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A triple-probe fluorescent *in situ* hybridisation screening strategy for risk stratified therapy of acute lymphoblastic leukaemia in low resource settings

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Abstract

Karyotyping along with a 3-probe fluorescent *in situ* hybridisation (FISH) strategy was used to risk stratify therapy in 303 children with B-cell precursor acute lymphoblastic leukaemia (BCP-ALL). Of the 166 patients risk stratified, karyotype identified 91 (55%). FISH identified all karyotypes accurately, with the exception of hypodiploidy, and risk-stratified an additional 75 patients. The frequency of *ETV6-RUNX1* is lower and high hyperdiploidy, higher than reported in the west. An adapted 3-probe FISH strategy identified two patients with *ETV6-ABL1* fusion who received Imatinib. In limited-resource settings, a 3-probe FISH approach provides a practical approach for risk-stratified therapy in childhood ALL.

Keywords

Cytogenetics of Leukaemia; Fluorescent in situ hybridisation; Acute Lymphoblastic Leukaemia; Cytogenetic risk stratification; karyotyping; Ph-like ALL

Introduction

B cell-precursor childhood acute lymphoblastic leukaemia (BCP-ALL) is characterised by prognostically significant cytogenetic subgroups. *ETV6-RUNX1* fusions and High

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Conflict of Interest

All authors report no conflict of interest,

Hyperdiploidy (HeH, 51-67 chromosomes) subgroups, observed in 40-50% of western patients, have cure rates exceeding 90% and are categorised as 'low-risk'. [1, 2] *KMT2A* gene rearrangements, *iAMP21* and *BCR/ABL1* fusions, seen in ~10%, identify a 'high-risk' cytogenetic subgroup. The rest occupy the 'intermediate-risk' category. Intensification of therapy, including addition of a tyrosine kinase inhibitor in *BCR/ABL1*, has improved outcomes in high-risk patients. [3] This pre-treatment cytogenetic risk stratification is a standard component of modern risk-adapted therapy in BCP-ALL. Cytogenetic analysis by karyotyping requires fresh samples, specialist laboratories and skilled scientists. In limited-resource countries, this is not readily available, resulting in suboptimal risk stratification and suboptimal therapy. Fluorescent *in situ* hybridisation (FISH) testing is rapid, does not require viable cells and is easily learnt. A triple-FISH strategy, with *ETV6/RUNX1*, *BCR/ABL1* fusion probes and a *KMT2A* break-apart probe reliably identifies major prognostic cytogenetic subtypes except low hypodiploidy/near triploidy(LH/NT).[4] The *ETV6/RUNX1* probe additionally identifies *iAMP21* and HeH, as multiple *RUNX1* signals in this context signify copy number change in the absence of a fusion. [5, 6] The discrete extra *RUNX1* signals here indicate HeH which is confirmed by FISH analysis using additional centromeric probes in the absence of karyotyping.

This triple FISH strategy has been adopted by the Indian Childhood Collaborative Leukaemia (ICiCLE) group to aid centres without dedicated cytogenetic facilities, as part of the risk stratification strategy to de-intensify therapy in low-risk BCP-ALL.

ABL1 rearrangements other than *BCR-ABL1* have been described in BCP-ALL and these cryptic fusions may respond to adjuvant Imatinib therapy [7]. Imatinib is widely available in the developing world at affordable prices. This systematic cytogenetic testing strategy indicated the presence of *ETV6-ABL1* fusion in two patients with newly diagnosed Pre-B ALL, enabling timely introduction of Imatinib therapy. Subsequent sequencing studies confirmed the presence of *ETV6-ABL1* fusions in both the patients. Here, we report our experience using 3-probe FISH analysis to therapeutically risk stratify BCP-ALL patients, coupled with an extended cytogenetic strategy to identify other *ABL1* fusions.

Patients and Methods

Consecutive paediatric patients (age <18 years) diagnosed with BCP-ALL, treated at the Tata Medical Center Kolkata between September 2011 and October 2016 were included in these analyses (n=303). Patients were treated after consent, on a standardised, risk-stratified, ethics-approved clinical protocol. Conventional karyotype and FISH analyses were performed on bone marrow aspirates using standard protocols as described. [8]. To reduce costs, FISH testing was performed as a two-step reflex study. Testing was performed first using the *ETV6/RUNX1* dual-colour dual-fusion FISH probe (Abbott, Illinois, USA). Samples with a negative result for *ETV6/RUNX1* fusion were sequentially probed with a *BCR/ABL1* dual-colour dual-fusion probe (Abbott) and a *KMT2A* dual-colour break-apart probe (Abbott), (Figure 1). Based on karyotype and triple-FISH results, patients were classified as standard and high risk or as B-others. In patients without successful karyotype results, the presence of discrete extra *RUNX1* signals served as a screening tool indicating HeH ALL. HeH was confirmed through FISH analysis using centromeric probes to identify

additional trisomies of chromosomes 4,10 and 17. The cytogenetic characterisation was complete within 5 days of receiving the sample. Patients with 9q34 rearrangement on karyotype and/or an extra *ABL1* FISH signal were further investigated with metaphase FISH and an *ABL1* dual-colour break-apart probe (Zytovision Bremerhaven, Germany) to identify *ABL1*-rearranged non-*BCR/ABL1* ALL. Metaphase FISH analysis was used to detect fusion partners of *ABL1*. FISH analysis using a *PDGFRβ* dual-colour break-apart probe (Zytovision) was performed in patients with poor day 7 prednisolone response and/or those with high end-induction minimal residual disease (MRD 10^{-4}).

Results

Three-probe FISH strategy complements karyotyping

Karyotyping was performed successfully in 252 of 303 BCP-ALL patients (83%), 196 of which were abnormal (Supplemental Figure 1). Karyotyping alone identified prognostically significant subgroups in 91 patients (30%), including HeH in 73, *KMT2A* rearrangements in 7, *BCR/ABL1* in 7 and LH/NT in 4. Three-probe FISH analysis identified all karyotype abnormalities except LH/NT. FISH analysis identified a further 75 patients (25%) with cytogenetic abnormalities, including 34 with *ETV6/RUNX1* fusions; 3 with iAMP21; 35 with HeH (including 8 with normal karyotype and 27 with failed karyotype); 2 with *BCR/ABL1* (1 with normal and the other with failed karyotype) and 1 with *KMT2A* rearrangement (normal karyotype). In all 35 patients with HeH, FISH analysis using centromeric probes for chromosomes 4,10 and 17 confirmed HeH. Thus, the combination of karyotyping and FISH identified standard risk cytogenetics in 142 patients (47%) and high-risk cytogenetics in 24 patients (8%), with the rest (137) classified as intermediate risk (Supplementary Table 1).

Three-probe FISH strategy serves as a screening tool to detect Ph negative *ABL1*-rearranged BCP-ALL

Extended cytogenetic analysis identified 2 patients with *ETV6/ABL1* fusions. In both these patients the *BCR/ABL1* fusion probe revealed an additional *ABL1* signal, which on metaphase FISH was observed to be on the short arm of chromosome 12 (12p). Metaphase FISH with cohybridisation of *ETV6/RUNX1* and *BCR-ABL1* probes on the same metaphase suggested an *ETV6/ABL1* fusion. Sequencing of the genomic fusion breakpoints in both the cases confirmed the *ETV6/ABL1* fusion (type B, genomic breakpoints in intron 5 of *ETV6* and intron 1 of *ABL1*) albeit with different break points.

Discussion

This study represents a large cohort of systematically cytogenetically characterised paediatric BCP ALL patients from India. [9, 10] Three-probe FISH analysis corroborated and complemented karyotyping, enabling cytogenetic categorisation in 166 of 303 patients (55%). FISH analysis alone additionally identified 25% patients with prognostically relevant cytogenetic subtypes. Hypodiploid ALL is however not detected using the 3-probe FISH strategy. In our setting, this limitation has been addressed by incorporating an inexpensive

flow cytometry-based DNA ploidy analysis as part of routine diagnostic immunophenotyping. [11]

The study findings are concordant with the reported lower frequency of *ETV6/RUNX1* fusion in Indian patients. [9] There was a higher incidence of HeH and thus the overall proportion of standard risk cytogenetics remains similar to those reported in the west. The frequency of high risk cytogenetic subtypes (*BCR/ABL1*, iAMP21, Hypodiploidy and *KMT2A* rearranged ALL) is similar to reported literature.

B-other ALL is a cytogenetically indeterminate group and constitutes nearly 50% of paediatric BCP-ALLs seen at our centre. In this cohort, 3-probe FISH analysis identified two patients with *ETV6/ABL1* fusion. In both patients, the detection of an extra *ABL1* signal on the short arm of chromosome 12 led us to suspect *ETV6/ABL1* fusion. Since commercial *ETV6/ABL1* fusion probes are not available, we further investigated by cohybridising *BCR/ABL1* and *ETV6/RUNX1* fusion probes on the same metaphase. In one of these patients an *ABL1* break-apart probe was used to confirm *ABL1* gene rearrangement. Where the *ABL1* break-apart probe is not available, metaphase FISH analysis using the *BCR/ABL1* probe can also detect *ABL1* gene rearrangements where, the extra *ABL1* signal will be observed on a different locus or on a different chromosome.

Contemporary risk-stratified approaches have improved outcomes in BCP-ALL to over 90%. Absence of suitable cost-effective modern diagnostic studies limits risk stratification in resource-constrained settings and contributes to global inequality in outcomes in childhood ALL. [12] Diagnostic approaches using readily available, easily standardised and relatively inexpensive techniques such as the proposed three-probe FISH testing have the potential to successfully address these disparities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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MP, MKS performed cytogenetics and analysed the data; RI and DS were involved in sample identification and performed, MLPA and molecular genetics experiments; MP, DKM, SK and VS were involved in designing the cytogenetic strategy and writing up the paper.

Abbreviation

BCP-ALL	B cell Precursor Acute Lymphoblastic Leukaemia
FISH	Fluorescent in situ Hybridisation
HeH	High Hyperdiploidy

LH/NT	Low Hypodiploidy/ Near triploidy
iAMP21	Intrachromosomal Amplification of chromosome 21

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Figure 1. FISH screening strategy to risk stratify precursor B cell ALL

Table 1
Cytogenetic risk stratification in 303 BCP-ALL patients based on karyotyping and FISH analysis

Cytogenetic Risk Group	Number (%)
Low	
High Hyperdiploidy	108 (36)
<i>ETV6-RUNX1</i>	34 (11)
High	
KMT2A rearrangement	8 (3)
<i>BCR-ABL1</i>	9 (3)
Low hypodiploid/Near triploid	4 (1)
iAMP 21	3 (1)
Intermediate	
Normal Karyotype	38 (12)
Chromosome9p abnormality*	21 (7)
TCF3-PBX1 fusion	15 (5)
Complex karyotype	16 (5)
Failed	13 (4)
6q deletion*	10 (3)
Abnormal 17p	5 (2)
1q duplication	8 (3)
-7/ 7p deletion*	7 (2)
14q32 rearrangement	1 (0.5)
t(8;14)(q24;q32)	1 (0.5)
13q	2 (1)