Frequency of *BCR-ABL* fusion transcripts (*e1a2, b2a2 and b3a2*) by RT-PCR in Guatemalan patients with acute lymphoblastic leukemia type B (B-ALL) and chronic myeloid leukemia(CML)

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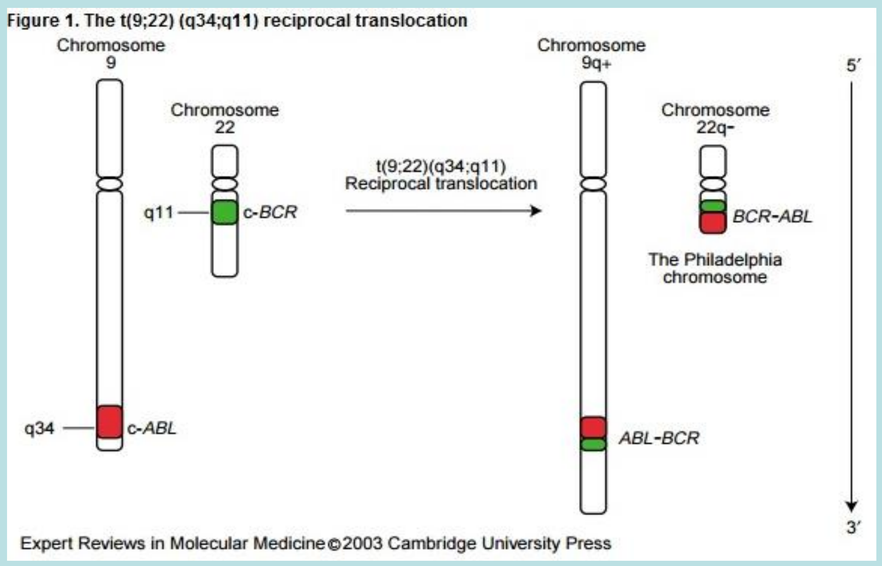
BACKGROUND

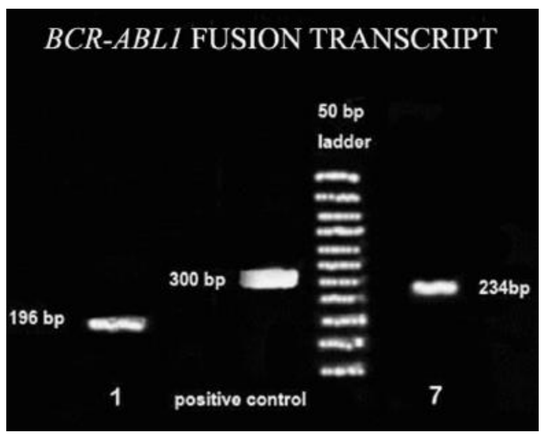
Chromosomal translocations in most leukemias result in fusion genes which produce mRNAs that encode chimeric proteins with different structural and functional properties than the normal constitutional proteins. The best-known case is that of the chimeric gene *BCR-ABL*1 (Philadelphia chromosome), which results from the translocation t(9;22)(q34;q11).

The genes involved in this translocation are the ABL1 gene on chromosome 9 and the BCR gene on chromosome 22. The 5´ portion of the BCR gene is fused to the 3´portion of the ABL1 gene, resulting in the chimeric gene *BCR-ABL1.* Depending on the point of breakage of the translocation, different transcripts may result producing PCR products of somewhat different lengths. These transcripts are present in acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML). It has been shown that there is a relationship between the type of pathological transcript and clinical-demographic characteristics of leukemic patients.



OBJECTIVES

- Describe the frequency of fusion transcripts *BCR-ABL* in bone narrow samples from patients with ALL-B and CML
- Determinate the first genetic characterization of *BCR-ABL* in leukemic Guatemalan population
- Determine the relationship between the type of pathological transcripts and the expressed type of leukemia associated with demographic characteristics and platelet count in leukemic patients



METHODS

Type of study

- Cross-sectional study

Sampling type

- Nonprobability sampling for convenience

Statistical analysis

The analysis was carried out with univariate analysis for frequency distribution, percentages and crossing of variables of interest (gender, origin, age group, platelet count, white blood cell and hemoglobin) using contingency tables based on the type of leukemia and type of fusion transcript. The relationship between variables of interest were determined using the nonparametric Kruskall Wallis test and for pairwise comparisons was used the Mann Whitney test to establish the statistical significance between the data. Results with P <0.05 were considered statistically significant. The software used was *SPSS version 14.0* (SPSS Inc., Chicago, IL, USA).

35 bone marrow samples from patients at diagnostic of ALL-B and CML referred from different national hospitals

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Extracting blasts from bone marrow was via RNAqueous® 4PCR Kit (Applied Biosystems, Ambion)

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The RNA was extracted with the RNAquous kit (Life Technologies, Grand Island, NY)

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To verify the quality of the cDNA obtained, the ABL1 control gene was amplified. The reagents for the PCR procedure were obtained from Novagen (EMD Millipore, Darmstadt, Germany)

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The methodology used in this study was based on studies conducted by Edmans, Kirk, Lee, & Radich, 1994; Lee, Kirk, Edmans, & Radich, 1995; Cerveira, Ferreira, Dória, Veiga, Ferreira et al., 2000.

RESULTS

Distribution of clinical factors by the type of fusion transcript of *BCR-ABL* in patients with ALL-B and CML

Fusion transcript	*e1a2*	*b2a2*	*b3a2*	P ♣
Total (%)	4 (11%)	15 (43%)	15 (43%)	0.004[1]
	♦e1a2 y b2a2 P= 0.010[1]	♦b2a2 y b3a2 P= 0.369[2]	♦e1a2 y b3a2 P= 0.001[1]	
Gender				
Female (F)	2 (5.5%)	6 (17%)	4 (11.5%)	0.612[2]
Male (M)	2 (5.5%)	9 (26%)	11(31.6%)	
Age Group	♦e1a2 y b2a2 P= 0.028[1]	♦b2a2 y b3a2 P= 0.221[2]	♦e1a2 y b3a2 P= 0.009[1]	
Mean ± SD	7 ± 0.82	40 ± 24.24	32 ± 15.46	0.022[1]
Range	7 (6-8)	46 (4-81)	34 (4-64)	
Coefficient of variation	0.12	0.61	0.48	
Platelet count (150,000-400,000 cel/mm³)	♦e1a2 y b2a2 P= 0.009[1]	♦b2a2 y b3a2 P= 0.604[2]	♦e1a2 y b3a2 P= 0.004[1]	
Mean ± SD	8,895 ± 6,641	199,806 ± 163,437	162,267 ± 91,257	
Range	7,970 (2,000-18,000)	184,000 (1,069-516,000)	143,000 (10,000-316,000)	
Q1 (25)	3,415	430,000	109,000	0.012[1]
Q2 (50)	7,970	184,000	143,000	
Q3 (75)	15,570	342,000	234,000	
Coefficient of variation	0.74	0.82	0.56	
White blood cell count (4,500-10,500 cel/mm³)				
Mean ± SD	119,423 ± 37,043	124,073 ± 158,099	22,384 ± 48,534	0.079[2]
Range	124,845 (73,000-155,000)	60,310 (1,050-567,740)	6,170 (1,500-185,000)	
Coefficient of variation	0.31	1.27	2.17	
Hemoglobin (F=12-16 y M=14-18 g/dl)				
Mean ± SD	8.20 ± 4.00	9.95 ± 2.85	11.96 ± 3.36	0.135[2]
Range	8.95 (2.7-12.2)	10.3 (5.6-15.8)	11.6 (5.8-19.1)	
Coefficient of variation	0.49	0.29	0.28	

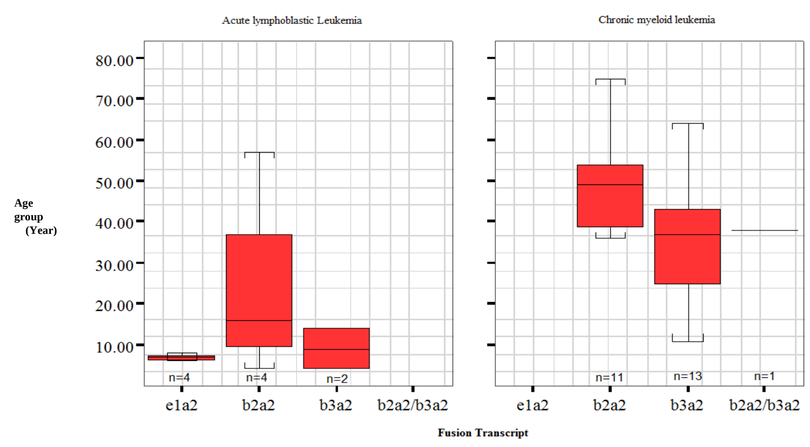
♦ = Mann Whitney ♣ = Kruskal Wallis 1 = significant difference 2 = no significant difference

Distribution of the type of fusion transcript *BCR-ABL* in patients with ALL-B and CML

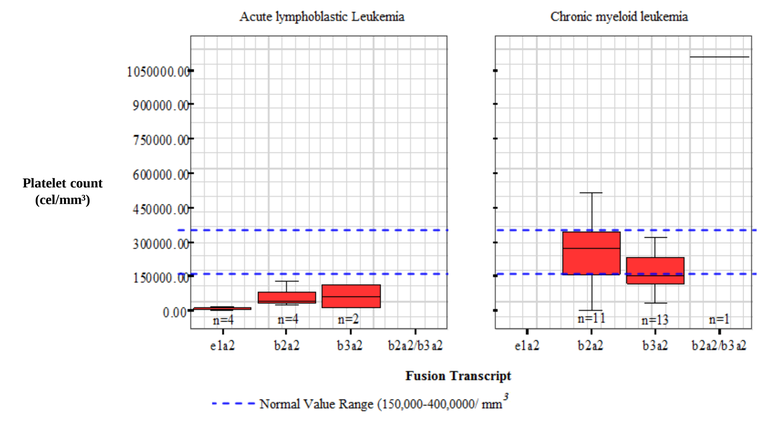
Leukemia	ALL-B		CML		Total (%)
Fusion Transcript	**n* (M/F)**	**M/F %**	**n* (M/F)**	**M/F %**	**n* (%)**
e1a2	4 (2/2)	5.5/5.5	0 (0/0)	0/0	4 (11%)
b2a2	4 (4/0)	12/0	11 (5/6)	14/17	15 (43%)
b3a2	2 (1/1)	2.9/2.9	13(10/3)	28.7/8.6	15 (43%)
b2a2/b3a2	0 (0/0)	0/0	1 (1/0)	3/0	1 (3%)
Total	**10 (7/3)**	**20.4/8.4**	**25(16/9)**	**45.7/25.6**	**35 (100%)**

= Case number , M= Male, F = Female *

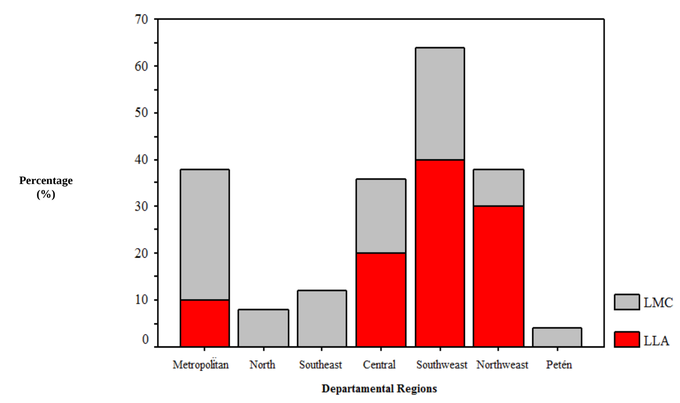
Distribution of age group by the type of fusion transcript of *BCR-ABL* in patients with ALL-B and CML



Distribution of the platelet count based on the type of fusion transcript of *BCR-ABL* in patients with ALL-B and CML



Distribution of geographic region by the type of fusion transcript of *BCR-ABL* in patients with ALL-B and CML



CONCLUSIONS

The frequency of expression of fusion transcripts of *BCR-ABL* gene in 35 bone marrow samples from patients with CML and ALL-B corresponds to a low frequency for *e1a2* transcript (11%) with respect to the frequency of the *b2a2* transcript (43%) and *b3a2* (43%) and with a frequency of 3% co-expression represented by *b2a2/b3a2* transcripts.

The frequency of expression of *BCR-ABL* fusion transcripts in CML patients was very similar to several studies conducted in Latin American countries whose population is mainly mestizo (b2a2 and b3a2 = 40-50%) and differs from that reported in Caucasian populations and Asian in which significant frequency differences between the b2a2 transcript expression (30-40%) compared with b3a2 expression (50-60%) are reported. This suggests that the Guatemalan population expressing leukemic fusion transcripts of *BCR-ABL* gene has a different biological behavior compared to that reported in Asian and Caucasian population product of genetic variability among populations as a possible explanation for the differences observed in this study.

The age group which corresponds to the expression of different chimeric gene transcripts BCR-ABL in CML patients (b2a2 and b3a2) are in the age range 30 to 40 years whereas patients with LAB (e1a2) belong to the ages of 5 to 10 years.

The presence of fusion transcripts of the chimeric BCR-ABL gene increases the thrombopoietic activity in patients with CML.

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