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ORIGINAL ARTICLE

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

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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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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.