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Flow cytometric analysis of myelomonocytic cells by a pattern recognition approach is sensitive and specific in diagnosing myelodysplastic syndrome and related marrow diseases: Emphasis on a global evaluation and recognition of diagnostic pitfalls

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Abstract

Published data on flow cytometry (FCM) in diagnosing myelodysplastic syndromes (MDS) varies greatly in analytic methods and interpretational approaches. We tested the diagnostic utility of the pattern recognition approach by a retrospective review of 180 MDS, 31 myelodysplastic/myeloproliferative disease (MDS/MPD), 37 non-MDS cytopenia and 20 myeloproliferative disease (MPD) cases. Cases were placed into "positive", "intermediate", and "negative" FCM categories based upon the antigenic aberrations observed on myelomonocytic cells. By exclusion or inclusion of the intermediate category as indicative of MDS or MDS/MPD, the overall sensitivity and specificity were 84% and 97% or 98% and 78%, respectively. The overall abnormalities detected by FCM correlated with the severity of morphological dysplasia and clonal cytogenetic abnormalities. MPD also demonstrated immunophenotypic aberrancy. Based on a global evaluation of myelomonocytic abnormalities, and recognition of diagnostic pitfalls and caveats, the pattern recognition approach of FCM is sensitive and reliable in diagnosing MDS and related myeloid diseases.

Keywords: Myelodysplasia; Myeloproliferative disorders; Flow cytometry

1. Introduction

The myelodysplastic syndromes (MDS) and myelodysplastic/myeloproliferative diseases (MDS/MPD) are a group of clonal bone marrow disorders characterized by peripheral cytopenia(s), ineffective hematopoiesis, morphologically apparent multilineage dysplasia and recurrent cytogenetic abnormalities [1]. Traditionally, the gold standard for the diagnosis of MDS has been based upon bone marrow morphologic examination and cytogenetic studies in conjunction with clinical history [2]. However, not all clinically suspected cases evince definitive morphologic dysplasia [3,4], and, not infrequently, the quality of bone marrow specimens is suboptimal. In addition, non-MDS cytopenia may mimic MDS morphologically [5]. Cytogenetic abnormalities are infrequent in low grade MDS [6]. Therefore, ancillary studies that could aid in the diagnosis of MDS would be informative, especially in morphologically intermediate and/or cytogenetically normal cases. Immunophenotyping using flow cytometry (FCM) is a potentially objective and reliable method to identify antigenic aberrancy of differen-

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tiating neoplastic cells. In the past 15 years, a number of studies have applied FCM to study MDS. Most of these studies [7–12] investigated a relatively small number of surface antigens and did not utilize FCM specifically for clinical diagnosis. In recent years, several studies have investigated the clinical usage of FCM in diagnosing MDS, some of which have focused on the blast population [13–16]; and some on the erythroid lineage [17,18]; but most studies have focused on myelomonocytic cells [19–25]. Applying a larger panel of antibodies to study the abnormal maturation patterns of myelomonocytic cells has shown diagnostic value in MDS [21–25]. However, past studies using pattern recognition approaches varied greatly in antibody panels, analytic methods and diagnostic criteria. The practical usefulness of such an FCM approach in the diagnosis of MDS has not yet been verified by multiple investigative groups and, therefore, has not yet reached general application.

In this study, we report our experience in the implementation and validation of the pattern recognition approach to test four-color FCM as a primary or adjunctive means to diagnose MDS and related myeloid diseases. The antibody panel design and data interpretation were based upon the recommendations of Stetler-Stevenson at the National Institutes of Health (NIH) [21], Kussick et al. [25] at University of Washington and Wells et al. at Hematologics Inc., the Fred Hutchinson Cancer Research Center [23], and modified with our experience in our laboratory. In the study, we emphasized the global evaluation of the antigenic alterations and recognition of non-specific changes and potential diagnostic pitfalls.

2. Materials and methods

2.1. Patients

Between December 2005 and November 2006, the bone marrow aspirates from 285 consecutive patients who presented with cytopenia, and/or monocytosis to rule out MDS and/or MDS/MPD were analyzed by FCM at UMass Memorial Medical Center (UMMMC). Twenty MPD cases that were "incidentally" analyzed with the same FCM panel were also included in the study. The FCM results were retrospectively correlated with morphology, cytogenetic results, and laboratory data with institutional review board (IRB) approval. In some cases, the final diagnosis was established by repeated clinical and laboratory examination.

2.2. Morphological diagnosis

In this study, the morphological evaluation was performed independently by at least 2 hematopathologists who are experienced in MDS diagnosis, in accordance with WHO criteria [2], without knowledge of the FCM findings. For each individual case, routine hematoxylin and eosin (H&E) histologic sections and well-prepared Wright–Giemsa-stained smears were evaluated, in the latter case including a 500 bone marrow nucleated cells differential. The Perls reaction for iron was performed on BM aspirate and silver impregnation stain for reticulin was performed on biopsy samples if necessary. To strictly define morphologic dysplasia, the features of dyserythropoiesis (DysE), dysgranulopoiesis (DysG) and dysmegakaryopoiesis (DysM) had to be present in at least 10% of cells of the respective lineage. Dysplasia was defined as unilineage if it was limited to a single lineage, while multilineage dysplasia was considered to be present when two or more lineages showed those features.

2.3. Cytogenetic analysis

Fixed preparations obtained from cultured (24 and 48 h) bone marrow samples were G-banded and a minimum of 20 metaphase spreads were examined. The criteria defined by the International System for Human Cytogenetic Nomenclature [26] were used for identification of abnormal clones.

2.4. Four-color flow cytometry analysis

For all specimens, four-color flow cytometry was performed on Coulter FC-500 instruments (Beckman Coulter [BC], Hialeah, FL) using commercially available reagents. In the processing of all specimens, red blood cells were lysed with buffered ammonium chloride (Beckman Coulter [BC], Hialeah, FL), and the remaining cells were washed once with phosphate-buffered saline–bovine serum albumin (PBS-BSA)-azide (pH 7.4), and resuspended to the desired cell concentration in PBS-BSA or minimum essential medium (MEM). Next, 100 μL of the cell suspension (5×10^5 to 1×10^6 cells) was incubated with appropriate amounts of titrated antibodies for 15 min at room temperature in the dark, washed once with PBS-BSA-azide, and resuspended in 0.1% paraformaldehyde. The instrument alignments, sensitivities, and spectral compensation were verified daily by standards, calibrators, procedural controls and normal peripheral blood samples prior to processing of patient samples. The data of normal fluorescence intensity and maturation patterns were collected from 10 lymphoma staging marrows, and used as references. For each patient sample, the lymphocyte population was used as an internal control to ensure the proper voltage and compensation, and changes in fluorescence intensity and maturation patterns were compared to the established data collected from normal bone marrows. In all cases, at least 50,000 cells were acquired from each tube and analyzed by gating on erythroblasts/blasts/lymphocytes, maturing myeloid cells and monocytes (Fig. 1). The myeloblasts were initially determined by CD45/SS but confirmed and enumerated by CD34, CD117, CD33, and CD13 antibodies. In the remaining tubes, the blast population was determined by CD45 intensity observed through above antibodies. All the antibodies were