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Letter to the Editor

Low-level *BCR-ABL1* transcripts in individuals without overt hematologic malignancy

Identifying chromosomal aberrations is an important tool for diagnosis, classification [1] and monitoring of various hematologic malignancies. The Philadelphia (Ph) chromosome resulting from translocation between chromosomes 9 and 22, is the hallmark for chronic myeloid leukemia (CML), some acute lymphoblastic leukemias (ALL) and rare acute myeloid leukemias (AML). The diagnosis of other myeloproliferative neoplasms specifically requires the absence of this translocation [1]. The BCR-ABL1 fusion protein, thus formed, is an active tyrosine kinase [2] and varies in size from 190 to 230 kDa. The majority of patients with CML express a 210-kDa BCR-ABL1 protein, while patients with Philadelphia positive ALL commonly express a 190-kDa BCR-ABL1 protein [3]. Low levels of *BCR-ABL1* transcript (both 190 and 210 kDa) have been reported with variable frequency in healthy individuals using ultra-sensitive nested PCR techniques [4–10]. PCR based assays commonly used in clinical testing for *BCR-ABL1* expression are typically much less sensitive than those used in the aforementioned studies. Thus, the significance of finding low-level *BCR-ABL1* transcripts using a standard clinical assay in patients without a hematologic disease characteristically associated with *BCR-ABL1* translocation is unknown. After having identified an index case that led to diagnostic confusion, 5 additional cases with reported low level *BCR-ABL1* were identified between 2009–2015 from archival records of Pathology Department, UPMC.

The original data was reviewed on all 6 cases. Repeat *BCR-ABL1* testing was performed in triplicate on stored RNA from the original specimen using a two-step quantitative reverse transcriptase PCR assay (sensitivity of 1 in 100,000). Primer sets and fluorescently labeled probes specific for the *BCR/ABL1* fusion transcript and *Beta-glucuronidase* (*GUS*) housekeeping gene were used (ABI 7500, Life Technologies, Carlsbad, CA). Comparison of patient *BCR-ABL1* expression to a clonal cell line expressing each fusion (K562 for the major breakpoint (e13/14a2), SupB15 for the minor breakpoint (e1a2)) was performed and results expressed as a percentage of the cell line expression. Classical G-banded cytogenetic karyotypes [11] and fluorescence in-situ hybridization studies (FISH) (200 nuclei analyzed) were performed using a *BCR/ABL1* Dual Color Dual Fusion translocation probe (VYSIS DNA probe, Abbott Molecular Inc., Des Plaines, IL; false positive < 1%).

The cohort consisted of 2 males and 4 females, 24–67 years old. The index patient, a 58-year-old female, with a history of colon carcinoma, status post chemotherapy, was noted to have erythrocytosis (hemoglobin – 15.5 g/dl, white cell count – 4.9 × 10^9/l and platelet count – 196 × 10^9/l), prompting bone marrow evaluation. The bone marrow was normocellular (40–50%) with trilineage hematopoiesis, normal myeloid:erythroid ratio, no increase in blasts or plasma cells and no significant morphologic abnormality including in the megakaryocytes (Fig. 1). A low-level *BCR-ABL1* transcript (e1a2, 3.4% relative to SupB15 expression, negative for e13/14a2) was identified on quantitative real time PCR with 3/60 cells with t(9;22) on classical cytogenetic studies and 1.5% cells by cytogenetic FISH studies (Fig. 1). Further work-up was negative for *JAK2* mutation (done at an outside facility). Molecular testing of multiple subsequent peripheral blood samples (only RNA available) over a 50-month period showed persistent *BCR-ABL1* positivity at low levels (Fig. 1). The patient is being followed without therapy and without clinical evidence of a hematologic neoplasm.

Among the other 5 cases, expression of the e13/14a2 transcript was identified in 4/5 cases (levels ranging from 0.002 − 0.007% K562) and the e1a2 transcript in 1/5 cases (0.003% SupB15). None of the cases with subsequent samples (4/5) demonstrated persistence of the *BCR-ABL1* transcript (Table 1). On repeat testing using stored RNA from the original sample, one case failed to show a positive result in any of the replicates. It is unclear if this was related to degradation of the stored RNA over time or a false positive in the original sample. None of the patients in the cohort developed overt hematologic malignancy associated with t(9;22) (mean 38.3 months, range: 12–84 months). Three had persistent thrombocytosis (*JAK2*V617 F mutation negative), 3 had persistent variable leukocytosis (including one with thrombocytosis). One patient (#6) who initially presented with leukocytosis and eosinophilia, later developed a skin lesion, which was suspicious for a T cell lymphoma with CD30 positivity.

Very few case reports have described incidental detection of t(9;22) on classical cytogenetic studies (CCG) and FISH in patients being worked up for unrelated diseases, which were then confirmed to have e13/14a2 *BCR-ABL1* transcripts on molecular studies [12–14]. These include a patient with IgG lambda monoclonal gammopathy of undetermined significance [12], a patient at 1 year follow-up for autologous stem cell transplant for IgA kappa multiple myeloma [14] and one who underwent staging bone marrow for a primary intraocular lymphoma [13]. Unfortunately, the molecular results are not entirely comparable to ours since either the methodology was not provided [12], is different [13] or the results were not converted to the standard international scale [14]. These cases probably do not represent low-level transcript, since the CCG and FISH had significant levels of posi-

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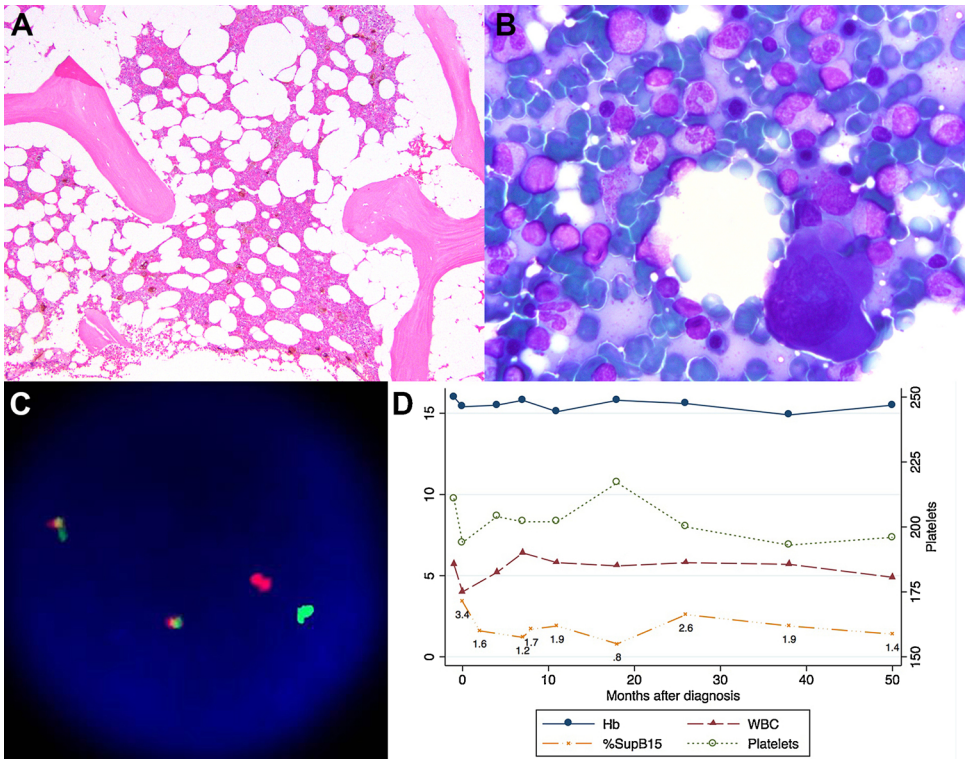

Fig. 1. (patient # 5): A- Bone marrow biopsy (Hematoxylin and eosin with FVIIIRA 10X) demonstrates about 40–50% cellularity, FVIIIRA highlights the megakaryocytes, B- Touch imprint demonstrates trilineage hematopoiesis, C- Cytogenetic FISH study for the *BCR-ABL1* fusion demonstrates two fusion signals, and one normal BCR and one normal ABL1 signal indicative of a *BCR-ABL1* fusion, D- Relative change in hemoglobin, WBC count, platelets and expression of *BCR-ABL1* minor transcript over time. The first two *BCR-ABL1* values (at diagnosis and at 2 months) are from bone marrow specimens. Subsequent tests were performed on peripheral blood.

tivity of the *BCR-ABL1* fusion (FISH (20%–33%) and CCG (25%–75%)). All these patients received treatment, either immediately or later due to increasing levels of the *BCR-ABL1* transcript. Our case with persistent *BCR-ABL1*, on the other hand, only had 1.5% cells positive on FISH and 5% on CCG, which has been stable over the past 50 months without any specific treatment.

The reported prevalence of *BCR-ABL1* carrying leukocytes in the blood of healthy individuals, using the ultrasensitive techniques, is less than 1–10 per 10^8 WBCs [6]. These studies, however, demonstrate significant variability in the reported frequencies of the various types of *BCR-ABL1* transcripts. The frequency of *BCR-ABL1* 210 kDa e13/14a2 product reported varies from 10%–54% in adults [5,6,8,10], 4.5–32% in children [5,8,10], and 0–16% in cord blood [5,10]. The frequency of *BCR-ABL1* 190 kDa e1a2 product reported varies from 0 to 77% in adults [6,8,10], 0–67% in children [8,10] and 42% in cord blood [10]. Some suggest a higher incidence in males [8] and increasing incidence with age [5,8]. The discrepancy in the reported frequencies may be due to differences in the patient demographics, and methodologic differences in the assays that affect their sensitivity.

The level of *BCR-ABL1* transcript in healthy individuals is usually below the sensitivity of the assays used clinically for diagnosis and monitoring of CML, ALL or AML and hence does not pose a clinical problem in most circumstances. The case in the current series with persistent low level *BCR-ABL*1 transcript did not fit the clinical and morphologic criteria for a definitive diagnosis of hematologic malignancy commonly associated with this gene abnormality. The significance of such a finding, in the aforementioned clinical scenario, is unknown and is a challenge for both the pathologists and the clinicians, without clear guidelines for management and follow up of the patient. This may represent a phenomenon akin to clonal hematopoiesis of undetermined potential. Bose et al [6], inferred that the incidence of such a positive result in an otherwise healthy individual in a clinical setting was 1 in 40 based on the differences in the sensitivity of the ultrasensitive method that they used and the one they used clinically. But, it is difficult to compare the sensitivity of the current clinically used method with those described in the literature. They also suggested that the probability for a persistently positive result would decrease further by chance on repeat testing. We cannot determine the incidence of a positive result in otherwise healthy individual since ours in not a population based study. Moreover, we selected cases based on how they were originally signed-out and did not evaluate all cases that underwent *BCR-ABL1* testing in the laboratory during that period. In our cohort, when the stored RNA from the original sample was tested again, 5/6 cases were positive in at least one of the replicates. However, when testing was performed on subsequent samples, 4 of the 5 cases were negative. Hence, the original result could represent a true low-level *BCR-ABL1* transcript at the border of our level of sensitivity; but could also be spurious due to low level contamination. These cases emphasize that unexpected particularly low-level positivity for *BCR-ABL1* transcripts requires replicate and follow-up testing on a subsequent sample prior to making any clinical conclusions.

Thus, low-level *BCR-ABL1* transcripts can be identified on clinical testing in the absence of overt hematologic neoplasms. A cautious report suggesting testing on a fresh sample is warranted, to avoid overdiagnosis, clinical confusion and inappropriate therapy. As identified in the cases studied here, repeat testing on a subsequent fresh sample, in most circumstances, is negative and resolves the issue clinically. However, it is important to recognize that rare cases may show persistent positive results in the absence of an overt malignancy. Clinical/laboratory follow-up including repeat *BCR-ABL1* testing in these patients is important to rule out evolution to an overt neoplasm.

Table 1
Characteristics of cases with low level *BCR-ABL1* transcript.

Case No	Age (yrs)/ sex	Clinical history/ indication for testing	WBC/Plt count (10^9/l) at the time of *BCR-ABL* testing	%K562 (SupB15 for minor transcript) (+ replicates/ total tested)	Repeat testing on same sample (%IS)	CCG	FISH	PCR on a subsequent sample (s)	Follow up	F/u (months)
MAJOR TRANSCRIPT										
1	48/F	Thrombocytosis	9.7/437	0.007 (1/2)	0/3	46,XX*	-*	NA	Persistent thrombocytosis (437-475). BM (one month later) –normocellular, TLH	84
2	24/F	Raynauds, thrombocytosis	6.4/636	0.002 (2/2)	3/3 (0.037)	46,XX*	-*	(-)x3 (at 12 months)	Persistent thrombocytosis (412-561), BM- normocellular, TLH	12
3	37/F	Leukocytosis with neutrophilia, basophilia, thrombocytosis	11/443	0.002 (2/2)	1/3 (0.021)	46,XX	–	(-)x2 (at 8 months)	Persistent leukocytosis (12.1-11.4), thrombocytosis (398-451), dental abscess, BM- normocellular, TLH	29
4	41/M	IgA nephropathy, chronic leukocytosis and anemia	18.3/266	0.004 (5/6)	2/3 (0.012)	46,XY	NA	(-)x3 (at 9 months)	Leukocyte count varied from 8.9-19.6. Renal transplant for IgA nephropathy. No hematologic malignancy.	22
MINOR TRANSCRIPT										
5	58/F	Colon carcinoma post chemotherapy, erythrocytosis	5.7/212	3.4 (2/2)	3/3	46,XX,t(9;22)(q34;q11.2)[3]/46,XX[57]	+ 4/260 cells (1.5%)	+ (multiple) (Fig. 1)	No evidence of CML, ALL or AML	50
6	67/M	malaria, chronic rash, eosinophilia (Absolute eosinophils 1.9 × 10^9/l)	13.2/359	0.003 (2/2)	3/3	45,X,-Y[8]/46,XY [12] *	-*	(-)x2 (at 2 months)	leukocyte count ranged from 6.8-16.9, eosinophil count 0.1-1.9), hyper IgE syndrome#, skin lesions suspicious for T cell lymphoma with CD30 positivity	33

Samples 1,2 and 6 were peripheral blood and 3, 4 and 5 were bone marrow.
*- subsequent bone marrow sample tested.
NA- Not available.
JAK2 V617 F mutation tested in 5 of 6 cases was negative. Patient 4 was not tested for *JAK2*.
#- subsequent bone marrow was negative for *CHIC2* deletion/*FIP1L1-PDGFRA* fusion and *KIT* mutation.
F/u- follow up, BM- Bone marrow, TLH-trilineage hematopoiesis, CCG- Classical cytogenetics.

Disclosures

The authors have no disclosures to make.

Conflict of interest

The authors declare no conflict of interest.

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RJL performed the research, and wrote the paper.

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