Optimal Strategy for Obtaining Chromosome (CA) and Fluorescence In Situ Hybridization (FISH) Results for Plasma Cell Dyscrasias (PCD) by Using Both Positive and Negative Fractions Of CD138 Enriched Plasma Cells (EPC)

Veronica Ortega, BA, CG(ASCP)^{CM}, Christina Mendiola, BS, CG(ASCP)^{CM}, Gihan Mohamed, BS, CG(ASCP)^{CM}, William Ehman, Jr., BS, CG(ASCP)^{CM}, Gopalrao V.N. Velagaleti, PhD

Department of Pathology, University of Texas Health Science Center, San Antonio, TX

Abstract

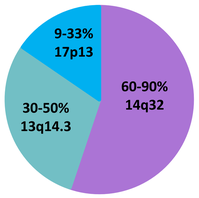
Plasma cell dyscrasias comprise a genetically diverse group of diseases such as monoclonal gammopathy of undetermined significance, plasmacytoma, smoldering myeloma, indolent myeloma, and plasma cell myeloma. FISH is superior to CA in detecting important prognostic genetic abnormalities in PCD; however, its sensitivity is hampered due to paucity of PCs in whole BM and often shows false-negative results when frequency of abnormal cells is below the cut-off values. Studies have shown that the abnormality detection rate in EPCs is greater than unselected plasma cells (UPC), but purification techniques are limiting to only FISH when bone marrow volumes are inadequate. The inability to perform CA may compromise patient care since CA is equally important for detecting non-PC related abnormalities, such as secondary myelodysplastic syndrome, when diagnosis is undefined. To resolve this critical issue and optimize limited quantity received, we designed a pilot study where an immnuno-magnetic CD138 enriched positive selection was used for FISH while the negative selection was used to retrieve other myeloid elements for CA. Parallel FISH studies were performed using UPCs and CD138 EPCs, while karyotyping was achieved using whole BM and discarded myeloid elements. Purity of EPC was confirmed by flow-cytometry (47.3% to 96.9%). Results showed that the abnormality rate of EPC (74%) was doubled compared to UPC (34%) for FISH, and CA displayed 100% (16/16) success rate using the discarded myeloid elements. PCD related chromosome abnormalities were confined to whole BM while non-PCD related abnormalities were found in both whole BM and discarded myeloid elements confirming effective removal of PCs by isolation and efficient utility of the supernatant for CA. Significantly higher frequency of IGH rearrangements (36%) and hyperdiploidy (24%) was observed in the EPC group compared to the UPC group (11% for both). Our results confirm the superior diagnostic potential of selecting PCs and demonstrate the feasibility of using the remaining myeloid elements for CA.

Introduction

- **Plasma cell dyscrasias are hematopoietic neoplasms that are produced as a result of malignant proliferation of a monoclonal population of plasma cells (Pozdnyakova et al, 2009).**
- **Also called Multiple Myeloma (MM), Plasma Cell Myeloma is the most advanced stage of all PCDs.**
- **It is the second most common hematological malignancy in the US comprising approximately 10-15% of all hematopoietic neoplasms (Mailankody et al, 2011; Hartmann et al, 2011; Christensen et al, 2007).**
- **Genomic abnormalities are important prognostic indicators. (Figure 1)**

Genomic Abnormalities

Incidence of Abnormalities Detected by FISH



- **IGH mixed prognosis**
- **RB1 poor prognosis**
- **p53 poor prognosis**

(Slovak et al, 2009)

Figure 1

Detection of Genomic Abnormalities

Detection can be achieved by CA and FISH using whole BM (WBM) samples. CA is restricted due to the slow proliferation rate of plasma cells and limited bone marrow infiltration resulting in 60-70% normal karyotypes and abnormalities may not be detectable until advanced stages.

FISH is superior to CA because it circumvents the need for cell division. It detects abnormalities in earlier stages of disease and can identify more cryptic changes of IGH, RB1 and p53, which is more informative than conventional cytogenetics (Pozdnyakova et al, 2009; Put et al, 2010).

Limitations of FISH using Unselected Plasma Cells (UPC)

Using WBM for FISH has limitations. Non-plasma cells comprise majority of the cell population in BM, so plasma cells may be underrepresented. Even in confirmed cases of PCD, the number of PC ranges from <10% in MGUS to >30% in myeloma. This in turn may show false-negative results when the frequency of abnormal cells is below the laboratory cut-off values (Fonseca et al, 2004; Pozdnyakova et al, 2009).

Limitations of Enriching for PCs (EPC)

Although several studies have shown EPC FISH is superior to UPC, the most significant limitation is sample size. For small samples, most labs prefer FISH on EPCs because of its higher sensitivity while sacrificing CA. The drawback of performing EPC FISH alone is that non-PC related abnormalities remain unidentified which may compromise patient care. Sacrificing CA may also risk accurate diagnosis of therapy-related myeloid malignancies.

Clinical Significance of CA

Secondary AML/MDS has been reported following myeloma for more than 4 decades. Studies show MM patients have an 8-11-fold increased risk of developing AML/MDS. Cytotoxic chemotherapy is known to induce unbalanced translocations involving chromosomes 5 or 7 and del(20q) is a common recurrent aberration in treated myeloma patients resulting from stem cell damage (Mailankody et al, 2008; Papanikolaou et al, 2011).

Materials and Methods

Hypothesis and Study

We hypothesized that EPCs are superior to UPCs by FISH analysis in detecting genomic abnormalities and that the remaining cellular components (RCC) after plasma cell isolation can be used for chromosome analysis to detect non-PC related abnormalities.

Testing the Hypothesis:

Study I: FISH results using UPCs vs. CD138 EPCs
UPC: *57 Samples (January-September 2010)*
EPC: *58 Samples (April-December 2011)*

Study II: Karyotype results using WBM vs. RCC
37 Samples

- **EasySep Human CD138 Selection Kit - *StemCell Technologies* (Figure 2)**
- **GSL10 CytoVision Scanning System – *Leica Microsystems***

Study I (FISH)

To isolate the CD138 enriched plasma cells, whole BM was mixed with Positive Selection Cocktail, then with Magnetic Nanoparticles containing CD138 antibodies. The tube was placed in the magnet and washed with buffer to remove unbound cells. The supernatant comprising the RCC was poured off leaving bound plasma cells inside the tube (StemCell Technologies, 2008).

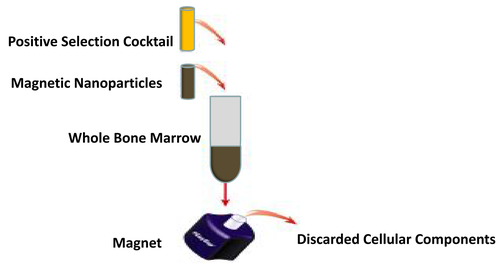


Figure 2

MM FISH Panel

Once isolated, plasma cells are harvested for FISH and hybridized using 5 probes, RB1, IGH/MAF, IGH/FGFR3, ATM and p53. (Figure 3)

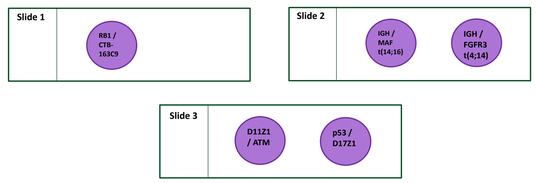


Figure 3

Study II (Karyotyping)

In the second study the negative fraction with RCC was used for CA. The supernatant was poured off but not discarded. It was centrifuged and a 24 hr culture was initiated. (Figure 4)

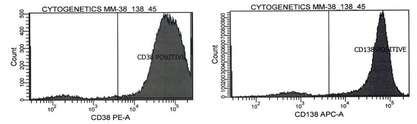
Negative Fraction Containing Discarded Cellular Components (Supernatant)

Figure 4

Results

Flow Cytometry

After isolation, purity of 90.6% CD138+ plasma cells was detected by flow cytometry. (Figure 5)



Tube: 38_138_45

Population	#Events	%Parent	%Total
All Events	30,000	####	100.0
CD38 POSITIVE	27,533	91.8	91.8
CD138 POSITIVE	27,172	90.6	90.6

Figure 5

Study I (FISH)

The top panel shows FISH scores from 2 techs apparently showing hyperdiploidy; 3 signals for ATM and p53 in 19 out of 200 cells. The bottom panel scores from the same patient after isolation of plasma cells demonstrates a significant increase. The frequency of abnormal cells rose by 82.5% . (Figure 6)

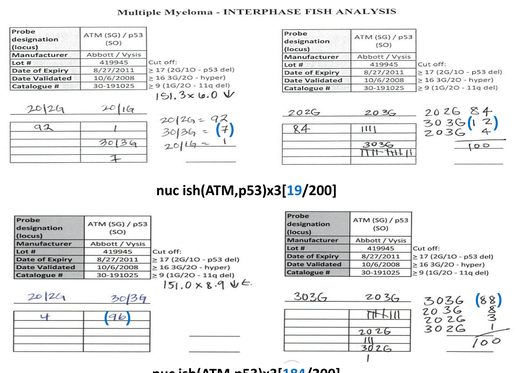


Figure 6

Abnormalities detected increased by ~40%
IGH (14q) abnormalities increased by ~20%
Hyperdiploidy and RB1 (13q) abnormalities increased by ~15% (Figure 7)

UPC vs. EPC FISH cases

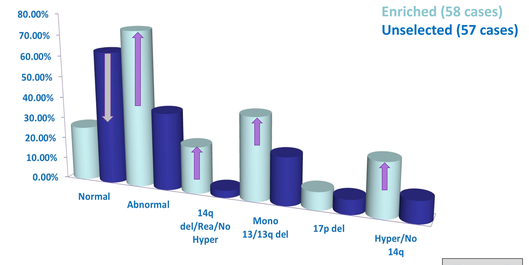


Figure 7

Study II (Karyotyping)

- **CA on the negative fraction was successful in 100% (37) of samples.**
- **Eight cases revealed interesting data with reference to our study using RCC.**
- **Karyotypes with abnormalities of unknown significance and loss of Y chromosome were found in both WBM and RCC. (Table 1, cases 1 & 2)**
- **Karyotypes with abnormalities of myeloid origin and other neoplastic processes were confined to only RCC. (Table 1, cases 3 & 4)**
- **Karyotypes with PCD related aberrations were seen only in the WBM and these aberrations have concordant FISH results on isolated PCs. (Table 1, cases 5, 6, 7 & 8)**

Study II (Karyotyping)

Table 1

Case	Routine Chromosome Analysis Results	WBM CA	RCC CA	EPC FISH Results
1	46,XY,der(9)dup(9)(p13)ins(9) (p11p13)[14]/ 45,idem,-Y [6]	Yes	Yes	Normal
2	45,X,-Y[3]/46,XY[17]	Yes	Yes	Normal
3	52,XX,+5,+add(5)(q31),+7,+del(7)(q22),+19,+19, -20,+22[2]/46,XX [19]	ND	Yes	Normal
4	46,XX,del(13)(q14q22)[10]/46,XX[10]*	ND	Yes	Monosomy 13 (99%)
5	46,XY,t(11;14)(q13.1;q32)[1]/46,XY[19]	Yes	No	IGH rearrangement (69.5%)
6	47~48,XY,dup(1)(q12q42),+add(3)(p11), add(6)(q23),+9,+11,-13,-15,add(17)(p11.2),-18, add(22)(p11.2),+mar1,+mar2,+mar3,+mar4[cp3]/ 46,XY[17]	Yes	No	Trisomy 11 (77.5%) 13q deletion (69.5%) p53 deletion (84%)
7	57~58,X,X,add(1)(p32),+add(1)(p32),+5, +add(6)(q15),+7,-8,-10,add(10)(q22),+11,+15,+19, +21,+21,+21,+mar1,+mar2,+mar3,+mar4[cp3]/ 46,XX[19]	Yes	No	Trisomy 11 (78%)
8	45,X,-Y[9]/45,X,-Y,del(13)(q14q22)[2]/ 46,XY,del(13)(q14q22)[2]/46,XY[9]**	Yes [del(13q),-Y]	Yes (-Y only)	13q deletion (73%)

*** Since the result of the MM EPC FISH study is ABNORMAL and showed a monosomy 13/13q deletion, additional FISH studies with the same RB1,CTB-63C9 probe were performed on the metaphases to determine whether the 13q deletion seen on chromosomes is the same clone as seen in the plasma cells. The results of these studies showed that the deletion observed on chromosomes is different to the one observed on plasma cells and as such may have originated in non-plasma cells. A 13q deletion is a nonspecific abnormality that can occur in either lymphoid or myeloid disorders.**

**** The monosomy 13/13q deletion found only in the WBM CA and confirmed by MM EPC FISH demonstrates effective removal of plasma cells from the RCC leaving only the –Y abnormality found in the RCC CA. Loss of Y is a nonspecific abnormality with unknown clinical significance.**

Conclusions

- **Data from Study I confirms that EPC FISH increases the detection rate of genomic abnormalities.**
- **Inadequate sample size is limiting and the inability to perform CA may compromise patient care since it is equally important for detecting non-PC related abnormalities in diagnostic and post-treatment samples.**
- **Results from Study II support the superior diagnostic potential of selecting PCs and demonstrate the feasibility of using the remaining cellular components (RCC) for CA.**
- **Retrieving the RCC from the negative fraction proves to be an innovative strategy for performing CA.**
- **An algorithm was developed to assist in the initiation process when CA and FISH are requested. Tests performed are based on sample volume as detailed in Figure 8. Any volume less than 1 ml is reduced to CA only to avoid compromising testing.**

Algorithm for Initiation

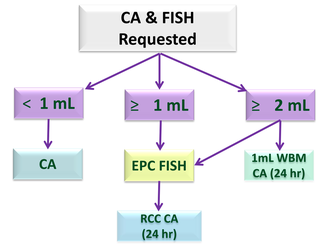


Figure 8

References

- Mailankody S, Pfeiffer R *et al*., Risk of Acute Myeloid Leukemia and Myelodysplastic Syndromes after Multiple Myeloma and its Precursor Disease (MGUS). Blood 2011;118: 4086-4092.
- Papanikolaou X, Barlogie B, Usmani S.Z. Therapy-related Myeloid Malignancies in Myeloma. Mediterr J Hematol Infect Dis 2011;3(1): e2011047.
- Hartman L, Biggerstaff J *et al*., Detection of Genomic Abnormalities in Multiple Myeloma: The Application of FISH Analysis in Combination With Various Plasma Cell Enrichment Techniques. Am J Clin Path 2011;136:712-720.
- Christensen J, Abildgaard N *et al*., Interphase Fluorescence in situ Hybridization in Multiple Myeloma and Monoclonal Gammopathy off Undetermined Significance without and with Positive Plasma Cell Identification: Analysis of 192 Cases from the Region of South Denmark. Cancer Gen and Cyt 2007;174:89-99.
- Pozdnyakova O, Crowley-Larsen P *et al*., Interphase FISH in Plasma Cell Dyscrasia: Increase in Abnormality Detection With Plasma Cell Enrichment. Cancer Gen and Cyt 2009;189:112-117.
- Put N, Lemmens H *et al*., Interphase Fluorescence in situ Hybridization on Selected Plasma Cells is Superior in the Detection of Cytogenetic Aberrations in Plasma Cell Dyscrasia. Gene, Chr & Cancer 2010;49:991-997.
- Slovak M, Bedell V *et al*., Targeting Plasma Cells Improves Detection of Cytogenetic Aberrations in Multiple Myeloma: Phenotype/Genotype Fluorescence in situ Hybridization. Cancer Gen and Cyt 2005;158:99-109.
- Fonseca R, Barlogie B *et al*., Genetics and Cytogenetics of Multiple Myeloma: A Workshop Report. Cancer Res 2004;64:1546.

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ORIGINAL ARTICLE

Optimal strategy for obtaining routine chromosome analysis by using negative fractions of CD138 enriched plasma cells

Veronica Ortega, Gihan Mohamed, William Ehman Jr., Meiqing Zhu, Christina Mendiola, Gopalrao Velagaleti *

Department of Pathology, University of Texas Health Science Center, San Antonio, TX, USA

Fluorescence in situ hybridization (FISH) is superior to routine chromosome analysis (RCA) in detecting important prognostic genetic abnormalities in plasma cell dyscrasia (PCD); however, its sensitivity is hampered due to paucity of plasma cells (PC) in whole bone marrow (BM). Studies showed that the abnormality detection rate in enriched plasma cells (EPC) is greater than unselected plasma cells (UPC), but purification techniques are limiting to only FISH when sample volumes are inadequate. Not performing RCA may compromise patient care since RCA is equally important for detecting non-PC related abnormalities when the diagnosis is undefined. To resolve this critical issue, we designed a study where an immuno-magnetic CD138 enriched positive selection was used for FISH while the negative fraction (NF) was used to retrieve other myeloid elements for RCA. Parallel FISH studies were performed using UPC and CD138 EPC, while karyotyping was achieved using whole BM and discarded myeloid elements from the NF. Results showed that the abnormality rate of EPC was doubled compared to UPC for FISH, and CA displayed 100% success rate using the NF. PCD related chromosome abnormalities were confined to whole BM while non-PCD related abnormalities were found in both whole BM and NF. Our results demonstrate the feasibility of using the NF for RCA.

Keywords Plasma cell dyscrasia, negative fraction, CD138, enriched plasma cells, unselected plasma cells

Introduction

Plasma cell dyscrasias (PCD) are hematopoietic neoplasms that are produced as a result of malignant proliferation of a monoclonal population of plasma cells. They consist of a genetically diverse group of diseases such as monoclonal gammopathy of uncertain significance (MGUS), plasmacytoma, smoldering myeloma, indolent myeloma and plasma cell myeloma (1). Plasma cell myeloma, also called multiple myeloma (MM), is the most advanced stage of all PCD and is the second most common hematological malignancy in the United States comprising approximately 10–15% of all hematopoietic neoplasms. The overall survival is a few months to more than 20 years depending on the significant prognostic genetic abnormalities and treatment (2–5). Detection of such vital prognostic genetic abnormalities by routine chromosome analysis (RCA) is limiting due to the slow proliferation rate of the plasma cells and limited bone marrow infiltration. This may result in normal karyotypes in about 60–70% of patients with PCD and abnormal clones are not detected until advanced stages of the disease (2,6). The advent of interphase FISH studies improved the detection rate of these genetic abnormalities since interphase FISH can circumvent the need for cell division and can also detect abnormalities in earlier stages of the disease (2). However, interphase FISH studies are also hampered by the limited number of PC in the bone marrow as the number of PC in patients with PCD is known to range from <10% in MGUS to >30% in MM. Given that non-PC comprise majority of the cell population in the bone marrow, PC may be underrepresented in early stages of PCD. This in turn may lead to false-negative results by interphase FISH analysis when the frequency of abnormal cells is below

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* Corresponding author.
E-mail address: Velagaleti@uthscsa.edu

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the laboratory established cut-off values (2,7). Several studies have conclusively shown that isolation and enrichment of plasma cells (EPC) significantly improved the abnormality detection rate compared to unselected BM samples and thus is the ideal method for identifying important prognostic genetic abnormalities (6–9). However, there are limitations involving isolation or the enrichment mechanism to select for PC. When the sample volume is inadequate (1 ml or less) both FISH on isolated PC and RCA cannot be performed since most laboratories require a minimum of 1 ml of whole BM sample for plasma cell isolation. In such situations, most laboratories prefer FISH on isolated PC because of higher sensitivity. The inability to perform RCA may compromise patient care since RCA is equally important for detecting non-PC related abnormalities. In cases where the diagnosis is still undetermined, which is the case for most laboratories when they receive the whole bone marrow sample for myeloma studies, RCA is crucial for diagnosis of non-PC related abnormalities. We hypothesized that instead of discarding the left-over negative fraction containing the non-plasma cell components from the BM after isolation of plasma cells, it can be used for RCA to detect non-plasma cell related chromosome abnormalities.

Materials and methods

The study included all BM samples received with a clinical indication of PCD from September 2012 through June 2014 and comprised a total of 153 samples. Of these 153 samples, 82 had RCA performed on both the NF (A culture) and whole BM (B culture). The remaining 71 samples had RCA performed only on the NF. The clinical indication and type of cultures set up for RCA on these samples are as follows: 10 samples (3 A only and 7 both A and B cultures) had unspecified PCD, 2 (1 A only and 1 both) had plasmacytoma, 23 (11 A only and 12 both) had MGUS, 9 (4 A only and 5 both) had lytic bone lesions, 5 (2 A only and 3 both) had amyloidosis, 99 (49 A only and 50 both) had multiple myeloma and 5 (1 A only and 4 both) had miscellaneous clinical indication. Initially, when the method was optimized and validated, the whole BM was used to set up a 48 hour (B) culture while the NF was used for 24 hour (A) culture. However, the study design was modified to set up only 24 hour cultures on both so that accurate evaluations can be made regarding clinical efficacy and diagnostic yield between the 2 methods. Thus a total of 65 of the 82 (79.2%) samples had both whole BM and NF studied on 24-hour cultures, while the remaining 17 (20.7%) samples were studied using a 24 hour NF culture and a 48 hour whole BM culture. All the samples were processed and plasma cell isolation was performed within 24 hours of sample receipt.

Plasma cells were isolated from the whole BM using the EasySep™ Human Whole Blood and Bone Marrow CD138 Positive Selection Kit (StemCell Technologies, Vancouver, BC, Canada). In brief, whole BM was mixed with Positive Selection Cocktail, then with Magnetic Particles containing CD138 antibodies. The tube containing the mixture was placed in the magnet and washed with RoboSep buffer to remove unbound cells. The supernatant comprising the NF was poured off into another sterile 15 mL centrifuge tube leaving bound plasma cells inside the tube. After the purification wash of bound plasma cells with RoboSep buffer 3 times, the bound plasma cells were re-suspended in 5 ml of fresh RPMI 1640 media. The cell suspension with isolated plasma cells was immediately processed for interphase FISH studies using the standard protocols while the cell suspension with the NF was used to set up a 24-hour culture along with the original whole BM. Purity of the isolated plasma cells was confirmed by flow cytometry whenever possible. Culture harvest and banding was performed using standard methods. RCA was carried out using a GSL10 Chromoscan Automated Metaphase Finding System (Leica Biosystems, Buffalo Grove, IL, USA). A total of 10 G-banded metaphases were analyzed from each culture, i.e., from the NF and from the whole BM. Interphase FISH analysis on the isolated plasma cells was carried out manually using five probes: FGFR3/IGH for detection of t(4;14), ATM/D11Z1 for detection of 11q deletion, RB1/CTB-163C9 for detection of monosomy 13/13q deletion, IGH/MAF for detection of t(14;16), and p53/D17Z1 for detection of 17p deletion (Rainbow Scientific, Windsor, CT, USA). A total of 200 interphase plasma cells were scored for each probe, 100 each by two experienced technologists in a blinded fashion.

Results

Of the 153 samples analyzed by RCA, chromosome abnormalities were identified in 15 patients (9.8%). An additional 10 patients showed loss of Y chromosome (6.5%), thus giving an abnormality rate of 16.3%. Of the 15 patients with abnormal chromosome results, 9 (60%) patients had both the NF (A) and whole BM (B) analyzed, while the remaining 6 patients (40%) had only the NF available for analysis (Table 1). Of the 10 patients with loss of Y chromosome, 6 patients (60%) had both cultures available for analysis while the remaining 4 (40%) only had the NF available for analysis (Table 1). Among the patients with both whole BM and NF cultures available for analysis, 3 patients (Table 1, patients 11, 14 and 15) showed non-myeloma related abnormalities in both cultures. Patient 11 had deletion of 5q which is often associated with myelodysplasia and not known to be associated with PCD, patient 14 similarly showed gain of chromosome 8 (trisomy 8) which is seen in both lymphoid and myeloid malignancies, but not in PCD, while patient 15 showed a small paracentric inversion on chromosome 1 in every cell which most likely represents a constitutional abnormality. Similarly, patients 7 and 8 (Table 1) showed PCD related abnormalities only in the whole BM and the absence of these abnormalities in the NF after plasma cell isolation suggests effective removal of plasma cells in the NF culture for these cases. Two patients (Table 1, patients 1 and 6) showed non-PCD related abnormalities in the NF (A culture). One patient (Table 1, patient 9) showed a deletion 20q in the whole BM (B culture) only and the abnormality was not detected in the NF (A culture). Only 1 cell with deletion 20q was observed in the whole BM culture and subsequent interphase FISH analysis on the whole bone marrow culture confirmed a low-level clone with a 20q deletion which could explain its absence in the cells analyzed from the NF (A culture).

In some cases our plasma cell isolation appeared to be less efficient as indicated by PCD related abnormalities seen in the NF (A culture) (Table 1, patients 2, 3, 4, 5, 10, 12, and 13). These results suggest that some residual plasma cells remained in the NF after isolation of CD-138 positive plasma cells.

Table 1 Karyotypic abnormalities detected on routine chromosome analysis

No.	Karyotype	Culture in which the abnormality is observed	Abnormality type
1	46,XY,t(6;12)(p21;p13)[1]/46,XY,der(6)t(6;12)(p21;p13)[1]/46,XY[20]	A only	Non myeloma—Lymphoid
2	**51**,XY,**+5,+7**,der(8)t(8;12)(q10;q10),**+9,+11,–13,+15,+15**,–16,**+19**,–20, **+21**,+mar[3]/46,XY[10]	A only	Myeloma
3	43,X,–X,**i(1)(q10)**,add(3)(q27),–4,i(6)(p10),**–13**,add(14)(q24), **add(16)(q12)**,+19,–21[2] 46,XX[19]	A only	Myeloma
4	**75**,XX,–X,–1,+3,add(4)(q12),+add(4)(q12),**+7**,+8,**+9,+11**,der(12)t(12;14) (q24.1;q24),**–13**,–18,+der(19)t(1;19)(q23;p13)x2,–20,**+21**,+mar, +mar[4]/76,idem,+7,+19[3]	A only	Myeloma
5	47,XX,der(2)t(2;6)(p13;p11.2),**+3**,del(3)(q21),der(3)t(3;9)(p31;q13),+6, del(6)(p23),dic(6;14)(q10;q10),–8,**der(16)t(1;16)(q21;q24),+19**[18]	A only	Myeloma
6	46,XY,del(16)(q22q24)[2]/46,XY[18]	A only	Non myeloma—Myeloid
7	**57~58**,X,–X,**add(1)(p32),+add(1)(p32),+5**,+add(6)(q15),**+7**,–8,–10, add(10)(q22),**+11,+15,+19,+21,+21,+21**,+mar1,+mar2,+mar3, +mar4[cp3]/46,XX[19]	B only	Myeloma
8	**51**,X,–X,**t(1;8)(p13;q24.3),+3**,add(3)(p11),**+5,+9,+11,der(13) del(13)(q12q14)add(13)(q32)**,–14,**+15,+19,+21,+21**[3]/46,XX[17]	B only	Myeloma
9	46,XY,del(20)(q11.2q13.1)[1]/46,XY[19]	B only	Non myeloma—Myeloid
10	46,XY,inv(1)(p21p36.3),**t(11;14)(q13;q32)**,add(17)(q25)[4]/46,XY[17]	A & B	Myeloma
11	46,XX,del(5)(q12q33)[6]/46,XX[14]	A & B	Non myeloma—Myeloid
12	40~42,X–X,**dup(1)(q21q42)**,add(3)(p21),add(4)(p12),**t(4;14)(p16;q32)**, –5,i(7)(q10),–10,–11,add(12)(p11.2),**–13**,del(14)(q22q24),–16,–17, del(19)(p13.3),–21,–22,–22,+mar1,+mar2,+mar3[cp17]/46,XX[3]	A & B	Myeloma
13	43,X,–Y,del(2)(q33),**–13**,–22[4]/46,XY[16]	A & B	Myeloma
14	47,XY,+8[14]/46,XY[6]	A & B	Non myeloma—Myeloid
15	46,XX,inv(1)(q22q31)c?[20]	A & B	Non myeloma—Constitutional

Among the patients with loss of Y chromosome (Table 2), the abnormality was found in the NF (patients 1–4) or in both the NF and whole BM cultures (patients 5–10). Since loss of Y chromosome is not specific to any particular lineage and seen in all cells, observation of this loss in all cultures validates our hypothesis of effective utility of the NF for RCA.

Discussion

Results from our study demonstrate the feasibility of using the NF for karyotyping to detect non-PCD related abnormalities in sample volumes that are inadequate for performing both RCA and isolation of plasma cells for FISH. Retrieving the remaining cellular components from the NF proves to be an innovative strategy for performing RCA, thus delivering a comprehensive cytogenetic analysis. Clinical laboratories routinely face the dilemma of deciding which test is optimal for patient care when an insufficient volume of sample is received. Due to the proven efficacy and superior diagnostic potential of FISH on isolated plasma cells, most laboratories select isolation of plasma cells for FISH and sacrifice RCA. Both *de novo* and secondary AML/MDS have been reported in patients with multiple myeloma and it has also been shown that patients with PCD are 8–11 times more likely to develop AML/MDS than the general population. Cytotoxic chemotherapy is known to

Table 2 Karyotypic abnormalities (loss of Y chromosome) detected on routine chromosome analysis

No.	Karyotype	Culture in which the abnormality is observed	Abnormality type	Age (years)	Gender
1	45,X,–Y[3]/46,XY[17]	A only	Non myeloma	63	Male
2	45,X,–Y[5]/46,XY[15]	A only	Non myeloma	78	Male
3	45,X,–Y[6]/46,XY[5]	A only	Non myeloma	85	Male
4	45,X,–Y[18]/46,XY[2]	A only	Non myeloma	80	Male
5	45,X,–Y[4]/46,XY[16]	A & B	Non myeloma	74	Male
6	45,X,–Y[3]/46,XY[17]	A & B	Non myeloma	78	Male
7	45,X,–Y[4]/46,XY[16]	A & B	Non myeloma	56	Male
8	45,X,–Y[10]/46,XY[10]	A & B	Non myeloma	77	Male
9	45,X,–Y[7]/46,XY[13]	A & B	Non myeloma	72	Male
10	45,X,–Y[8]/46,XY[12]	A & B	Non myeloma	63	Male

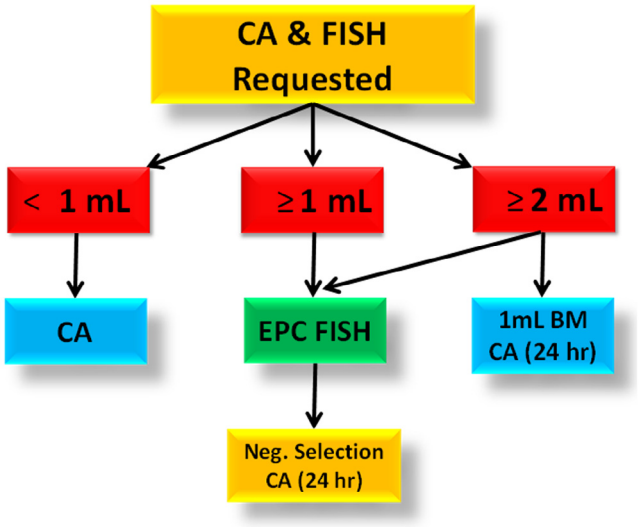

Figure 1 Algorithm showing the process for optimizing the sample volume to achieve optimal patient care.

induce unbalanced structural rearrangements involving chromosomes 5 and 7 and deletion 20q is known to be a common recurrent aberration in treated PCD patients (2,10,11). For these reasons, sacrificing RCA may result in suboptimal patient care and using the remaining cellular components from the NF, based on our preliminary results, appears to be an effective strategy to overcome the issue of sample inadequacy. In our study, we detected 6 cases of non-plasma cell related abnormalities using the NF. In 5 of the 6 cases, the detected abnormalities were clinically significant, especially for detecting co-existing myeloid disorders (Table 1, cases 6, 9, 11 and 14). The remaining case showed what appears to be a constitutional rearrangement with implications for reproductive health.

We have developed an algorithm to achieve optimal patient care based on sample volume (Figure 1). In our experience, less than 1 ml of sample is inadequate for plasma cell isolation. In such patients, we perform only RCA while sample volumes of 1 ml or more are subjected to EPC FISH analysis and the remaining cellular components from NF following the plasma cell isolation are used for RCA. When 2 ml or more of sample is received, 1 ml is used for EPC FISH analysis and RCA using NF, and the remaining 1 ml of whole BM is used to initiate a 24-hour culture for RCA. Isolation procedures did not seem to affect the ability to culture and obtain metaphase chromosomes from NF as proven by 100% complete success in obtaining metaphase chromosomes.

The abnormality rate of 16.3% in our study is similar to what has been reported by other groups using conventional cytogenetics, especially short-term cultures of 24- or 48-hours without any mitogen stimulation (12–14). The length of culture (24- vs 48-hour) did not appear to influence the detection of abnormal clones in our study as myeloma related clones were detected both at 24- and 48-hour cultures with equal frequency, and our results are in agreement with Dewald et al. (12), who also reported that culture duration did not influence the detection rate of abnormal clones in their study. Of the 15 cases with abnormal karyotypes, 9 were interpreted as PCD related based on the abnormalities detected (Table 1, patients 2–5, 7, 8, 10, 12 and 13) and interphase FISH on selected plasma cells showed 100% concordant results except for one patient (patient 4) where the volume of the sample was <1 ml and the isolation was not successful. In the remaining 6 cases where non-PCD related abnormalities were detected on RCA, FISH on EPC showed PCD related genetic abnormalities in 5 cases (Table 1, patients 1, 6, 9, 11 and 15) indicating that these patients probably have co-existing malignancies/genetic abnormalities. In one patient (patient 14) where RCA showed trisomy 8, FISH analysis on EPC showed normal result. This case highlights the drawbacks of not performing RCA when sample volume does not permit performing both EPC FISH and RCA. Even in cases where RCA detected only loss of the Y chromosome, FISH analysis on EPC showed PCD related genetic abnormalities except for one case where the result was normal. Loss of Y chromosome, considered being an age related phenomenon rather than malignancy related, was seen in both NF and whole bone marrow cultures. As majority of our patients are considered advanced in terms of age, this loss of Y chromosome was not considered significant regarding their disease status (Table 2). The presence of these non-PCD related abnormalities in both the NF that is devoid of any plasma cells and in whole BM confirms our hypothesis that the NF can be used to detect non-PCD related abnormalities.

Although there appeared to be some residual plasma cells in some cases as evidenced by the PCD related abnormal clones detected in the NF after plasma cell isolation (Table 1, patients 2, 3, 4, 5, 10, 12, and 13), the diagnostic efficacy was not compromised as demonstrated by the interphase FISH analysis on EPC in these patients. In all but one case, interphase FISH on EPC showed PCD related prognostic genetic abnormalities (Supplementary Table S1). Whenever possible, the purity of isolated plasma cells was confirmed by flow cytometry which detected 90.6% of the cells isolated as CD138+, thus proving the accuracy of our isolation. The "spillover" of plasma cells in the NF could be due to the delay in processing the samples for isolation. It is well known that CD138 expression in plasma cells is volatile and time-lag dependent with delay in isolation leading to loss of CD138 expression *in vitro*, and ultimately resulting in less than optimal isolation of PCs (14). When CD138 is used as a plasma cell marker for isolation, it is recommended that the sample is processed without delay (15). In the 7 cases where PCD related abnormalities were detected in NF, 2 samples were delayed more than 24-hours in processing for isolation. However, the sample size is too small to derive any definite conclusions as to this "spillover".

In summary, we present a novel strategy for performing both RCA and FISH on EPC when the BM sample volume is low. Our results show that this strategy is feasible and efficient in detecting both PCD related and non-PCD related abnormalities and essential to providing optimal patient care.

Authorship and conflict of interest

The authors declare no conflict of interest and declare no competing financial interests.

VO and GV designed the study, compiled the data and contributed to writing the manuscript. GM, WE, MZ and CM analyzed the cases, scored the FISH results, performed the RCA studies and helped in manuscript editing.

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Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.cancergen.2015.12.008.

References

1. Pozdnyakova O, Crowley-Larsen P, Zota V, et al. Interphase FISH in plasma cell dyscrasia: increase in abnormality detection with plasma cell enrichment. Cancer Genet Cytogenet 2009;189:112–117.
2. Mailankody S, Pfeiffer RM, Kristinsson SY, et al. Risk of acute myeloid leukemia and myelodysplastic syndromes after multiple myeloma and its precursor disease (MGUS). Blood 2011;118:4086–4092.
3. Hartmann L, Biggerstaff JS, Chapman DB, et al. Detection of genomic abnormalities in multiple myeloma. Am J Clin Pathol 2011;136:712–720.
4. Christensen JH, Abildgaard N, Plesner T, et al. Interphase fluorescence in situ hybridization in multiple myeloma and monoclonal gammopathy of undetermined significance without and with positive plasma cell identification: analysis of 192 cases from the Region of Southern Denmark. Cancer Genet Cytogenet 2007;174:89–99.
5. Sawyer JR. The prognostic significance of cytogenetic and molecular profiling in multiple myeloma. Cancer Genet 2011;204:3–12.
6. Put N, Lemmens H, Wlodarska I, et al. Interphase fluorescence in situ hybridization on selected plasma cells is superior in the detection of cytogenetic aberrations in plasma cell dyscrasia. Genes Chromosomes Cancer 2010;49:991–997.
7. Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. Cancer Res 2004;64:1546–1558.
8. Lu G, Muddasani R, Orlowski RZ, et al. Plasma cell enrichment enhances detection of high-risk cytogenomic abnormalities by fluorescence in situ hybridization and improves risk stratification of patients with plasma cell neoplasms. Arch Pathol Lab Med 2013;137:625–631.
9. Shin SY, Jang S, Park C-J, et al. Application of an immune-magnetic cell sorting method for CD138-positive plasma cells in FISH analysis of multiple myeloma. Int J Lab Hematol 2012;34:541–546.
10. Papanikolaou X, Barlogie B, Usmani SZ. Therapy-related myeloid malignancies in myeloma. Mediterr J Hematol Infect Dis 2011;3:e2011047.
11. Slovak ML, Bedell V, Pagel K, et al. Targeting plasma cells improves detection of cytogenetic aberrations in multiple myeloma: phenotype/genotype fluorescence in situ hybridization. Cancer Genet Cytogenet 2005;158:99–109.
12. Dewald GW, Kyle RA, Hicks GA, et al. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. Blood 1985;66:380–390.
13. Sawyer JR, Waldron JA, Jagannath S, et al. Cytogenetic finding in 200 patients with multiple myeloma. Cancer Genet Cytogenet 1995;82:41–49.
14. Smadja NV, Bastard C, Brigaudeau C, et al. Hypodiploidy is a major prognostic factor in multiple myeloma. Blood 2001;98:2229–2238.
15. Dorwal P, Thakur R, Rawat S. CD138 expression in plasma cells is volatile and time-lag dependent. Egypt J Haematol 2014;39:258–259.