Detection of Genomic Abnormalities in Multiple Myeloma

The Application of FISH Analysis in Combination With Various Plasma Cell Enrichment Techniques

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Abstract

Multiple myeloma (MM) is a hematopoietic neoplasm characterized by malignant plasma cells (PCs) that accumulate in the bone marrow. A number of different genomic abnormalities are associated with MM; however, detection of these by fluorescence in situ hybridization (FISH) can be limited by the percentage of PCs in the specimen. In this study, we tested 20 bone marrow specimens with known MM and a low concentration of monoclonal PCs for the presence of genomic abnormalities using FISH in combination with various PC enrichment techniques: magnetic cell sorting, targeted manual scoring, and automated image analysis. In addition, flow cytometric cell sorting of PCs in combination with FISH analysis was also tested for minimal residual disease applications. Different parameters were evaluated when assessing the detection efficiency of each approach. FISH results are highly dependent on the chosen enrichment method. We describe the evaluation of different techniques applicable for various laboratory settings and specimen parameters.

Multiple myeloma (MM) comprises about 10% to 15% of all hematopoietic neoplasms; it is characterized by the presence of monoclonal plasma cells (PCs) that accumulate in the bone marrow. In 2007, more than 20,000 new cases of MM were diagnosed in the United States.[1] The disease is still considered to be incurable, with a median survival of 3 to 4 years.[2] Diagnostic criteria for MM include the presence of M protein in serum or urine, monoclonal bone marrow PCs, and related organ or tissue impairment such as bone lesions.[1,3]

A number of genomic aberrations are associated with MM, many of which confer prognostic significance. Translocations involving the *IGH* locus on chromosome 14q32 have been reported to occur in 55% to 70% of MM cases. The most common partner genes are *CCND1* (11q13), *FGFR3/MMSET* (4p16), and *MAF* (16q23); the former is associated with a good prognosis and the latter 2 with a poor prognosis.[1] Hyperdiploidy showing nonrandom gain of chromosomes 3, 5, 7, 9, 11, 15, and 21 has been reported in a large percentage of cases and has been suggested as a good prognostic indicator in the absence of a *TP53* gene deletion.[4] Deletions of or monosomy 13 (which are detected in almost 50% of MM cases) had previously been associated with a poor prognosis and early stages of the disease.[5,6] Recent studies, however, show that loss of 13q sequences does not add additional prognostic significance and only the presence of t(4;14), deletion of *TP53*, and/or gain of 1q are associated with short overall survival.[7-9]

Metaphase cytogenetic analysis often fails to detect genomic abnormalities in MM owing to the low proliferation rate of PCs.[10] It has been suggested that the finding of abnormal metaphases during cytogenetic analysis is

reflective of an increased PC proliferation rate, which has a negative impact on prognosis.[11] Interphase fluorescence in situ hybridization (FISH) does not depend on cell proliferation, but its detection sensitivity is limited by the percentage of PCs in whole bone marrow. Polyclonal PCs normally constitute between 0.2% and 2.2% (mean, 1.8%) of the bone marrow.[12] Although this percentage might increase in patients with MM, the monoclonal PC concentration can be close to this low level early in the disease or after treatment. In cases with a low percentage of monoclonal PCs, abnormal cells may be too rare to detect during analysis, leading to a false-negative result, ie, a FISH scoring result that simply falls below the laboratory's established cutoff values, precluding reporting. It has been suggested that FISH should be performed in combination with PC targeting strategies.[8,10] Techniques currently available for PC targeting and enrichment vary in reproducibility and impact on abnormality detection rate, workload, and test cost.

To maximize the detection efficiency of genomic abnormalities in MM, we compared 4 PC-enrichment approaches for FISH analysis in a clinical laboratory setting. These are magnetic cell sorting (MACS), fluorescence-activated cell sorting (FACS), targeted manual scoring, and customized automated image analysis. We conclude that PC-enrichment techniques greatly increase the rate of detected cytogenetic abnormalities in interphase cells and should therefore be performed in all cases with a low monoclonal PC concentration. Different techniques may be more or less suitable depending on specific laboratory needs and specimen parameters.

Materials and Methods

Specimens

We evaluated 20 heparinized bone marrow aspirates with known MM for genomic abnormalities. The percentage of monoclonal PCs in the specimen varied between 0.2% and 6.8% (mean, 2.4%) according to flow cytometric analysis. All specimens were deidentified, and the study was performed in accordance with institutional review board exemption issued by the Western Institutional Review Board (Olympia, WA).

Magnetic Cell Sorting

Magnetic cell separation of PCs was performed using the Whole Blood CD138 MicroBeads, Whole Blood Column Kit, and the QuadroMACS Separation Unit (Miltenyi Biotec, Auburn, CA) according to the manufacturer's protocol. Briefly, 1 mL of heparinized bone marrow specimen was passed over a 200-μm preseparation filter (Miltenyi Biotec) to remove cell clumps and bone fractions; 50 μL of MicroBeads were added to the cell suspension and incubated for 15 minutes at 4°C. Unbound antibodies were removed by a washing step before the cell suspension was transferred to the separation column. After removal from the magnetic field, the immunomagnetic-labeled cells were eluted from the column.

Flow Cytometric Analysis of MACS Enrichment

The MACS enrichment was verified by flow cytometric analysis. The unpurified bone marrow sample and enriched PC population were evaluated for their PC percentage. Each fraction was stained with CD56-fluorescein isothiocyanate (FITC), CD38-phycoerythrin (PE; selectively in some cases with CD138-PE), and CD45-allophycocyanin for 20 minutes at room temperature. Flow analysis was carried out on the FACSCalibur (Becton Dickinson, Franklin Lakes, NJ) with CellQuest software (Becton Dickinson). Data analysis was performed using WinList software (Verity Software house, Topsham, ME; http://www.vsh.com). PCs were identified by their high expression of CD38.

FISH Procedure

The MM FISH panel included the following probes: LSI D13S319 (13q14.3)/LSI 13q34 (LAMP1), IGH/FGFR3 Dual Color Dual Fusion Translocation, LSI TP53/CEP17, and MLL Dual Color Break Apart Rearrangement (Abbott Molecular, Abbott Park, IL). The probes are designed to detect deletion 13q/monosomy 13, the t(4;14)(p16.3;q32.2) translocation or alternate *IGH* gene rearrangements, loss of *TP53*/monosomy 17, and 11q abnormalities (loss or gain), respectively. If FISH results were normal for these probes, a second probe set was used to evaluate the specimen for gain of chromosomes 3, 5, 7, 9, and/or 15.

For each bone marrow sample, FISH was performed on the separated PCs (MACS FISH) and whole specimen (direct-FISH). Cells for direct-FISH were obtained by a standard lymphocyte harvest procedure (0.075 mol/L potassium chloride, 37°C) followed by fixation in Carnoy (3:1 methanol/acetic acid). Slides for FISH were prepared in a temperature- and humidity-controlled chamber (Thermotron, Holland, MI). Before hybridization, slides were aged in 2× saline sodium citrate [SSC] buffer at 37°C for 5 minutes, followed by a pepsin treatment (0.05% pepsin, 0.01N hydrochloric acid) for 10 minutes. The slides were subsequently dehydrated in an ethanol series (70%, 85%, and 100% ethanol) for 2 minutes each. Probe mixture (3.5 μL Vysis Hybridization Buffer [Abbott Molecular], 0.5 μL labeled DNA probe, and 1μL purified water) was applied on the target area, coverslipped, and sealed with rubber cement. Codenaturation of probe and target DNA at 74°C for 2 minutes and hybridization at 37°C were accomplished using a StatSpin ThermoBrite S500 (Abbott Molecular). After overnight hybridization, slides were incubated in wash

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1 (0.4× SSC/0.3% NP-40, 73°C) for 2 minutes followed by wash 2 (2× SSC/0.1% NP-40, ambient temperature) for 30 seconds. Nuclei were counterstained with DAPI II (Abbott Molecular). Slides were stored at –25°C for at least 20 minutes before examination.

Standard Manual FISH Analysis

Two technicians analyzed 100 cells each using an Olympus Reflected Fluorescence System, model No. BX41TF with a 100× objective (Olympus, Center Valley, PA). Touching and overlapping cells were excluded, and cells were analyzed regardless of their shape and size. During a previous validation study, sensitivity cutoff values were calculated by using the Excel (Microsoft, Redmond, WA) statistical function CRITBINOM (n, p, α) with a confidence level of 95%.[13] A specimen was considered "abnormal" if scores from both technicians independently exceeded the sensitivity cutoff values for 1 or more signal patterns. In cases of discrepant analysis results, analysis was performed by a third technician.

Targeted Manual Scoring of PCs

Direct-FISH slides were evaluated as described in the preceding section; however, the technicians focused their analysis on large and mononuclear cells, with the intent to target PCs. Cells with irregularly shaped nuclei, such as neutrophils or monocytes, were excluded.

Automated Image Analysis

The automated slide scanning platform Metafer (MetaSystems, Waltham, MA) was used to analyze direct-FISH slide preparations. A total of 220 cells were captured for each specimen. The detection software classifier was specifically customized to increase the number of PCs using cell selection by shape and size. Briefly, cells were captured and analyzed if they fulfilled the following characteristics: a minimum area of 30.0 μm^2 and a maximum aspect ratio of 1.85 (length of long axis divided by short axis). Automated image analysis and signal detection was accomplished with MetaCyte software (MetaSystems). The results were reviewed to ensure accurate categorization of signal patterns and appropriate cell shapes.

Flow Cytometric Cell Sorting

A FACSVantage SE cell sorter with CellQuest software (Becton Dickinson) was used for PC sorting. Cells were labeled with CD56-FITC and CD38-PE. PCs (n = 1,500) were directly sorted onto microscope slides. The applied sorting rate varied between 700 and 2,000 cells per second. After a fixation step, slides were dehydrated in an ethanol series (70%, 85%, and 100% ethanol) for 2 minutes and processed for FISH as described in the preceding sections.

Results

Flow Cytometric Analysis of MACS-Enriched Cell Populations

We verified the MACS enrichment of PCs in 9 specimens by flow cytometry. The initial concentration on PCs in the bone marrow samples varied between 0.08% and 3.81%. For all cases, enrichment was accomplished. The purity of the resulting separated PC population ranged from 1.06% to 78.29% **Table 1**.

In the first 2 cases, fewer than 10% PCs were present in the MACS-separated fraction; however, both specimens were 5 days old when MACS was performed. We therefore evaluated the success of PC enrichment in correlation with specimen age. One bone marrow sample was subjected to PC enrichment at 3 different time points. The first separation was performed on the second day after specimen collection, the second on day 4, and the last separation on day 8. The percentage of PCs in the enriched population decreased significantly with specimen age. The specimen contained about 58% PCs on day 2 and only about 13% on day 8 **Figure 1**.

Table 1
Verification of Plasma Cell Enrichment*

Plasma Cell Concentration (%)		
Unpurified Specimen	**After Magnetic Cell Sorting**	**Specimen Age at Separation (d)**
0.17	1.06	5
0.88	2.09	5
0.08	13.22	3
0.17	15.79	2
0.32	34.93	3
2.62	43.62	4
3.81	57.77	2
1.39	60.88	3
1.7	78.29	2

* Flow cytometric analysis was performed to evaluate the percentage of plasma cells before and after magnetic cell separation with CD138 MicroBeads.

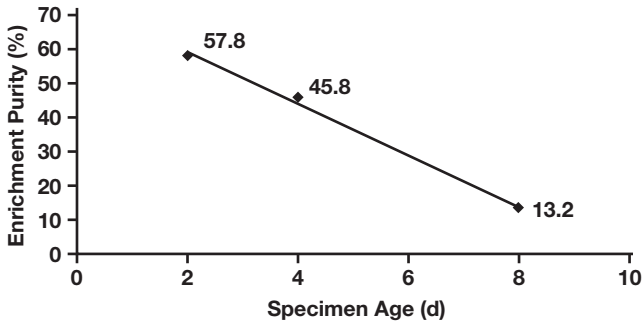

Figure 1 Time dependency of plasma cell enrichment. Plasma cells were isolated from a single specimen at 3 time points after collection. $R^2 = 0.9949$.

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FISH Results Without PC-Enrichment Strategies

We evaluated 20 bone marrow aspirates with a known monoclonal plasma population ranging from 0.2% to 6.8%, as detected by flow cytometric analysis, for genomic aberrations. In standard FISH analysis without PC targeting on unpurified samples, genomic abnormalities were detected in 4 of 20 specimens tested **Table 2**. The remaining 16 evaluated specimens were normal by FISH analysis, although monoclonal PCs were present.

FISH Results After MACS and Targeted Scoring (Manual and Automated) Approaches

The same 20 specimens were subjected to FISH analysis before and after PC enrichment by MACS (Table 2) **Figure 2**. Detected FISH abnormalities after MACS purification and after applying targeted manual or automated scoring techniques without prior MACS purification are shown in Table 2.

In a blinded manner, all direct-FISH slides were manually reanalyzed. During the second evaluation, only round and relatively large cells were examined to target PCs. This technique detected abnormal cells in 12 specimens. The percentage of abnormal cells ranged from 2% to 27% **Table 3** (Figure 2A).

By using an automated scanning system with customized image analysis for PCs, we detected genomic abnormalities in all direct-FISH samples (2 specimens not analyzed) (Table 2, Figure 2B). The percentage of abnormal cells varied between 0.5% and 22.8% (Table 3).

FISH analysis of PCs isolated by MACS detected genomic abnormalities in all 20 evaluated samples (Table 2, Figure 2C). The most frequent aberrations present were gain of MLL or trisomy 11 (8 cases [40%]), monosomy 13 (5 cases [25%]), and gain of *IGH* (14q32) signal (5 cases [25%]). Other abnormalities detected include deletion of *TP53, FGFR3/IGH* gene rearrangement t(4;14), trisomy 3

Table 2
Detailed FISH Analysis Results for Different Plasma Cell Enrichment Techniques

			Percentage of Detected Abnormal Cells			
Case No.	Monoclonal Plasma Cells (%)*	Genomic Aberration	Standard FISH	Targeted Manual Scoring	Automated Slide Analysis	MACS-FISH
2	0.2	Gain IGH	0	2	6.6	56
		Trisomy 11	0	0	5.2	59
3	0.2	Monosomy 13	0	3	0.5	58
10	0.3	Trisomy 11	0	†	0.9	33
8	0.4	Trisomy 11	0	0	2.6	30
17	0.5	Gain IGH	0	0	3.9	18.5
12	0.5	Monosomy 13	0	0	0.9	56.5
20	1.2	t(4;14)	1	7	†	86
18	1.4	Gain IGH	0	3	6.5	19
11	1.5	Monosomy 13	0	4	4.8	61
4	1.8	Trisomy 11	0	5	4.5	38
13	2.1	Trisomy 11	0	0	3.1	58.5
5	2.3	Trisomy 7	0	4	8.1	55
7	2.7	Trisomy 11	0	2	2.5	78
1	2.8	Monosomy 13	0	0	6.5	25
19	3.0	Deletion 13q	0	0	†	35.5
6	3.7	Trisomy 3	0	4	8.6	94
		Trisomy 5	0	11	7.3	94
9	5.1	Monosomy 13	4.3	†	3.6	21
		Deletion 13q	9.3	†	21.8	30
		Deletion IGH	5.0	†	22.8	49
15	5.7	Trisomy 11	4	0	6.3	19
		Tetrasomy 11	19	22	6.3	62.5
		Gain IGH and FGFR3	0	0	3.6	4
		Gain IGH	0	0	15.8	18
		Gain TP53 and CEP17	3	0	4.5	7
		Gain CEP17	4	7	13.6	34.5
14	6.0	Deletion TP53	10.5	27	18.8	88
16	6.8	Trisomy 11	0	13	6.1	89
		Gain IGH	0	2	5.8	44.5

FISH, fluorescence in situ hybridization; MACS, magnetic cell sorting.
* According to flow cytometric analysis.
† Not analyzed.

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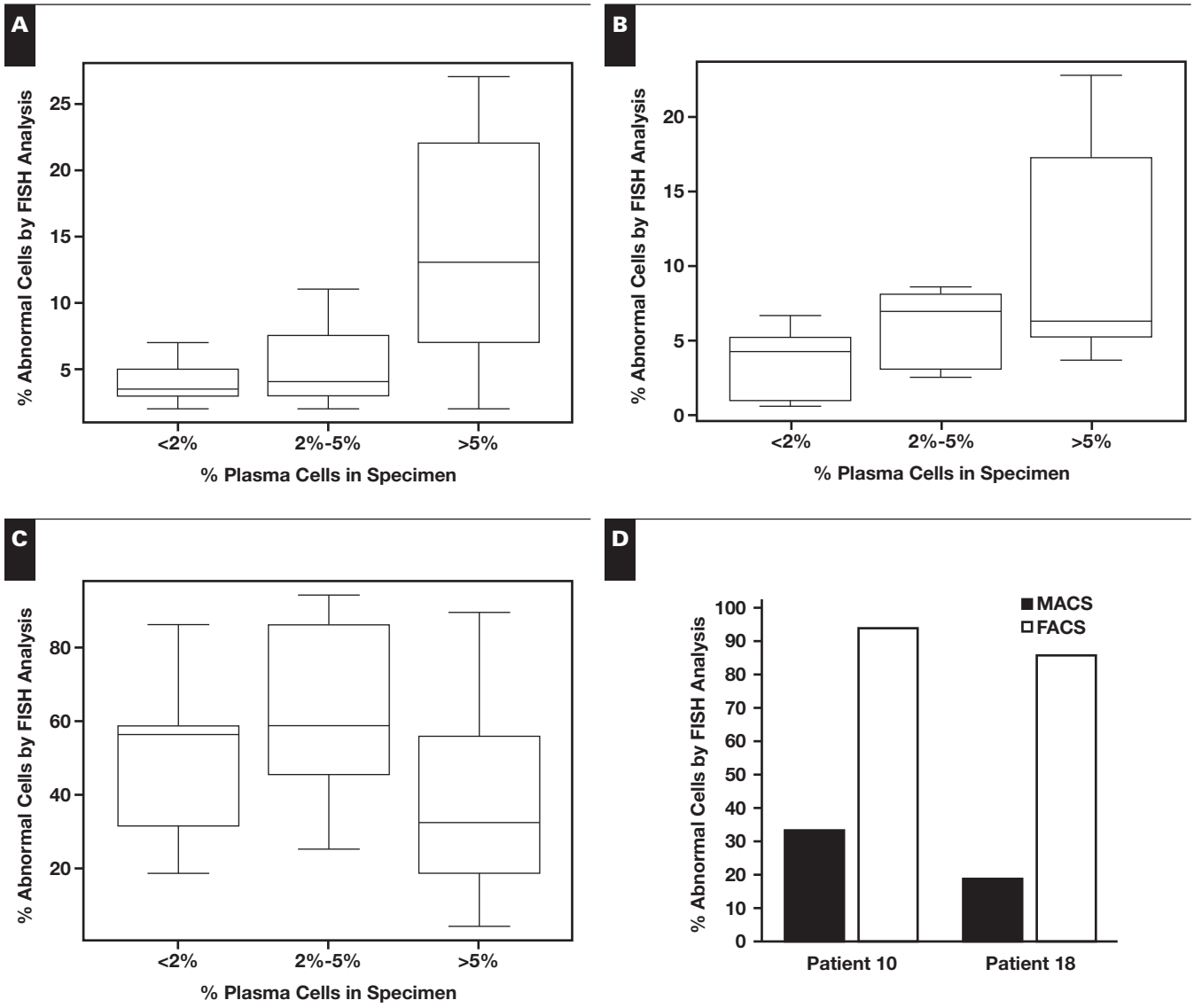

Figure 2 Distribution of fluorescence in situ hybridization (FISH) analysis results according to enrichment technique and initial plasma cell concentration in 20 bone marrow aspirate specimens with estimated monoclonal plasma cell concentrations by flow cytometric evaluation (detailed findings in Table 2). **A**, Percentage of detected abnormal cells by targeted manual FISH analysis. **B**, Percentage of detected abnormal cells by automated slide analysis. **C**, Percentage of detected abnormal cells by magnetic cell sorting (MACS)-FISH analysis. **D**, Comparison of FISH results after plasma cell enrichment by MACS and fluorescence activated cell sorting (FACS). Box plots illustrate the 25th and 75th percentile (bottom and top of box, respectively), the median (band within box), and minimum and maximum (whiskers) of abnormal cells detected.

Table 3
Comparison of FISH Strategies for the Detection of Abnormal Plasma Cells in Known Multiple Myeloma Bone Marrow Aspirate Specimens

Method	Number of False-Negative Test Results	Percentage of Abnormal Cells
Standard FISH	21/30 (70)	1-19 (mean, 6.7)
Manual analysis with plasma cell targeting	11/26 (42)	2-27 (mean, 7.7)
Automated slide analysis with plasma cell targeting	0 (0)	0.5-22.8 (mean, 7.2)
MACS-FISH	0 (0)	4-94 (mean, 47)

FISH, fluorescence in situ hybridization; MACS, magnetic cell sorting.

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and 5, and trisomy 7 in fewer than 10% of the evaluated specimens. The percentage of abnormal cells in the MACS-purified samples varied from 4% to 94% (Table 3).

FACS in Combination With FISH

Two specimens (10 and 18; Table 2) with MM and a known low disease burden were used for flow cytometric PC sorting followed by FISH analysis. In the first case (0.3% monoclonal PCs in the unpurified specimen), MLL gain or trisomy 11 was detected in 94% of the PCs obtained by FACS. The second case (1.4% monoclonal PCs) showed 3 *IGH* signals in 86% of the flow cytometric sorted cells. In addition, PC MACS was performed on both specimens. With this technique the same genomic aberrations were found in 33% and 19% of cells, respectively (Figure 2D). Standard FISH analysis of both specimens showed no evidence for gain of *MLL* or *IGH* signals from unenriched bone marrow aspirates.

Discussion

For MM specimens with low percentages of monoclonal PCs, the combination of FISH analysis with PC-enrichment techniques is important for achieving a high level of confidence for the detection of genomic abnormalities. In this study, we evaluated and compared various methods specifically targeting the cells of interest in MM.

Flow Cytometric Analysis of Plasma Cell Populations

Flow cytometric evaluations were used to assess and compare PC concentrations in the present study. PCs are known to often be aggregated or lost in flow cytometric acquisition. Whereas quantitative PC concentrations determined by flow cytometric analysis are useful for the comparison and quality control of different PC-enrichment strategies, their values are known to be consistently lower in comparison with conventional morphologic enumeration. Paiva et al[14] reported a median difference of 23% between flow cytometric and morphologic PC counts in addition to a positive correlation between the 2 techniques.

We and others observed that the final PC purity fluctuates even in samples with similar initial concentrations of PCs.[15-18] A side-by-side study by Ahmann et al[18] evaluated the effect of specimen shipping and delayed PC harvest. The results showed that purity and PC recovery are higher from specimens that are immediately subjected to MACS. This comparison supports our observation about the negative correlation between PC-enrichment success and increasing specimen age. According to Jourdan et al,[19] PCs rapidly lose the CD138 marker once they are separated from the bone marrow environment. Due to MicroBeads targeting the PC-specific CD138 marker, MACS should be performed as soon as possible to ensure sufficient PC yield. Flow cytometric quantification of CD138-expressing PCs can provide a quality control step if implemented before each plasma cell separation to ensure adequate CD138 surface expression. In addition, flow cytometric analysis targeting CD38 can be used as a postenrichment control step to confirm adequate plasma cell yield for subsequent FISH analysis.

Standard FISH Analysis

Standard FISH analysis of whole bone marrow specimens detected genomic aberrations in only 4 of 20 specimens. For the majority of these positive cases, scoring results only slightly exceeded the established normal reference cutoff, resulting in low confidence for calling a FISH result abnormal. This may cause difficulties in detecting loss of 1 hybridization signal for the *TP53* region on chromosome 17. The potential overlap of 2 normal *TP53* signals in addition to a low signal intensity of this probe set are the reasons for an often higher laboratory-specific cutoff value to detect *TP53* deletion. Because loss of the *TP53* tumor suppressor gene is one of the most important prognostic factors in MM, it is crucial to provide accurate analysis results for these loci, even for specimens with a low level of monoclonal PCs.

FISH-Targeted Scoring Results

Targeted PC scoring can be performed on unpurified specimens. Technologists focus their analysis on large mononuclear cells to increase detection sensitivity for PC abnormalities. According to our comparative analysis, the increase in detection sensitivity by targeted manual FISH analysis is low in comparison with MACS enrichment. The ability to detect abnormalities by using manual targeted analysis only decreased the false-negative test result rate from 70% to 42% in our study, with a percentage of detected abnormal cells ranging from 2% to 27%. Other possible disadvantages for this technique include the additional training necessary for PC recognition by technologists and the prolonged analysis time. In addition, this technique introduces a certain degree of subjectivity and lack of reproducibility. Consequently, target PC scoring should be considered as an option only if other techniques have not yet been or cannot be established in the laboratory.

The best results for evaluation of unpurified specimens were obtained when FISH was combined with automated image analysis. Genomic aberrations could be identified in all cases (false-negative result rate of 0%). The use of an automated scanning system has a number of advantages, including faster analysis time, accurate and detailed results documentation, as well as precise and reproducible scoring results due to objective analysis algorithms. However, it has to be considered that the system's capability to detect genomic abnormalities in cases of very low tumor burden may still be

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limited. The high purchase price and time commitment necessary to develop and validate customized software algorithms for targeted PC detection also have to be considered.

FISH Results After MACS

In this study, PCs enriched by immunomagnetic anti-CD138 beads (MACS) revealed 0% false-negative FISH results in addition to higher abnormal cell rates in comparison to manual and automated scoring techniques evaluating unseparated specimens. We detected genomic aberrations using MACS enrichment at similar frequencies in comparison with findings published by others.[15,20,21] The high percentage of abnormal cells (4%-94%; mean, 47%) allows strong confidence for making a negative or positive "call" and/or confirming the presence of minimal residual disease (MRD), if that is the clinical question being asked.

From a technical viewpoint, MACS can be easily established in different laboratory settings owing to its relatively low setup cost and straightforward procedure. It is, however, also necessary to be aware of the disadvantages and limitations of MACS. We estimated material cost of $35 for each separation at the time of publication, and so far there is no Current Procedural Terminology (CPT) code available to bill for this process. Magnetic separation is more time-consuming than a direct harvest and might challenge the established daily routine of a clinical laboratory without an increase in technical staff. Quality control steps have to be developed to guarantee successful PC isolation and to rule out low PC purity or even loss of the enriched population. In addition, it can be a challenge to develop slide preparation techniques suitable for MACS-purified cells, reproduce good FISH hybridization quality obtained by standard specimens in a clinical laboratory for all FISH probes used, and avoid loss of cells. The major technical drawback of MACS seems to be the age of the specimen at the time of separation. The decreasing PC yield of MACS with increasing specimen age hampers the ability to perform "add-on" tests at a later time and still obtain accurate FISH results.

FACS in Combination With FISH

As an alternative to MACS, we demonstrated FISH analysis on flow cytometric–sorted PCs. The use of a set of various antibodies (eg, anti-CD45, anti-CD56, and anti-CD38) in combination with assessment of the light scattering properties of the cells allows more specific purification of PCs. We compared FISH analysis results of PCs separated by MACS and FACS. The difference in the percentage of abnormal cells confirms that FACS enables a more pure PC enrichment. This technique does not depend entirely on the presence of CD138 and is therefore especially suitable for specimens with decreased expression of this surface protein. With our technique we were able to detect genomic abnormalities by sorting as few as 1,500 PCs. Hence, the method is highly valuable for monitoring MRD using patient-specific genomic abnormalities that were identified at diagnosis. There are also drawbacks for the application of this technique in routine clinical practice, the first and foremost of which is that few laboratories have access to a FACS sorter validated for clinical use. Moreover, flow cytometric cell sorting of single specimens is labor-intensive, cannot be automated, and is expensive due to the costs of monoclonal antibodies, capital equipment, and maintenance.

Other Approaches

In addition to the methods described, other options for PC enrichment are available. In this study, we were unable to include cytoplasmic immunoglobulin FISH (cIg-FISH) in the group of evaluated methods due to the lack of this platform in our laboratory.

cIg-FISH entails the targeted analysis of PCs from an unpurified specimen that are identified by light chain–specific immunofluorescence.[22-24] Several studies have shown an increase in the rate of abnormality detection using cIg-FISH comparable to MACS.[22-24] Due to the κ or λ staining, this method is more specific than the manual technique of targeting PCs by visual assessment alone in whole marrow preparations.

Nevertheless, it has to be taken into account that cIg-FISH requires additional time and expertise, which may present obstacles for its integration into a conventional FISH laboratory. Extra processing steps are required in comparison with routine FISH testing, including cytocentrifuged slide preparations followed by immunostaining. Furthermore, the cIg-FISH scoring process includes cytomorphologic assessment to exclude non-PCs that stain positively for cIg.[18] Additional technologist training and analysis time per case are required for this technique. Immunostaining of PCs may be variable, and κ and λ staining may have to be performed if the light chain restriction is unknown. The interference of cytoplasmic immunostaining fluorescence with FISH signals in the nucleus has to be ruled out by careful optimization of wet-lab and analysis procedures. Moreover, nonproducer myeloma cases (no cIg synthesis) or PCs harboring t(11;14) with a lymphoplasmacytic morphologic appearance may be difficult to detect. PCs have to be identified manually in cIg-FISH in a large background of other hematopoietic cells, whereas PC enrichment processes (MACS, Miltenyi Biotech, and EasySep, Stemcell Technologies, Vancouver, Canada) can be automated to accommodate higher specimen throughput. Direct PC enrichment or selection can also be used for downstream DNA isolation if additional follow-up studies are required (eg, B-cell gene rearrangement studies for clonality assessment or microarray analysis for chromosomal copy number aberrations in the case of normal cytogenetic studies).[25]

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One important advantage of cIg-FISH is that specimens of advanced age with loss of CD138 expression can still be successfully analyzed, if FACS separation of PCs is unavailable as an alternative approach to magnetic bead enrichment.

As additional techniques for minimal disease confirmation and monitoring in MM, clonality assessment by B-cell gene rearrangement and real-time polymerase chain reaction (PCR) analysis have to be considered. Quantitative real-time PCR analysis is currently the most sensitive and specific MRD monitoring approach available for monoclonal B-cell populations. However, setup requires the design and development of patient-specific oligonucleotide PCR assays.[26] The clonal *VDJH* gene rearrangement signature has to be sequenced for every patient, followed by primer design and assay validation; this method is currently cost- and time-prohibitive for routine clinical use. As an alternative approach for MRD monitoring, PCs can be sorted by flow cytometry, and standard gene rearrangement PCR analysis can then be used to confirm the presence of patient-specific clonal signatures that are known from the time of diagnosis.[27]

Conclusions

To avoid false-negative FISH results, target cell–specific enrichment techniques are highly recommended in the case of low monoclonal PC tumor burden. We demonstrated that FISH results are dependent on the enrichment method used. The percentage of abnormal cells detected is associated with the initial PC concentration if targeted manual or automated analysis is applied on unpurified specimens. In contrast, CD138 expression and specimen age will affect the accuracy of FISH results when performing MACS or FACS. Therefore, benefits and limitations of each PC targeting technique have to be considered before choosing which method(s) to validate for clinical use and in what clinical circumstances each method would be most accurate **Table 4**.

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HematoLogics is a for-profit clinical reference laboratory and offers multiple myeloma FISH analysis on plasma cell–enriched specimens.

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Table 4
Comparison of Different Plasma Cell Targeting Strategies

Criteria	MACS	Manual Slide Analysis With PC Targeting	Automated Slide Analysis With PC Targeting	FACS
Sensitivity increase	High on fresh specimens	No. of detected abnormal cells is low	Sensitivity varies with percentage of PCs and hybridization quality	Very pure separation of PCs, even in MRD cases
Operating expenses	$35 for each separation	Additional analysis time	Equal to standard FISH analysis	Additional cost for monoclonal antibodies
Capital cost	Low	No additional capital costs	High purchase price for instrument and software	High purchase price for instrument and software
Technical effort	Low; straightforward procedure	Extensive training required	Low	High
Time requirement	Additional ~1.5 h for separation	Additional ~15 min additional analysis time	Faster data acquisition	~1 h for labeling; ~1 h for instrument setup and sorting
Reproducibility and objectivity	Good	Low; technique very subjective	High owing to analysis algorithms	Good
Specimen requirements	Fresh specimen with high CD138 expression	Relatively high percentage of plasma cells (estimated >5% by flow cytometry)	Relatively high percentage of plasma cells (estimated >5% by flow cytometry)	No special requirements
Conclusion	High detection efficiency for fresh specimens with sufficient expression of CD138	Low detection confidence; not an alternative for MACS or FACS in cases of low PC percentages	Precise for specimens with medium percentages of PCs	Especially valuable in cases of MRD or specimens with increased age or low CD138+ expression; difficult integration into daily clinical workflow

FACS, fluorescence activated cell sorting; FISH, fluorescence in situ hybridization; MACS, magnetic cell sorting; MRD, minimal residual disease; PC, plasma cell.

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