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Letters to the Editor

Detection of *NUP214-ABL1* translocation using *BCR-ABL1* dual color FISH probes in T-cell acute lymphoblastic leukemia – an illustrative report and review of literature

TO THE EDITOR: *NUP214-ABL1* translocation is probably the most common tyrosine kinase inhibitor (TKI)-targetable cytogenetic abnormality observed in T-cell acute lymphoblastic leukemia (T-ALL), accounting for 5–6% of the T-ALL cases [1]. However, there are only few case reports and small series in the literature, possibly indicating the underdiagnosis of this entity. Unlike in B-cell acute lymphoblastic leukemia, testing for gene fusions using reverse transcriptase polymerase chain reaction (RT-PCR) or fluorescent in-situ hybridization (FISH) is often avoided in T-ALL because of the absence of distinct associations with prognosis or targeted therapy, especially in resource-limited settings. However, TKI-targetable abnormalities have also been described in T-ALL, including *NUP214-ABL1, BCR-ABL1*, and mutations in *STAT5b* [2, 3]. Among these, FISH testing using *BCR-ABL1* dual-color dual-translocation probes can help to identify not only *BCR:ABL1* but also *NUP214-ABL1* based on the characteristic patterns in FISH testing. This is an illustrative case highlighting an easy and cost-effective approach for routine detection of cytogenetic abnormalities.

A developmentally normal male child, appropriately immunized for his age, presented with continuous moderate-grade fever, progressive pallor, and mild hepatosplenomegaly since 10 days. Peripheral blood examination revealed that the hemoglobin level was 71 g/L; total leukocyte count, 45.3×10^9/L; and platelet count, 65×10^9/L. The peripheral blood film revealed 72% blasts, 9% neutrophils, 16% lymphocytes, and 3% monocytes. The blasts were negative for myeloperoxidase. Flow cytometry confirmed T-ALL [positive for cluster of differentiation (CD) 7, CD4, CD5, CD2, cytoCD3, and terminal deoxynucleotidyl transferase (TdT);

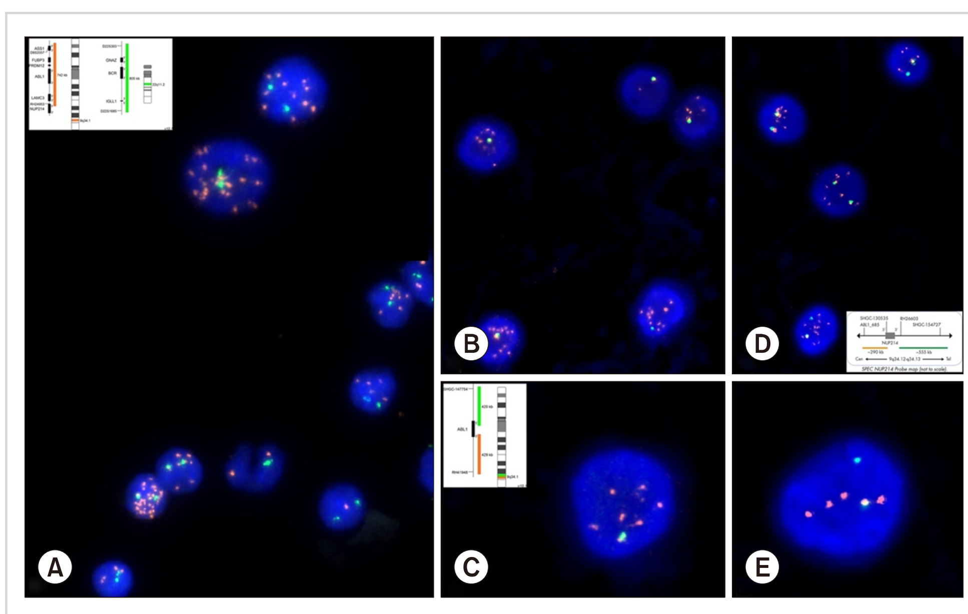

Fig. 1. **(A)** FISH using *BCR-ABL1* dual-color dual-fusion probe showing two green signals (normal *BCR* gene) and multiple orange signals (3–50 copies), indicating amplification of the *ABL1* gene; **(B, C)** FISH using *ABL1* break-apart probe showing one fusion (normal *ABL1* gene) and multiple orange signals, indicating amplification of the 3′ region of the *ABL1* gene along with deletion of the 5′ region of the *ABL1* gene; **(D, E)** FISH using *NUP214* break-apart probe showing one fusion (normal *NUP214* gene), one green (normal 3′ region of the other *NUP214* allele), and multiple orange signals, indicating amplification of the 5′ region of the *NUP214* gene.
Abbreviation: FISH, fluorescent in-situ hybridization.

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negative for surface CD3, CD1a, CD8, T-cell receptor (TCR) αβ, TCRγδ, B cell, and myeloid antigens]. FISH testing using *BCR-ABL1* dual-color dual-fusion probe (Metasystems, Germany) showed amplification of *ABL1* (3–50 copies) using the protocol as previously described [4]. Further testing using *ABL1* (Metasystems, Germany) and *NUP214* (Zytolight, Germany) dual-color break-apart probes showed a characteristic signal pattern confirming *NUP214-ABL1* translocation (Fig. 1). Details of immunophenotyping and FISH cytogenetics are summarized in Table 1.

Bone marrow examination was not performed, as a confirmatory diagnosis could be made from the peripheral blood investigation, and the child was not willing to undergo bone marrow examination. Conventional cytogenetics of the peripheral blood did not show metaphase. Augmented Berlin-Frankfurt-Munich protocol plus imatinib was administered. Post-induction bone marrow was hypocellular with 2% blasts, and no measurable residual disease was detected using 10-color flow-cytometric immunophenotyping. The delayed intensification phase 2 was completed uneventfully, and the child is now in the maintenance phase.

Both *ABL1* and *NUP214* genes are located at 9q34.1, with the latter on the telomeric side. The fusion of these genes results from extrachromosomal episome formation and amplification of both genes. The episome containing the fused gene exists autonomously and freely replicates in the cytoplasm or integrates with the chromosome and replicates with it. This episomal amplification, varying between 5–50 copies/cell, can be visualized using FISH, multiplex ligation-dependent probe amplification, or chromosomal microarray; however, it is undetectable with conventional cytogenetics. Amplification of *ABL1* does not appear to be the only mechanism involved in the pathogenesis of T-ALL; there have been reports of associated alterations of other genes, such as *CDKN2A*, *TLX1*, *TLX3*, and *NOTCH1*. These observations indicate a multigene contribution to the pathogenesis of T-ALL with *NUP214-ABL1* fusion. *NUP214-ABL1* fusion is found predominantly in men, and these patients usually present with high-risk factors, including elevated leukocyte count, mediastinal mass, or extramedullary involvement, often with early relapse and dismal outcomes [5]. While an occasional patient has survived for more than 194 months, the median overall survival reported in previous series is only 18 months. These patients are reported to benefit from TKI, especially dasatinib; hence, it is imperative to diagnose this entity in the clinics [6]. However, the long-term benefit of adding TKI

Table 1. Hematological and laboratory parameters at diagnosis and after induction therapy.

	Diagnosis	After induction
Peripheral blood		
Total leukocyte count	45.3×10^9/L	4.9×10^9/L
Hemoglobin	71 g/L	119 g/L
Platelet count	65×10^9/L	195×10^9/L
Peripheral blood blasts	72%	0%
Bone marrow blasts	Not done	2%
Immunophenotyping (flow cytometry)		
Gated events	CD45-dim low side scatter events (progenitors) -90% of viable events	CD7-positive low side scatter events -5% of viable events
Positive[a,b] markers on gated cells	CD2 (39.2%), CytoCD3 (98%), CD4 (79.5%), CD5 (56%), CD7 (98%), CD10 (70%), CD81 (98%), CD33 (28.7%), CD45 (dim), CD58 (93.4%), CD38 (72%), Tdt (55%)	Surface CD3 (86%), cytoplasmic CD3 (100%), CD4 (42.8%), CD8 (40.9%), CD5 (84%), CD56 (6.8%), CD45 (100%)
Negative markers on gated cells	Surface CD3, CD8, CD13, CD19, CD20, CD34, CytoCD79a, CytoCD22, CD86, CD117, CD56, TCRαβ, TCRγδ, anti-MPO, CD14, CD36, CD64, CD15, CD123, HLA-DR	CD34, CD38
FISH cytogenetics	XL *BCR/ABL1* dual color dual fusion probes,	Not done
Probes tested	Cytotest LSI *KMT2A* dual color break apart rearrangement probe, and Zytolight SPEC *NUP214* dual color break-apart probe	
Pattern	nuc ish (*ABL1*3−50, *BCR*×2)[160/200], (5′*KMT2A*×3,3′*KMT2*A×3)(5′*KMT2A* con 3′*KMT2A*)×3[120/200], (5′*NUP214*×3-50,3′*NUP214*×2)(5′*NUP214* con 3′*NUP214*)×1	

[a)] ≥20% positive, [b)]CD2, CD10, CD81, CD13, CD33, CD117, CD34, CD58, and Tdt were not included in post-induction measurable residual disease analysis. Only T-cell tube was studied for measurable residual disease testing.
Abbreviations: CD, cluster of differentiation; FISH, fluorescent in-situ hybridization; HLA-DR, human leukocyte antigen-DR isotype; MPO, myeloperoxidase; TCR, T-cell receptor; Tdt, terminal deoxynucleotidyl transferase.

in the treatment remains unclear owing to the lack of randomized controlled trials. A summary of the cases reported until now is presented in Table 2.

The limited number of reports and absence of definite treatment guidelines may indicate underdiagnosis of this entity. Amplification of *ABL1* (9q34) is an indirect indicator of *NUP214-ABL1* fusion, which can easily be detected by routine testing of *BCR-ABL1* using FISH in T-ALL cases. *NUP214-ABL1* fusion can be further confirmed using *NUP214* break-apart FISH testing or RT-PCR. In addition to *BCR-ABL1* translocation, this dual-color probe FISH helps to detect other *ABL1*-related and possibly TKI-responsive cytogenetic abnormalities associated with T-ALL, such as *EML1-ABL1* and *ETV6-ABL1* fusions or 9q34 duplication associated with therapeutic resistance [7]. Although *BCR-ABL1*-positive T-ALL is rare, the frequency of its detection has increased, with approximately 30 cases reported in the literature and pediatric cases accounting for more than 40% of the total cases. In most reported cases, the prognosis was poor with a median survival of only 7 months (range, 0.1–60 mo), and nearly 50% of the patients died by the time of the last follow-up [8-15].

To summarize, this illustrative report highlights the utility of incorporating routine FISH testing using a *BCR/ABL1* dual-color probe in the workup for T-ALL in resource-constrained settings, especially in the absence of advanced testing, such as ribonucleic acid -sequencing.

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Table 2. Summary of the cases of *NUP214-ABL1*-positive T-cell acute lymphoblastic leukemia [1,5,9-15].

Variable	Value
Total N of cases	65[a)]
Male:female	52:13
Age, median (range; IQR) in years	18 (1–68; 10–28.5)
Total leukocyte count, median (range; IQR)$\times 10^9$/L	54 (1.8–480; 18–122)
Blast % (peripheral blood/bone marrow), median (range; IQR)	86 (10–98; 78–93)
Conventional cytogenetics	
Not available/failed	17 (26.2%)
Normal karyotype	18 (27.7%)
Abnormal karyotype	30 (46.1%)
Method of detection of *NUP214-ABL1* [b)]	
FISH using *BCR/ABL1* probe	45 (69.2%)
FISH using *NUP214* break-apart probe	28 (43%)
Sanger sequencing	7 (10.7%)
Reverse transcriptase PCR	24 (36.9%)
Mate pair sequencing	1 (1.5%)
Overall outcome	
Complete response	32
Relapse	15[c)]
Death	8[c)]
No data	11
N of patients receiving tyrosine kinase inhibitor	4 (6.1%)
Complete response	4 (6.1%)
Relapse	NIL
Death	NIL
Overall survival (N=64), median (range; IQR) in months	17.7 (1 week–194; 7–39.4)
Event free survival (N=46), median (range; IQR) in months	16.8 (1–125; 9.3–36.1)

[a)]Including present case; [b)]Some cases were detected by multiple methods; [c)]Includes patients who had complete response but subsequently relapsed/died.
Abbreviations: FISH, fluorescent in-situ hybridization; IQR, interquartile range; NIL, none; PCR, polymerase chain reaction.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Rapid development of lower leg compartment syndrome following firearm injury in a patient with moderate hemophilia B

TO THE EDITOR: Hemophilia is the most common inherited bleeding disorder caused by factor VIII (hemophilia A) or factor IX (FIX) (hemophilia B) deficiency. The incidence of hemophilia A is 1 in 5,000, and that of hemophilia B is 1 in 30,000 live male births. Increased bleeding tendency is the main clinical manifestation of the disease; however, the bleeding pattern may be highly variable depending on the residual activity of the missing factor. In patients with a severe form of the disease (residual activity <1 IU/dL), spontaneous joint and muscle bleeding are common. In patients with residual factor activity >1 IU/dL, prolonged bleeding after trauma or surgery may predominate in clinical presentation [1].

Firearm injuries in patients with hemophilia, even if treated immediately, undoubtedly have uncertain outcomes. Only two cases of firearm injury in patients with hemophilia have been reported, with one being fatal [2, 3]. Therefore, the clinical course and treatment of these injuries in patients with hemophilia are unknown.

Herein we present a patient with moderate hemophilia B who sustained a gunshot wound to the right lower leg that was complicated by acute limb compartment syndrome (ACS) development.

The patient, born in 1983, was diagnosed with moderate hemophilia B at the age of 10 years after prolonged bleeding following tooth extraction. In the initial finding, the FIX level was 4 IU/dL. The patient never experienced significant spontaneous hemorrhage and received FIX replacement therapy on one occasion in 2015 for pilonidal sinus surgery.

The patient was brought to the emergency department of our institution on January 6, 2019, with a penetrating gunshot wound in the right lower leg, which was sustained after a bullet from a 7.65 mm caliber pistol backfired.

Immediately after the injury, the bleeding was intense, so the patient pressed the wound with gauze to stop the bleeding, as he did not have FIX concentrate at home. Emergency medical personnel were called, and they continued to apply pressure, which was enough to control the bleeding. Owing to harsh winter conditions, it took approximately 3 h until the patient was brought to the emergency