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PLASMA CELL ENRICHMENT: MANUAL VERSUS AUTOMATED METHODS

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ABSTRACT

Multiple myeloma is a cancer of the plasma cell characterized by overproduction of monoclonal immunoglobulin, renal damage, and osteolytic lesions as a complication of diffuse osteoporosis. Although there is currently no cure, there are specific cytogenetic and FISH abnormalities that help determine the prognosis of the disease. Advances in the field of cell separation have led to plasma cell enrichment (PCE), which isolates the cells of cancerous origin. This enriched sample can then be analyzed with higher specificity. The purpose of this study was to compare automated and manual methods of PCE as a function of abnormality detection and cost. Is the higher capital investment for an automated PCE instrument prove to be more cost effective with extended use? This study included a side-by-side analysis of twenty-two patients using automated and manual methods, a comparison of fifty different patients using either approach and a cost analysis. The data from this study including the cost analysis is presented below. In summary, our investigation supports the use of automation to: 1) enrich plasma cell samples; 2) increase the detection rate of genetic abnormalities in plasma cell disorders; 3) to save budget dollars; and, 4) increase efficiency allowing technologists time to do other tasks in the laboratory.

INTRODUCTION

Multiple myeloma is a malignant plasma cell neoplasm characterized by overproduction of monoclonal immunoglobulin, hypercalcaemia, anemia, and renal damage, as well as diffuse osteoporosis with advancement to osteolytic lesions.1 There are other plasma cell neoplasms closely related to multiple myeloma. These include monoclonal gammopathy of undetermined significance (MGUS), and primary PC leukemia (PCL). Currently, there is no cure for these plasma cell disorders; however, there are specific cytogenetic and FISH abnormalities which aid in the characterization of the disease in terms of severity, staging, and prognosis. These include 13q deletions, 17p deletions, and rearrangements of the IGH locus.2-4 It is well known that the majority of MM cases produce normal karyotypes under conventional cytogenetics, thus Fluorescent in situ Hybridization (FISH) is often used to identify the disease-related abnormalities in interphase cells.5 Advances in the field of cell separation have led to plasma cell enrichment (PCE), which isolates the cell of cancerous origin.6,7 This enriched sample can then be analyzed with more specificity.8 The purpose of the study was to evaluate and compare the manual methods of PCE to a relatively new automated method (Auto-MACS; Miltenyi Biotec) as a function of abnormality detection and cost. This investigation included a side-by-side study of twenty-two patients using automated and manual methods, a comparison of the results of fifty different patients using either approach and a cost analysis. The question or hypothesis was: would the capital investment of an automated instrument prove to be more cost effective with extended use and would automation better isolate the plasma cell population, thus increasing the rate of interphase FISH abnormality detection?

METHODS

1. Manual Plasma Cell Enrichment

Bone marrow specimens were received in heparinized vaccutainers and logged into the lab according to specimen volume and quality. An aliquot of 1mL sample was isolated per patient, then diluted with a phosphate buffered saline (PBS) and layered using Ficoll and 400g centrifugation. This isolates the white blood cells into a single layer, which can then be removed and inoculated with CD 138+ antibodies attached to magnetic microbeads. The samples were then resuspended in a phosphate-buffered saline solution and loaded onto a magnetic separation (MS) column that was attached to a MACS separator magnet (MACS; Miltenyi Biotec). The isolated plasma cells were then eluted into centrifuge tubes and a subsequent direct harvest was performed. The fixed cells were dropped and probed the same day using class I Analyte Specific Reagents (ASR's) for chromosome 13 deletions, deletions of 17p, IGH/MAF t(14:16), IGH/FGFR3 t(4:14), and IGH/CCND1/BCL1 t(11;14). These probes are locus-specific probes.

2. Automated Plasma Cell Enrichment

Bone marrow specimens were received in heparinized vaccutainers and logged into the lab according to specimen volume and quality. Aliquots of approximately 2mL of sample were placed into 15mL centrifuge tubes and inoculated with CD 138+ antibodies attached to magnetic microbeads. The samples were then resuspended in PBS and centrifuged. After centrifugation, samples were loaded onto the automated machine (Auto-MACS; Miltenyi Biotec) and run under the possel_wb program with rinses in between. The isolated plasma cells were then eluted into centrifuge tubes and a subsequent direct harvest was performed. The fixed cells were dropped and probed the same day using class I Analyte Specific Reagents (ASR's) for chromosome 13 deletions, deletions of 17p, IGH/MAF t(14:16), IGH/FGFR3 t(4:14), and IGH/CCND1/BCL1 t(11;14). These probes are locus-specific probes.

3.Scoring Interphase FISH

The assays were scored using a per cell nuclei approach, with 200 interphase nuclei counted per assay by two technologists. The probes were validated in the laboratory by analyzing 20 specimens from cytogenetically normal patient referred for myeloid disease. Cutoffs were calculated by using beta distribution with a 95% confidence limit. Specific cutoffs are as follows: 14;16- 1R1G2F=0.5%, 1R1G1F=10.5%; 13q- 1R2G=3.97%, 1R1G=1.08%, 4R4G=3.40%, 2R1G=1.54%; p53- 1R=10.2%; 4;14- 1R1G2F=0.5%, 1R1G1F=10.5%; 11;14- 1R1G2F=0.5%, 1R1G1F=10.5%, 3R2G=3.15%.

4. Cost Analysis

The cost analysis was administrated under the assumption of bulk purchasing, average technologist time at average technologist hourly wage plus employer costs, and average material use per test. A reference of 15 patients per week was used to establish cost of reusable equipment. The total cost per test was calculated for both manual and automated methods and then compared on a financial platform for overall savings. The investment of the automated machine was calculated into the overall cost for that approach, assuming a 7 year depreciable life span, and a payback period was determined by dividing the initial investment by the anticipated annual savings.

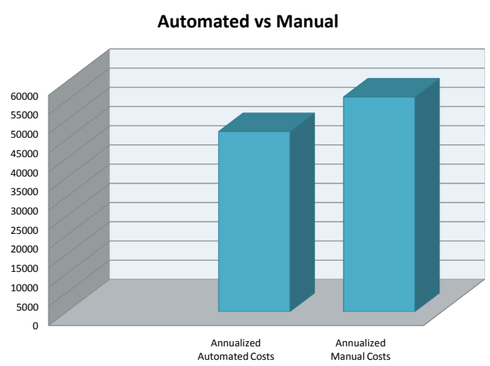

Figure 2: Cost analysis. The bar graph demonstrates the annualized savings of the automated approach versus the manual approach.

	13q		4;14		p53		14;16		11;14	
	OLD	NEW	OLD	NEW	OLD	NEW	OLD	NEW	OLD	NEW
1) FT09-012563	-	-	-	-	-	-	-	-	-	-
2) FT09-012565	-	-	-	-	-	-	-	-	1% = 2F	5.5% = 2F
3) FT09-012569	-	-	-	3% = 2F, 24% = 3G	-	-	-	-	1% = 2F	5.5% = 2F
4) FT09-012056	-	no cells	-	no cells	-	no cells	-	-	5.6% = 2F	46.4% = (2+)fusions
5) FT09-012050	-	-	-	-	-	-	-	-	41% = 2F	69% = (2+) fusions
6) FT09-012567	9% = polysomy	2.0% = del / 30% = (polysomy)	5% = IgH break	4.5%= IgH break	-	-	3% = del 16q	23% = del 16q	10% = polysomy	41% = polysomy
7) FT09-012503	-	-	-	-	-	-	-	-	11% = poly chrom 11	51% = poly chrom 11
8) FT09-012001	2% = del/4% = monos	2.5% = del / 7.8% = monos	-	-	-	-	-	-	-	-
9) FT09-012003	-	-	-	-	7.5% = del	8.0% = del	15% = 3G	17% = 3G	58% = 2F / 14% = 1F	69% = 2F/24% = 1F
10) FT09-012863	-	-	-	-	41.5% = polysomy	28% = polysomy	-	-	-	-
11) FT09-011997	-	-	40% = 3R	20% = 3R	38.0% = polysomy	10.5% = polysomy	-	-	38% = 3R	19% = 3R
12) FT09-012048	-	-	-	-	39% = 3R	8.5% = 3R	-	-	-	33% = 3R
13) FT09-012859	-	-	-	-	-	-	-	-	76% = poly 11	81.5% = poly 11
14) FT09-012867	-	-	-	-	-	-	-	-	-	-
15) FT09-012052	27% = monosomy	27% = monosomy	-	-	-	-	-	-	7% = (2+) fusion	25% =(2+) fusion
16) FT09-012868	-	7.5% = del	-	-	-	-	-	-	23% = 3R	54.5% = 3R
17) FT09-012872	-	-	-	-	-	del = 15%	-	-	33.5% = poly 11	42% = poly 11
18) FT09-012287	-	-	-	-	-	-	-	-	27% = poly 11	16.5% = poly 11
19) FT09-012629	-	-	-	-	-	-	-	-	1% = 2F / 24% = 1F	8% = 1F
20) FT09-012639	-	-	-	-	-	-	-	-	5% = poly 11	9% = poly 11
21) FT09-012632	-	-	19% = 1F	1F = 6.0%	-	-	-	-	-	-
22) FT09-012107	-	-	-	-	-	-	-	-	-	-

Figure 1: Side-by-Side study of automated and manual plasma cell enrichment (PCE). The table shows the results from 22 patient samples receiving both automated and manual PCE.

RESULTS

The side-by-side study showed a 20% increase in the overall abnormality detection among probe sets using the automated approach. Included in the count were four assays in which an abnormality was detected using the automated method and was not detected using manual PCE. The opposite was not found. Figure 1 illustrates the results of the side-by-side comparison study. In some instances the automated method produced cells showing more than one abnormal signal pattern, compared to a single abnormal signal pattern from the cells obtained through manual means. In addition, the most common abnormal results were observed using the t(11;14) probe set, with 72.7% of patients expressing abnormalities at this locus.

The distribution of detected abnormalities for the 100 patient studies is presented in figure 2. The most prevalent abnormalities were those associated with 13q deletions. A 67% and 71% distribution of 13q abnormalities was found for manual and automated PCE, respectively. There were more p53 deletions associated with the manually enriched plasma cell samples at 24%, compared to 5% using the automated approach. The 4;14 and 14;16 abnormalities were more prevalent in the patient samples that underwent automated plasma cell enrichment at a distribution of 23%, compared to 8% of the abnormalities detected from manually enriched samples. For the automated method, there were twice as many patient samples with low cellularity.

The cost analysis revealed an annualized savings of $9,012.74 by utilizing the Auto-MACS machine (Figure 3). The reusable separation columns and the reduction of technologist time factored in to reduce the majority of the cost. The payback period of the initial capital investment was determined to span 5.16 years.

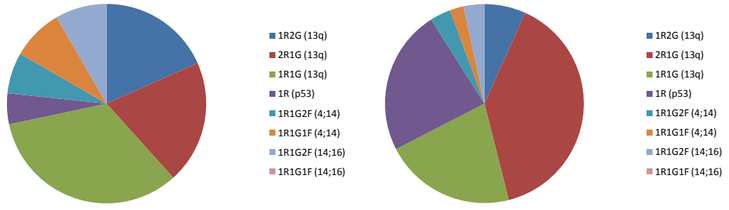

Figure 3: Overall distribution of interphase FISH signal abnormalities for automated and manual methods. The pie charts show relative distribution of abnormal signal patterns from each of the fifty patients surveyed for either automated or manual PCE. Each piece represents the proportion of abnormal signals showing its corresponding pattern.

DISCUSSION

By evaluating the side-by-side study, it becomes evident that using the automated approach to plasma cell enrichment does indeed increase the rate of interphase FISH abnormality detection. This indicates a more authentic population of plasma cells isolated using automated PCE. The large proportion of patients with t(11;14) abnormalities coincides with findings from previous studies, as this abnormality has been associated with all three disease stages of plasma cell neoplasms.9 Although direct comparisons cannot be made between the fifty patients analyzed using manual PCE and those by the automated method, correlations can be made in terms of the distribution of signal abnormalities. The most prevalent abnormalities were associated with a 13q deletion, which is consistent with findings in previous studies.4 However, the incidence of 13q deletions in all three disease stages of plasma cell neoplasms has been a point of difference amongst prior reports.9, 10 The alteration of the p53 tumor suppressor gene is found in cancers of a wide spectrum of tissues.11 The p53 inactivation is considered a rare event in multiple myeloma, usually found in late stage disease.12 Therefore, the large distribution of cells with p53 mutations from the manually enriched patient samples could indicate a more heterogeneous mix of cell types, thus expressing p53 mutations more than the cells isolated from the automated approach. Rearrangements with the IGH locus, specifically t(4;14) and t(14;16) are particular to plasma cell disease.4 The increased distribution of both abnormalities from the automated patient samples would also indicate a more pure population of plasma cells. Of concern was the decrease in overall cellularity of the plasma cell enrichment using automated methods. Plasma cells comprise less than 5% of total bone marrow involvement, and a diagnosis of multiple myeloma is assigned when the plasma cell population reaches 10% or more.1 If the cells isolated from PCE are true plasma cells, one can deduce that a small sample will be obtained from the majority of cases, thus leading to cellularity concerns. Lastly, the cost analysis attempted to quantify the savings associated with the use of the automated instrument. The reusable separation columns were assumed to be utilized for a 15 patient per week volume, however, the columns can last for up to 100 patients before they need to be changed implying the potential for additional savings with increased testing.

CONCLUSION

Our investigation supports the use of PCE automation to better enrich samples, detect abnormalities, save money as well as provide more time for the technologist to do other tasks. However, more studies are indicated including analysis of the limitations of the plasma cell population within the marrow sample, confirmation of plasma cell purity through cell sorting and staining techniques, comparing the effect of different anticoagulants on plasma cell enrichment, and the effect of sample volume or quality on the eluted plasma cell suspension. Although there is much to explore in the field of automated plasma cell enrichment, it is clear that the enrichment of plasma cells to detect interphase FISH abnormalities is warranted. As more is learned about this field, implications for automated testing will also increase.

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