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Genomic imbalances in peripheral blood confirm the diagnosis of myelodysplastic syndrome in a patient presenting with non-immune hemolytic anemia

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ABSTRACT

Myelodysplastic syndrome (MDS) is a clonal stem-cell disorder characterized by dyshematopoiesis. We report a patient who presented with cytopenias and microangiopathic hemolytic anemia. Chromosome microarray analysis (CMA), using single nucleotide polymorphism arrays, on peripheral blood revealed genomic imbalances indicative of MDS, which was confirmed by bone marrow examination. This report highlights the importance of suspecting MDS in patients with cytopenias and microangiopathic hemolytic anemia. CMA of peripheral blood may assist in the preliminary diagnosis of MDS, representing a comparatively less invasive diagnostic procedure and may aid bone marrow evaluation when an aspirate sample is insufficient for conventional cytogenetic analysis.

Introduction

Myelodysplastic syndrome (MDS) is an acquired, clonal stem-cell disorder characterized by dyshematopoiesis and stem-cell dysplasia of single or multiple blood cell lineages. MDS leads to varying degrees of cytopenias and, paradoxically, hypercellular bone marrow with potential evolution to acute myeloid leukemia (AML) or marrow failure [1–3]. As a result of dyserythropoiesis, red blood cell (RBC) morphological abnormalities, such as anisocytosis, poikilocytosis, macrocytosis and sometimes elliptocytosis, are often observed in MDS [1,2]. Schistocytes refer to fragmented RBCs and are commonly associated with causes of microangiopathic hemolytic anemia (MAHA). However, schistocytosis with a high reticulocyte count in the peripheral blood smear is a rare and unusual manifestation of MDS [1].

Karyotypic analysis is commonly used in the diagnostic work up of various hematological diseases. Some chromosome abnormalities are specifically associated with certain diseases or disease subtypes [4,5]. The detection of various chromosomal alterations not only assists in diagnosis, but also helps in the prognostication of patients [6]. However, due to technical limitations, such as the need for sufficient number of dividing malignant cells and the limited resolution of light microscopy, karyotypic analysis is able to detect such abnormalities in only about 50% of MDS cases [7].

Whole genome scanning technologies, such as chromosomal microarray analysis (CMA), using single nucleotide polymorphism (SNP) arrays, do not require dividing cells, have a much greater resolution than karyotypic analysis, and allow for detection of loss of heterozygosity (LOH) as well as changes in genomic copy number [7]. SNP-based CMAs also permit the detection of acquired copy-neutral LOH (cn-LOH), which is common in hematological malignancies [8], but undetectable by conventional cytogenetics [7,9].

Here we describe severe anemia and marked schistocytosis as a manifestation of MDS in an elderly patient admitted at our cancer center. Initial bone marrow examination was inadequate for detailed histopathological evaluation and cytogenetics. However, application of SNP-based CMA on peripheral blood uncovered genomic imbalances indicative of MDS, which was confirmed upon a subsequent bone marrow examination.

Materials and methods

CMA was performed essentially as described in detail elsewhere [10]. Briefly, total genomic DNA from blood was digested with *Nsp*I restriction enzyme, ligated with an adaptor complimentary to polymerase chain reaction (PCR) primer, PCR

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amplified, purified using magnetic beads, fragmented, biotin labeled, and hybridized to an Affymetrix CytoScan HD array. The hybridized array was washed and scanned with a GeneChip Scanner 3000 7G. Intensities of probe hybridization were analyzed using Affymetrix GeneChip Command Console, and copy number and genotyping analyses were performed using Affymetrix Chromosome Analysis Suite software.

Case summary and results

A 70-year-old woman was admitted with a 2-month history of exertional dyspnea associated with weakness and fatigue, decreased appetite and weight loss. Her complaints had been worsening during the previous 2 weeks, and she had also noticed purple lesions on both knees at that time. She denied any history of hematemesis, hemoptysis, melena, hematochezia or hematuria.

Past medical history was significant for right-sided triple negative breast cancer, treated by lumpectomy followed by local radiation therapy ~11 years prior to this encounter. She also had a history of stage 1A uterine cancer, for which she underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy, followed by 3 cycles of chemotherapy with carboplatin and paclitaxel with radiation therapy to the pelvis 3 years prior to this visit.

Physical examination showed no apparent distress. Other than conjunctival pallor and palpable purpuric lesions on both knees, no bruises or petechiae were noted on any other part of her body. The rest of the systemic examinations were unremarkable.

Blood work on presentation showed pancytopenia (hemoglobin: 6.2 g/dL, leukocytes: 3.5×10^9/L and platelets: 57×10^9/L) with reticulocyte count of 10.15% and hyperbilirubinemia with significant schistocytosis, elliptocytosis, tear drop cells and basophilic stippling, abnormal immature myeloid cells, hyposegmented hypogranular neutrophils and abnormal nucleated RBCs (Fig. 1A), pointing towards hemolysis. A work up for hemolytic anemia was ordered. Her blood counts had continued to fall. Her coagulation work up was normal. Direct and indirect Coombs tests were negative. Bilirubin was increased to 3.7 mg/dL, with normal direct bilirubin and normal liver enzymes. LDH 683 U/L, haptoglobin was < 10 mg/dL, and her ESR was 100 mm. Serum folate, B12, iron and total iron binding capacity were within normal limits. Erythropoietin was elevated: 49.9 mU/mL. Chest and abdominal CT of lungs and liver ruled out metastasis from previous malignancies that could account for shortness of breath and elevated bilirubin levels. Paroxysmal nocturnal haemoglobinuria was also excluded based on normal CD55 and CD59 expression on flow cytometry.

Initial bone marrow biopsy was inadequate in size for detailed examination and had severe crush artifact. The preserved focus of marrow showed hypercellularity with large, multinucleated, dysplastic megakaryocytes and megaloblastoid changes in erythroid series (Fig. 1B and C). There was no increase in reticulin fiber content in the marrow biopsy, making a myelophthisic process as the underlying cause of schistocytosis unlikely. Blood vessels were not appreciated in the bone marrow biopsy due to severe crush artifact. A skin biopsy showed deep subcutaneous hemorrhage and no evidence of microangiopathic changes. The marrow aspirate was also insufficient for further analysis by flow cytometry or cytogenetics. CMA of peripheral blood revealed multiple genomic imbalances in a mosaic state, including losses of portions of chromosome arms 5q, 7q and 13q, isodisomy of 17p, and gains of chromosomes/chromosomal segments 1p, 5p, 6, 8, 19, 20p, 21 (Fig. 2), findings consistent with the diagnosis of MDS. Conventional cytogenetic analysis performed on a repeat bone marrow sample revealed an abnormal karyotype: 47-50,X,-X,der(?)t(?;1)

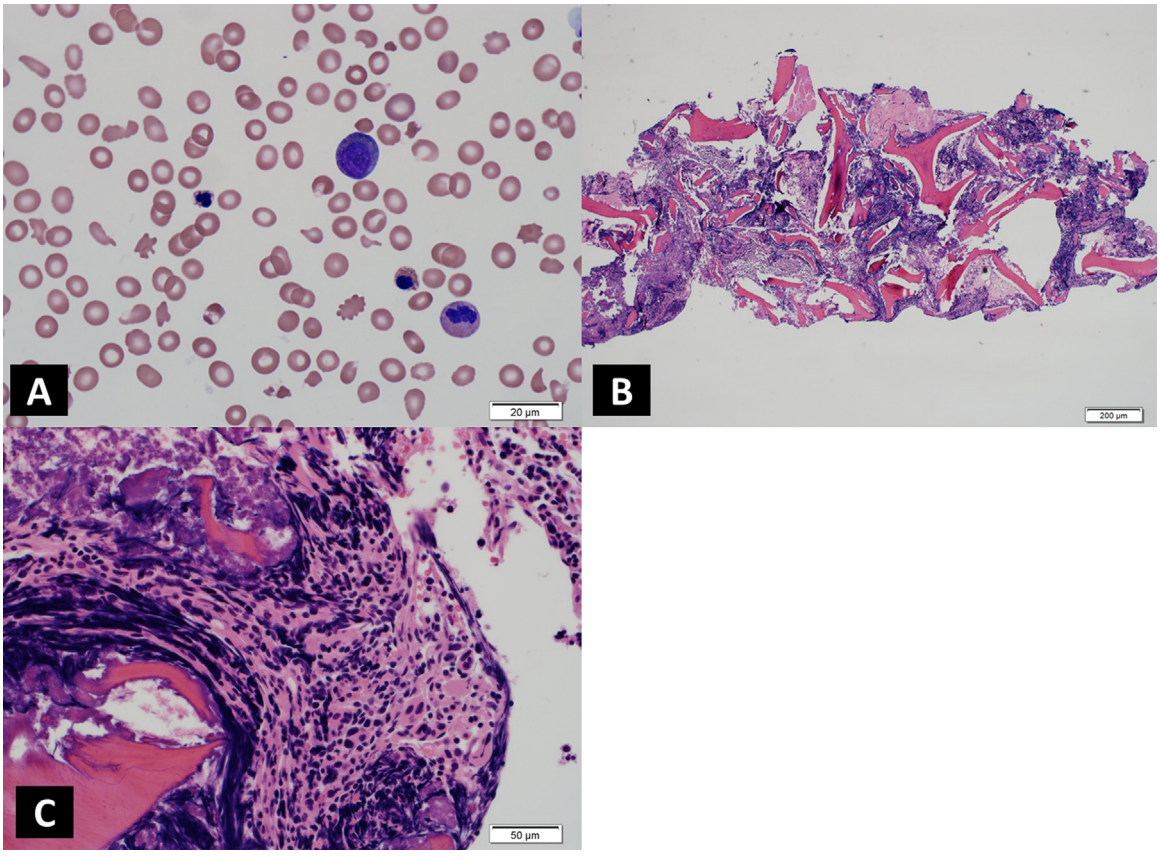

Fig. 1. A) Peripheral blood film showing marked schistocytosis, anisocytosis and nucleated red blood cells as well as myelocytes and dysplastic neutrophils. B) Bone marrow biopsy with severe crush artifacts. C) Bone marrow biopsy with severe crush artifacts showing cluster of dysplastic megakaryocytes.