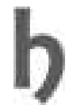
Multiple Myeloma



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Fluorescent polymerase chain reaction and capillary electrophoresis for IgH rearrangement and minimal residual disease evaluation in multiple myeloma

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Background and Objectives. Several polymerase chain reaction (PCR)-based techniques for tracking minimal residual disease (MRD) in B-lymphoproliferative disorders have been recently proposed. These procedures show significant variation in sensitivity and specificity. We describe an alternative assay based on fluorescent–PCR combined with capillary electrophoresis and GeneScan analysis, to identify the monoclonal immunoglobulin heavy chain (IgH) rearrangement in multiple myeloma (MM) and to provide a semi-quantitative evaluation of MRD by limiting dilutions.

Design and Methods. Different sets of family specific primers derived from the leader region and from the framework-1 of IgH were used, with a unique reverse fluorescent primer JH. The malignant clone was identified by GeneScan and sequenced. Two tumor primers, mapping in the complementarity determining regions CDRII and CDRIII, were designed for each patient. A comparison between the nested-PCR approach and direct fluorescent PCR was performed for three patients in complete clinical remission after autologous or allogeneic bone marrow transplantation.

Results. Thirty-six consecutive patients with MM were screened and monoclonality was identified in about 70% of the cases. Molecular MRD evaluation was performed in 18 patients using tumor primers. This method allowed identification of 1 neoplastic cell among 10^4-10^6 normal cells. In three cases, negative by nested-PCR and agarose gel electrophoresis, gene scanning showed persistence of the neoplastic clone, despite the negativity of the immunofixation.

Interpretation and Conclusions. Capillary electrophoresis of fluorescent fragments with gene scanning provides a simple, rapid and reproducible method to detect IgH rearrangement and to evaluate MRD. Furthermore, the sensitivity reached is up to 1 log higher than that of the conventional approach with nested-PCR, even though two steps of specificity are maintained.
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Key words: multiple myeloma, IgH-CDR3, MRD, gene scan, capillary electrophoresis.

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Multiple myeloma (MM) is an incurable B-cell neoplasia arising from the clonal expansion of a plasma cell (PC) precursor in the bone marrow. High-dose melphalan followed by a peripheral blood stem cell (PBSC) autologous transplantation has provided discrete tumor regression, longer survival and up to a 50% rate of complete remission (CR) for patients aged less than 70 years.[1-3] However, although most patients initially respond to therapy, relapse is unavoidable.[4,5]

The VDJ rearrangement of the immunoglobulin heavy chain (IgH) provides a unique clonotypic marker for MM plasma cells and there is no evidence of clonal evolution during the course of the disease; thus, quantification of the residual myeloma cells may be used as a prognostic factor and a marker of efficacy of chemotherapy.[6] Polymerase chain reaction (PCR) IgH gene amplification, followed by hybridization of sequence-specific radiolabeled probes, has led to the development of highly sensitive methods for determining monoclonality and for monitoring minimal residual disease (MRD), with a sensitivity of 1 myeloma cell in 10^4-10^5 normal cells.[7] To bypass the hybridization step and to avoid radioactivity, nested-PCR strategies, with allele-specific oligonucleotide (ASO) probes, derived from the CDRs (complementarity determining regions) of patient's tumor, have been proposed.[8,9] These approaches are time-consuming, laborious and furthermore, the nested-PCR technique increases the risk of contamination and of false positive results, due to post-PCR manipulation.

Recently, real-time PCR using TaqMan technology has been proposed as a powerful and highly sensitive system to quantify residual neoplastic cells.[10,11] This approach needs expensive analytical equipment, is not available in the majority of laboratories and moreover requires the construction of both an allele-specific oligonucleotide (ASO) primer and an ASO dual-labeled fluorogenic probe (ASO TaqMan probe) specific for the V_H family or

for each patient. To date, few studies have been published about MM.[12,13] Fluorescent PCR followed by automated GeneScan fragment analysis (GSFA) has been reported to improve IgH monoclonal detection rate.[14-16]

This paper describes the application of this strategy to investigate monoclonality in 36 MM patients. Furthermore, for 18 of them, a semi-quantitative estimation of MRD by limiting dilutions was made, using two patient-specific primers in a direct fluorescent-PCR approach. For three patients in CR, the results of GeneScan analysis are compared with those of the nested-PCR technique and conventional agarose gel detection.

Design and Methods

Patients and samples for molecular analysis

Between January 1999 and July 2000, 36 consecutive MM patients, aged 35-66 years, (median 56 years) were enrolled in this study. All patients were included in a therapeutic program consisting of PBSC autologous transplantation after 2 to 3 courses of VAD (vincristine, adriamycin, dexamethasone) or 2 cycles of VAD followed by 2 cycles of DCEP (dexamethasone, cyclophosphamide, etoposide, cis-platin). PBSC were harvested after cyclophosphamide (4-7g/m^2) or DCEP. The patients' clinical stage was classified according to the Durie and Salmon criteria.[17] Twenty-five patients were newly diagnosed, 6 were in relapse, and 5 had received oral therapy and had progressive disease. Clinical response was assessed according to the EBMT criteria.[18]

Bone marrow (BM) samples were collected at diagnosis or at relapse/progression of the disease and subsequently during chemotherapy, before PBSC infusion, every 3 months after transplantation for the first year and every 6 months thereafter. A sample of PBSC was also evaluated. DNA was obtained by cell lysis and phenol extraction according to standard procedures.[19] In 4 cases (#2, 5, 14, and 30; Table 1) DNA was extracted from smears by lysing scraped cells. The quality of DNA and the absence of Taq polymerase inhibitors were assessed by β-globin amplification.[20] The real number of PC in samples used to detect monoclonal rearrangement was assessed by flow cytometry using the following monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated CD138 (Immuno Quality Products, Groningen, The Netherlands), phycoerythrin (PE)-conjugated CD38 (Becton Dickinson Immunocytometry Systems, San José, CA, USA), peridinine chlorophyll protein (PerCP)

Table 1. Detection of VDJ monoclonal rearrangement by fluorescent PCR.

Pat	*Status*	*PC% in BM smear*	*PC% at FACS*	*VH.LB-JH3'*	*VH.FD-JH3'*
1	diagnosis	15%	3%	ni	VH.FD3
2	diagnosis	65%	60%	ni	VH.FD3
3	diagnosis	90%	nv	VH4.LB	VH.FD4a
4	diagnosis	60%	2.5%	ni	ni
5	diagnosis PD	60%	nv	VH3.LB	VH.FD3
6	relapse	90%	nv	VH3.LB	ni
7	diagnosis	10%	10%	ni	VH.FD3
8	diagnosis	75%	1.5%	VH1.LB	ni
9	relapse	65%	nv	VH2.LB	VH.FD2
10	diagnosis	25%	3%	VH.FD3	VH.FD3
11	diagnosis	10%	9%	ni	VH.FD4a
12	diagnosis	95%	11%	ni	ni
13	diagnosis	8%	1%	ni	ni
14	treated	25%	nv	ni	ni
15	relapse	70%	nv	VH1.LB	ni
16	diagnosis	5%	0%	ni	ni
17	treated	15%	nv	VH4.LB	ni
18	diagnosis	80%	nv	VH5.LB	VH.FD5
19	diagnosis	80%	nv	VH3.LB	VH.FD3
20	diagnosis	20%	8%	ni	VH.FD3
21	diagnosis	25%	10%	ni	VH.FD3
22	diagnosis	40%	18%	VH4.LB	VH.FD4a
23	diagnosis	10%	1.5%	ni	ni
24	diagnosis	65%	38%	ni	ni
25	diagnosis	40%	15%	ni	ni
26	treated	20%	5%	ni	ni
27	diagnosis	60%	nv	ni	VH.FD5
28	diagnosis	0%	nv	ni	ni
29	relapse	80%	15%	VH3.LB	VH.FD3
30	treated	95%	nv	ni	ni
31	relapse	55%	nv	ni	ni
32	diagnosis	60%	3.5%	VH3.LB	VH.FD3
33	treated	25%	12%	VH4.LB	VH.FD4a
34	relapse	90%	nv	VH4.LB	VH.FD4a
35	diagnosis	40%	35%	VH2.LB	VH.FD2
36	diagnosis	20%	3%	VH1.LB	VH.FD1

Abbreviations: Pat, patient; PC, plasma cells; PD, disease progression; ni, not identified.

CD19 (Caltag, San Francisco, CA, USA) and CD56 PE (Becton Dickinson). Lymphocytes were stained according to U.S.-Canadian recommendations;[21] a minimum of 50,000 events on the total population gate was acquired using a Becton Dickinson FACScan with CELLQuest v3.1 software. Malignant PC were identified as $CD138^+$/$CD38^{++}$/$CD56^+$/CD19.[22,23] Flow cytometry analysis was not performed on samples obtained from scraped cells.

Tumor VDJ rearrangement identification by PCR

PCR reactions were performed using a set (VH.LB) of 6 family specific consensus forward primers from

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the IgH leader region and 2 sets (VH.FD and VH.FS) of 7 forward consensus primers from the framework 1 (FR1). A common JH3′reverse primer 5′ labeled with 6-FAM fluorochrome was used.[24]

DNA at diagnosis or at relapse (500 ng–1 μg) was amplified using 1.5 mM $MgCl_2$, 2.5 μM of each dNTP, 0.25 μM of each primer, and 1.25 U AmpliTaq Gold (PE Applied Biosystems, Monza, Italy) in a final volume of 50 μL. Thermocycler conditions were: 95°C for 10 min, followed by 40 cycles: 94°C for 1 min, 62°C for 1 min and 72°C for 1 min; for the combination of VH.FD4a-JH3′, VH.4LB-JH3′ and VH.FS4a -JH3′ primers the annealing temperature was 58°C. The last cycle was performed at 72°C for 15 min. PCR products were separated on 3% agarose gel (Nusieve FMC BioProducts, Rockland, ME, USA) and stained with ethidium bromide.

Gene-Scan fragment analysis (GSFA)

For GSFA, 2 μL of the PCR product were added to 13 μL of deionized formamide (Molecular Sigma Biology St. Luis, MO, USA) and 0.5 μL of the internal size standard Gene Scan™ 500 ROX (ABI – PE Applied Biosystems). This mixture was denaturated at 95°C for 5 min, cooled on ice for at least 5 min and subjected to capillary electrophoresis on an ABI PRISM 310 following the specifications in the user's manual. The electrophoresis was run for 24 min for FR1 fragments and 30 min for the longer leader fragments. Data were elaborated with 310 GeneScan 2.1 Software. Polyclonal samples show a Gaussian-like distribution of the peaks in the range of 300-350 bp for FR1 fragments and 450-550 bp for leader fragments; monoclonal cases displayed a single or a predominant peak (Figure 1). A signal was taken as positive if the level of fluorescence was at least 250 absorbance units.

Tumor VDJ sequencing

To sequence the tumor-specific IgH CDRs, monoclonal samples, identified by GSFA, were amplified using the appropriate forward family primer and a non-fluorescent reverse JH3′ under the same PCR conditions as described above. The target band was purified from a 3% Nusieve agarose gel and sequenced on GeneAmp 9600 (PE Applied Biosystems) using a BigDye™ terminator kit (PE Applied Biosystems) protocol: 96°C for 10 sec, 50°C for 5 sec, 60°C for 4 min for 25 cycles.

After removal of the excess dye terminators, 4 μL of product were resuspended in 16 μL of H_2O and run without denaturation on the ABI PRISM 310 as follows: 10 sec injection, 2 kV injection and 15 kV run voltage at 45°C for 18 min, using polymer POP 4 and module Seq POP 4 E.

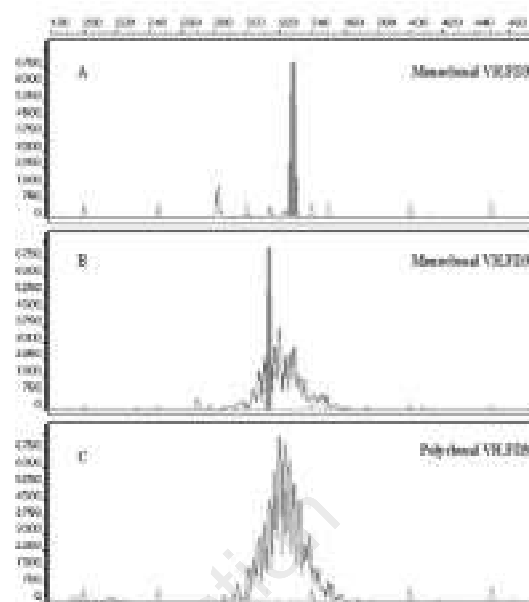

Figure 1. Amplification of VDJ rearrangement by VH.FD-JH3′ fluorescent primers. A monoclonal pattern is represented by a single discrete peak (A) or by a dominant peak (B). The polyclonal pattern shows a Gaussian-like distribution of peaks separated by 3 bp (C). Peaks spread in a range of 300-350 bp. Continuous line: sample tested; dotted line: internal size standard GeneScan 500 Rox.

Sequences were examined by the FASTA program (available at *http://www.ncbi.nlm.nih.gov/igblast*).

Two tumor-specific primers were designed for each patient in CDRs sequences, using the Primer Express Program (PE Applied Biosystems).

Sensitivity of the method

The sensitivity of the method was assessed for each patient by preparing three different serial dilutions, from 10^{-1} to 10^{-6} by diluting DNA at diagnosis (11 cases), at relapse (5 cases) and during treatment (2 cases) with DNA from a donor pool. The recorded sensitivity was the median value obtained from the three dilutions. Individual PCR conditions were selected in order to avoid any background from the control DNA. For each patient, the three serial dilutions and all the samples collected during therapy and follow-up were tested at the same time by direct PCR, using patient-specific primers. Reactions consisted of one step at 95°C for 10 min, followed by 40 cycles: 94°C for 1 min, annealing from 55°C to 65°C (depending on the patient primers) for 1 min, 72°C for 1 min; the final extension was carried out at 72°C for 15 min. Products were analyzed by GeneScan as

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previously described.

For the nested-PCR approach, all the samples were initially amplified using the family VH primer of interest, as previously described; 1μL of the first PCR was re-amplified with patient CDR primers, under the same conditions optimized for the MRD evaluation with GSFA. Amplicons were detected by agarose gel electrophoresis (AGE).

Results

Clinical status of patients

Between June 1999 and March 2001, 29/36 (80.5%) patients completed high-dose chemotherapy and PBSC autotransplantation and were observed for a period of 3-30 months during the follow-up. Seven patients achieved complete remission (24%) and twenty-two achieved a partial remission (75.9%). Of these, six patients relapsed (20.7%) and four died from progressive disease within 6 months. In seven patients transplantation was not carried out due to poor clinical conditions.

Identification of patient-specific IgH sequences and primer selection

In order to identify myeloma-specific VDJ rearrangements, DNA samples were first amplified using VH.FD primers from the FR1 and subsequently with the VH.LB primer set from the leader region; if the monoclonal population failed to be identified, VH.FS primers were used. In order to avoid false results, three independent PCR reactions were performed for each sample.

Monoclonality was identified in 24/36 cases (66.7%), with 1.5-90% of BM plasma cells, as assessed by flow cytometry (Table 1). In three samples (8.3%) a clonal rearrangement was not detectable due to the small number of PC. In the remaining cases (25%) the analysis failed. VH.FD-JH3′ PCR led to the identification of a single clear band in twenty patients (55.5%); four additional cases were resolved by VH.LB-JH3′ PCR, increasing the detection rate to 66.7%. VH.FS PCR failed to give additional results. Finally, eighteen patients showed a polyclonal pattern. In 20/24 samples monoclonality was detectable both by AGE and GSFA; four samples were identified only by gene scanning. In one case, considered as monoclonal by AGE, gene scanning analysis showed a biclonal pattern with two clear peaks differing by 3bp. The VH3 family was represented in 11 cases, the VHD4 family in 6 cases, and VH1, VH2, and VH5 in 2 cases each. Family VH6 was not found.

The sequence of the rearrangements was successfully obtained for all patients using the forward V_H primer. For eighteen patients, CDRII forward and CDRIII reverse primers were constructed.

Table 2. Comparison of nested PCR/AGE detection and direct fluorescent PCR/GeneScan analysis in three patients in complete remission.

	Patient #3		Patient #5		Patient #17	
Samples	AGE	GeneScan	AGE	GeneScan	AGE	GeneScan
Diagnosis	+	+	+	+	+	+
Dilution 10^{-3}	+	+	+	+	+	+
Dilution 10^{-4}	+	+	+	+	**–**	**+**
Dilution 10^{-5}	**–**	**+**	+	+	–	–
Post-VAD	+	+	+	+	+	+
PBSC	–	–	+	+	+	+
CD34+	nd	nd	**–**	**+**	nd	nd
Pre-AT	nd	nd	+	+	+	+
Post-Apl.	nd	nd	–	–	–	–
1 month Post AT	+	+	+	+	**–**	**+**
3 months AT	nd	nd	**–**	**+**	**?**	**+**
6 months Post AT	**–**	**+**	+	+	–	–
9 months Post AT	nd	nd	nd	nd	nd	nd
11 months Post AT	–	–	nd	nd	nd	nd
12 months Post AT	nd	nd	nd	nd	+	+
17 months Post AT	+	+	nd	nd		
Pre-allo-T			+	+		
1 months Post allo-T			+	+		
2 months Post allo-T			+	+		
3 months Post allo-T			–	–		
5 months Post allo-T			+	+		
7 months Post allo-T			+	+		
9 months Post allo-T			+	+		

AT: autologous transplantation; allo-T: allogeneic transplantation; +: PCR positive signal; -: PCR negative signal; ?: difficult interpretation; nd, not determined; bold signs indicate discordant results between the two techniques.

Comparison of nested PCR and AGE detection with direct fluorescent PCR and GSFA for a semiquantitative evaluation of MRD

The results of GeneScan analysis in the three patients who achieved CR were compared to the results obtained by nested PCR followed by AGE detection (Table 2).

The sensitivity reached by the nested-PCR strategy was 10^{-5} in two cases and 10^{-4} in the other. Direct fluorescent-PCR combined with capillary electrophoresis always increased the sensitivity up to 1 log and allowed the persistence of the malignant clone to be verified in samples that resulted negative by the nested PCR technique. Figure 2 (A-B) shows the results obtained in patient #3.

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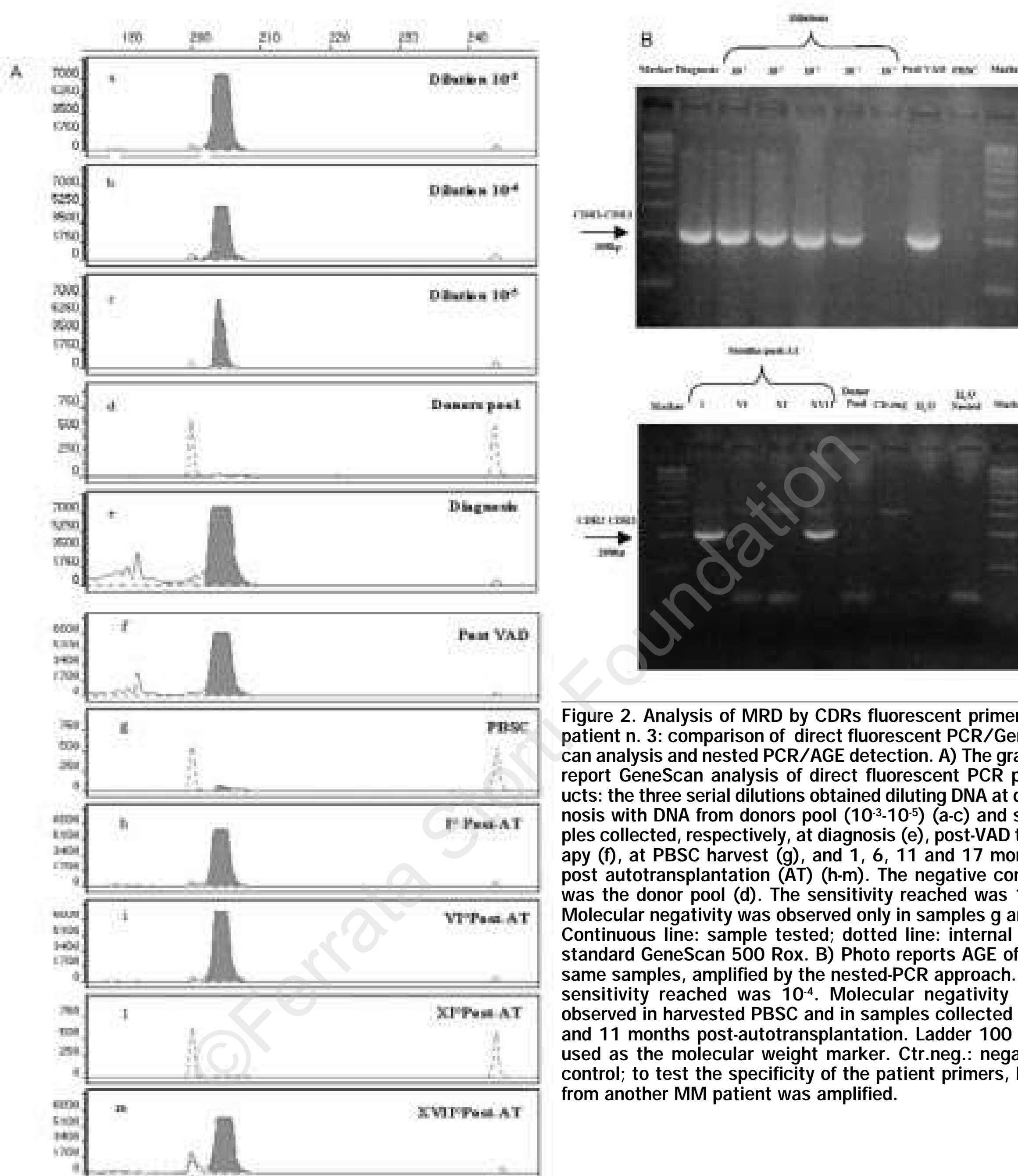

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Figure 2. Analysis of MRD by CDRs fluorescent primers in patient n. 3: comparison of direct fluorescent PCR/GeneScan analysis and nested PCR/AGE detection. A) The graphs report GeneScan analysis of direct fluorescent PCR products: the three serial dilutions obtained diluting DNA at diagnosis with DNA from donors pool (10^{-3}-10^{-5}) (a-c) and samples collected, respectively, at diagnosis (e), post-VAD therapy (f), at PBSC harvest (g), and 1, 6, 11 and 17 months post autotransplantation (AT) (h-m). The negative control was the donor pool (d). The sensitivity reached was 10^{-5}. Molecular negativity was observed only in samples g and l. Continuous line: sample tested; dotted line: internal size standard GeneScan 500 Rox. B) Photo reports AGE of the same samples, amplified by the nested-PCR approach. The sensitivity reached was 10^{-4}. Molecular negativity was observed in harvested PBSC and in samples collected at 6 and 11 months post-autotransplantation. Ladder 100 was used as the molecular weight marker. Ctr.neg.: negative control; to test the specificity of the patient primers, DNA from another MM patient was amplified.

Discussion

The significance of molecular monitoring in MM patients remains controversial.[25] However, the future perspective of disease eradication by high-dose chemotherapy or immunotherapy would require methods of greater sensitivity to detect neoplastic cells, and methods easily applicable in large clinical studies. The efficiency of IgH-PCR in detecting monoclonal rearrangements in MM is markedly lower than in other B-cell malignancies.[26-28] Literature reports a 95-100% positive detection rate in ALL and CLL, but a rate ranging from 17% to 80% in MM.[26,29-30] The high percentage of false-negative results observed at diagnosis is the critical point of molecular analysis in MM and is mainly due to the occurrence of somatic mutations and of deletions in the primer target sites, generated under antigen selection. Further-

more, IgH PCR can fail because the malignant clone involves VH genes rarely used in VDJ rearrangements and not included in the design of the consensus primer chosen.[31] The background, derived from the co-amplification of admixed polyclonal B-lymphocytes, could be an additional cause of failure to detect a monoclonal pattern.[30] Nevertheless, rearrangement at the IgH locus is the clonality marker of choice for investigating MRD in MM, because of the absence of characteristic cytogenetic aberrations.[32]

Different molecular approaches have been proposed, based on the use of FR1, FR2 or FR3 forward primers and a reverse primer mapping in FR4, in order to amplify the hypervariable domain CDRIII and to synthesize patient-specific primers for MRD evaluation.[24,33] A positivity of 89% has been obtained by combining different sets of forward primers, even with 5 different reverse JH consensus;[33] however, this procedure is applicable to a limited number of patients. Furthermore FR2 and FR3 strategies require laborious and time-consuming cloning procedures in order to obtain the sequence of the tumor CDRs; moreover, the FR3 method allows the construction of only one tumor-specific primer. An interesting novel strategy based on the amplification of tumor rearrangement with two sets of primers, derived from the leader region of IgH and FR1, has recently been proposed.[24] The amplification of a longer segment allows the identification of both CDRII and CDRIII sequences, so that two internal patient-specific primers could be designed. MRD was then monitored using a nested-PCR approach. This method is a valid alternative to hybridization procedures based on ASO probes;[8-9] however it is associated with an increased risk of false positive results caused by cross-contamination.

In addition to optimal primer selection, the application of different detection methods could increase the clonality detection rate. Besides the conventional ethidium bromide-stained agarose or polyacrylamide gel electrophoresis, previous reports described the advantage derived from the analysis of fluorescently labeled PCR fragments.[14-15, 34] The main purpose of the present study was to design a simple, practical and reproducible method that takes advantage of the use of fluorescence-labeled primers and capillary electrophoresis with GeneScan analysis. The fluorescent method has been employed both to identify clonal VDJ rearrangement at diagnosis and to provide a semi-quantitative evaluation of MRD by the direct use of two patient-specific primers during the follow-up.

Samples collected at diagnosis were screened by using the family primers from the leader region and FR1, in conjunction with a single fluorescent reverse primer JH3'. In this way mono-, oligo- and polyclonal samples were clearly discriminated. We were able to identify a monoclonal population in 66.7% of patients tested (68% at diagnosis, 83.3% at relapse, 40% treated) whereas such a population was detected in only 55.5% by the standard procedure with agarose gel electrophoresis. In all but one case we used a smaller amount of DNA (500 ng vs 1 μg) than that previously reported.[8,24,34]

The failure of VDJ identification was due to the small number of PC (< 1%) in two patients of the selected group; in the remaining it could have depended on problems that prevented the annealing of the primers, as explained before. As reported in Table 1, there was often a striking discrepancy in PC numbers recorded by BM morphologic quantification and flow cytometry analysis of the same sample. This may be because the sample for cytometry was diluted by peripheral blood, whereas the estimation of PC numbers in the bone marrow smear was done in the context of tissue particles.

If we consider only the subset of patients with a >1% percentage of bone marrow monoclonal PC by flow cytometry, the identification of monoclonal rearrangement was successful in 70.6% of cases. It should be noted that previous studies fixed a minimum of 10% PC in the sample at diagnosis to enable detection of the VDJ rearrangement.[8,24]

In order to evaluate the residual tumor cells, we amplified all the samples collected during therapy and follow-up directly with the CDRII and the fluorescent CDRIII patient-specific primers. The sensitivity of the reaction, calculated as the median value of three serial dilutions of the DNA at diagnosis with control DNA, was 10^{-5} for 14 patients and 10^{-4} for 4 patients. The variability observed depends mainly on the percentage of PC at diagnosis but also on patient to patient differences.[8]

Martinelli *et al.*[35] fixed a stringent definition for molecular remission as *two consecutive PCR negative results*, but they used standard AGE detection. Only three of the patients included in this study obtained molecular remission in keeping with the above mentioned definition. However, fluorescence PCR at gene scanning did not confirm this result and showed that MCR was only transitory, even in consecutive samples proved to be negative by immunofixation. This observation raises a crucial point pertaining to the sensitivity of the method used to assess MRD. Indeed, the results depend strictly on the detection threshold and no clinical

studies are available to establish the desirable sensitivity, although it seems reasonable to prefer the most sensitive available method. In one patient we were able to anticipate progression of disease, the other two are currently in CR despite having reverted to molecular positivity.

From our experience, we can confirm that direct fluorescent PCR with capillary electrophoresis by GeneScan has many technical advantages. Firstly, with this method, the sensitivity of MRD detection is up to 1 log greater than that of nested PCR and conventional agarose gel.[15] Secondly it avoids the cross-contamination associated with the nested-PCR strategy and maintains two steps of specificity because of the use of two patient-specific primers. Thirdly, it allows the size of the monoclonal band to be identified with a maximum variability of 1-2 nucleotides. A further advantage of this technique is that the procedure could be easily applied to large clinical studies; it is time-saving, requiring only 24h whereas nested PCR takes at least 72h, and is relatively cheap since the automated sequencing apparatus can be used for both fluorescence fragment analysis and for sequencing. Quantitative PCR using TaqMan technology represents the gold standard procedure for monitoring MRD in lymphoproliferative disorders; however, although the proposed method is only semi-quantitative, it seem similarly sensitive and surely less expensive than quantitative PCR which requires sophisticated equipment for real-time PCR in addition to the automated sequencing apparatus. Furthermore this method is easier to perform in clinical laboratories.

In conclusion, the method presented could be considered a valid alternative to the nested PCR approach for IgH rearrangement detection and for a semi-quantitative evaluation of MRD by limiting dilution, as soon as new therapeutic protocols are able to control or eradicate this neoplasia. At this point, it could be useful to adopt a strategy combining different sets of VH and JH primers to improve the clonality detection rate and to increase the number of patients who could be monitored for MRD as previously described.[33]

Contributions and Acknowledgments

EN and IG were the main investigators, involved in the design of the study, analysis of the data and interpretation of the results. EN wrote the manuscript. IG contributed to drafting the paper. FE performed nested PCR, was involved in the molecular analysis, and collected and analyzed the clinical data. DM was responsible for flow cytometry analysis and supervised the technical protocols. AP contributed to the collection and analysis of the clinical data. GC and FR supervised the study, critically reviewed the manuscript and gave final approval for its submission. The authors are grateful to Dr. P. Salgarelli for technical support. EN: prime responsibility for the paper publication; AP: responsible for Table 1; IG: responsible for Figures 1and 2A; FE: responsible for Figure 2B; EN: responsible for Table 2.

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Disclosures

Conflict of interest: none.

Redundant publications: no overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

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What is already known on this topic

The possibility of obtaining the sequence of rearranged immunoglobulin genes in multiple myeloma is already well documented.

What this study adds

A detection system based on capillary electropheresis is original.

Potential implications for clinical practice

Faster analysis of minimal residual disease in B-cell tumors.

Paolo Corradini, Associate Editor (Milan, Italy)