Leukemia Research Reports 3 (2014) 46–48

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Case report

Translocation (8;21) acute myeloid leukemia presenting as severe aplastic anemia

Enkhtsetseg Purev*, Bogdan Dumitriu, Christopher S. Hourigan, Neal S. Young, Danielle M. Townsley

National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20814, United States

ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form
31 March 2014
Accepted 13 April 2014
Available online 11 June 2014

Keywords:
t(8;21)
Aplastic anemia
Acute leukemia
Comparative genomic hybridization

ABSTRACT

We report a case of t(8;21) acute myeloid leukemia presenting as severe aplastic anemia. While initial bone marrow biopsy lacked any cytogenetic abnormalities in 20 analyzed metaphases, repeat bone marrow biopsy eight days later demonstrated this translocation. Initial cytogenetic analysis of 20 metaphases was therefore insufficient to make the diagnosis of hypocellular acute myeloid leukemia. We discuss that further complementary molecular tests, such as CGH, would likely provide a more robust diagnosis of hematopoietic diseases.

1. Introduction

Aplastic anemia (AA) is a rare disease characterized by pancytopenia and a hypocellular bone marrow. Specific cytogenetic abnormalities, monosomy 7 and trisomy 8, are frequently associated with clonal evolution [1]. Clonal cytogenetic abnormalities also may be present with hypocellular bone marrow morphology: some experts have used them to differentiate AA from hypoplastic myelodysplastic syndrome (MDS) [2], while others accept certain aberrations as consistent with AA [3]. Although cryptic *RUNX1/AML1* lesions have been reported in patients with Fanconi anemia and MDS [4], the (8;21) translocation is not observed by standard cytogenetic methods in bone marrow failure diseases.

2. Case report

A 23-year-old Ecuadorian homemaker, resident in the United States, presented to medical attention with a peritonsillar abscess. Laboratory studies at presentation showed white blood cells 1.38 k/μL, absolute neutrophil count 0 k/μL, hemoglobin 7.4 g/dL, absolute reticulocyte count 5 k/μL, and platelets 38 k/μL. Initial bone marrow biopsy revealed 5% cellularity with minimal trilineage hematopoiesis. Cytogenetics showed normal female karyotype 46,XX in all 20 analyzed metaphases. She was referred to the National Institutes of Health (NIH) for evaluation and consideration for immunosuppressive therapy (IST) and eltrombopag on a clinical trial (NCT01623167). There was no evidence of an inherited bone marrow failure disorder on physical examination or from a detailed family history. Tests for Fanconi anemia (diepoxybutane stress) and telomere disorders (leukocyte telomere length) were normal. Flow cytometric evaluation for paroxysmal nocturnal hemoglobinuria (PNH) was negative. Repeat bone marrow analysis performed immediately prior to IST (as required by protocol) demonstrated 10% cellularity, no dysplasia, nor increased number of blasts. Less than 1% of cells were CD34 positive. Severity of neutropenia (ANC < 200/μL) prompted immediate treatment on protocol, beginning with equine antithymocyte globulin (ATG) and cyclosporine, standard for severe AA. However, two days after completion of IST, standard cytogenetic analysis returned t(8;21)(q22;q22) in 3 out of 20 metaphases, with confirmation by FISH (Fig. 1A). The patient was placed "off protocol" and did not receive the study drug, eltrombopag. This significant cytogenetic abnormality was unexpected and was inconsistent with the marrow morphology. Three months after initial presentation at NIH, confirmatory bone marrow evaluation showed 2/2 metaphases with t(8;21) abnormality. Focally increased CD34+ cells were observed by IHC (Fig. 1B, C) but not by flow cytometry. Her clinical status was unchanged after receiving IST: she remained severely pancytopenic and transfusion-dependent. Chemotherapy for AML was withheld due to the severe pancytopenia, hypocellularity, and

* Correspondence to: National Heart Lung and Blood Institute, National Institutes of Health, 10 Center drive, Rm 4-5140, Bethesda, MD 20814, United States. Tel.: +1 301 335 4085; fax: +1 301 594 1290.
E-mail address: pureve@mail.nh.gov (E. Purev).

http://dx.doi.org/10.1016/j.lrr.2014.04.002

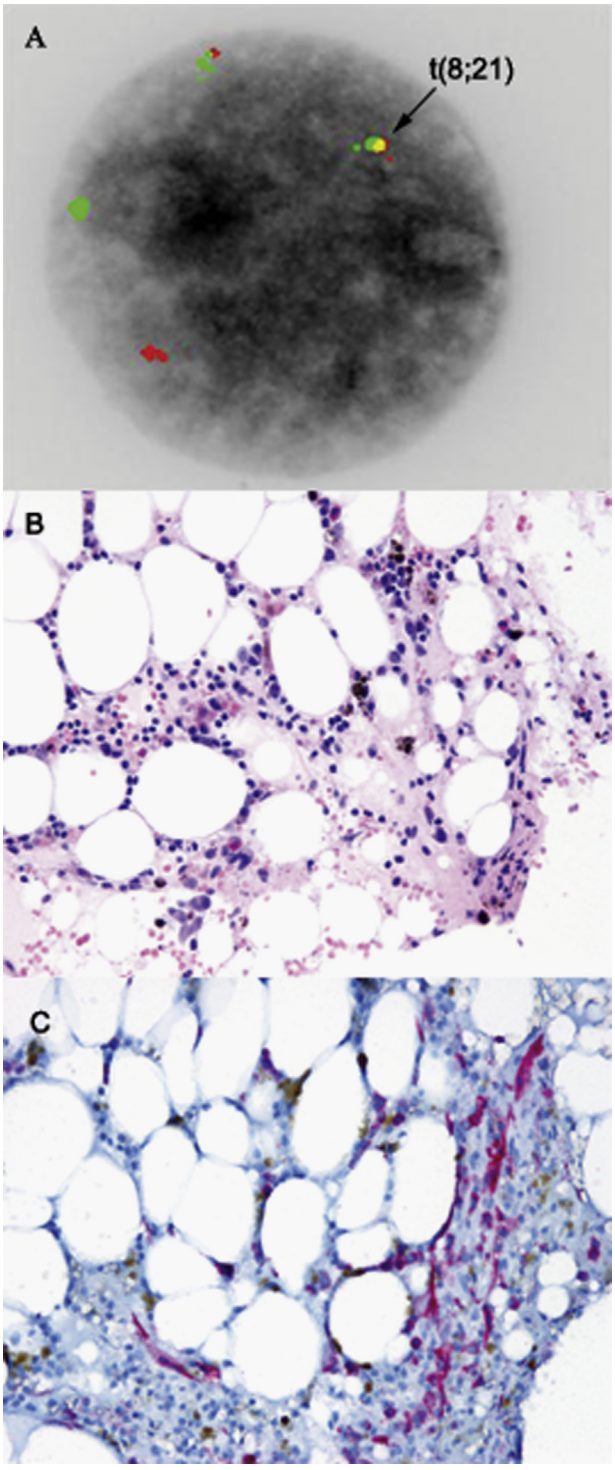

Fig. 1. (A) Bone marrow aspirate was analyzed in co-hybridization experiment of *RUNX1T1* (*ETO*; 8q22) and *RUNX1* (*AML1*; 21q22.1). (B) H+E staining of bone marrow at 200× magnification. (C) Representative IHC demonstrating cluster of CD34+ cells.

absence of increased blasts. Progression to frank leukemia with circulating blasts did not occur until *eight* months following initial presentation. She underwent allogeneic stem cell transplantation from a matched sibling donor. She had minimal grade chronic GVHD of the skin, that was successfully treated with steroids. Eight months after successful bone marrow transplant, the patient is in remission.

Table 1
Association of LOH detected in patient and reported gens involved in evolution of mutations in AML.

Chromosome LOH	Genes associated with clonal evolution
2 (q24.2)	*TTN*
3 (p21.2)	*FOXP1*
7 (q34)	*MLL3, CUL1, EZH2*
12 (q23.2)	*PTPN11*
16 (p11.2)	*TP53TG3b, TP53TG3*

The core binding factor leukemia involving translocation of 8;21 (q22;q22), generating a fusion of *RUNX1* and *AML1* genes, is considered leukemia-defining, regardless of the number of blasts [5]. This unusual case prompted us to perform comparative genomic hybridization (CGH). Single nucleotide polymorphism (SNP) based CytoScan high-density microarray [6] was performed on DNA derived from the patient's bone marrow cells. Sample obtained at NIH at initial diagnosis had no identifiable gains or losses of more than 100,000 bp. However, multiple, large regions of copy neutral loss of heterozygosity (LOH, also referred to as uniparental disomy) were identified, ranging in size from 3 to 29 Mbp on multiple chromosomes. CGH did not demonstrate any large regions of copy neutral LOH in 10 patients with acquired severe AA with normal cytogenetics, nor in 35 healthy controls. Emerging data show that SNP arrays can detect abundant copy neutral LOH amongst select hematologic malignancies and are associated with the duplication of oncogenic mutations [7]. Addition of SNP arrays to standard cytogenetics increases the ability to identify a clonal marker from 50% to 80% [6]. When we compared locations of LOH in our patient with reported genes involved in evolution of mutations in AML we found several important overlaps (Table 1). These results suggest SNP based CGH arrays may be useful in distinguishing hypocellular AML from AA.

3. Discussion

To our knowledge, we report for the first time t(8;21) acute myeloid leukemia presenting as severe aplastic anemia. t(8;21) (q22;q22) is present in 5–10% of all AMLs, and includes variety of subtypes with varying pathology, and clinical presentations, ranging from circulating blast in peripheral blood to absent blasts forms [5,8,9]. However, it is important to note that t(8;21) is not associated with bone marrow failure disorders. Our patient developed frank leukemia *eight* months following diagnosis; however, one may have predicted leukemia at the time of diagnosis of AA. While initial bone marrow biopsy lacked any cytogenetic abnormalities in 20 analyzed metaphases, repeat bone marrow biopsy eight days later demonstrated t(8;21) in 3 out of 20 metaphases. In this regard, analyzing 20 metaphases was not sufficient. Further complementary molecular tests, such as CGH, would likely provide a more robust diagnosis of hematopoietic diseases.

Cytopenias are common in AML for unclear reasons. One accepted mechanism is physical replacement of normal hematopoietic stem cells (HSC) by myeloblasts, although blood counts may be affected in the absence of marrow morphology showing abundant blasts. Recently, Miraki-Moud et al. reported that leukemic cells inhibited hematopoiesis by producing cytokines that suppress differentiation of normal HSC and possibly reducing cycling of HSC [10]. Indeed, our patient showed severe bone marrow failure in the absence of myeloblasts. More sensitive assessment of chromosome aberrations likely will reveal similar cases.

Conflict of interest

We have no conflicts of interest to disclose.

Acknowledgments

This work was supported by the Intramural Research Program of the National Heart, Lung, and Blood Institute of the National Institutes of Health (grant no. NCT01623167).

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