Relationship Between the Type of BCR-ABL Rearrangement and Bone Marrow Histopathological Features in Chronic Myeloid Leukemia

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The aim of the present study was to characterize the type of BCR-ABL transcript and to correlate the molecular feature with bone marrow histology. For this purpose, we analysed the BCR-ABL rearrangement in 26 patients with chronic myeloid leukemia (CML) in the chronic phase by RT-PCR, and we also classified the bone marrow histology according to the predominance of granulocylic (GRAN) or granulocytic and megakaryocytic (GRAM/MEG) proliferation, after analysis of two independent observers. We did not find any significant difference in survival of patients presenting b^2-a^2 and b^3-a^2 transcripts or GRAN and GRAN/MEG bone marrow types, nor did we find any significant correlation of the type of BCR-ABL transcript with the bone marrow histological subgroups GRAN and GRAN-MEG (Fisher's test = 0.31). Thus, we conclude that the presence of exon b^3 is not correlated to bone marrow histology in CML.

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the presence of a reciprocal translocation between chromosomes 9 and 22 in at least 95% of the cases, resulting in a 22q- or Philadelphia (Ph1) chromosome (1, 2). The Ph1 translocation results in the activation of the ABL oncogene of chromosome 9, which becomes contiguous with the 5′ portion of the BCR gene in chromosome 22 (2–4). In chromosome 22, the breakpoint occurs in a region of 5.8 Kb of the BCR gene, called major breakpoint cluster region (M-BCR). The majority of breakpoints occur between exons 2 and 3 (b^2-a^2 rearrangement) or 3 and 4 (b^3-a^2 rearrangement) of M-BCR (5). The consequences of the BCR-ABL translocation are two variants of a hybrid mRNA which may either include or exclude exon b^3 and differ in size by 75 bp. Both transcripts encode a p210 protein with high tyrosine kinase activity (6).

Many authors have reported a significantly shorter median duration of chronic phase or survival in patients whose molecular rearrangement includes exon b^3 (7, 8). However, other studies have reported no significant statistical difference in the chronic phase duration among patients with or without exon b^3 (9–12).

In CML patients, bone marrow morphology can be classified according to the predominance of granulocytic (GRAN) or granulocytic and megakaryocytic (GRAN/MEG) proliferation (13) and it may have a prognostic importance in survival (13–15). Frisch et al. (14) observed a shorter survival in the GRAN type but Lorand-Metze (15) observed a worse prognostic in the GRAN/MEG type. Megakaryocytic proliferation may represent a pre-myelofibrotic state or a sign of future evolution to acceleration in CML (16) and reticulin or collagen fibrosis are thought to be associated with a significant shortening of life expectancy (17). In addition, the subgroup GRAN/MEG seems to be three times more frequent than GRAN type (15).

Previous reports have demonstrated that exon b^3 is involved in thrombopoietic activity (18, 19). However, there are other studies which do not confirm these findings (20–22).

Thus the aim of this study was to characterize the type of BCR-ABL transcript and to investigate if exon b^3 was correlated to bone marrow histology at the CML chronic phase.

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MATERIAL AND METHODS

We analysed 26 CML patients with a follow-up of 7 to 94 months (median 29 months). There were 13 male and 13 female patients and the median age was 48.5 years (ranging from 15 to 77 years). Diagnosis was based on clinical, laboratorial and bone marrow features. Cytogenetic analysis was performed using 24-h culture and G banding (23).

The BCR-ABL rearrangement was detected by reverse transcriptase-polymerase chain reaction (RT-PCR), using samples collected from bone marrow or peripheral blood during chronic phase.

RNA was obtained by guanidine thiocianate method (24). c-DNA was transcribed using 2 μg of total RNA; 100pM abl-RT primer; 6.4 mM dNTP; 10 U RT AMV (USB @-10.000U/ml); 5X RT buffer; 3.72 U RNAsin (Pharmacia @-37.200U/ml); sterile water with 0.1% DEPC to 30 μl. Ten μl of c-DNA were used in 50 μl of PCR with 100pM of 5′ and 3′ primers described by Roth et al. (25). A second reaction using 10 μl of the first PCR product was performed using internal primers (nested PCR) (20). To avoid contamination during the PCR, we used negative controls in all experiments. The PCR product was separated on 1.5% agarose gel and stained by ethidium bromide. The b^2-a^2 rearrangement was identified as a 276 bp fragment and the b^3-a^2 rearrangement as a 351 bp fragment (Figure). All positive samples were double checked by a second RT-PCR reaction.

Bone marrow biopsy was performed at posterior iliac crest, using a Jamshidi needle, during the chronic phase, and the tissue was paraffin-embedded. The 4 μm thick sections were stained by hematoxilin-eosin (H&E), Giemsa and silver impregnation. They were then classified as GRAN or GRAN/MEG types (13) after analysis by two independent observers. GRAN type was characterized by neutrophils proliferation, groups of immature granulocytic precursors, atypical megakaryocytic proliferation (with micro-megakaryocytes) and evident reticulin fibrosis.

The analysis of 2×2 table which correlated bone marrow histological subgroups with BCR-ABL transcripts, was performed using Fisher's test (26). Median survival analysis was calculated by use of Kruskal-Wallis test (26).

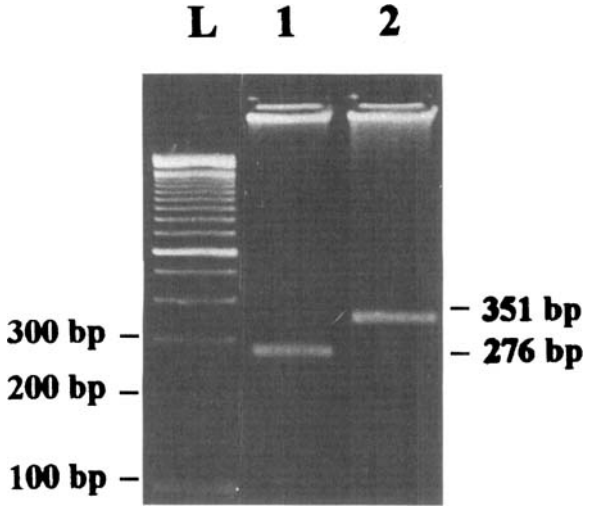

Figure. Agarose gel electrophoresis (1.5%), stained by ethidium bromide, of BCR-ABL nested PCR. (1) b2-a2 rearrangement (276 bp); (2) b3-a2 rearrangement (351 bp).

RESULTS AND DISCUSSION

When this study came to an end (July 1995), 17 patients were alive (one of them 5 months after allogeneic bone marrow transplantation), 7 had died (3 patients after allogeneic bone marrow transplantation, one post-septicemia and 3 after evolution to blast crisis) and 2 had left treatment during the chronic phase (with 7 and 21 months of follow-up, respectively).

Cytogenetic analysis was performed in 23 out of 26 cases (88.5%) and Ph1 chromosome was present in 82.6% of them. The t(9;22) was absent in one case and mitosis were not available in 3(13.1%) of the cases. Molecular analysis showed BCR-ABL rearrangement in all the cases studied.

The possible implications of the presence of exon b^3 in M-BCR region during the formation of Ph1 chromosome have been extensively studied. Its importance in the chronic phase duration, median survival and its correlation with clinical features has been previously described, but the conclusions are still conflicting (7–12). Considering that megakaryocytic proliferation and fibrosis might be related to the acceleration of CML and unfavourable prognosis (17) and a possible involvement of exon b^3 in thrombopoietic activity (18, 19) we attempted to correlate bone marrow histology with the type of BCR-ABL transcripts. As far as we know, this is the first study comparing these aspects.

The frequencies of b^2-a^2 and b^3-a^2 rearrangement were 30.8% (8/26) and 69.2% (18/26) respectively. Thus, our study showed a predominance of the type b^3-a^2, different from the findings of another population (8, 10) but in accordance to the results of Inokuchi et al. in Japan (18).

Concerning the bone marrow histology, type GRAN was detected in 38.5% (10/26) and GRAN/MEG in 61.5% (16/26). Bone marrow histology of those cases treated with allogeneic bone marrow transplantation (4/26 or 15.4%) revealed 3 cases of GRAN/MEG and one case of GRAN type. There was no significant difference in the median survival of patients presenting type GRAN and GRAN/MEG ($p = 0.3836$).

Among the GRAN subgroup, 2 cases (7.7%) presented b^2-a^2 and 8 cases (30.8%) presented b^3-a^2 rearrangement. For GRAN/MEG subgroup, 6 cases, (23.15%) presented b^2-a^2 and 10 (38.4%) b^3-a^2 rearrangement. However, we did not find significant correlation between the type of BCR-ABL rearrangement and bone marrow histology (Fisher's test = 0.31) (Table).

Table

Correlation between bone marrow histology (GRAN and GRAN/MEG) and BCR-ABL rearrangement

BCR-ABL rearrangement	Bone marrow histology		Total
	GRAN	GRAN/MEG	n (%)
b^2-a^2	2 (7.7%)	6 (23.1%)	8 (30.8%)
b^3-a^2	8 (30.85)	10 (38.4%)	18 (69.2%)
Total	10 (38.5%)	16 (61.5%)	26 (100.0%)

Fisher's test = 0.31

Previous reports have suggested that the type of BCR-ABL rearrangement may be responsible for the duration of the chronic phase of CML (7, 8). On the other hand, recent studies failed to demonstrate a correlation between breakpoint and evolution of CML (9–12). Thus, it may be possible that the molecular variability of the BCR-ABL rearrangement in CML represents a genetic polymorphism without any clinical and prognostic importance.

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