

First Reported Case of Aplastic Anemia Occurring in a Patient after Acute Promyelocytic Leukemia in Remission

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History of Present Illness

A 32-year-old Hispanic male with a history of acute promyelocytic leukemia (APL) in complete remission presented with increasing fatigue and mild dyspnea. His previous diagnosis of APL was made 2 years ago (Figure 1). Cytogenetic evaluation at the time of diagnosis demonstrated the presence of a reciprocal translocation between chromosomes 15 and 17, t(15;17), and the promyelocytic/retinoic acid receptor (PML/RAR) alpha gene rearrangement by reverse transcriptase polymerase chain reaction (RT-PCR). Complete remission was achieved after induction therapy with all-trans-retinoic acid (ATRA) and idarubicin. Three cycles of consolidation treatment were performed with ATRA, idarubicin, and mitoxantrone, with subsequent maintenance therapy on 6-mercaptopurine, methotrexate, and ATRA. After completing 2 years of maintenance therapy, the patient felt well, had normal blood counts, and peripheral blood RT-PCR was negative for PML/RAR alpha.

Approximately 7 months after completion of chemotherapy, the patient presented with fatigue and shortness of breath. He developed pancytopenia with a white blood cell (WBC) count of $2.1 \times 10^9/L$, hemoglobin of 12.6 g/dL, and platelets of $50 \times 10^9/L$. His physical exam at the time was unremarkable without evidence of splenomegaly. Bone marrow biopsy revealed hypoplasia (Figure 2), PCR of the peripheral blood was negative for PML/RAR alpha, and flow cytometry revealed expression of human leukocyte antigens (HLA)-DR and CD34, providing evidence against APL. There was concern of

an evolving myelodysplastic syndrome or an unusual presentation of APL, as well as other causes of bone marrow failure. However, a repeat bone marrow biopsy confirmed hypoplasia without evidence of promyelocytes or myelodysplasia (Figure 3). CD55 and CD59 were present in normal amounts precluding paroxysmal nocturnal hemoglobinuria (PNH). Nutritional deficiencies were considered but transglutaminase immunoglobulin G (IgG) and IgA levels less than 3 U/mL made suspicion for Celiac disease low, and folate, vitamin B12, homocysteine, methylmalonic acid, zinc, and copper were all within normal limits. Thyroid stimulating hormone and adjusted T4 were within normal limits. Infectious causes were pursued, but parvovirus IgG and IgM and HIV were undetectable by enzyme-linked immunosorbent assay, and hepatitis B and C, Epstein-Barr virus, and cytomegalovirus

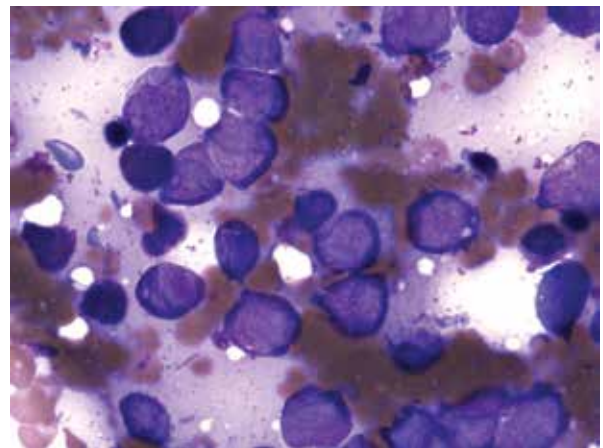


Figure 1. Acute promyelocytic leukemia touch prep of biopsy at 50x.

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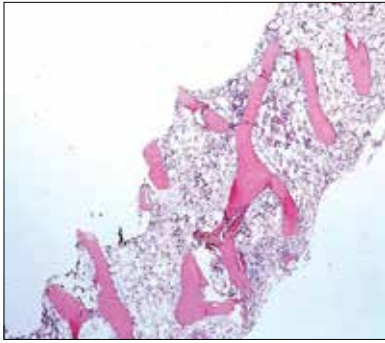


Figure 2. Aplastic anemia core biopsy #1 at 4x.

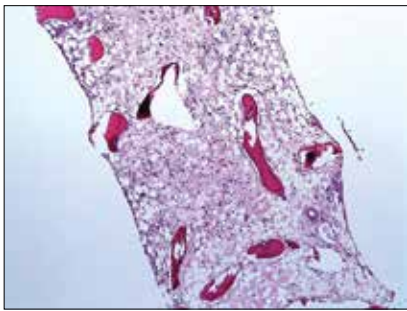


Figure 3. Aplastic anemia core biopsy #2 at 4x.

were negative. No mediastinal/thymic mass was noted. Over a 6-week period, the patient's blood counts continued to drop, and required periodic blood and platelet transfusions. A third bone marrow biopsy continued to demonstrate bone marrow hypoplasia with limited marrow cellularity. WBC count had decreased to $0.7 \times 10^9/L$, hemoglobin had dropped to 7.7 g/dL, and platelets fell to $6 \times 10^9/L$. These findings met the diagnostic criteria for severe aplastic anemia (AA).¹

The patient's only brother was a 10/10 HLA match. Approximately 2 months after the onset of the patient's AA, he underwent a hematopoietic allogeneic stem cell transplant from his sibling. The conditioning regimen incorporated cyclophosphamide, antithymocyte globulin with methotrexate on days 1, 3, 6, and 11 after transplant, and cyclosporine for graft-versus-host-disease prophylaxis.² The initial post-transplant course has been generally uneventful. A complete blood count 183 days after transplant showed a WBC count of $8.8 \times 10^9/L$, with a hemoglobin of 15.1 g/dL and a platelet count of $223 \times 10^9/L$. Bone marrow biopsy on day 92 revealed focal hypoplasia and granulocytopenia, but since the absolute neutrophil count was 4,114/uL on that day, it

was clear that there was adequate granulocyte maturation elsewhere. The biopsy was also negative for PML/RAR alpha on fluorescence in situ hybridization analysis.

Discussion

APL is a type of acute myeloid leukemia (AML) that was classified in the French-American-British classification system as AML-M3.³ In the newer World Health Organization classification, it is classified as APL with $t(15;17)(q22;q12)$.⁴ Symptoms are generally the result of pancytopenia, and may include fatigue, shortness of breath, epistaxis, gingival bleeding, and varying infections.⁵ The molecular genetics of APL are almost always characterized by a reciprocal translocation between the long arms of chromosomes 15 and 17. This translocation results in a fusion gene, PML/RAR-alpha. The RAR-alpha gene is located on chromosome 17 and may impair promyelocyte differentiation and apoptosis.⁶ Morphologically, atypical promyelocytes will have nuclei that appear folded, creased, or dumb-bell shaped. These characteristic morphologic features along with demonstration of the usual $t(15;17)$ or the resulting product, PML/RAR-alpha, is suggestive of APL. This product has a reduced sensitivity to retinoic acid, which prevents maturation of promyelocytes to terminally differentiated neutrophils.⁷ A combination of ATRA, which surmounts this reduced sensitivity, plus cytotoxic chemotherapy, the strategy used in our patient, appears to prolong event-free survival over ATRA or chemotherapy alone.^{8,9}

AA is a clinical syndrome of bone marrow hypocellularity in the setting of peripheral pancytopenia. AA may be inherited or acquired. Inherited syndromes leading to aplastic anemia, such as Fanconi anemia and Diamond-Blackfan anemia, are thought to be due to impaired DNA damage repair mechanisms, telomerase regulation, and ribosomal function. Acquired AA, as in our patient, is thought to be due to immune activation against hematopoiesis. This situation leads to immune-mediated stem cell destruction. Generally, treatment consists of immunosuppressive therapy with anti-thymocyte globulin and cyclosporine, or stem cell transplantation if the patient is young (<40 years old) and has an available matched related donor, which was the case with our patient.¹⁰ AA is a deadly disease if it remains untreated or poorly responsive to therapy due to risks of bleeding and infection. Transplantation offers a 10-year overall survival of 64%.¹¹

Secondary APL occurring after treatment for other malignancies and blood disorders has been well documented.¹² One of the major reasons this is thought to occur is due to the use of topoisomerase II-targeted medications such as anthracyclines. Secondary AML

after AA has also been well recognized.¹³ An association between AA, myelodysplastic syndrome, AML, and PNH has been supported by cellular, cytogenetic, and molecular analyses and their inherent clonality. It has also been theorized that telomere shortening plays a role in the pathogenesis of both myelodysplastic syndrome and AA.

We believe this is the first reported case of severe aplastic anemia occurring after APL, and AML for that matter, as opposed to the well-described evolution of AA to AML. Brodsky and Jones have theorized a relationship between APL, AA, and chronic myeloid leukemia dubbed, the “field leukemogenic effect.” They postulate that a generalized insult to the bone marrow could lead to multiple abnormal cell lines, with one dominant clone and the others remaining present but below the level of detection. Targeted therapy against the dominant disease entity, such as ATRA in APL, would have no activity against the other abnormal clones essentially unmasking them to expand and become detectable.¹⁴ This theory may explain the phenomenon that occurred in our patient.

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Review

Aplastic Anemia Surfacing After Treatment of Acute Promyelocytic Leukemia: the Dameshek Riddle

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Pham and colleagues¹ report on a 32-year-old Hispanic man who underwent treatment for acute promyelocytic leukemia (APL) and developed aplastic anemia (AA) a few months after completion of his APL maintenance program. The patient's induction therapy consisted of all-trans-retinoic-acid (ATRA) and idarubicin. This was followed by 3 cycles of consolidation therapy using ATRA and anthracyclines. Subsequently, he underwent 2 years of maintenance therapy using 6-mercaptopurine, methotrexate, and ATRA. Seven months after completion of maintenance therapy, he presented with pancytopenia without any molecular evidence of APL recurrence. A bone marrow biopsy and aspirate failed to reveal any morphologic evidence of myelodysplasia (MDS). Although cytogenetic findings are not reported, we assume that the patient had normal cytogenetics to allow the authors to appropriately exclude MDS. An exhaustive evaluation ruled out any cause of secondary AA including viral etiologies, paroxysmal nocturnal hemoglobinuria (PNH), nutritional deficiencies, endocrine disorders, or unusual manifestation of APL recurrence. Three bone marrows were performed confirming the diagnosis of AA. The patient subsequently underwent matched sibling allogeneic stem cell transplantation (SCT) with adequate hematologic recovery. A bone marrow biopsy performed at day 92 from SCT confirmed remission.

The introduction of ATRA alongside the exquisite sensitivity of APL cells to anthracyclines has transformed this lethal disease into the most curable acute leukemia in adults.² Tallman and colleagues reported the long-term results of the North American Intergroup study

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that employed maintenance ATRA as an essential part of the treatment program. Disease-free survival advantage, which was maintained with induction and maintenance ATRA, was estimated as 75% at 5 years.³ While some areas of APL treatment remain controversial due to the lack of randomized studies, recent expert consensus suggests that patients should undergo ATRA-based induction followed by ATRA/anthracycline/cytarabine to be followed by maintenance therapy.⁴ Pham and colleagues treated their patient using conventional methods, though they did not use cytarabine as part of their treatment scheme.

AA is a rare life-threatening disease characterized by failure of hematopoiesis, leading to pancytopenia and its inherent complications.⁵ Current management strategies utilize SCT in younger patients with matched donors with or without potent immunosuppressive therapies. These modalities have transformed AA from a uniformly fatal disease to a manageable disease with some patients being long-term survivors.^{6,7}

A subset of AA is considered acquired, affecting younger adults with a second peak at the age of 60 years. Several factors have been implicated in acquired AA such as drugs, benzene exposure, insecticides, and viral etiologies.⁸ The pathophysiology of acquired AA has evolved over the years from an old theory that suggested a direct insult to the hematopoietic stem cells to a novel one that involves an autoimmune basis for the destruction of hematopoietic elements.⁹

Diagnosis of AA is usually made on the basis of peripheral blood cytopenias in the presence of a hypoplastic bone marrow. To affirm an appropriate diagnosis, one must exclude hypoplastic MDS based on presence or lack of cytogenetic abnormalities. MDS patients usually carry chromosomal translocations, whereas AA patients lack such abnormalities. In addition to clonal cytogenetic abnormalities, a higher proportion of CD34 positive cells (>0.3%) is more suggestive of hypoplastic MDS.⁹ Of importance, young adults who have a positive family history might have Fanconi's anemia as a manifestation, and accordingly diepoxybutane or mitomycin tests need to be performed to detect the increased chromosomal breakage seen in this disorder.¹⁰

The case described by Pham and colleagues raises the obvious question as to whether the chemotherapy given as part of the APL treatment program had any causative effect on developing AA, or whether this is a random secondary marrow event occurring in the same unfortunate patient. To our knowledge, there have been no published reports of secondary AA with either 6-mercaptopurine or methotrexate even when these agents were used for other disorders. However, secondary clonal disorders following treatment of APL have been suggested. Latagliata and coworkers reported on 77 consecutive patients with

APL treated with standard induction and consolidation, of whom 6.5% developed MDS, acute myeloid leukemia (AML), or both.¹¹ Lobe and coauthors confirmed that MDS might be a long-term complication from APL therapy, although these authors reported a much lower incidence of 1% in 677 treated patients.¹² Importantly, reported MDS cases usually occur much later than 6 months after stopping APL therapy, and most patients demonstrate complex karyotype. In the case report described, the patient presumably had no evidence of cytogenetic abnormalities, although this was not clearly mentioned in the report. In addition, the severe hypoplasia occurred at 6 months from stopping treatment, arguing against MDS as a possible explanation.

How can we be sure that the patient did not have 2 clones at the time of his initial APL diagnosis, with AA occurring much later in his disease course? Can we attribute AA in his case to APL treatment with certainty? To some extent, this might be the first case of AA after APL therapy; however, there are some caveats and some other possible suggestions. This case could represent the riddle postulated by Dameshek as to the commonality between AA, APL, PNH, and hypoplastic MDS and AML.¹³ In actuality, this case report might validate the hypothesis put forward by Brodsky and colleagues that such clonal disorders represent different manifestations of a general "insult" to the bone marrow.¹⁴ This insult leads to several clones, with one clone dominating while others remain at levels below detection, but not for long. Treatment of the dominant clone leads to the surfacing of the other clones that subsequently manifest themselves. This theory called the "field leukemogenic effect" is similar to the "field cancerization effect" described in solid tumors.^{15,16}

In conclusion, what Dameshek described in his editorial back in 1967 is nicely illustrated in this case of AA surfacing after APL treatment. Another interesting explanation maybe that the AA is a consequence of an ongoing immune reaction against APL—a theory that has been reported by Nissen and associates and demonstrated in both hematologic malignancies and solid tumors.¹⁷ Regardless, this case report is important and of scientific interest. Future case reports would likely shed more light on the pathophysiology of such events.

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