Malignant clonal evolution from high proportion of monocytes in patients with aplastic anemia: a case report

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Background: Aplastic anemia (AA) is a heterogeneous group of hematopoietic failure diseases, characterized mainly by immune hyperfunction, impaired immune tolerance, the hematopoietic microenvironment, and hematopoietic stem or progenitor cell deficiency. Oligoclonal hematopoiesis and clonal evolution make the disease more complicated, and extremely challenging to diagnose. After immunosuppressive therapy (IST) and granulocyte colony-stimulating factor (G-CSF) treatment, AA patients have a risk of developing acute leukemia.

Case Description: Here we report a patient with a relatively high proportion of monocytes, and all other tests were consistent with severe aplastic anemia (SAA). Monocytes increased rapidly after G-CSF treatment and were eventually diagnosed as hypo-hyperplastic acute monocytic leukemia 7 months later. A high proportion of monocytes may predict malignant clonal evolution in patients with AA. In combination with the literature, we recommend paying close attention to monocytes' elevation in patients with AA for clonal evolution and accurately selecting treatment options.

Conclusions: The proportion of monocytes in the blood and bone marrow of AA patients should be closely monitored. Hematopoietic stem cell transplantation (HSCT) should be performed as early as possible once monocytes continue to increase or are associated with phenotypic abnormalities or genetic mutations. The unique value of this study is that although there were case reports about AA-derived acute leukemia, we suggested that an early high proportion of monocytes may predict malignant clonal evolution in patients with AA.

Keywords: Aplastic anemia (AA); leukemia; clone evolution; granulocyte colony-stimulating factor (G-CSF); case report

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Introduction

Aplastic anemia (AA) is an immune-mediated bone marrow aplasia that is strongly associated with clonal hematopoiesis (1). Some studies have shown that, due to clonal hematopoiesis and evolution, about 5–15% of cases with acquired AA may be susceptible to myelodysplastic syndrome (MDS) and leukemia (2-5). Possible risk factors include patient age, disease severity, long-term use of recombinant human granulocyte colony-stimulating factor (rhG-CSF), and repeated courses of immunosuppressive therapy (IST) treatment (2-5). Here, we report a patient with a relatively high proportion of monocytes, and all other tests are consistent with severe aplastic anemia (SAA). Informed written consent was obtained from the patient. Monocytes increased rapidly with granulocyte colony-stimulating factor (G-CSF) treatment and dropped back to the normal level after stopping G-CSF treatment. Patient was diagnosed with acute monocytic leukemia 7 months later. A high proportion of monocytes in patients

with AA may predict malignant clonal evolution. We present this article in accordance with the CARE reporting checklist (available at: https://sci.amegroups.com/article/view/10.21037/sci-2022-049/rc).

Case presentation

A young male (28 years old) was referred to our hospital for gingival bleeding for 3 months without any history of chronic diseases, such as hypertension, diabetes, or hepatitis. The complete blood count (CBC) at the local hospital showed that the white blood count (WBC) was 2.62×10^9/L, the absolute neutrophil count (ANC) was 0.15×10^9/L, the lymphocyte ratio was 88.5%, the monocyte ratio was 5.1%, the red blood count (RBC) was 1.84×10^9/L, hemoglobin (Hb) was 59 g/L, the platelet count (PLT) was 11×10^9/L, and the reticulocyte (Ret) ratio was 0.7%. A bone marrow aspirate and biopsy showed decreased bone marrow hyperplasia. He was diagnosed with AA and treated with cyclosporine 400 mg and testosterone undecanoate 120 mg per day for 3 months; however, the patient did not respond to this combination regimen.

The physical examination showed an obvious anemic appearance, gingival bleeding, and hemorrhage spots in the skin and mucous membrane. The CBC on admission showed WBC 2.62×10^9/L, ANC 0.09×10^9/L, lymphocyte 86.9%, monocytes 5.1%, Hb 69 g/L, PLT 10×10^9 /L, and the ratio and absolute counts of Ret were 0.21% and 6.3×10^9/L, respectively. The results are approximately in line with the SAA, in which whole blood cells and Ret decreased while lymphocyte ratio increased. The proportion of monocytes was not significantly increased, but it was relatively higher compared with neutrophils. No obvious abnormalities were found in these tests, including urine and stool examination, liver and kidney function, folic acid, vitamin B12, the rheumatism complete set, the extractable nuclear antigen (ENA) antibody profile, the paroxysmal nocturnal hemoglobinuria (PNH) clone, Coomb's test, and DNA quantification of the EB virus and the human cytomegalovirus (HCMV). The lactate dehydrogenase (LDH) was 268 U/L and ferritin was 1,960.8 ng/mL. A chest computed tomography (CT) showed inflammation in the upper lobe of the left lung. A bone marrow aspiration (BMA) of the ilium showed bone marrow hyperplasia was severely reduced, and the ratio of granulocytic cells to erythroid cells was 7. A bone marrow biopsy (BMB) showed a hypoplastic bone marrow with cellularity <10% as well as fatty infiltration, and with a small number of granulocytic cells and erythroid cells scattered or in clusters of distribution, but no megakaryocytes were seen. Lymphocytes and plasma cells were scattered and showed hemosiderin deposits. A flow-cytometry analysis of peripheral blood was negative for PNH.

The proportion of lymphocytes was significantly increased to 86.1%, and the proportion of $CD3^+$ T cell was 77.9%. The ratio of $CD4^+$ T cells to $CD8^+$ T cells was 1.34, which was in the normal range, and the antigen expression of T cells was generally normal. The $CD20^+$ cells accounted for 18.4%, and the $CD56^+$ natural killer (NK) cells accounted for 10.7%, which were within normal ranges. The $CD34^+CD117^+$ early myeloid cells accounted for 0.4%, with normal proportion and phenotype. The percentage of granulocytes decreased significantly to 6.1%, mainly mature granulocytes. The side scatter (SSC) decreased, suggesting decreased cytoplasmic particles. Monocytes were increased to 5.1%, mainly mature monocytes with a normal phenotype. The phenotype of the mildly elevated monocyte was normal, which was not sufficient for the diagnosis of MDS. The fluorescence in situ hybridization (FISH) test showed no cytogenetic abnormalities. Because of bone marrow dysplasia, the chromosome karyotype has no mitotic image, and interphase FISH is required to exclude the common abnormal karyotype in MDS. The next generation sequencing (NGS) showed that the gene for congenital hemopoietic failure was negative and that no gene closely related to leukemia was detected. No gene mutation was found in the myeloma-related mutation gene screening. The *TET2* gene was found to be polymorphic.

The patient was diagnosed with AA and received supportive treatment, such as transfusions of red blood cells and platelets. Due to the lack of suitable donors, this patient did not get the hematopoietic stem cells transplantation. Cyclosporin and G-CSF were used prior to the antithymocyte globulin (ATG) treatment, However, the monocytes increased at a rapid rate, reaching 16.5% and 41.4% at day 1 and 5 days after the administrations of G-CSF, respectively. The increased monocytes were confirmed as mature monocytes by morphology and flow cytometry, but had an abnormal phenotype, expressing CD15, partially expressing CD16, and partially missing human leukocyte antigen-DR (HLA-DR). G-CSF, cyclosporine A (CsA), and all other drugs were immediately discontinued. Before the BMA or BMB examination was done this patient took traditional Chinese herbal medicine for treatment at the local hospital. Six months later, the patient came back for further treatment. The CBC

test results showed WBC 1.1×10^9/L, ANC 1.1×10^9/L, lymphocytes 27%, monocytes 21%, Hb 98 g/L, PLT 13×10^9/L, and blast cells 16%. BMA examination revealed that bone marrow hematopoiesis was severely reduced, with 18% primitive monocytes, 8.5% monocytic blasts, and 26% mature monocytes. Flow cytometry revealed 44.3% of monocytes with an immunophenotype of HLA-DR$^+$, CD33$^+$, CD117$^-$, CD34$^-$, CD4$^+$, CD64^{bright}, CD11b$^+$, CD13$^+$, CD15$^-$, CD56$^+$, CD36$^+$, CD14$^+$. NGS gene examination revealed positive fusion genes of acute myeloid leukemia (AML)1-MDS1/EVII. Acute monocytic leukemia was subsequently diagnosed according to the National Comprehensive Cancer Network (NCCN) guidelines. The daunorubicin, etoposide, and cytarabine (DEA) regimen of chemotherapy was given for induction with no remission. Then decitabine, combined with cytarabine, aclamycin, and G-CSF (CAG), was given for another round of induction chemotherapy. No CBC recovery was observed. Eventually, 6 weeks after chemotherapy, the patient died from a cerebral hemorrhage.

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the editorial office of this journal.

Discussion

Clonality evolution occurs in AA and can be transformed into PNH, MDS, or AML. Many studies have explored and confirmed the mechanism. The persistent "abnormal immune response" leads to hematopoietic failure and dominant clonal screening, making oligoclonal hematopoiesis and clonal evolution inevitable (3,6,7). Its essence is oligoclonal expansion on the basis of hematopoietic failure. In the long-term survival competition, the "survival of the fittest" selects the dominant clones. G-CSF, thrombopoietin (TPO), immunosuppressors, and other factors may accelerate this process and promote the expansion of some clones (2-5). For example, the -7-clone showed dominant amplification after G-CSF, due to the abnormal expression of the G-CSF IV receptor. Several cases of the leukemia transformation from AA were reported in the literature (4,6,8,9).

The bone marrow failure was severe in this patient, with a hematopoietic area of <10%, low hyperplasia, and granulocyte deficiency. The monocytes were relatively high at the beginning, and even increased to 41% after G-CSF treatment, indicating that there was a dominant monocytic clonal evolution or even at the pre-leukemia stage. At this monument, there was a suspicion about the possibility of leukemia. The CsA and G-CSF treatments were stopped immediately. However, the diagnosis was not confirmed due to the patient's voluntary leave for alternative treatment at the local hospital without a BMA or BMB examination for six months. Six months later, when the patient came in for another hospital visit, it was clear that he had a typical monocytic leukemia. If identified early, this patient could have the opportunity to undergo hematopoietic stem cell transplantation (HSCT). Some gene mutations, such as *ASXL1*, *RUNX1*, splicing factor mutations, and others, when detected at 6 months after IST in AA patients may suggest a poor efficacy of IST and a high incidence of malignant clonality evolution and predict the evolution to MDS/AML. By contrast, the predictive value of isolated mutations in genes like *TET2* and *DNMT3A*, which are frequently mutated in age-related clonal hematopoiesis, was lower (9). In this case, most scholars recommend choosing HSCT for treatment.

Another AA patient had been observed in our hospital, whose monocytes increased to 13% after G-CSF application and converted to MDS 4 months later. There was a total of 283 cases of patients with increased monocytes reported with different diseases, including chronic myelomonocytic leukemia (CMML), AML, myeloproliferative neoplasms (MPNs), MDS, and undecided diagnosis, reported in the literature (2,5), in which the incidence of genetic mutations was up to 79%. For patients with increased monocytes with nondiagnostic bone marrow features, the gene mutation rate was as high as 57%. On multivariate analysis, age, ASXL1, CBL, DNMT3A, NRAS, and RUNX1 mutations retained significance. Furthermore, the presence of a mutation was associated with a progressive decrease in hemoglobin/platelet levels and increasing monocyte counts compared with mutation-negative patients (1). The immunophenotype and overall survival (OS) were similar to those of CMML. Hb and PLT decreased, while monocytes continued to increase compared with those without genic mutations (2,5).

The standard chemotherapy treatment scheme according to the type of leukemia is usually selected as the induction of remission treatment for AA-transformed MDS and leukemia patients. However, these patients usually have poor responses to the chemotherapy treatment and a poor prognosis. This is one of the characteristics of these

patients. Once complete remission is achieved, patients should get HSCT as soon as possible due to its poor prognosis. However, a HSCT should be performed if there is any intention of clonal evolution evidence, such as in this case, where the monocytes rapidly increase. Most idiopathic cases of AA appear to be immune-mediated (10,11). However, there is still much to investigate about the mechanism of AA transformation into leukemia.

Conclusions

In conclusion, the proportion of monocytes in the blood and bone marrow of AA patients should be closely monitored. HSCT should be performed as early as possible once monocytes continue to increase or are associated with phenotypic abnormalities or genetic mutations.

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Footnote

Reporting Checklist: The authors have completed the CARE reporting checklist. Available at https://sci.amegroups.com/article/view/10.21037/sci-2022-049/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://sci.amegroups.com/article/view/10.21037/sci-2022-049/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the editorial office of this journal.

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