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Detection of *bcr/abl* mRNA in a case of chronic myelogenous leukemia in long-term remission: CML or sensitivity of detection?

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Abstract

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder which, after a chronic phase which lasts an average of 3 years, evolves into an acute disease which is resistant to chemotherapy. Nevertheless, a few studies have reported cases in which partial or complete hematologic, cytogenetic and/or molecular remission of the disease were observed either spontaneously or after non intensive chemotherapy, with or without medullar aplasia. Some of these patients later relapsed into a blast crisis. We report a case of CML with clinical and hematologic remission for 19 years after two cycles of busulphan not causing medullar aplasia, negative for the BCR/ABL gene by Southern blot but with the gene's mRNA detectable by hot start nested RT-PCR.
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Key words: chronic myelogenous leukaemia, BCR/ABL, nested RT-PCR, Southern blot, remission, prolonged survival

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder which presents a biphasic pattern, beginning with a chronic stage with a mean duration of 3 years followed by an acute phase, similar to acute leukemia, which is resistant to chemotherapy.[1,2] More than 95% of patients with CML bear the Philadelphia chromosome (Ph1) which originates from the reciprocal translocation t(9;22)(q34;q11), which gives rise to rearrangement of the BCR and ABL genes.[1] We describe a case of CML with complete clinical and haematologic remission accompanied by a negative value for the BCR/ABL gene by Southern blot although the gene's mRNA was detected by hot start nested RT-PCR at 19 years evolution after two cycles of busulphan without medullar aplasia.

Case Report

A 42-year-old woman was referred to our hospital in March 1978 on detecting in a routine hemogram 120×10^9/L leucocytes (WBC) (polymorphonuclear neutrophils 56%, bands 14%, eosinophils 1%, basophils 4%, metamyelocytes 10%, myelocytes 4%, promyelocytes 1%, lymphocytes 8%, monocytes 2%), hemoglobin (Hb) 14.7 g/dL and a platelet count of 280×10^9/L. Physical examination revealed splenomegaly extending 8 cm below the costal margin. The patient's bone marrow was markedly hypercellular and the amount of fat was greatly reduced. Granulopoiesis was dominant, erythropoiesis was considerably decreased, the megakaryocyte count was normal, there was an increase in basophils and sea-blue histiocytes were present. The cytogenetic study revealed Ph1 cells in the bone marrow. The patient received 5 mg/day of busulphan for 5 weeks. The lowest peripheral blood counts (PB) achieved during treatment and in the following two weeks were: WBC 7×10^9/L, Hb 12.5 g/dL, 160×10^9/L platelets; the splenomegaly disappeared. The patient remained stable with normal PB levels until March 1979 when her leucocytes increased to 18×10^9/L. Busulphan treatment was reinitiated, 0.5 mg/day for 5 weeks and the WBC decreased to 4.9×10^9/L. There were no signs of bacterial infection at any time. From this moment on the patient has maintained normal PB levels (WBC between 4 and 5×10^9/L and platelet count 100-200×10^9/L in most of the tests).

After diagnosis, the bone marrow was aspirated in May 1989 but this could not be repeated because of the patient's refusal. This bone marrow showed no cytogenetic alterations, or rearrangement of the BCR and ABL genes detectable by Southern blot, performed as described previously.[3] Rearrangement of the BCR/ABL gene was not detected in repeats of Southern blot carried out on peripheral blood cells in July 1992 and October 1994. In March 1997, 19 years after the diagnosis, hot start nested RT-PCR was performed, using the technique described elsewhere,[4] on peripheral blood cells. this technique revealed the presence of the P210 b3a2 type of BCR/ABL mRNA (a 268 bp band) (Figure 1). At the same time a weak 190 bp band corresponding to a P190 hybrid BCR/ABL transcript (e1a2 junction) was detected (Figure 1). None of the negatives controls were contaminated. Table 1 shows how the patient has remained in hematologic and clinical remission over 18.5 years.

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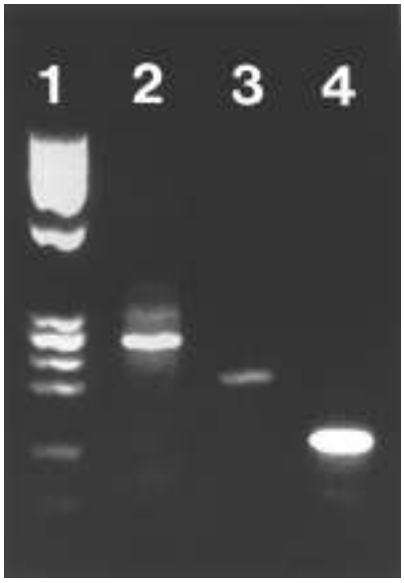

Figure 1. cDNA amplification of the patient's PB analyzed by hot start nested RT-PCR. Line 1: Molecular weight marker (DNA of phiX174 digested with Hae III). Line 2: Amplification of the P210 type of BCR/ABL transcript showing the positivity for the b3a2 junction. Line 3: Amplification of the P190 type of BCR/ABL (e1a2 junction) showing the patient's positivity. Line 4: Amplification of the non rearranged ABL gene (it is always used as internal control). Positive and negative controls were included in all the analyses but are not shown.

Discussion

In our CML case, it appears that a low level of BCR/ABL positive cells, below the detection capacity of Southern blot,[3] persisted and mRNA of BCR/ABL was detected in peripheral blood by nested hot start RT-PCR, a procedure which is sensitive enough to detect one BCR/ABL positive cell in 10^7 BCR/ABL negative cells (Figure 2). However, long-term remission, as defined by normal hematologic and clinical features, was maintained over 18.5 years, even six months after the RT-PCR analysis. This remission was achieved with only two 5-week periods of normal dose of busulphan which did not signs of produce medullar aplasia.

In some cases the Ph1 chromosome can be made negative by allogeneic bone marrow transplantation, interferon-α or intensive chemotherapy, although the only potentially curative option is the first.[5,6] Isolated cases of cytogenetic and molecular remissions confirmed by Southern blot have been described in patients receiving long-term non-intensive chemotherapy.[7] In some cases cytogenetic remission was achieved after a period of medullar hypoplasia.[8] In other cases, both cytogenetic and molecular remissions were spontaneous with a decreased Ph1-positive metaphase,[9,10] or disappearance of the rearrangements detected by Southern blot,[11] although 2 of these patients died after 3 and 8 years follow-up after a blast crisis.[9,11] Also, another recent study reported

Table 1. The patient's hematological and biological data.

					Differential count									
Date	*Spleno-megaly*	*Hb g/dL*	*Plts x10⁹/L*	*WBC x10⁹/L*	*Band forms %*	*Segm. cells %*	*Baso-phils %*	*Eosino-phils %*	*Lympho-cytes %*	*Mono-cytes %*	*Others %*	*Karyotype*	*Southern blot*	*RT-PCR*
3/3/78	8 cm	14.7	280	120	14	56	4	1	8	2	15*	t(9;22)		
3/13/79	NO	14.8	131	18.1	2	55	4	1	31	7	0			
4/15/80	NO	15.5	141	4.1	1	59	1	0	33	6	0			
4/23/81	NO	16.3	130	5	0	51	3	0	38	8	0			
4/27/83	NO	15.9	99	4.6	0	47	0	0	40	13	0			
2/27/85	NO	15.4	78	4.3	0	45	1	0	50	4	0			
6/24/87	NO	15	148	4.4	0	40	1	1	56	2	0			
5/24/89	NO	16	144	4.8	0	50	0	0	44	6	0	normal	BCR/ABL negative	
4/24/91	NO	15.6	155	4.4	0	42	0	0	51	7	0			
6/24/92	NO	15.9	145	4.2	0	47	0	0	47	6	0		BCR/ABL negative	
10/18/94	NO	15.6	177	4.3	0	44	0	0	49	7	0		BCR/ABL negative	
4/10/96	NO	15.5	161	4.3	0	46	0	1	43	10	0			
3/17/97	NO	15.8	155	4.3	0	44	0	0	48	8	0			P210 P190
11/5/97	NO	15.7	136	4.4	0	64	0	0	30	6	0			

**Metamyelocytes 10%, myelocytes 4%, promyelocytes 1%.*

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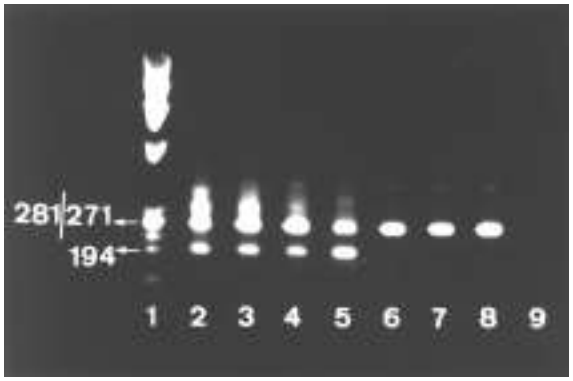

Figure 2. Estimation of the sensitivity of the hot start RT-PCR for mRNA of P210 type of BCR/ABL. Electrophoresis in 1.5% agarose gel of 12 µL of the amplification product. Line 1: Molecular weight marker (DNA of phiX174 digested with Hae III). Line 2: RT-PCR analysis of a mixture with 1 µL of RNA from a known b3a2 (268 bp band) and b2a2 (193 bp band) P210 positive lymphoblastic leukemia with a concentration of 1 µg/µL diluted in 9 µL from a known BCR/ABL negative lymphoblastic leukemia RNA with a concentration of 1 µg/µL. Line 3: Amplification product of a mixture with 1 µL of the former mixture diluted in a 9 µL from the BCR/ABL negative lymphoblastic leukemia RNA (dilution of 1 in 10^2). Line 4: RT-PCR analysis of the dilution of 1 in 10^3. Line 5: RT-PCR analysis of the dilution of 1 in 10^4. As in the previous dilutions we can see a band of 268 bp corresponding to the b3a2 type of junction and a fragment of 193 bp corresponding to the b2a2 junction. Line 6: RT-PCR analysis of the dilution of 1 in 10^5. Line 7: RT-PCR analysis of the dilution of 1 in 10^6. Line 8: RT-PCR analysis of the dilution of 1 in 10^7. The last three lines show the presence of the 268 bp fragment only. Line 9: Negative control.

a case of spontaneous hematologic remission with disappearance of the Ph1 chromosome and the BCR/ABL hybrid measured by Southern blot and RT-PCR.[6] In another two patients, after administration of hydroxyurea for 10 years to one and busulphan for 18 months to the other, cytogenetic and molecular remission according to Southern blot and RT-PCR techniques were achieved. The hematologic follow-up was only 6 months in the first patient[12] and 8 years in the second.[13] These cases seem to indicate the possibility of remission, occurring either spontaneously or with the aid of therapy, permitting the patient to be asymptomatic with a normal hematologic profile. Nevertheless the techniques used were not sufficiently sensitive to exclude the possible existence of a low level of the disease. Even in the three cases in which RT-PCR was used the presence of positive BCR/ABL cells below the sensitivity threshold of the technique used can not be ruled out, especially since nested PCR was only employed in the last one (but even then the method used only had a sensitivity of detecting one malignant cell in 10^5 normal cells).[13] In fact, evidence of the disease persists in some of these patients and/or might reappear with the onset of blast crisis. In the remaining cases perhaps follow-up has not been long enough to rule out possible reappearance of the disease. In one patient, with a follow-up of 27 years, clinical and haematologic remission occurred. The Ph1 chromosome remained at a low level accompanied by rearrangement of the BCR/ABL gene after two cycles of busulphan.[14] From this case one could infer that, at least in some patients, the pathologic clone is maintained at low levels over a prolonged period.

Biernaux *et al.*[15] have detected m-RNA of BCR/ABL at very low levels in some healthy individuals. Nevertheless, the case described here is a patient with CML with t(9;22) at diagnosis.

Recently, a mathematical model has been postulated which predicts that three mutations in the stem cell are required to cause CML.[2] On the other hand, persistence of cells expressing BCR/ABL mRNA after allogeneic bone marrow transplantation has been described.[1,16]

These facts seem to indicate that under some circumstances part of the leukemic clone can disappear and another clone which has markers of this, such as the BCR/ABL gene, can persist, but not other lesions which determine or contribute to the proliferative advantage and/or survival of the leukemic cells. This could possibly have occurred in the case we describe although the patient's follow-up must continue. With time, it would be hypothetically possible that clonal alterations which appear to have been lost would recover and that the clonal course of the disease would proceed.

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Contributions and Acknowledgments

EA was responsible for the design of the study and made the molecular analysis by means of PCR, he wrote the paper with AV; AV did the cytology study and took part in the conception of the study. FAG did Southern blot analysis. JDM and EP followed the patient clinically. DE took part in the conception of the study and gave the final approval of the version to be published. The criteria for the order in which the name of the authors appear is based on the importance of their contribution to the analysis, design and execution of the study.

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Disclosures

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