A RAPID METHOD FOR DETECTION OF BTG1 DELETIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is characterized by impaired differentiation of lymphoid lineage cells. In the genetic context, BCP-ALL is caused by initiating lesions followed by secondary events. In this sense, studies have shown that concomitant *BTG1* and *IKZF1* deletions are associated with a worse prognosis as well as reduced glucocorticoid therapy response in patients with BCP-ALL . Due to the clinical relevance of *BTG1* deletions, our study aimed at developing a multiplex PCR protocol (M-PCR) to identify the different types of recurrent *BTG1* deletions and correlated it with patients clinical-laboratory characteristics.

METHODS

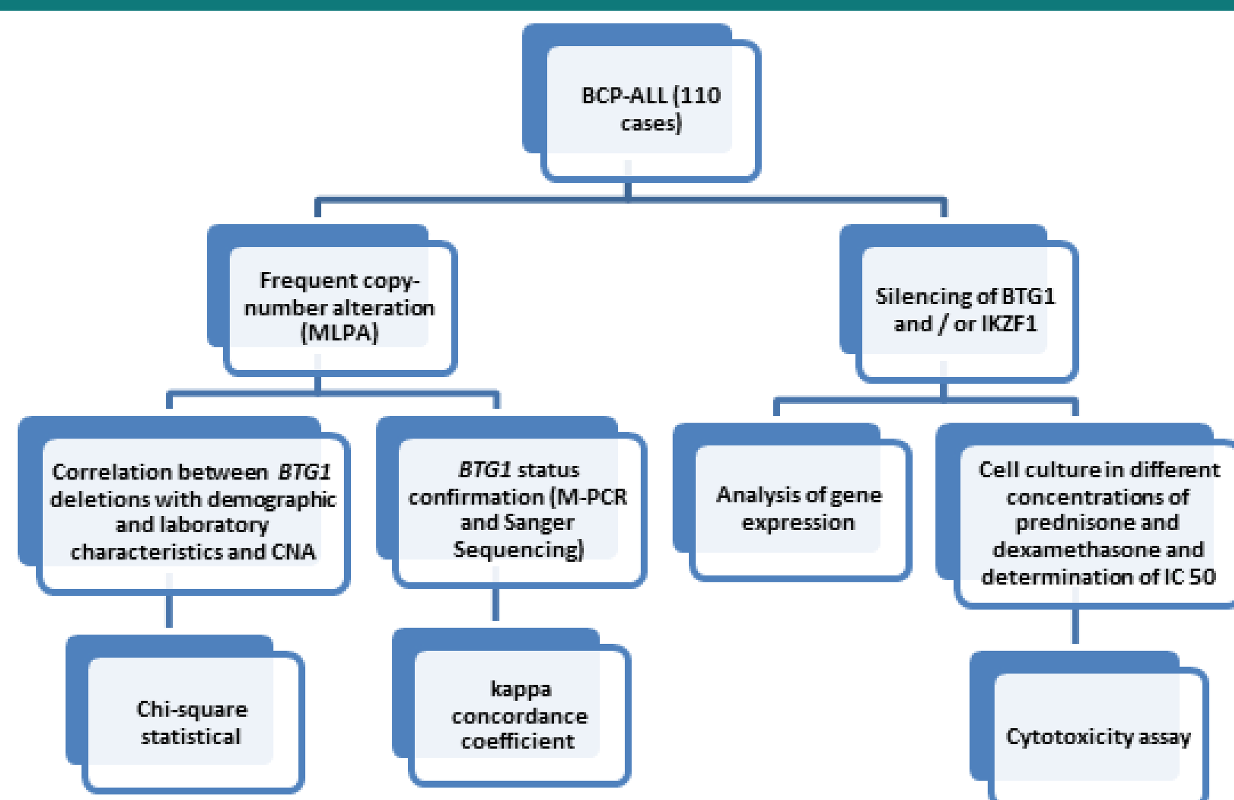

Fig. 1: Flowchart describing the steps of this study, including their methodologies.

RESULTS

BTG1 deletions were found in 10.9% (12/110) of patients. No difference was observed in clinical-laboratory characteristics according to *BTG1* status. On the other hand, *BTG1* deletions were associated with additional alterations in BCP-ALL, including deletions within the pseudoautosomal region 1 (PAR1: represented by *CRLF2, IL3RA, CSF2RA, SHOX* genes) and *ETV6* (P < 0.05). Performing a serial dilution of DNA from REH cell line, we observed that *BTG1* deletions were detected even in a low-blast count scenario (1.25% of REH). M-PCR was able to identify 3 of the 8 known *BTG1* deletions, including: type III deletion (66.6%), IV (16.7%) and V (16.7%). Kappa coefficient revealed a moderate agreement between MLPA and M-PCR techniques (κ = 0.59).

Table 1. Clinical-laboratory data of patients with BCP-ALL.

	All cases n=602	*BTG1* non-deleted	*BTG1* deleted	P-value
	Cases (%)	Cases (%)	Cases (%)	
Sex				.446
Male	62 (56.4)	54 (55.1)	8 (66.67)	
Female	48 (43.6)	44 (44.9)	4 (33.3)	
Age at diagnosis (months)				.620
<12	1 (0,9)	1 (1.0)	0 (0.0)	
13-119	84 (76.4)	76 (77.6)	8 (66.7)	
>120-216	4 (33.3)	71 (21.4)	4 (33.3)	
WBC ($x10^9/L$)				.125
<50	83 (76.2)	76 (78.4)	7 (58.3)	
≥50	26 (23.8)	21 (21.6)	5 (41.7)	
NCI risk				.259
Standard	62 (56.9)	57 (58.8)	5 (41.7)	
High	47 (43.1)	40 (41.2)	7 (58.3)	

Abbreviations: WBC, white blood cell count; NCI, National Cancer Institute of US; BCP-ALL, B-cell precursor acute lymphoblastic leukemia.

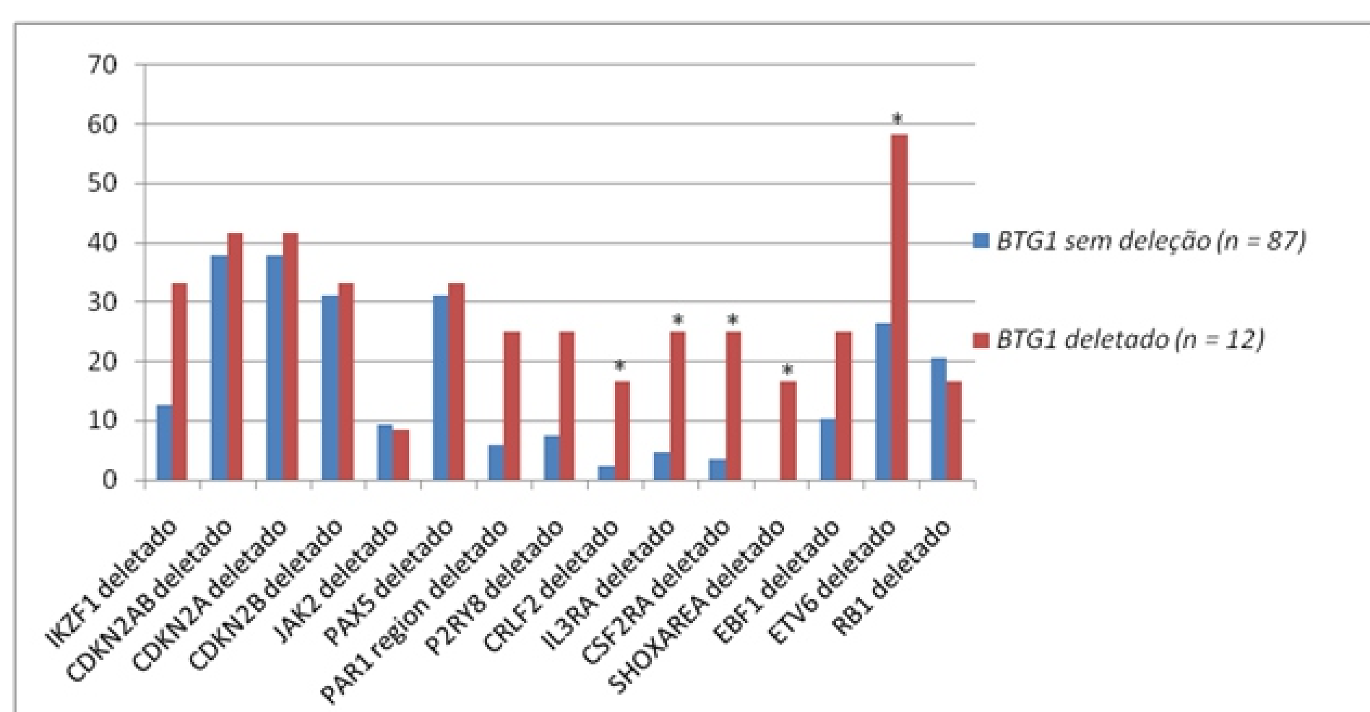

FIG.2: Correlation between *BTG1* status and other additional alterations in BCP-ALL.

[a].*KZF1*status was determined using MLPA (P335 and P202) and M-PCR. All the other alterations were determined solely using MLPA (P335) technique.

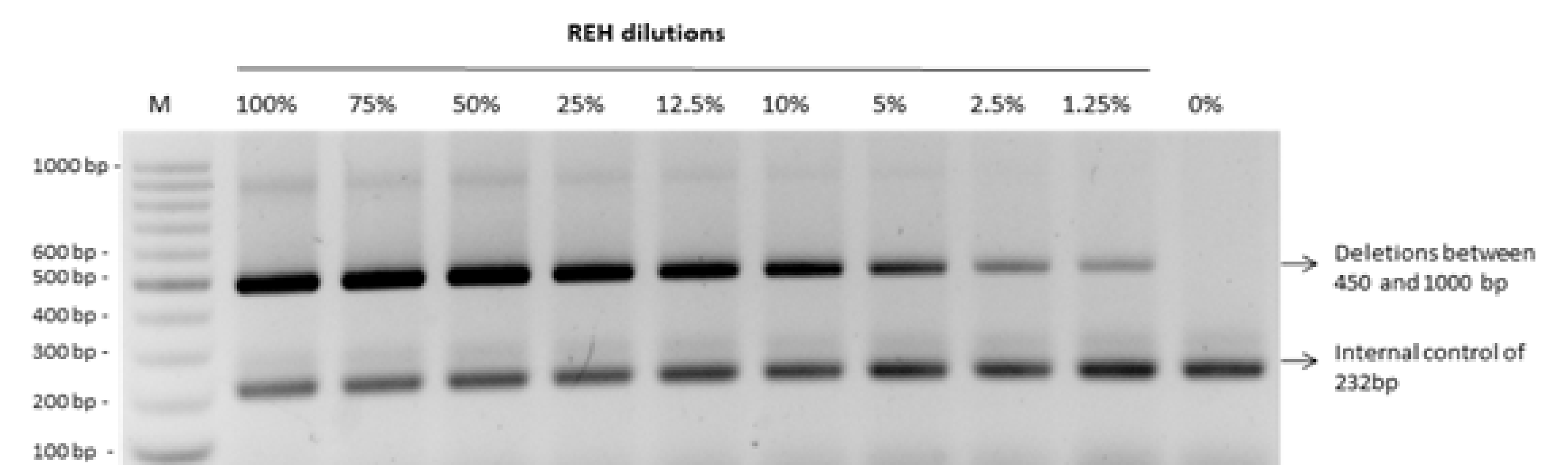

Fig. 3: Agarose gel. M-PCR test of sensitivity by dilution of REH (positive control) and healthy individual DNAs. DNA detection in a low-blast count scenario (1.25% of REH).

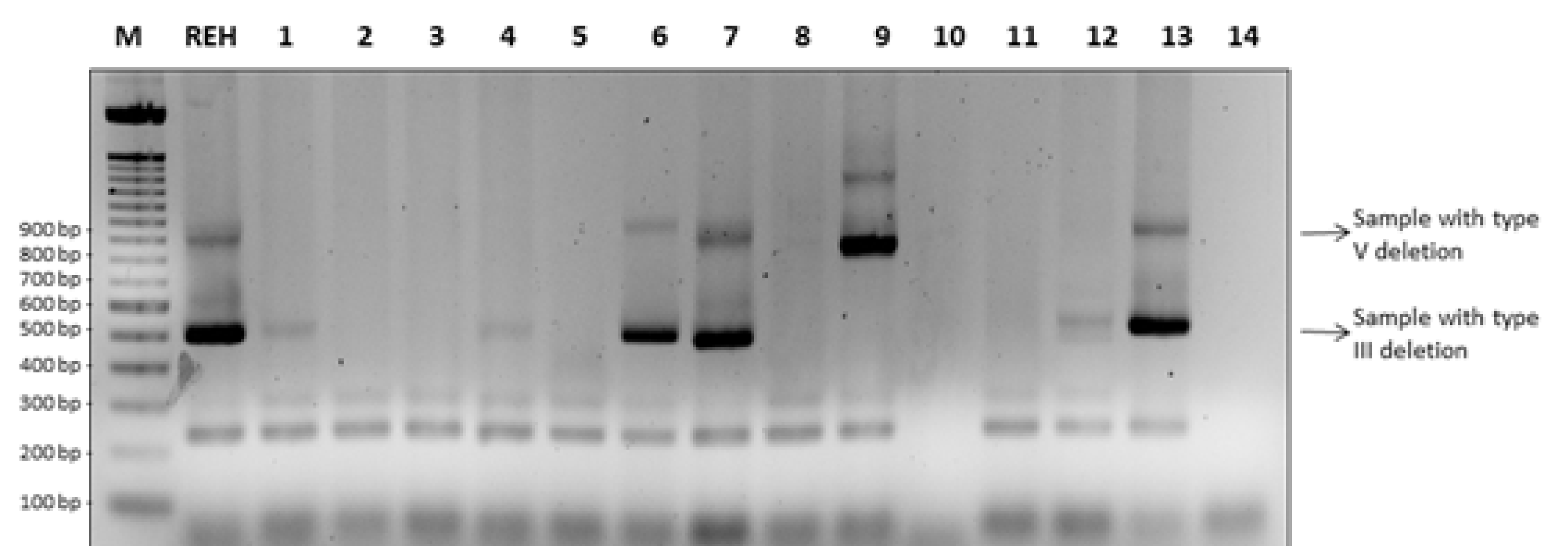

Fig. 4: Agarose gel. Eletrophoresis including REH cell line (positive control to *BTG1* deletions) and patients' samples, showing the detection of different types of deletions.

		M-PCR		
MLPA		*BTG1* non-deleted	*BTG1* deleted	Total
	BTG1 non-deleted	87	4	91
	BTG1 deleted	5	8	13
	Total	92	12	104

Kappa geral	0.591
P-valor geral	< 0.001

Fig. 5: Kappa coefficient revealed a moderate agreement between MLPA and M-PCR techniques (κ = 0.59).

CONCLUSION

We suggest that both MLPA and our newly developed M-PCR should be combined for the evaluation of *BTG1* deletions. As perspectives, we will perform *in vitro* experiments to identify genes related to the glucocorticoid resistance in ALL cases with *BTG1* and *IKZF1* deletions.

Acknowledgment:

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

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