormal uptake [maximum standardized uptake value (max SUV), 7.1] in the lymph nodes and the lesion (Fig. 2b), which was suspected to be malignant. Since these observations suggested that the severe, persistent leukopenia could have been caused by immune-mediated or paraneoplastic syndrome, we decided to try immunosuppressive therapy. Further, we considered performing thymectomy because of the possible association between pure white cell aplasia and the presence of thymoma (9). However, we later rejected that approach because the FDG uptake in the lymph nodes was higher than that in the anterior mediastinal tumor, which suggested the absence of a thymoma. Thus, we initiated high-dose in-

| Table. Blood Examination Findings of the Patient on Admission. |
|---------------------------------|---------------------------------|---------------------------------|
| Normal range                    | Normal range                    | Normal range                    |
| WBC (×10^9/L)                   | UN (mg/dL)                      | ANA ×640                        |
| 1.4                             | 15                              | Speckled pattern                |
| Stabs, segs (%)                 | Cre (mg/dL)                     | RF Negative                     |
| 0                               | 0.88                            | Negative                        |
| Lymphocytes (%)                 | CRP (mg/dL)                     | Anti-centromere Ab (index)      |
| 99                              | 7.57                            | 175                             |
| Monocytes (%)                   | Ferritin (ng/mL)                | Anti-dsDNA Ab (IU/mL)           |
| 1                               | 329.2                           | <5.0                            |
| Eosinophils (%)                 | Vit B12 (pg/mL)                 | Anti-Sm Ab                      |
| 0                               | 838                             | Negative                        |
| Basophils (%)                   | Folate (ng/mL)                  | LA (RATIO)                      |
| 0                               | 8.3                              | 1.2                             |
| RBC (×10^12/L)                  | sIL-2 R (U/mL)                  | Cardiolipin Ab (U/mL)           |
| 3.43                            | 1,180                           | <8.0                            |
| Hb (g/dL)                       | Free T3 (pg/mL)                 | HBsAg                           |
| 9.5                             | 2.1                             | Negative                        |
| HCT (%)                         | Free T4 (pg/mL)                 | HBsAb (mIU/mL)                  |
| 29.5                            | 1.16                            | 13.2                            |
| RETIC (%)                       | TSH (IU/mL)                     | HBcAb                           |
| 6.1                             | 1,020                           | Negative                        |
| PLT (×10^9/L)                   | IgG (mg/dL)                     | HB virus-DNA                    |
| 263                             | 1,829                           | Negative                        |
| TF (g/dL)                       | IgA (mg/dL)                     | HCV Ab                          |
| 7.1                             | 240                             | Negative                        |
| Alb (g/dL)                      | IgM (mg/dL)                     | EBV VCA IgG                      |
| 3.3                             | 103                             | <x10                            |
| T-Bil (g/dL)                    | PT-INR                          | EBV VCA IgM                      |
| 0.4                             | 1.00                            | <x10                            |
| AST (IU/L)                      | APTT (s)                        | EBNA                            |
| 16                              | 45.2                            | Indeterminable                  |
| ALT (IU/L)                      | Fibrinogen (mg/dL)              | CMV antigenemia                 |
| 16                              | 534                             | Negative                        |
| γ-GTP (IU/L)                    | FDP (μg/mL)                     | HPB B19-IgM                      |
| 87                              | 5.7                             | Negative                        |
| ALP (IU/L)                      | Cross mixing test Inhibitory pattern |
| 421                             | 5.7                             | Negative                        |
| LDH (IU/L)                      | TB-INF-γ                        |
| 125                             | 115-229                         | Negative                        |

TP: total protein, Alb: albumin, T-Bil: total bilirubin, AST: aspartate transaminase, ALT: alanine transaminase, γ-GTP: gamma-glutamyl transpeptidase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, UN: urea nitrogen, Cre: creatinine, CRP: C-reactive protein, Vit B12: vitamin B12, sIL-2R: soluble interleukin-2 receptor, TSH: thyroid-stimulating hormone, RF: rheumatoid factor, TMB: total bilirubin, TB-Inf: thymus. Abnormal values are in bold type.
travenous methylprednisolone (mPSL) therapy (1 g/day for 3 days) from Day 20 and then administered PSL (1 mg/kg), while also continuing the G-CSF therapy. During the treatment, she tested positive for anti-neutrophil antibodies by the granulocyte immunofluorescence test (GIFT) (10). However, we did not detect the anti-neutrophil antibody in other techniques, and the correspondent antigen was not identified (11-14). At Day 40 (19 days after corticosteroid initiation), the neutrophil count increased to 312/μL and then gradually returned to the normal range. However, despite the administration of broad-spectrum antibiotics and anti-mold drugs, the patient died of Aspergillus pneumonia and cerebral hemorrhage, possibly due to disseminated intravascular coagulation (DIC) on Day 46. An autopsy revealed the thymic tumor to be a cyst, and no findings of malignancy were observed in the lymph nodes, spleen, or other tissues.

Discussion

We herein describe a case of anti-neutrophil antibody-positive severe agranulocytosis that did not seem to be drug-induced. The administration of G-CSF alone for 16 days was ineffective. The neutrophil counts recovered by Day 40 (19 days after corticosteroid administration); however, the patient died of a mold infection and cerebral hemorrhage, possibly due to DIC prior to granulocyte recovery. The ear-
The administration of G-CSF for agranulocytosis is controversial (4). On the other hand, its use for treating primary AIN in pediatric patients is recommended. In adult patients, its effectiveness depends on the underlying diseases (23). The optimal treatment for AIN in cases that are refractory to G-CSF has not yet been established, but the use of corticosteroids, immunosuppressants, and intravenous gamma-globulin has been reported (20). In patients with etiology-unknown agranulocytosis, who tested positive for anti-neutrophil antibody as was seen in this case, immunosuppressive therapy including high-dose corticosteroids should be attempted while carefully managing fungal infections, because not only profound prolonged neutropenia, but also the administration of corticosteroids are well-known risk factors for fungal infections (24).

Some limitations associated with this case report include the fact that we did not perform biopsies of the skin, liver, and thymic gland when the patient was alive and we used high-dose mPSL with the understanding that steroids may mask the manifestations of underlying autoimmune disease. However, the masking of manifestations, which indicates a very high efficacy of immunosuppressive therapy, may suggest the presence of immune-mediated neutropenia.

In conclusion, we herein described an adult case of steroid-responsive agranulocytosis with anti-neutrophil autoantibody suspected of having an immune-related pathophysiology. Our findings suggest that the empiric administration of high-dose mPSL might be useful for such cases with severe agranulocytosis that fail to respond to G-CSF administration.

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

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