To the editor:

Calreticulin mutated prefibrotic-stage myelofibrosis and PMF represent an independent clone from coexisting CLL

JAK2^{V617F}-positive myeloproliferative neoplasms (MPNs), especially polycythemia vera (PV), may occur concomitantly with chronic lymphocytic leukemia (CLL). This association has not been described with a variant of essential thrombocythemia (ET), prefibrotic-stage myelofibrosis (preF-MF) or primary myelofibrosis (PMF) or with calreticulin (*CALR*)-mutated MPNs. *JAK2*^{V617F} has been reported in MPNs, but not in CLL clones.[1,2] We previously reported 3 unrelated women with concomitant PV and CLL, in which these 2 diseases used different X chromosome alleles, indicating their origin from different pre-*JAK2*^{V617F} hematopoietic stem cells.[2] Herein, we present additional observations in 2 patients suggesting an independent relation between MPN and coexisting CLL clones.

The first is a 67-year-old man with thrombocytosis who presented 9 years ago with a platelet count of 707 × 10^6/μL, a leukocyte count of 9.55/μL (lymphocytes 19%), and a hemoglobin level of 13.5 g/dL, but no history of thromboembolic events, aquagenic pruritus, erythromelalgia, lymphadenopathy, or splenomegaly. There was no evidence of reactive thrombocytosis or detectable *JAK2*^{V617F} or the thrombopoietin receptor (cMPL) mutations. Marrow was 90% cellular and consistent with preF-MF (Figure 1A-B). Although no increased lymphocytes were appreciated by marrow morphological evaluation, marrow and blood flow cytometry revealed a small CD5, CD19, dim CD20, CD23, dim κ light chain-positive B-cell clone accounting for ~4% of lymphoid cells. This clone resembled CLL but was deemed to be of unknown clinical significance. Imaging studies failed to detect any evidence of lymphoproliferative disorder. The propositus remained asymptomatic and in hematological remission on hydroxyurea for the subsequent 9 years. On 3 successive occasions, the same small B-lymphocyte clone was consistently detectable at a low level in blood. After 9 years, the patient developed progressive lymphocytosis (white blood cells of 25.12 × 10^3/μL, with 72% lymphocytes and 23% neutrophils, platelet count of 281 × 10^6/μL, and hemoglobin level of 12 g/dL) and a moderately enlarged spleen but was asymptomatic with no lymphadenopathy. Marrow showed persistent morphology of preF- MF, with expansion of the previous B-cell clone to 30% of marrow cells (Figure 1C) and increased fibrosis, now judged "moderate" (2+). Flow cytometry again revealed the same B-cell clone, now comprising 48% of leukocytes and consistent with CLL.

The second case is a 69-year-old man with a 14-year history of CLL who recently was referred to us. A year ago, while his CLL was in complete remission after chemotherapy, he developed transfusion-dependent anemia and progressive splenomegaly

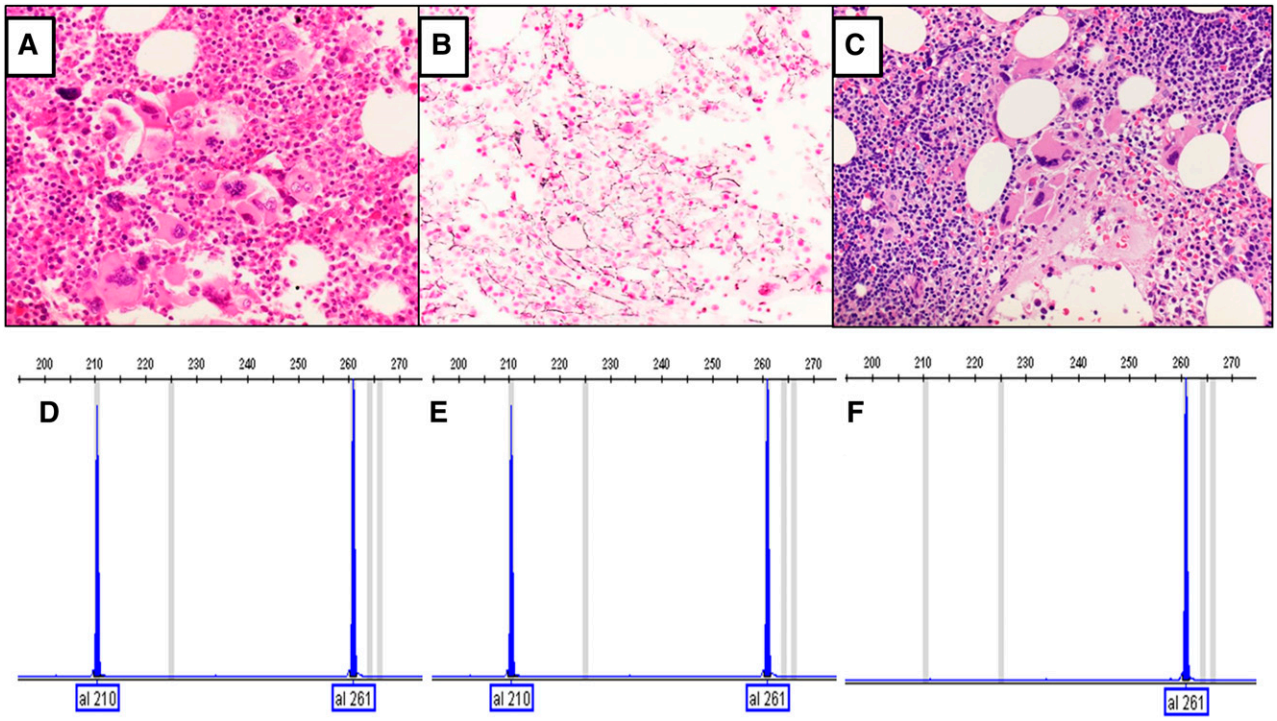

Figure 1. Analyses of *CALR* mutations and bone marrow morphology of the first subject. (A) Hematoxylin and eosin stain of initial marrow core biopsy and hypercellular marrow with increased megakaryocytes with variable size and shape and hyperchromatic hypolobate nuclei that are arranged in dense clustering with no overt CLL cells. (B) Reticulin stain of initial marrow biopsy with no significant increase in reticulin fibers. (C) Hematoxylin and eosin stain of follow-up marrow core biopsy after 9 years, showing megakaryocytic histotopography consistent with the prefibrotic stage of primary myelofibrosis. An increased number of small lymphocytes in aggregates are present in the upper right and lower left corners of the field, compatible with CLL (original magnification, ×40). Analysis of *CALR* mutations in exon 9 was performed by fragment analysis, which is a semiquantitative analysis.[3] (D) Fragment analysis of *CALR* from granulocytes obtained 3 years after diagnosis of type 1 *CALR* deletion. (E) Fragment analysis data of current granulocyte DNA (9-year follow-up after diagnosis), with the same *CALR* deletion. (F) Fragment analysis of DNA from CD19$^+$ cells; the type 1 *CALR* deletion is not present.

and underwent a splenectomy resulting in a decreased but persistent red cell transfusion requirement. On referral, we found leukoerythroblastic peripheral blood morphology with dacrocytes, and his marrow also indicated PMF, without JAK2 or cMPL mutations.

Somatic *CALR* mutations have been identified in patients with ET and MF who lacked *JAK2*^{V617F} or *cMPL* mutations.[3,4] We detected a *CALR* 52-bp deletion type 1 mutation[3] in the granulocytes of both patients, but the CD19-positive sorted CLL cells had no detectable *CALR* mutation (Figure 1D-F). It is not known if *CALR* mutations in MF and ET are disease-originating mutations or more similar to the *JAK2*^{V617F}-positive MPNs, wherein other somatic or germ line mutations are thought to precede the MPN phenotypes.[5]

CALR mutations are reported to be associated with a lower risk in MPNs and thus are likely to be acquired earlier,[3,4] raising the possibility that preF-MF and CLL may arise from the same pluripotent stem cell. However, the lack of *CALR* mutation in these patients' CLL cells suggests that they represent independent clones. However, as the X chromosome–based clonality assays can be performed only in women, a common precursor origin of MPN and CLL clones cannot be completely excluded in these male patients.

Mohamed E. Salama
Associated Regional University Pathologists Laboratories, Salt Lake City, UT
Department of Pathology, University of Utah, Salt Lake City, UT

Sabina I. Swierczek
Division of Hematology, School of Medicine, University of Utah, Salt Lake City, UT

Tsewang Tashi
Division of Hematology, School of Medicine, University of Utah, Salt Lake City, UT

Christy A. Warby
School of Medicine, University of Utah, Salt Lake City, UT

N. Scott Reading
Associated Regional University Pathologists Laboratories, Salt Lake City, UT

Josef T. Prchal
Associated Regional University Pathologists Laboratories, Salt Lake City, UT
Department of Pathology and Division of Hematology, School of Medicine, University of Utah, Salt Lake City, UT

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Correspondence: Mohamed E. Salama, Associated Regional University Pathologists Laboratories and University of Utah, Department of Pathology, Salt Lake City, UT 84108; e-mail: Mohamed.salama@aruplab.com; or Josef T. Prchal, Hematology, SOM 5C310, University of Utah School of Medicine, Salt Lake City, UT 84132-2408; e-mail: josef.prchal@hsc.utah.edu.

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Response

Platelets do not generate activated factor XII—how inappropriate experimental models have led to misleading conclusions

Interestingly, for the research on the role of platelets in contact activation of coagulation, 2 articles on the topic using the same polyphosphate (polyP) preparation have been published.[1,2] In our paper in *Blood*, "Putting polyphosphates to the test: evidence against platelet-induced activation of factor XII,"[1] we found no evidence for platelet-induced activation of factor XII (FXII). Conversely, the paper in *Cell*[2] concluded the following: "PolyP represents the long sought 'foreign' surface that triggers fibrin formation by activated platelets linking primary to secondary hemostasis and critically contributing to 'procoagulant' platelet activity." Obviously, these 2 papers contradict each other, and it is useful for progress in the field to consider why. To support their position that platelet-derived polyP activates FXII, some of the authors of the *Cell* paper[2] tried to explain our negative findings in a letter to the editor.[3] Unfortunately, their letter contains statements based on factual errors and unfounded assumptions. Therefore, we wish to correct these errors and present possible explanations to the discrepancies that could be valuable for researchers in the field.

To support their claim that platelet-derived polyP activates FXII,[2] it is incorrectly assumed in the letter[3] that lack of FXII activation in our paper[1] can be explained by use of an old preparation and thus degraded polyphosphates. However, it is important to note that all activity measurements were performed within 1.5 months from receiving the substance donated by the Renné laboratory. It is also incorrectly stated that our findings were a result of using polytetrafluoroethylene filters, with the claim that such filters activate FXII. However, this claim is based on a citation that mentions neither contact activation nor FXII.[4] In fact, polytetrafluoroethylene is extensively used in blood-contacting biomaterials and shows very low procoagulant activity.[5] Regardless, polytetrafluoroethylene filters were not used in our paper. Figure 1A demonstrates that plasma filtration with the Minisart filters used[1] does not generate detectable FXIIa.

Their letter[3] further dismisses our results that were obtained using the sensitive fluorogenic substrate Boc-Gln-Gly-Arg-AMC (7-Amino-4-methylcoumarin) to measure FXIIa, claiming potential