Diagnostic and therapeutic implications of genetic heterogeneity in myeloid neoplasms uncovered by comprehensive mutational analysis

Sarah M. Choi\textsuperscript{a}, Ben Goldenson\textsuperscript{b}, Lo Ann Peterson\textsuperscript{c}, Shira Dinner\textsuperscript{d}, Brady L. Stein\textsuperscript{d}, Amir Behdad\textsuperscript{c,⁎}

\textsuperscript{a} Department of Pathology, University of Michigan, Ann Arbor, MI, USA
\textsuperscript{b} Feinberg School of Medicine, Northwestern University, Chicago, IL, USA
\textsuperscript{c} Department of Pathology, Northwestern University, Chicago, IL, USA
\textsuperscript{d} Department of Medicine/Division of Hematology/Oncology, Northwestern University, Chicago, IL, USA

A R T I C L E   I N F O

Keywords:
BCR-ABL1
JAK2
Myeloproliferative neoplasm
CML

A B S T R A C T

While growing use of comprehensive mutational analysis has led to the discovery of innumerable genetic alterations associated with various myeloid neoplasms, the under-recognized phenomenon of genetic heterogeneity within such neoplasms creates a potential for diagnostic confusion. Here, we describe two cases where expanded mutational testing led to amendment of an initial diagnosis of chronic myelogenous leukemia with subsequent altered treatment of each patient. We demonstrate the power of comprehensive testing in ensuring appropriate classification of genetically heterogeneous neoplasms, and emphasize thoughtful analysis of molecular and genetic data as an essential component of diagnosis and management.

Through the expanding usage of high throughput sequencing analysis combined with traditional cytogenetic and molecular techniques, myeloid neoplasms are becoming increasingly defined and characterized by the genetic alterations they harbor. In some instances, such as with BCR-ABL1, t(9;22) in chronic myelogenous leukemia (CML), the genetic alterations are considered to drive and define the disease.

However, we are now beginning to realize that the genetic landscape, even within a single disease entity, can be quite complex and continues to evolve as a disease progresses, with ensuing diagnostic and therapeutic implications [1,2].

Mutations of genes such as JAK2, CALR and BCR-ABL1 are considered drivers (i.e. causative mutations which drive oncogenesis and often provide a growth advantage to cells harboring them) in the pathogenesis of myeloproliferative neoplasms (MPN) and have generally been thought to be mutually exclusive. However, there are rare reports of cases with co-occurring BCR-ABL1, JAK2 and/or CALR mutations, suggesting a more complex picture than previously thought [3–5]. The spectrum of mutations and the order in which they are acquired can influence the character and nature of disease progression [6]. Here, we report two cases of myeloid neoplasms that exhibit genetic heterogeneity, highlighting the potential for diagnostic misinterpretation in the face of multiple disease-associated/defining mutations and emphasizing the need for thoughtful, and comprehensive genetic analysis of these neoplasms.

Patient 1. A 74-year-old woman presented in 2001 with a platelet count of 834,000/µL, normal white blood cell count (WBC), and normal hemoglobin. She underwent observation, and her platelet count continued to increase. In 2006, her WBC was 21,000/µL. Molecular testing for JAK2 (V617F) was negative. Her first bone marrow biopsy in 2013 showed a hypercellular marrow, with granulocytic hyperplasia, increased megakaryocytes with hyperchromatic forms and marked reticulin fibrosis (Fig. 1A,B). A qualitative BCR-ABL1 molecular assay was positive for a p190 transcript. Fluorescent in situ hybridization (FISH) and karyotype were negative for t(9;22). A diagnosis of CML was considered. Imatinib and subsequent dasatinib therapies were ineffective with no hematologic response. In 2015, the patient presented with a left breast mass, a biopsy of which showed myeloid sarcoma. FISH was positive for an 11q23 KMT2A(MLL) translocation. The bone marrow demonstrated acute myeloid leukemia in a background of extensive fibrosis (Fig. 1C). Genetic analysis of the bone marrow was negative for BCR-ABL1 p190 and p210 and KMT2A(MLL) translocation, but was positive for both IDH2 and CALR mutations. The patient received 7 + 3 induction chemotherapy with idarubicin, followed by decitabine and radiation therapy without a response. Her peripheral blood blast count continued to rise, and she expired 4 months after the diagnosis of AML.
Retrospective molecular testing of the 2006 blood sample was positive for the CALR mutation, but wild type for IDH2. The initial 2013 bone marrow was positive for BCR-ABL1, CALR and IDH2 mutations. Examination of the mutant allele frequencies over time suggests that the CALR was the earliest genetic event and the founder mutation of patient’s MPN (Fig. 1D). The transiently positive BCR-ABL1 p190, initially interpreted as evidence of CML, may have arisen in a non-dominant clone and/or one that disappeared with tyrosine kinase inhibitor therapy.

Patient 2. A 68-year-old male with presented with two year history of chronic phase CML on dasatinib. Review of his diagnostic blood and bone marrow biopsy material showed some unusual features for CML, including absolute monocytosis, lack of basophilia, and dysplastic neutrophils. The bone marrow demonstrated a predominance of myeloid progenitors and prevalent hypolobated megakaryocytes. However, occasional dysplastic megakaryocytes with nuclear separation that are often present in myelodysplastic syndromes (MDS) were also seen (Fig. 2). Molecular studies reportedly showed the presence of both a BCR-ABL1 p210 fusion transcript and JAK2 (p. V617F) mutation. FISH confirmed t(9;22), but cytogenetic analysis reportedly showed normal karyotype. A diagnosis of MDS/MPN was favored. Quantitative BCR-ABL1 using PCR-based methods showed 2 copies of BCR-ABL1 per 1000 copies of ABL1 (0.22% international scale). A targeted next generation sequencing analysis for the frequently mutated genes in myeloid neoplasms demonstrated JAK2 (p. V617F) mutation with low allele frequency (4%); and a SRSF2 (p. P95H) mutation with high mutant allele frequency (37%). SRSF2 mutation has recently been described in chronic myelomonocytic leukemia (CMML), MDS, and less frequently in primary myelofibrosis [7,8]. This myeloid neoplasm demonstrated a heterogeneous mixture of mutation, among which SRSF2 had the highest mutant allele frequency, possibly representing the initiating event.

Because of the retrospective nature of this analysis, some of the historic material was not available from each patient for further testing. However, a few aspects of these cases are worth noting. The initial diagnosis of CML was understandably made in both patients based on the presence of a BCR-ABL1 fusion. In the first case, it could be argued that the level of transcript was quite low, as it was detected by PCR methods only and not FISH or karyotype. BCR-ABL1 fusion in the second case was detected by FISH but not karyotype. However, this case had clinical and pathologic features that are not typical for CML, including the presence of dysplasia, monocytosis, and absence of basophilia. Detection of SRSF2 mutation, while not definitive, would further support a diagnosis of MDS/MPN in this case, were it not for the BCR-ABL1 fusion. Even the most recent 2016 update WHO classification [9] has not changed the diagnostic criteria for CML, MDS/MPN, or MPNs with regard to inclusion and exclusion of BCR-ABL1 fusion. Given that we know, however, that low levels of BCR-ABL1 transcript can be present in the blood of normal individuals [10], it may be worth re-examining whether cases that have unusual morphologic features for CML or mutational profiles more commonly associated with other neoplasms should be more appropriately classified as a non-CML entity. On a biologic level, it is also unclear in the current cases whether the genetic abnormalities are generally present within the same clonal populations as has been described [3-5] or separate ones exhibiting different mutations. Regardless of the initial distribution, the proportions of each mutation would theoretically be able to shift over time and/or with therapy, possibly also influenced by acquisition of additional mutations, such IDH2 in the first case. Given this possibility, the benefit of traditional CML therapies, including tyrosine kinase.
inhibitors, as well as combined therapy with more recently studied treatments for MPNs, such as ruxolitinib, needs to be further investigated.

These cases provide examples of genetic heterogeneity that challenge our assumptions regarding exclusivity of driver mutations in myeloid neoplasm pathogenesis. It also highlights an important diagnostic pitfall: that BCR-ABL1 positivity does not necessarily equate a diagnosis of CML and careful, contextual interpretation of molecular testing results is required. Lastly, these findings emphasize the need for prospective, comprehensive molecular testing in myeloid neoplasms that can potentially impact the diagnosis and therapy of patients with these diseases.

References

[1] L. Ding, T.J. Ley, D.E. Larson, C.A. Miller, D.C. Koboldt, J.S. Welch, J.K. Ritchey, M.A. Young, T. Lamprecht, M.D. McLellan, J.F. McMichael, J.W. Wallis, C. Lu, D. Shen, C.C. Harris, D.J. Dooling, R.S. Fulton, L.L. Fulton, K. Chen, H. Schmidt, J. Kalicki-Veizer, V.J. Magrini, L. Cook, S.D. McGrath, T.L. Vickery, M.C. Wendl, S. Heath, M.A. Watson, D.C. Link, M.H. Tomason, W.D. Shannon, J.E. Payton, S. Kulkarni, P. Westervelt, M.J. Walter, T.A. Graubert, E.R. Mardis, R.K. Wilson, J.F. DiPersio, Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing, Nature 481 (7382) (2012) 506–510.

[2] M.J. Walter, D. Shen, L. Ding, J. Shao, D.C. Koboldt, K. Chen, D.E. Larson, M.D. McLellan, D. Dooling, R. Abbott, R. Fulton, V. Magrini, H. Schmidt, J. Kalicki-Veizer, M. O’Laughlin, X. Fan, M. Grillot, S. Witowski, S. Heath, J.L. Frater, W. Eades, M. Tomason, P. Westervelt, J.F. DiPersio, D.C. Link, E.R. Mardis, T.J. Ley, R.K. Wilson, T.A. Graubert, Clonal architecture of secondary acute myeloid leukemia, New Engl. J. Med. 366 (12) (2012) 1090–1098.

[3] M. Bornhauser, B. Mohr, U. Oelschlaegel, P. Bornhauser, S. Jacki, G. Ehninger, C. Thiede, Concurrent JAK2(V617F) mutation and BCR-ABL translocation within committed myeloid progenitors in myelofibrosis, Leukemia 21 (8) (2007) 1824–1826.

[4] X. Cabagnols, J.M. Cayuela, V. Vainchenker, A CALR mutation preceding BCR-ABL1 in an atypical myeloproliferative neoplasm, New Engl. J. Med. 372 (7) (2015) 688–690.

[5] S. Loghavi, N. Pemmaraju, R. Kanagal-Shamanna, M. Mehrotra, L.J. Medeiros, R. Luthra, P. Lin, Y. Hub, H.M. Kantarjian, J.E. Cortes, S. Verstovsek, K.P. Patel, Insights from response to tyrosine kinase inhibitor therapy in a rare myeloproliferative neoplasm with CALR mutation and BCR-ABL1, Blood 125 (21) (2015) 3360–3363.

[6] C.A. Ortmann, D.G. Kent, J. Nongalia, Y. Silber, D.C. Wedge, J. Grinfeld, E.J. Baxter, C.E. Massie, E. Papaemmanual, S. Menon, A.L. Godfrey, D. Dimitropoulos, P. Guglielmelli, B. Bellosillo, C. Besses, K. Dohner, C.N. Harrison, G.S. Vassiliou, A. Vannucci, P.J. Campbell, A.R. Green, Effect of mutation order on myeloproliferative neoplasms, New Engl. J. Med. 372 (7) (2015) 601–612.

[7] M. Meggendorfer, A. Roller, T. Häfnerlach, C. Eder, F. Dicker, V. Grossmann, A. Kohlmann, T. Alpermann, K. Yoshiha, S. Ogawa, H.P. Koeffler, W. Kern, C. Häfnerlach, S. Schnittger, SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CML-M0), Blood 120 (15) (2012) 3080–3088.

[8] S.J. Wu, Y.Y. Kuo, H.A. Hou, L.Y. Li, M.H. Tseng, C.F. Huang, F.Y. Lee, M.C. Liu, C.W. Liu, C.T. Lin, C.Y. Chen, W.C. Chou, M. Yao, S.Y. Huang, B.S. Ko, J.L. Tang, W. Tsai, H.F. Tien, The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution, Blood 120 (15) (2012) 3106–3111.

[9] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C.D. Bloomfield, M. Cazzola, J.W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 127 (20) (2016) 2391–2405.

[10] C. Biernaux, M. Loos, A. Sels, G. Huez, P. Straykman, Detection of major bcr-abl gene expression at a very low level in blood cells of some healthy individuals, Blood 86 (8) (1995) 3118–3122.

Fig. 2. Patient 2: (A): Rare dysplastic neutrophils are noted in the peripheral blood smear. (B): Bone marrow aspirate smear shows occasional dysplastic megakaryocytes with nuclear separation.