References

1. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med 1981; 304:1529-33.
2. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Kolb HJ, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 1990; 75:555-62.
3. Russell JA, Larratt L, Brown C, Turner AR, Chaudhry A, Booth K, et al. Allogeneic blood stem cell and bone marrow transplantation for acute myelogenous leukemia and myelodysplasia: influence of stem cell source on outcome. Bone Marrow Transplant 1999; 24:1177-83.
4. Ringden O, Labopin M, Gluckman E, Reiffers J, Vernant JP, Jouet JP, et al. Strong antileukemic effect of chronic graft-versus-host disease in allogeneic marrow transplant recipients having acute leukemia treated with methotrexate and cyclosporine. The Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Transplant Proc 1997; 29:733-4.
5. Blaise D, Kuentz M, Fortanier C, Bourhis JH, Milpied N, Sutton L, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Société Française de Greffe de Moelle. J Clin Oncol 2000; 18:537-46.
6. Brunet S, Urbano-Ispizua A, Ojeda E, Ruiz D, Moraleda JM, Diaz MA, et al. Favourable effect of the combination of acute and chronic graft-versus-host disease on the outcome of allogeneic peripheral blood stem cell transplantation for advanced haematological malignancies. Br J Haematol 2001; 114:544-50.
7. Powles R, Mehta J, Kulkarni S, Treleaven J, Millar B, Marsden J, et al. Allogeneic blood and bone-marrow stem-cell transplantation in haematological malignant diseases: a randomised trial. Lancet 2000; 355:1231-7.
8. Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 2001; 344:175-81.
9. Vigorito AC, Azevedo WM, Marques JF, Azevedo AM, Eid KA, Aranha FJ, et al. A randomised, prospective comparison of allogeneic bone marrow and peripheral blood progenitor cell transplantation in the treatment of haematological malignancies. Bone Marrow Transplant 1998; 22:1145-51.
10. Schmitz N, Bacigalupo A, Hasenclever D, Nagler A, Gluckman E, Clark P, et al. Allogeneic bone marrow transplantation vs filgrastim-mobilised peripheral blood progenitor cell transplantation in patients with early leukaemia: first results of a randomised multicentre trial of the European Group for Blood and Marrow Transplantation. Bone Marrow Transplant 1998; 21:995-1003.

Younger age and shorter chronic phase in b2a2-positive chronic myeloid leukemia adults with high white blood cell count at diagnosis

Controversial results between the bcr-abl mRNA type and its relationship to clinical data at presentation in chronic myeloid leukemia (CML) have been reported. We analyzed 71 adults with chronic phase CML using reverse transcription polymerase chain reaction (RT-PCR) validated by sequencing and observed that in a subgroup with elevated white blood cell (WBC) count at diagnosis, younger patients and shorter chronic phase were more commonly registered in b2a2-positive individuals.

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CML is a myeloproliferative disorder characterized by a neoplastic expansion of the granulocytic and megakaryocytic lineages,[1] as well as by the presence of the Philadelphia chromosome as the result of a reciprocal translocation between chromosome 9 and 22 in more than 90% of CML patients.[2] At the molecular level, the 5´ sequences of the bcr gene on chromosome 22 become fused to the 3´ sequences of the abl proto-oncogene on chromosome 9, generating either b2a2 or b3a2 mRNA.[3]

Discrepancies between various studies on the bcr-abl chimeric mRNA type and its possible association with differences in the clinical features observed during the course of CML generated a debate protracting for several years,[4,5] with some evidence in favor[6] and some against.[7] In order to gain more information on this apparent association, we analyzed 71 patients with CML in first chronic phase. The diagnosis of CML was established on the basis of bone marrow aspirates, supported by cytochemical analysis and low alkaline phosphatase activity in granulocytes. We standardized a RT-PCR technique to co-amplify the bcr-abl junc-

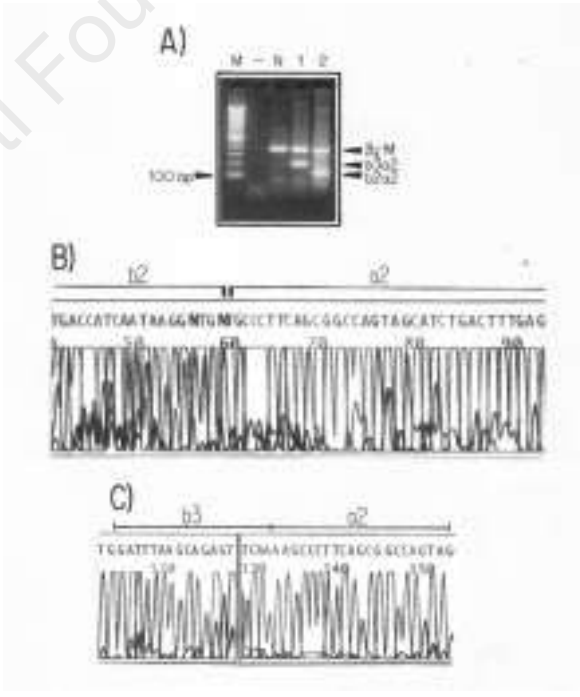

Figure 1. Detection of the bcr-abl rearranged mRNA from RNA samples of chronic myeloid leukemia (CML) adults by RT-PCR. (A) A representative gel that shows results of the RT-PCR from a healthy individual (lane N) and two CML patients in chronic phase (lanes 1 and 2). The patients carried a b3a2 (lane 1; 194-bp fragment) or b2a2 (lane 2; 119-bp fragment) fusion transcript. A fragment of 370 bp was co-amplified from the β_2-microglobulin gene (β_2-M) as an internal control. Water was used as a negative control instead of RNA in the reverse transcription reaction (lane -). Lane M: 100-bp DNA ladder. Nucleotide sequence of the RT-PCR amplified mRNA containing the *bcr-abl* junction region from two CML patients who expressed a b2a2 (B) or b3a2 (C) transcript type.

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Table 1. Clinical parameters at diagnosis of CML patients in chronic phase (CP) expressing the b2a2 or b3a2 type of the bcr-abl rearranged mRNA.

			Stratification of the patients on the basis of WBC count			
			WBC < 100×10⁹/L		*WBC > 100×10⁹/L*	
	b2a2	*b3a2*	*b2a2*	*b3a2*	*b2a2*	*b3a2*
Parameter	*n = 32*	*n = 38*	*n = 7*	*n = 8*	*n = 25*	*n = 30*
Male/Female	19/13	19/19	4.3	5.3	15.10	14/16
Age (yrs)						
Mean±SEM	39.7±2.89	47.1±2.47	48.4±7.21	43.9±5.2	37.2±3.0*	48±2.83*
Median (range)	36 (18-75)	44 (22-74)	40 (28-75)	42.5 (22-69)	36 (18-67)	47 (22-74)
WBC×10⁹/L						
Mean±SEM	218.3±27.3	239.4±24.9				
Median (range)	201 (18-750)	230 (10-694)				
Platelets ×10⁹/L						
Mean±SEM	470.2±62.7	545.7±54.9				
Median (range)	356 (39-1692)	465.5 (120-1395)				
Risk profile by Sokal						
Low	10	12				
Intermediate	9	17				
High	13	9				
Risk index (high/low)	1.3	0.75				
	n = 23	n = 25				
CP duration (months)						
Mean ± SEM	37.6±4.9	49.2±4.2				
Median (range)	35 (10-95)°	51 (12-81)°				

**p = 0.012 (unpaired Student t test); °p= 0.044 (Mann-Whitney rank sum test).*

tion region and the β_2-microglobulin gene (β_2-M) as an endogenous control to confirm RNA integrity, and applied this technique on one microgram of RNA extracted from Ficoll-separated peripheral blood mononuclear cells by the acid guanidinium thiocyanate/phenol-chloroform method.[8] Appropriate controls were carried out for each RT-PCR assay. Amplified fragments from the *bcr-abl* rearranged mRNA generated by RT-PCR were visualized on a 2% agarose gel containing ethidium bromide (Figure 1A). To validate the RT-PCR specificity, the amplified material of some CML cases were directly sequenced on both strands with the same primers employed for PCR, using a BigDye Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturer's instructions. Obtained nucleotide sequences were compared with the reported sequence for the major breakpoint *bcr-abl* junction region and, as expected, the 119-bp and 194-bp amplification bands corresponded to the b2a2 (Figure 1B) or b3a2 (Figure 1C) junction type, respectively.

Once the RT-PCR technique had been validated, the consecutive patients were classified on the basis of the PCR product size. As a result, the b2a2 variant was detected in 32 (45.1%) cases, 38 (53.5%) patients had b3a2 and only one patient (1.4%) carried both types of transcripts. The clinical data at diagnosis of patients of the b2a2 and b3a2 groups were compared: i.e. sex, age, WBC count, platelet count, risk profile and chronic phase duration (Table 1). No statistically significant difference in any clinical parameter was found between the two groups of patients, except for a tendency of the b2a2 individuals to be younger ($p = 0.052$). Interestingly, when the few patients whose WBC count was < 100×10⁹/L were omitted from the statistical analysis, the difference in the mean age at diagnosis between the two groups was statistically significant ($p = 0.012$), suggesting that patients with the b2a2 variant are younger than those expressing b3a2. Although the risk index is higher in b2a2 than b3a2 patients, the stratification of the patients into three risk categories according to the Sokal criteria[9] is unrelated to bcr-abl transcript type ($p = 0.24$).

On the other hand, the patients were treated with hydroxyurea or busulphan and followed during disease progression to determine the length of the chronic phase. Altogether 22 of 71 patients were excluded from this follow-up study because they had inadequate clinical information, due to change in the treatment or because they continued in chronic phase. The clinical follow-up of the remaining 49 patients showed a difference in the median duration of chronic phase among the b2a2 (35 months) and b3a2 (51 months) individuals ($p = 0.044$), again suggesting a greater risk for b2a2 patients.

Although the present study includes a small number of patients and a large multicenter study is required, our data indicate that in a subgroup of patients in chronic phase CML with a high WBC count at the time of diagnosis, younger patients and shorter chronic phase duration were more commonly registered in the group that expressed the b2a2 mRNA.

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References

1. Lichtman M, Liesveld J. Chronic myelogenous leukemia and related disorders. In: Beutler E, Lichtman M, Coller B, Kipps T, Seligsohn U, eds. Williams Hematology, 6th ed. New York: McGraw-Hill, 2001:1085-23.
2. Rowley J. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 1973; 243:290-3.
3. Deininger M, Goldman J, Melo J. The molecular biology of chronic myeloid leukemia. Blood 2000; 96:3343-56.
4. Lee S. Chronic myelogenous leukaemia. Br J Haematol 2000; 111:993-1009.
5. Melo J. The diversity of Bcr-Abl fusion proteins and their relationship to leukemia phenotype. Blood 1996; 88:2375-84.
6. Bianchi C, Cairoli R, Marenco P, Muti G, Del Monte U, Perego R. Detection by polymerase chain reaction of bcr/abl transcripts in myeloproliferative diseases at time of diagnosis and for monitoring chronic myelogenous leukaemia patients after bone marrow transplantation. Eur J Cancer 1995; 31A:197-201.
7. Shepherd P, Suffolk R, Halsey J, Allan N. Analysis of molecular breakpoint and mRNA transcripts in a prospective randomized trial of interferon in chronic myeloid leukaemia: no correlation with clinical features, cytogenetic response, duration of chronic phase, or survival. Brit J Haematol 1995; 89:546-54.
8. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156-9.
9. Sokal J, Cox E, Baccarani M, Tura S, Gomez G, Robertson J, et al. Prognostic discrimination in good-risk chronic granulocytic leukemia. Blood 1984; 63:789-99.

Significance of real-time quantitative polymerase chain reaction detection of p16 gene deletions in childhood acute lymphoblastic leukemia

We show that real-time polymerase chain reaction can detect the majority (40/55; 80%) of patients with homozygous deletion of the p16 gene as well as those with germline p16 configuration. The remaining samples (11/55; 20%) should be interpreted with caution. Patients with p16 deletion show a worse prognosis and TEL/AML1-positive children do not accumulate in any p16-defined group.

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Deletion of p16ink4A gene is one of the most frequent genetic alterations in childhood acute lymphoblastic leukemia (ALL) with a particularly high incidence in T-ALL.

Recently, an effort has been made to examine this abnormality in ALL patients by new technology, real-time quantitative polymerase chain reaction (RQ-PCR).[1-3] The published data concerning the usefulness of this method are to some extent contradictory. Carter *et al.*[1] are strongly convinced that RQ-PCR is able to distinguish not only between samples with germline and homozygously deleted p16 genes but, moreover, can reveal those patients with hemizygous deletion. The alternative view of Einsiedel *et al.*[2] suggests that the RQ-PCR technique is suitable only for the detection of homozygous deletion. The data published in the study by M'Soka *et al.*[3] also show some technical ambiguity.

We used RQ-PCR with LightCycler™ technology to examine 56 children with ALL (54 newly diagnosed patients and 2 children at relapse) diagnosed in 1999 in the Czech Republic. The genomic DNA (gDNA) and complementary DNA (cDNA) levels of p16 gene were successfully analyzed in 55 and 54 patients, respectively. The amount and quality of gDNA and cDNA were determined using RQ-PCR amplification of β-2-microglobulin (β2M) gene in two separate systems. Sequences of oligonucleotides used for RQ-PCR are listed in Table 1.

Examination of genomic DNA and normalized values of [gDNA p16]/[gDNA β2m] ratio adjusted to the percentage of leukemic blasts at diagnosis divided patients into three groups: (1) patients with a low p16 content in leukemic blasts (<20%) due to homozygous deletion (22/55 patients; 40%); (2) patients with normal p16 content (>80%) and germline p16 (22/55 patients; 40%); (3) patients with a medium p16 content (30-65%) (11/55 patients; 20%). The distribution of p16 gDNA subgroups among patients with different immunophenotype was as follows: mature B-ALL (n=1): medium p16 content; pre-B and common ALL (n=43): low p16 content 14/43=33%, medium 9/43=21%, normal 20/43=47%; pro-B ALL (n=2): low p16 content 2/2=100%; T-ALL (n=9): low p16 content 6/9=67%, medium 1/9=11%, normal 2/9=22%.

We next analyzed the presence of TEL/AML1 and BCR/ABL fusion genes in our patients. Whereas both BCR/ABL-positive patients had normal p16 status, TEL/AML1-positive patients were distributed homogeneously within all three groups (4, 2 and 4 patients with low, medium and normal p16, respectively). Five patients from our cohort had an *event* (early death n=3; death in remission n=1; relapse n=1). All but one belonged to the p16 low gDNA group; the remaining *event* was early death of a BCR/ABL-positive patient with a normal content of genomic p16. There were only two patients examined at relapse in our original cohort and both had low p16 gDNA. This is in agreement with previously published data.[4]

Table 1. The primers and probes for RQ-PCR amplification of p16 and β2M genes.

Oligonucleotide	*5'_3' sequence*	*Application*
p16-F	tggacctggctgaggagct	primer
p16-gDNA-R	tcctcacctgagggaccttcc	primer
p16-cDNA-R	tcaatcggggatgtctgag	primer
p16-LC	ctgcctctggtgccccccgc	probe
p16-FL	gcggcatctatgcgggcatggtt	probe
β2m-F	ccagcagagaatggaaagtc	primer
β2m-gDNA-R	agtgggggtgaattcagtgtag	primer
β2m-cDNA-R	gatgctgcttacatgtctcg	primer
β2m-LC	atgaaacccagacacatagcaattcag	probe
β2m-FL	ttcttcagtaagtcaacttcaatgtcgga	probe