sepsis.[10,11] These effects are possibly a result of increased provider experience, improved adherence to standard guidelines, adequacy of supportive staff and ancillary services, and greater financial capacity and resources.[10,11] In the context of cancer chemotherapy, higher-volume hospitals may have more experienced house staff capable of recognizing and managing chemotherapy complications at an earlier stage, leading to fewer deaths.

It is notable that the overall inpatient mortality among patients undergoing chemotherapy for AML was quite low compared with an early mortality rate of 12.2% reported by the Southwest Oncology Group.[12] This can be attributed to our inability to distinguish induction vs consolidation chemotherapy among the study cohort. Consolidation therapy involves the use of fewer and less-toxic chemotherapies and is associated with fewer complications and mortality.

Our study has several limitations. We were unable to separately analyze the impact of hospital volume on induction vs consolidation chemotherapy. Second, we were unable to analyze factors affecting prognosis such as cytogenetic and molecular profile, type of chemotherapy, performance scores, and use of supportive therapy such as growth factors. However, our study, being the first of its kind, generates a hypothesis that the volume-outcome relationship may hold true for chemotherapy for AML as well and should be explored in future studies.

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References

1. Hillner BE, Smith TJ, Desch CE. Hospital and physician volume or specialization and outcomes in cancer treatment: importance in quality of cancer care. *J Clin Oncol.* 2000;18(11):2327-2340.
2. Wang L. The volume-outcome relationship: busier hospitals are indeed better, but why? *J Natl Cancer Inst.* 2003;95(10):700-702.
3. Classification of Diseases, Functioning, and Disability. International classification of diseases, ninth revision, clinical modification (ICD-9-CM). http://www.cdc.gov/nchs/icd/icd9cm.htm. Accessed December 20, 2014.
4. HCUP Nationwide Inpatient Sample (NIS). Healthcare Cost and Utilization Project (HCUP). 2009-2011. Agency for Healthcare Research and Quality, Rockville, MD. http://www.hcup-us.ahrq.gov/nisoverview.jsp. Accessed December 20, 2014.
5. Hannan EL, Radzyner M, Rubin D, Dougherty J, Brennan MF. The influence of hospital and surgeon volume on in-hospital mortality for colectomy, gastrectomy, and lung lobectomy in patients with cancer. *Surgery.* 2002;131(1):6-15.
6. Yao SL, Lu-Yao G. Population-based study of relationships between hospital volume of prostatectomies, patient outcomes, and length of hospital stay. *J Natl Cancer Inst.* 1999;91(22):1950-1956.
7. Schafer JL, Kang J. Average causal effects from nonrandomized studies: a practical guide and simulated example. *Psychol Methods.* 2008;13(4):279-313.
8. Lipscomb J. Transcending the volume-outcome relationship in cancer care. *J Natl Cancer Inst.* 2006;98(3):151-154.
9. Begg CB, Cramer LD, Hoskins WJ, Brennan MF. Impact of hospital volume on operative mortality for major cancer surgery. *JAMA.* 1998;280(20): 1747-1751.
10. Ross JS, Normand SL, Wang Y, et al. Hospital volume and 30-day mortality for three common medical conditions. *N Engl J Med.* 2010;362(12):1110-1118.
11. Shahul S, Hacker MR, Novack V, et al. The effect of hospital volume on mortality in patients admitted with severe sepsis. *PLoS ONE.* 2014;9(9): e108754.
12. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood.* 2006;107(9):3481-3485.

To the editor:

Insights from response to tyrosine kinase inhibitor therapy in a rare myeloproliferative neoplasm with *CALR* mutation and *BCR-ABL1*

Calreticulin (*CALR*) mutations have been reported primarily in the context of *JAK2* and *MPL* wild-type essential thrombocythemia and primary myelofibrosis.[1-5] *CALR* mutations are exceedingly rare in the setting of t(9;22)/*BCR-ABL1*,[4,5] with only a single report in the literature describing a case of an atypical myeloproliferative neoplasm (MPN) in which *CALR* mutation preceded *BCR/ABL1* fusion.[6]

Here, we describe a patient with *CALR* mutation seen in the context of an MPN with the Philadelphia chromosome. The patient was a 67-year-old man found to be hypertensive on routine physical examination. His workup revealed an elevated white blood cell (WBC) count of 25×10^9/L (normal, 4×10^9/L) that prompted bone marrow (BM) aspiration and a biopsy at the referring institution, which was interpreted as suggestive of the patient having an MPN. Conventional cytogenetics and real-time reverse transcriptase polymerase chain reaction (RT-PCR) performed at the referring institution were positive for t(9;22) and *BCR-ABL1* fusion, respectively. The patient was treated with dasatinib as frontline therapy and was referred to our institution for further evaluation and management. A complete blood count revealed a WBC count of 39.9×10^9/L (Figure 1A), hemoglobin of 14.1 g/dL,

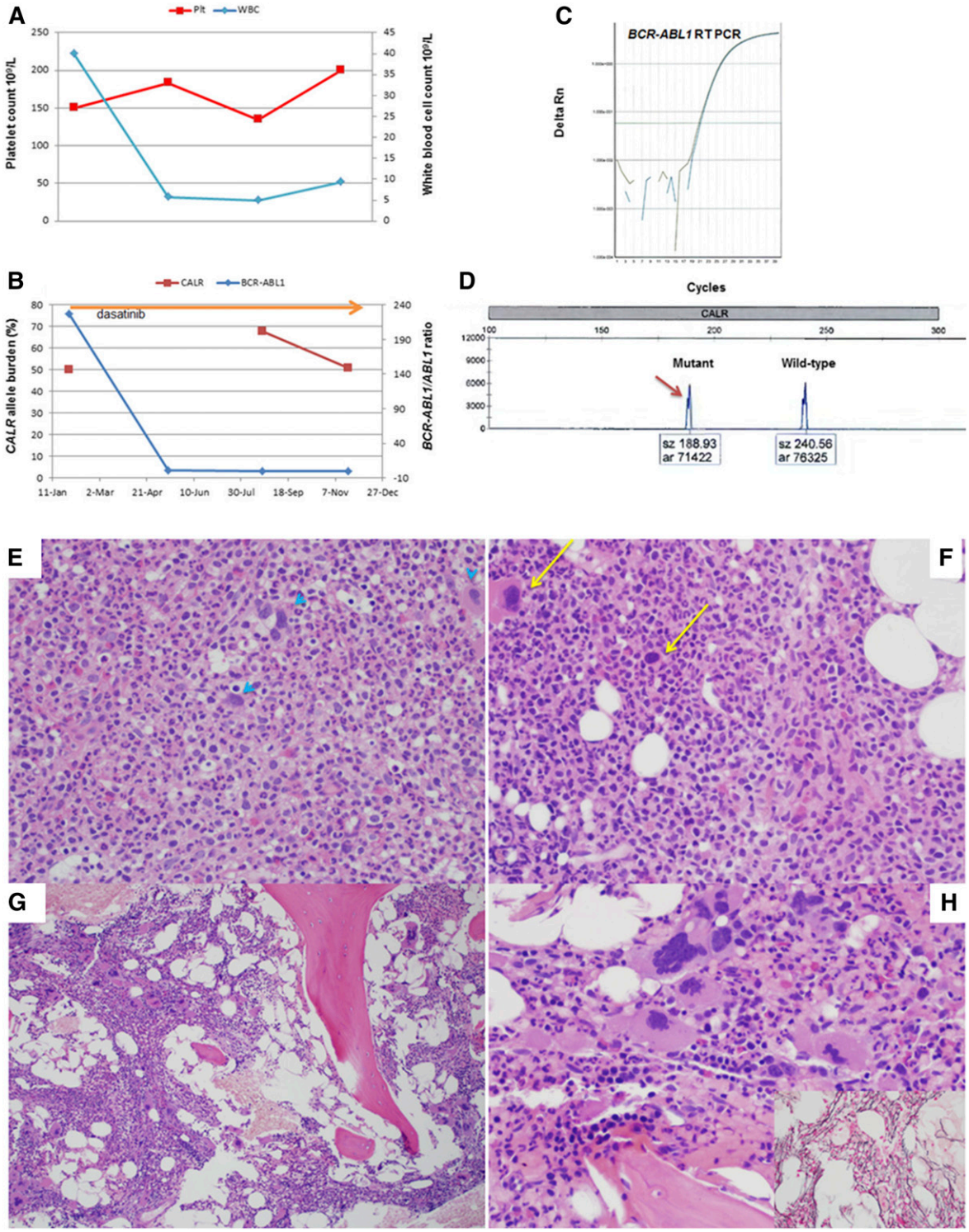

Figure 1. Laboratory and pathologic features of this MPN with concurrent *BCR-ABL1* and *CALR* mutation during the course of therapy with dasatinib. (A) The patient had marked leukocytosis and no significant thrombocytosis during his initial presentation to us. The WBC count continued to decrease as the patient received dasatinib, whereas the platelet count demonstrated an upward trend. The *BCR/ABL1* to *ABL1* ratio was >100% at presentation (B-C) and dropped to a nadir of 0.07% at last follow-up, whereas the allele frequency of the *CALR* mutation remained in the range of 51% to 68% (B). *CALR* mutation screening was performed using PCR followed by standard capillary electrophoresis on a Genetic Analyzer (Applied Biosystems, Foster City, CA) and Sanger sequencing for confirmation. The frequency of the mutant allele (red arrow) was calculated by dividing the mutant peak area by the sum of the mutant and wild type peak areas. (D) The initial bone marrow (BM) biopsy at presentation to our institution showed a hypercellular bone marrow with marked granulocytic hyperplasia and variably distributed megakaryocytes including a mixture of small and large forms, some with hyperchromatic nuclei (yellow arrows) and others without significant nuclear hyperchromasia (blue arrowheads). The morphologic findings were in keeping with the diagnosis of chronic myeloid leukemia, although the presence of occasional large, hyperchromatic megakaryocytes was unusual. (E-F) A repeat bone marrow biopsy after 7 months of therapy with dasatinib showed a moderately hypercellular BM with prominent megakaryocytic clustering and mild osteosclerosis (hematoxylin and eosin [H&E] stain, original magnification ×100). (G) Notably, there was a predominance of large megakaryocytes with hyperlobulated nuclear contours and prominent nuclear hyperchromasia (H&E stain, original magnification ×100). (H) A reticulin stain showed moderate reticulin fibrosis characterized by coarse bundles of reticulin with many interconnections (H&E stain, original magnification ×100; inset, reticulin; original magnification ×100).

and platelet count of 150×10^9/L. A BM aspiration and biopsy revealed a hypercellular (80%-90%) BM with left-shifted granulocytic hyperplasia and mild megakaryocytic hyperplasia, including a mixture of small and large forms and some with hyperchromatic nuclei (Figure 1E-F). No megakaryocytes with classical "dwarf"[7] morphology were identified. Moderate reticulin fibrosis was present and characterized by a diffuse and dense increase in reticulin fibers forming extensive intersections.[8] Conventional cytogenetics revealed a Philadelphia chromosome–positive clone, 46,XY,t(9;22)(q34;q11.2)[14], and an additional Philadelphia chromosome–negative clone with a complex karyotype, 46,XY,der(2)t(2;5)(p21;q15),der(5)t(2;5) del(2)(p22p24),add(7)(q22),der(12)t(7;12)(q22;p11.2)[6]. Fluorescence in situ hybridization studies showed *BCR/ABL1* fusion signals in 78% of the interphases analyzed. RT-PCR performed on peripheral blood showed a *BCR/ABL1* to *ABL1* ratio of >100% (Figure 1B-C). A diagnosis of chronic-phase chronic myeloid leukemia was rendered. The patient was continued on dasatinib therapy. His WBC count and *BCR/ABL1* fusion transcript continued to decrease (Figure 1A-B). Follow-up BM aspiration and biopsy performed 7 months later showed strikingly distinctive features compared with the initial BM biopsy specimen, characterized by marked atypical megakaryocytic hyperplasia including many large forms with hyperchromatic and hyperlobulated nuclei and frequent clusters with moderate reticulin fibrosis,[8] and mild osteosclerosis and no substantial granulocytic hyperplasia, in keeping with PMF (Figure 1G-H). RT-PCR was positive for *BCR-ABL1* fusion transcripts (decreased ratio of 0.21) and conventional cytogenetics showed a Philadelphia chromosome–negative clone with a complex karyotype identical to the previous sample. The findings prompted additional mutational workup including *JAK2* V617 analysis, which was wild-type, and *CALR* analysis, which revealed a type 1 mutation (52 bp deletion)[5] with a mutant allele frequency of 68% (Figure 1B). We retrospectively analyzed the peripheral blood sample from the time the patient initially presented; we detected a *CALR* mutation with a mutant allele frequency of 51% (Figure 1B,D).

Our findings and the genetic trends observed in this patient (Figure 1A-B) further confirm the observations made by Cabagnols and colleagues,[6] indicating that although the Philadelphia-positive clone is sensitive to dasatinib, the *CALR*-mutant clone persists throughout the course of therapy. This case is of interest because it shows that the BM morphologic features were dominated by the presence of *BCR/ABL1* fusion in the initial sample, despite the fact that the *CALR* mutation was also present. The *CALR* mutation morphologically declared itself subsequent to tyrosine-kinase inhibitor therapy, as shown by the emergence of markedly atypical megakaryocytic hyperplasia, supporting the diagnosis of PMF. Of note, the patient never showed thrombocytosis, before or after dasatinib therapy, potentially because of the presence of moderate myelofibrosis at the time of presentation. Notably, the patient reported by Cabagnols and colleagues[6] also had moderate myelofibrosis at the time of diagnosis.

Given that the *CALR* mutant allele burden was 51% and fluorescence in situ hybridization studies showed *BCR/ABL1* fusion signals in 78% of interphases analyzed in the initial sample, it is reasonable to assume that a *BCR/ABL1*-positive subclone arose from a dominant clone, with a heterozygous *CALR* mutation rendering the *BCR/ABL1* fusion a secondary genetic event in this case. This observation is similar to that described earlier by Cabagnols et al.[6]

This study emphasizes the importance of BM morphology and expanded molecular testing in the workup of patients with an MPN.

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References

1. Tefferi A, Lasho TL, Finke C, et al. Type 1 vs type 2 calreticulin mutations in primary myelofibrosis: differences in phenotype and prognostic impact. *Leukemia.* 2014;28(7):1568-1570.
2. Rumi E, Pietra D, Ferretti V, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. JAK2 or

CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. *Blood.* 2014;123(10): 1544-1551.

3. Vannucchi AM, Rotunno G, Bartalucci N, et al. Calreticulin mutation-specific immunostaining in myeloproliferative neoplasms: pathogenetic insight and diagnostic value. *Leukemia.* 2014;28(9):1811-1818.

4. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med.* 2013; 369(25):2391-2405.

5. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013;369(25): 2379-2390.

6. Cabagnols X, Cayuela JM, Vainchenker W. A CALR mutation preceding BCR-ABL1 in an atypical myeloproliferative neoplasm. *N Engl J Med.* 2015; 372(7):688-690.

7. Vardiman JW, Melo JV, Baccarani M, Thiele J. Chronic myelogenous leukaemia, BCR-ABL1 positive. In: Swerdlow SH, Campo E, Harris NL, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008:32-37.

8. Thiele J, Kvasnicka HM, Facchetti F, Franco V, van der Walt J, Orazi A. European consensus on grading bone marrow fibrosis and assessment of cellularity. *Haematologica.* 2005;90(8):1128-1132.