original paper
Hematologica 1997; 82:156-160

EARLY DETECTION OF BONE MARROW ENGRAFTMENT BY AMPLIFICATION OF HYPERVARIABLE DNA REGIONS

GIOVANNI MARTINELLI, ELISABETTA TRABETTI*, PATRIZIA FARABEGOLI, NICOLETTA TESTONI, GIUSEPPE BANDINI, MARIA ROSA MOTTA, ALESSANDRA VITTONE, CAROLINA TERRAGNA, PIER FRANCO PIGNATTI,* SANTE TURA

*Institute of Hematology and Medical Oncology "Seragnoli", University of Bologna; *Institute of Biological Sciences, University of Verona, Italy*

ABSTRACT

Background and Objective. After allogeneic bone marrow transplantation (BMT) it is important to be able to distinguish between the host and donor origin of cells in order to monitor the engraftment process. However, identifying whether the hematopoietic stem cells are of donor or recipient origin may be a difficult task. DNA studies using Southern blotting techniques or the amplification by PCR of regions in the human genome with high polymorphic neutral sequence variation showing Mendelian inheritance as variable number of tandem repeats (VNTR) can detect the origin of host bone marrow after BMT. We have tried to apply these sensitive systems of detection to the early stages of BMT when small numbers of regenerated cells are available for analysis.

Methods. We used *in vitro* polymerase chain reaction (PCR) amplification of three single-locus simple repetitive DNA sequences, all of which vary extensively in their repeat number among different individuals (VNTR D1S80, ApoB, and D17S5), to evaluate post transplant engraftment in six patients who showed no signs of peripheral blood engraftment at 2-3 weeks after transplant. We tested 2 patients with chronic myelogenous leukemia (CML), 2 with B-acute lymphoblastic leukemia (B-ALL), 1 with T-acute lymphoblastic leukemia (T-ALL), and 1 with aplastic anemia (SAA), all in prolonged aplasia following allogeneic bone marrow transplantation (BMT).

Results. In a sequential analysis protocol with the different loci, the donor was distinguishable from the recipient in all pairs with at least one of the three markers used. After 16 days (median 16.2; range 15 to 20 days) we found that complete chimerism was present in 5 patients: 4 of donor origin (= engraftment) and one of host origin (= rejection), this last case being one of mixed chimerism. In the 2 cases in which the presence (one complete and one partial) of host DNA was detected, rejection of the donor bone marrow followed, and in 1 patient a second BMT was necessary. The other 4 patients with complete chimerism of donor origin achieved hematological reconstitution and we documented complete engrafment of donor bone marrow a few months later.

Interpretation and Conclusions. Utilizing PCR to document early post-transplant engraftment and chimerism in the first month after BMT has the advantage over Southern blotting of being more sensitive and requiring small amounts of sample. It may also be useful for guiding subsequent therapeutic decisions.

Key words: bone marrow transplantation, PCR, VNTR

Following allogeneic bone marrow transplantation (BMT) it is important to be able to distinguish between the host and donor origin of cells in order to monitor the engraftment process. Hemopoietic cell chimerism is known to be involved in rejection and in relapse of residual disease.[1-4] Furthermore, successful engraftment has been associated with stable chimerism in which all the bone marrow cells are of donor origin after BMT. However, establishing whether hematopoietic stem cells are of donor or recipient origin may be a difficult task, even when blood group or karyotypic differences between donor and recipient are known.[5,6] In cases in which there is no sign of renewed growing hematopoiesis after a few days, it becomes imperative to find out whether the few cells are of donor or recipient origin.[8,9]

The small number of cells available at early time points after BMT may be studied only by strategies involving polymerase chain reactions (PCR). We and others have demonstrated that DNA studies using Southern blotting techniques or the amplification by PCR of regions in the human genome with high polymorphic neutral sequence variation

Correspondence: Dr. Giovanni Martinelli, Istituto di Ematologia e Oncologia Medica "Seràgnoli", Policlinico S. Orsola, via Massarenti 9, 40138 Bologna, Italy. Tel. international +39.51.6364075. Fax. international +39.51.398973.

Acknowledgements: this work was supported by Associazione Italiana per la Ricerca sul Cancro (A.I.R.C), by Italian C.N.R A.C.R.O. no. 93.0220.PF39, by 95.2206 and by "30 ore per la vita" target projects. We wish to thank Mr. Robin Cooke for checking the English version of the manuscript.
Received September 5, 1996; accepted January 3, 1997.

showing Mendelian inheritance as variable number of tandem repeats (VNTR) could detect the origin of host bone marrow after BMT.[7-12] We previously reported on three different VNTRs[13-15] which were found to be useful in monitoring hematopoietic cell chimerism after BMT.[16-20] We have now tried to apply these sensitive detection systems to the early stages of BMT when small numbers of regenerated cells are available for analysis. For this purpose, we sequentially amplified three VNTRs to assess hematopoietic cell origin after BMT. Using these techniques, the presence of donor and recipient cells after BMT can be detected at levels approaching 1% by Southern blotting and 0.01% by PCR,[9-11] respectively.

Materials and Methods

Patients

Six allogeneic BMT donor-recipient (D/R) pairs were studied. Clinical data, disease status, donor and recipient sex, transplant conditioning regimens, GvHD prophylaxis, and outcome of BMT are shown in Table 1. All D/R pairs were matched at the HLA A, B, C, and DR loci and were non-reactive in mixed lymphocyte cultures (MLC). One of these patients (#4 in Table 1) was transplanted with a serological and molecular HLA-class I and HLA-DRB1, DQA and DQB typed identical unrelated donor;[12-14] a DP gene mismatch between donor and recipient was present.

Transplant conditioning regimens

After informed consent, all patients received a combination of chemotherapy. Conditioning chemotherapy included busulphan (BU in Table 1), cyclophosphamide (Cy in Table 1), etoposide (VP16 in Table 1) and total body irradiation was performed as reported.[15] Unrelated donor bone marrow was not T-cell depleted prior to transplant to prevent graft versus host disease (GvHD). All patients received irradiated leukocyte-depleted blood products throughout the course of their BMT. Post BMT anti-GvHD treatment consisted of cyclosporin A (CsA) in three patients, and CsA plus methotrexate (MTX) in the remaining three patients (Table 1).

PCR analysis

The time of bone marrow aspirates and the DNA markers used are indicated in Table 2. After informed consent high molecular weight DNA was extracted from bone marrow samples as previously described.[24] The genomic regions studied were: locus D1S80, with a 16 bp repeat detected by probe MCT 118, located on the short arm of chromosome 1;[25] ApoB, with a 14-16 bp repeat located about 0.5 kb on the 3' side of the last amino acid codon in the apolipoprotein B gene;[18] D17S5, with a 70 bp repeat detected by probe YNZ22, on the short arm of chromosome 17.[15] Analyses on ApoB, D1S80, D17S5, were performed as described.[24] The primers used are reported in the literature.[17-19,24,26,27] Briefly, the cycling parameters were as follows: 94°C, 20 sec; 55°C, 30 sec, and 72°C, 1 min, for 30 cycles, as previously described.[24,26,27] Observed allele number and heterozygosities in the Italian population for these polymorphisms are given elsewhere.[26,27] Spermidine 0.25 mM final concentration was added in the PCR reaction mix[24] to increase the specific product yield. After amplification, 1/10 of the sample volume was electrophoresed through standard non-denaturing 8% polyacrylamide gels (D1S80, ApoB) or 2% agarose gels (D17S5). Gels were subsequently stained with ethidium bromide and photographed under UV light. All primers used in this study were synthesized on an Applied Biosystems 391 PCR MATE DNA synthesizer (Applied Biosystems, Foster City, CA, USA) and used without further purification. Negative controls were included in all PCRs.[28-30] In the two CML cases, the presence of bcr-abl neoplastic transcripts was investigated by RT-PCR using a very sensitive procedure.[31] Conditions for preventing and for revealing possible contamination were used as previously described.[29-31] Total RNA extraction, cDNA synthesis and PCR were performed as previously reported.[32,33]

Cytogenetics

Samples from the CML patient were collected at varying time intervals post BMT and karyotypes were analyzed using standard and previously reported techniques.[5,6] The Y chromosome was detected using quinacrine banding.[5]

Definitions

Cases exhibiting complete donor hematopoiesis with all markers tested at all times were defined as donor chimeras having complete chimerism of donor (CC) or of recipient origin (CC*). Patients who exhibited mixed populations of donor and host cells with at least one DNA marker were considered as exhibiting mixed chimerism (MC).

Results

Assessment of chimerism using hypervariable DNA polymorphisms

Details of chimerism evaluation of the D/R pairs investigated with two or more of the hypervariable DNA markers D1S80, ApoB and D17S5 amplified

Table 1. Clinical data from the six transplanted patients.

No.	UPN	Name	Diagnosis	Disease status	Donor/ recipient	First transplant conditioning	WBC at +20 ($x10^9$/L)	PLTs at +20 ($x10^9$/L)	GVHD prophylaxis	GVHD grade	Acute GVHD	Survival	2nd BMT	HLA class I donor/ recipient	HLA class II donor/ recipient
1	159	FA	SAA	Aplastic P	M/F	Cy	0.2	40	CsA	0	neg	44 mo	yes	M	M
2	173	NL	B-ALL	Relapse	M/M	Bu+Cy	0.6	40	CsA	0	neg	3 mo	no	M	M
3	161	BE	T-ALL	II CR	M/M	TBI+Cy	0.36	28	CsA+MTX	1	neg	23 mo	no	M	M
4	170	DM	CML	Blast	M/M	TBI+Cy VP-16	0.1	40	CsA+MTX	2	2	4 mo	no	M	DP mismatch
5	145	MF	CML	CP	F/M	Bu+Cy+VP16	1.4	80	CsA	3	3	4 mo	yes	M	M
6	167	TD	B-ALL	Relapse	M/M	TBI+Cy+VP16	0.31	28	CsA+MTX	1	1	36 mo	no	M	M

Abbreviations: No., number of patient; Name, inital of patient's name; M, male; F, female; Diagnosis, disease classification; SAA, severe aplastic anemia; B-ALL, acute lymphoblastic leukemia of B lineage; CML, chronic myelogenous leukemia; T-ALL, acute lymphoblastic leukemia of T lineage; Disease status, disease phase at BMT; Aplastic P, aplastic phase; II CR, second complete remission; Blast, blast phase; CP, chronic phase; mo, months; CsA, cyclosporin A; MTX, methotrexate; GvHD, graft versus host disease grade; M, method; neg, negative; Bu, busulphan; Cy, cyclophosphamide; VP16, etoposide.

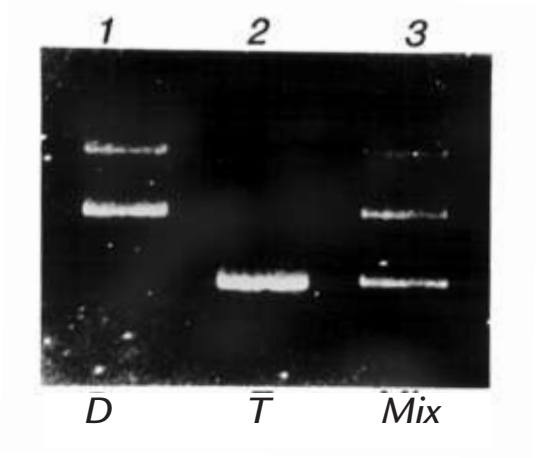

Figure 1. Complete chimerism of donor origin detected with PCR of D17S5 locus on bone marrow samples from D/R pair no. 3: D = donor DNA sample; T = transplanted: the patient's DNA fragments after BMT; R = recipient: DNA from the patient before BMT. 1, 2, 3 and 4 are arbitrary DNA fragment numbers. The patient's post-transplant pattern (T) (1, 4) shows donor-specific (D) (1, 4) DNA fragments are not present in patient (R) DNA before BMT (2, 3).

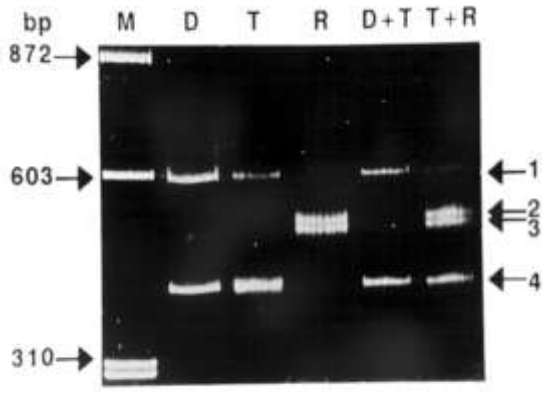

Figure 2. PCR amplification of the ApoB locus on bone marrow samples from D/R pair #5 (M.F.). There was failure of the first transplantation, identified as (CC*): complete chimerism of patient origin, after which a second procedure was performed, and this was followed by CC of donor origin. Mix = DNA from donor and patient amplified separately for control. Post-transplant DNA fragments (T) show the patient pattern. The two samples were mixed just prior to electrophoresis.

in vitro are reported in Table 2. Figure 1 shows an example of mixed chimerism detected with D17S5; Figure 2 illustrates complete chimerism (T.D. in Tables 1 and 2) detected with ApoB polymorphism. In the D/R couple formed by unrelated individuals (D.M.), as expected, no identical polymorphism was evidenced with either of the 2 markers tested. Polymorphisms which could identify chimerism were found in all 6 (100%) pairs of patients who underwent allogeneic transplantation (Table 2). In 6/6 (100%) pairs, D1S80, the first marker used, was sufficient for identification (so-called *informative*. The second (ApoB) was informative in 5/6 (83%) cases; and the last polymorphism employed, D17S5, was also able to distinguish between donor and recipient in case #2 (Table 2).

Of the 5 patients who became chimeras, one (M.F. in Tables 1 and 2) displayed a CC of recipient origin (CC* in Table 2) and experienced graft failure (= rejection); bcr-abl mRNA detection by RT-PCR confirmed leukemia relapse at the molecular level. Also, cytogenetic analysis for the Ph+ and Y chromosomes was positive for relapse or non engraftment. After a long period of aplasia (> 40 days) during which only a few cells of host origin were detectable by PCR, this patient underwent a second transplant from his brother. Fifteen days after this second BMT his hematopoiesis was of donor origin (CC), but the patient died of complications due to infection four months after the first BMT. No mixed chimerism, Ph+ metaphases or bcr-abl mRNA (minimal residual disease) was docu-

Table 2. Molecular data from the six transplanted patients. Cytogenetic and molecular evaluation of bone marrow origin by PCR analysis is reported.

No.	UPN	Name	Diagnosis	Day post-BMT when VNTR analysis was performed	D1680	Apo B	D17S5 (YNZ22)	Cytogenetic of Y chr. (range 15-20 days after BMT)	Clinical status at the date of VNTR analysis	Clinical status at 2 months from BMT	VNTR status after 3 months with the informative VNTR	PCR of bcr-abl status at 15-20 days after BMT
1	159	FA	SAA	15 days	CC	CC	ND	ND	Not engrafted	Engrafted	CC	–
2	173	NL	B-ALL	15 days	MC	MC	MC	ND	Not engrafted	Engrafted	MC/Died	–
3	161	BE	T-ALL	17 days	CC	Neg	ND	ND	Not engrafted	Engrafted	CC	–
4	170	DM	CML	20 days	CC	CC	ND	ND	Not engrafted	Engrafted	CC	pos
5	145	MF	CML	15 days	CC*/CC	CC*/CC	ND	CC*	Not engrafted	Not engrafted	Died	pos*/neg
6	167	TD	B-ALL	15 days	CC	CC	ND	ND	Not engrafted	Engrafted	CC	–

Abbreviations as in Table 1. CC, complete chimerism; CC, complete chimerism (results related to the first BMT) means that in case #5 there was failure of the first transplantation after which a second procedure was performed, and this was followed by CC of donor origin; MC, mixed chimerism.*

mented for the second BMT in this CML patient.

Patient F.A. (#1 in Tables 1 and 2) received a first BMT from her brother and after 20 days of aplasia was found to possess a few hematopoietic cells of donor origin (CC). After a prolonged aplastic phase without engraftment (no take), an additional late decline in peripheral blood leukocyte count was noted (leukocyte number under 0.2×10^9/L), raising clinical suspicion of graft rejection. She was again conditioned and transplanted from the same donor and achieved an engraftment of donor origin which had lated for 44 months so far.

The other patients had bone marrow donor engraftment confirmed at the molecular level that lasted 3, 23, and 36 months. Patient D.M. died 4 months after BMT of GvHD complications in clinical but not in molecular remission. Patients T.D. and B.E. died of ALL relapse 36 and 23 months after BMT, respectively.

Mixed chimerism was documented 20 days after BMT in patient N.L., who had undergone BMT for B-ALL. After 3 months of molecularly detectable mixed chimerism, together with a complete hematological remission following a rapid increase of host hematopoiesis and clinical relapse of B-ALL, this patient died.

Discussion

This study confirms the use of a PCR-based method to monitor engraftment following allogeneic BMT. We describe the use of polymorphic regions to document engraftment or rejection in 6 patients post BMT at time points when peripheral leukocyte counts were low.

Amplified sequence polymorphisms are a useful tool for evaluating hematopoietic chimerism, and they have also been used by others[15,21-24] for determining the origin of leukemic cells in patients with recurrent disease after BMT and for the study of marrow engraftment after allogeneic BMT. When limited quantities of DNA are obtainable from an aplastic or still leukopenic patient immediately following BMT, DNA analysis by Southern blot is not always possible. The PCR method overcomes this problem by allowing detection of mixed chimerism at levels below 1%.[23,34] We did not attempt to quantify our results using the quantitative PCR strategies reported;[34] however, the random distribution of the hypervariable loci on the human genome allows us to assess chimerism even in the presence of chromosome rearrangements or genetic losses, such as monosomies or deletions, which are frequently reported in leukemias and after hematological disorders.[35-38,40]

It has been reported that many different markers need to be employed in order to achieve complete characterization of transplanted tissue origin.[23] In our study, all patients obtained a diagnostic result through the use of only two VNTRs of DNA polymorphism. Our finding that D1S80, a highly polymorphic marker, proved to be the most useful for chimerism detection is in accordance with the high heterozygosity of this locus previously described in a larger sample of Italians.[27] This finding is important because it indicates that 70% or more of the BMT cases in our population may be investigated with only one PCR assay, which reduces the cost and time required for analysis. When, as should always be possible, the polymorphism of the locus is determined prior to BMT, there should be no need to take other polymorphisms into consideration after BMT,[24] thus reducing the analysis time.

Whereas PCR polymorphism gave useful information in all patient pairs, karyotypic analysis was possible in only two patients and on samples collected from 11 to 30 days after BMT. Another advantage of the PCR-based method is that it is applicable to all cases, even when no cytogenetic markers are present (DNA from all nucleated cells is available for analysis by PCR, while cytogenetic analysis is restricted to actively dividing sample cells).

Most microsatellite assays use radioactively labeled PCR products and denaturing acrylamide gels. Our method allows direct analysis of PCR products on ethidium bromide soaked gels. This technique has the advantages of speed, sensitivity, and ease of analysis because it involves only two steps: amplification and electrophoresis.

Detection of engraftment as early as day 7 has been reported with leukocyte counts as low as 0.2×10^9/L.[39] Our results suggest late engraftment or failure to engraft. In this study, engraftment was documented in four cases and early graft rejection was documented in one.

In conclusion, our results confirm the previously reported advantages of PCR-based analysis of engraftment following BMT and extend the application of this technique to the early phase of BMT. The method described here provides increased speed, sensitivity and ease of analysis of polymorphic sequences since only two steps (amplification and gel electrophoresis) are required. Finally, it should be noted that this method might prove suitable for other forms of engraftment, including liver transplantation.

References

1. Santos GW. Bone marrow transplantation in leukemia. Cancer 1984; 54: 2732-6.
2. Blume KG, Beutler E, Bross KJ, Schimidt GM, Spruce WE, Teplitz RL. Genetic markers in human bone marrow transplantation. Am J Hum Genet 1980; 32:414-8.
3. Zaccaria A, Rosti G, Testoni N, et al. Cytogenetic events after bone marrow transplantation for Philadelphia chromosome positive myeloid leukemia. Leuk Res 1991; 15:289-93.
4. Carlo Stella C, Tabilio A. Stem cells and stem cell transplantation. Haematologica 1996; 81:573-87.
5. Zaccaria A, Rosti G, Testoni N, et al. Cytogenetic follow-up of 100 patients submitted to bone marrow transplantation for Philadelphia

chromosome-positive chronic myeloid leukemia. Eur J Haematol 1988; 40:50-4.
6. Wang L, Jujit T, Tokunanga K, et al. Brief report: polymorphic microsatellite markers for the diagnosis of graft-versus-host disease. N Engl J Med 1994; 330:398-402.
7. Gasparini P, Martinelli G, Trabetti E, Ambrosetti A, Benedetti F, Pignatti PF. Bone marrow transplantation monitoring by DNA analysis. Bone Marrow Transplant 1989; 4:157-9.
8. Vannucchi AM, Bosi A, Laszlo D, Guidi S, Saccardi R, Rossi Ferrini P. Treatment of a delayed graft failure after allogeneic bone marrow transplantation with IL-3 and GM-CSF. Haematologica 1995; 80: 341-3.
9. Majolino I, Buscemi F, Scimé R, et al. Treatment of normal donors with rhG-CSF 16 µg/kg for mobilization of peripheral blood stem cells and their apheretic collection for allogeneic transplantation. Haematologica 1995; 80:219-26.
10. Petz LD, Yam P, Wallace RB, et al. Mixed hematopoietic chimerism following bone marrow transplantation for hematologic malignancies. Blood 1987; 70:1331-5.
11. Bretagne S, Vidaud M, Kuentz M, et al. Mixed blood chimerism in T cell-depleted bone marrow transplant recipients: evaluation using DNA polymorphisms. Blood 1987; 70:1692-6.
12. Martinelli G, Buzzi M, Farabegoli P, et al. New strategies for selection of unrelated bone marrow donors. Bone Marrow Transplant 1993; 11:31-2.
13. Martinelli G, Zaccaria A, Testoni N, Buzzi M, Tura S. Fingerprinting of HLA-DQA by polymerase chain reaction and heteroduplex analysis. Mol Cell Probes 1996; 10:123-7.
14. Mantovani V, Bragliani M, Martinelli G, et al. Molecular analysis of HLA genes for the selection of unrelated bone marrow donor. Bone Marrow Transplant 1995; 16:3290-5.
15. Benini C, Bandini G, Motta MR, et al. Donor origin of hematopoiesis after a case of allogenic transplantation with cryopreserved marrow. Haematologica 1993; 78:414-5.
16. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 1988; 239:487-91.
17. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 1991; 48:137-41.
18. Boerwinkle E, Xiong W, Fourest E, Chan L. Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: Application to the apolipoprotein B3' hypervariable region. Proc Natl Acad Sci USA 1989; 86:212-6.
19. Horn GT, Richards B, Klinger KW. Amplification of a highly polymorphic VNTR segment by the polymerase chain reaction. Nucleic Acids Res 1989; 17:2140-4.
20. Min GL, Hibbin J, Arthur C, Apperley J, Jeffreys A, Goldman J. Use of minisatellite DNA probes for recognition and characterization of relapse after allogeneic bone marrow transplantation. Br J Haematol 1988; 68:195-9.
21. Lawler M, McCann SR, Conneally E, Humphries P. Chimærism following allogeneic bone marrow transplantation: detection of residual host cells using the polymerase chain reaction. Br J Haematol 1989; 73:205-9.
22. Ugozzoli L, Yam P, Petz LD, et al. Amplification by the polymerase chain reaction of hypervariable regions of the human genome for evaluation of chimerism after bone marrow transplantation. Blood 1991; 77:1607-11.
23. Browne PV, Lawler M, O'Riordan J, Humphries P, McCann SR. Early detection of leukæmic relapse after bone marrow transplantation using the polymerase chain reaction. Bone Marrow Transplant 1991; 7:167-71.
24. Martinelli G, Trabetti E, Zaccaria A, et al. In vitro amplification of hypervariable DNA regions for the evaluations of chimerism after allogeneic bone marrow transplantation. Bone Marrow Transplant 1993; 12:115-20.
25. Kasai K, Nakamura Y, White R. Amplification of a variable number of tandem repeats VNTR locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science. J Forensic Sci 1990; 35:1196-200.
26. Trabetti E, De Leo D, Gasparini P, Pignatti PF, Marigo M. DNA amplification (PCR) of several hypervariable loci in disputed paternity cases. In: Berghaus G, Brinkmann B, Rittner C, Staak M, eds. DNA-technology and its forensic application. Berlin:Springer-Verlag; 1991. p. 92.
27. Trabetti E, Galavotti R, Pignatti PF. Genetic variation in the Italian population at five tandem repeat loci amplified in vitro: use in paternity testing. Mol Cell Probes 1993; 7:81-4.
28. Hughes TP, Ambrosetti A, Barbu V, et al. Clinical value of PCR in diagnosis and follow-up of leukaemia and lymphoma: report of the third Workshop of the Molecular Biology/BMT study group. Leukemia 1991; 5:448-52.
29. Hughes TP, Goldman JM. Improved results with PCR for chronic myeloid leukaemia. Lancet 1990; 336:812-6.
30. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989; 339:237-41.
31. Zaccaria A, Martinelli G, Buzzi M, Farabegoli P. Will molecular biology contribute to refine prognosis and to select treatment? Leuk Lymph 1993; 11:81-9.
32. Guerrasio A, Martinelli G, Saglio G, et al. Minimal residual disease status in transplanted chronic myelogenous leukaemia patients: low incidence of polymerase chain reaction positive cases among 48 long disease-free subjects who received unmanipulated allogeneic bone marrow transplants. Leukemia 1992; 6:507-12.
33. Saglio G, Guerrasio A, Rosso C, et al. New type of Bcr/Abl junction in Philadelphia chromosome-positive chronic myelogenous leukemia. Blood 1990; 76:1819-23.
34. Rudert WA, Kocova M, Rao AS, Trucco M. Fine quantitation by competitive PCR of circulating donor cells in posttransplant chimeric recipients. Transplantation 1994; 58:964-8.
35. Santucci MA, Trabetti E, Martinelli G, et al. Host origin of bone marrow fibroblasts following allogeneic bone marrow transplantation for chronic myeloid leukemia. Bone Marrow Transplant 1992; 10:255-9.
36. Martinelli G, Sessarego M, Gasparini P, et al. An assessment of chimeric transcript detection in CML patients after bone marrow transplantation. Bone Marrow Transplant 1991; 7:21.
37. Martinelli G, Lemoli RM, Farabegoli P, et al. Persistence of non clonal hematopoietic progenitor cells in blastic phase chronic myelogenous leukemia (CML). Haematologica 1994; 79:445-7.
38. Martinelli G, Zaccaria A, Tassinari A, et al. Molecular variability in acute Philadelphia positive leukemia. Haematologica 1991; 76:32.
39. Ginsburg D, Antin JH, Smith BR, Orkin SH, Rappeport JM. Origin of cell populations after bone marrow transplantation. Analysis using DNA sequence polymorphisms. J Clin Invest 1985; 75:596-600.
40. Santini V, Zoccolante A, Bosi A, et al. Detection of bcr/abl transcripts by RT-PCR and their colorimetric evaluation in chronic myeloid leukemia patients receiving allogeneic marrow transplantation. Haematologica 1996; 81:201-7.