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Original article

Validation of interphase fluorescence *in situ* hybridization (iFISH) for multiple myeloma using CD138 positive cells

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ABSTRACT

Background: Multiple myeloma is a plasma cell neoplasm with acquired genetic abnormalities of clinical and prognostic importance. Multiple myeloma differs from other hematologic malignancies due to a high fraction of low proliferating malignant plasma cells and the paucity of plasma cells in bone marrow aspiration samples, making cytogenetic analysis a challenge. An abnormal karyotype is found in only one-third of patients with multiple myeloma and interphase fluorescence *in situ* hybridization is the most useful test for studying the chromosomal abnormalities present in almost 90% of cases. However, it is necessary to study the genetic abnormalities in plasma cells after their identification or selection by morphology, immunophenotyping or sorting. Other challenges are the selection of the most informative FISH panel and determining cut-off levels for FISH probes. This study reports the validation of interphase fluorescence *in situ* hybridization using CD138 positive cells, according to proposed guidelines published by the European Myeloma Network (EMN) in 2012.

Method: Bone marrow samples from patients with multiple myeloma were used to standardize a panel of five probes [1q amplification, 13q14 deletion, 17p deletion, t(4;14), and t(14;16)] in CD138^{+} cells purified by magnetic cell sorting.

Results: This test was validated with a low turnaround time and good reproducibility. Five of six samples showed genetic abnormalities. Monosomy/deletion 13 plus t(4;14) were found in two cases.

Conclusion: This technique together with magnetic cell sorting is effective and can be used in the routine laboratory practice. In addition, magnetic cell sorting provides a pure plasma cell population that allows other molecular and genomic studies.

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Introduction

Multiple myeloma (MM) is a disease characterized by clonal proliferation of plasma cells (PCs) in bone marrow, which leads to bone marrow failure, skeletal lesions, suppression of normal immunoglobulin synthesis and production of a monoclonal protein.[1] This disease accounts for between 10% and 15% of hematological cancers. The median age at diagnosis is 60 years, and the evolution is heterogeneous, with the survival time varying from a few months to more than a decade.[2,3]

MM shows acquired genetic abnormalities of clinical importance. In about half of the cases, the initial genetic process involves a reciprocal translocation between the immunoglobulin heavy (IgH) gene (14q32) and many target genes including *CCND1* (11q13), *FGFR3/MMSET* (4p16) and *MAF* (16q23).[4,5]

The study of cytogenetic abnormalities by karyotyping is limited because of the low mitotic index of the malignant PCs.[6–8] Only 20–50% of the cases show clonal abnormalities by G banding karyotype. However, the presence of hypodiploidy or monosomy of chromosome 13 predicts poor survival.[9–13]

Molecular studies show that most MM cases present genetic abnormalities with interphase fluorescence *in situ* hybridization (iFISH) being the most useful cytogenetic tool for their investigation.[7,14,15] However, iFISH testing requires previous identification or selection of PCs by morphology, immunophenotyping or sorting. Cell selection using the anti-CD138 antibody can be performed using magnetic columns or sorting. The major limitation of this approach is the considerable loss of cells during the purification process. In the cytoplasmic immunoglobulin (Clg) fluorescence *in situ* hybridization (FISH) technique, PC detection is carried out using fluorescent anti-Kappa or anti-Lambda antibodies in the PC cytoplasm and analysis is performed only using this population.[4,5,16]

The other challenges in MM testing with FISH are probe selection, the determination of cut-off levels and number of PCs to be scored. The European Myeloma Network (EMN) has organized two workshops on iFISH in MM. In 2012, they published some technical recommendations herein transcribed from the paper:

(1) Material should be part of the first draw of the aspirate;
(2) Samples should be sent at suitable times to allow for the lengthy processing procedure;
(3) Most importantly, PCs must be purified or specifically identified;
(4) Cut-off levels should be relatively conservative: 10% for fusion or breakapart probes, and 20% for numerical abnormalities;
(5) Informative probes should be combined for best effect;
(6) In specialist laboratories, a single experienced analyst is considered adequate;
(7) At least 100 PCs should be scored;
(8) Essential abnormalities to test for are t(4;14), t(14;16) and 17p13 deletions;
(9) Suitable commercial probes should be available for clinically relevant abnormalities;
(10) The clinical report should be expressed clearly and must state the percentage of PC involved and the method used for identification".[4]

Objective

This study aimed to standardize an iFISH panel test for MM for its incorporation in laboratories as a routine cytogenetic test.

Methods

This research was evaluated by the Ethics Committee of the Hospital (SGPP number 169913 – "Validation of laboratory tests for Clinical Pathology Laboratory"). The study was conducted in accordance with the Helsinki Declaration as revised in 2008.

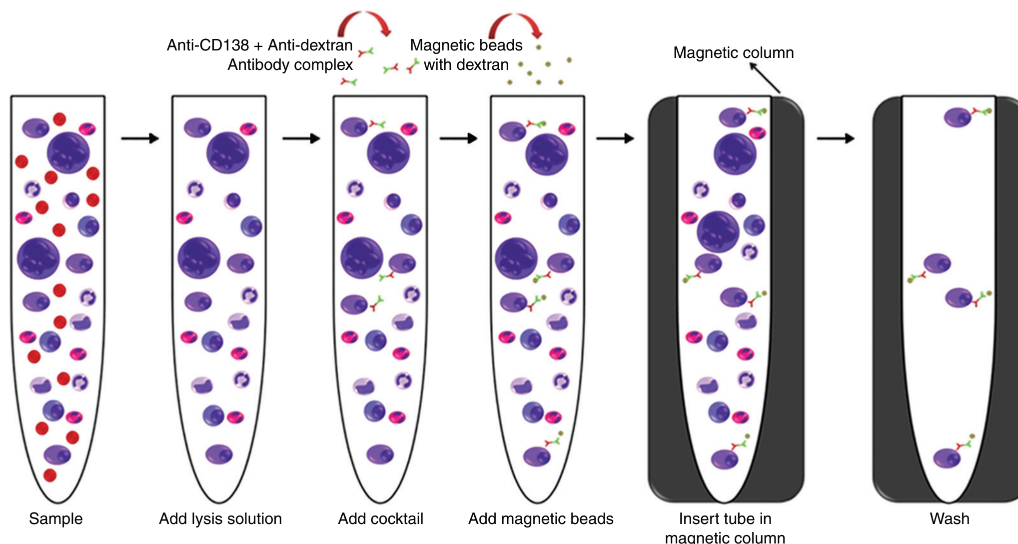

Figure 1 – Magnetic cell sorting (MACS) of CD138+ cells using the kit *EasySepTM Human WB and BM CD138 Positive Selection Cocktail* (Stemcell TechnologiesTM).

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