Utility of peripheral blood for cytogenetic and mutation analysis in myelodysplastic syndrome

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Key Points
- There is 100% concordance in the cytogenetic and mutation profile between PB and BM in myelodysplastic syndrome.

Introduction

The myelodysplastic syndromes (MDSs) are clonal disorders of hematopoiesis that occur predominantly in the elderly (median age 72 years) and are characterized by morphologic dysplasia, ineffective hematopoiesis, peripheral blood (PB) cytopenias, chromosomal aberrations, and propensity to myeloid leukemic transformation. The advent of high-throughput and high-resolution techniques for genetic analysis has shown that more than 80% of MDS patients harbor somatic mutations and/or genomic aberrations in their bone marrow (BM), which provide pathogenetic as well as diagnostic and prognostic insights into this disease.1-4 Frequent BM aspirates may be required for morphological5 and genetic assessment, especially after BM transplant. In addition, in a significant patient proportion, the BM is hypocellular (10% to 15%)6 and/or fibrotic (17%),7 making the aspiration procedure painful and uncomfortable, especially in the elderly. In MDS the molecular analysis of copy number changes and genetic mutations has been done primarily on BM-derived DNA. Recent studies have shown that more than 80% of bone marrow (BM) samples from patients with myelodysplastic syndrome (MDS) harbor somatic mutations and/or genomic aberrations, which are of diagnostic and prognostic importance. We investigated the potential use of peripheral blood (PB) and serum to identify and monitor BM-derived genetic markers using high-resolution single nucleotide polymorphism array (SNP-A) karyotyping and parallel sequencing of 22 genes frequently mutated in MDS. This pilot study showed a 100% SNP-A karyotype concordance and a 97% mutation concordance between the BM and PB. In contrast, mutation analysis using Sanger sequencing of PB and serum-derived DNA showed only 65% and 42% concordance to BM, respectively. Our results show the potential utility of PB as a surrogate for BM for MDS patients, thus avoiding the need for repeated BM aspirates particularly in elderly patients and those with fibrotic or hypocellular marrows. (Blood. 2013;122(4):567-570)

Study design

Genomic DNA from PB and BM was extracted (Qiagen) from frozen cell pellets and 100 ng was whole genome amplified (WGA; Qiagen), both per manufacturer’s protocols. Serum DNA was purified from 200 μL of serum using a modified sodium iodide/Triton-based lysis followed by isopropanol precipitation as described.12 Affymetrix SNP 6.0 array (SNP-A) karyotyping and 454-PS of all exons of DNMT3a, RUNX1, CEBPa, TP53, EZH2, and ZRSR2 and mutation “hot spots” for NPM1, FLT3, ASXL1, IDH1, IDH2, MPL, JAK2, BRAF, cCBL, NRAS, KRAS, C-KIT, SF3B1, SRSF2, and U2AF35 were performed and analyzed as previously described.13,14 TET2 was analyzed using Sanger sequencing. Independent validation for all mutations was performed using Sanger sequencing of unamplified genomic DNA. Polymerase chain reaction (PCR) conditions for serum were identical to those for PB; however, a second 10-cycle PCR reaction using nested primers (US1–GTAGTGCGATGGCCAGT, US2–CAGTGTCGACCGATGAC) was required to provide adequate amplification yield for Sanger sequencing. The study was approved by the local research ethics committee under project 0033 and conducted in accordance with the Declaration of Helsinki.

Results and discussion

Karyotype analysis

Karyotype aberrations were assessed using SNP-A on PB samples from 31 MDS patients, from whom metaphase cytogenetics (MC) and BM SNP-A karyotypes were available. These consisted of the

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following: normal karyotype (n = 9), del5q (n = 9), del7q/-7 (n = 5), trisomy 8 (n = 2), complex (n = 4), isodisomy 13 (n = 1), and t(2;4)(q33;q27) (n = 1).

Concurrent BM and PB samples were available for 22 patients; nonconcurrent PB samples, median of 364 (14 to 1379) days after initial BM aspirate, were available for the remaining 9 patients. SNP-A karyotyping showed 100% concordance between BM and PB karyotype, except for 1 sample from a patient (USN1902) with a complex karyotype comprising 30 chromosomal aberrations in the BM, of which only 15 could be detected in the PB, suggesting the presence of multiple mosaic clones (Figure 1A; supplemental Table 1, available on the web site). 

In conclusion, our study showed excellent concordance both for SNP-A karyotype and mutational analyses between BM and PB, albeit with a lower clonal burden in PB, using FISH, SNP-A, and 454-PS that was independent of the differential PB profile of the patient cohort (supplemental Table 2). Patient serum is not recommended for mutation detection. The detection of regions of UPD in both PB and BM provides additional prognostic information because UPD on chromosome 7q has been associated with more aggressive clinical behavior in MDS and UPD on 5q may identify patients with potential response to lenalidomide. 

To further study the utility of these procedures, we isolated DNA from serum of 12 patients with a total of 19 mutations in their BM. Analysis using electrophoresis and DNAOK! reagent (Web Scientific) showed highly fragmented serum-derived DNA unsuitable for SNP-A analysis. Sanger sequencing correctly identified only 8 mutations with no preference for any of the genes (42% concordance); 6 mutations were identified as wild type and 5 samples failed to amplify (Table 1).

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Our results show the feasibility of using PB for initial genetic diagnostic screening of patients with MDS and sequential monitoring of
disease clones following treatment, thus forming a prelude for validation in larger studies.

Acknowledgments

We thank Rajani Chelliah for assisting with sample processing and tissue separation.

We acknowledge Leukaemia Lymphoma Research (UK)/British Society for Haematology for supporting A.K. and King’s College London for funding the King’s College Haemato-Oncology Tissue Bank, from which all local samples were processed.

Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.

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