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Flow cytometric differentiation of abnormal and normal plasma cells in the bone marrow in patients with multiple myeloma and its precursor diseases

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ABSTRACT

Flow cytometric (FC) enumeration of abnormal plasma cells (APCs) for diagnosis and prognostication of plasma cell dyscrasias (PCD) is challenging. We studied antigen expression in normal plasma cells (NPC) ($N = 34$) and APC in a series of unselected PCD ($N = 59$). NPC subpopulations often demonstrated CD19(−), CD20(+), CD45(−) or dim and CD56(+), an immunophenotype observed in PCD. However abnormal CD81 was only observed in APCs (APC detection sensitivity 95%; specificity 100%). We evaluated differences in antigen expression patterns among MGUS ($N = 14$), SMM ($N = 35$) and MM ($N = 10$), finding the combination of CD45 and CD56 helpful in differentiating MGUS from SMM and MM ($p = 0.0002$).

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1. Introduction

Plasma cell dyscrasias (PCD) are a heterogeneous group of disorders with a spectrum of clinical presentations from asymptomatic monoclonal gammopathy of uncertain significance (MGUS) and smoldering multiple myeloma (SMM) to the symptomatic multiple myeloma (MM) with its high morbidity and diminished quality of life. Although diagnosis is based upon serum M spike, extent of plasma cell (PC) involvement of the BM and presence of end organ damage, flow cytometric (FC) characterization and quantification of abnormal plasma cells (APCs) has been used in the diagnosis, prognostication and monitoring of PCD [1–5]. FC determination of the percentage of total bone marrow PCs that are phenotypically aberrant (% APC) versus the percent of total PCs with a normal immunophenotype (NPC) allows risk stratification of progression of MGUS and SMM patients to overt MM and can be used for prognostication in MM [6–12]. FC studies can also help predict response to autologous stem cell transplantation [12–14].

All of the FC studies in PCD rely on an accurate differentiation of APC from NPC. FC analysis provides a consistent and stringent method for differentiating APCs from their normal counterpart based on the expression of a variety of surface antigens and demonstration of intracellular light chain restriction. Aberrant surface antigen expression can be demonstrated in the majority of MGUS, SMM and MM cases, with typical aberrant antigen profiles including expression of CD56, CD20 or CD117, diminished CD38, and complete absence of CD19 and/or CD45 [6,15,16]. The simultaneous analysis of CD38, CD56, CD19, CD45 and CD138 expression has been reported to detect a significant APC population in most patients with myeloma, even in the absence of

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intracytoplasmic immunoglobulin detection [4,5,15–17]. The precise enumeration of APCs in the presence of NPCs, however, remains challenging, especially in specimens with low numbers of PCs (e.g. MGUS). Panels with greater specificity for differentiating APCs from NPCs are needed to be able to accurately assess prognosis and minimal residual disease. CD81 is strongly expressed on the surface of NPCs but MM cell lines are shown to underexpress CD81, making it a potentially useful marker in differentiating APCs from NPCs [18]. Furthermore, levels of CD81 expression may correlate with prognosis in myeloma [19]. However, little information is available regarding its expression in the APCs of MM, SMM and MGUS patients. In the current study we evaluated the expression of CD19, CD20, CD38, CD45, CD56, CD81 and CD138 as well as intracellular immunoglobulin light chain (kappa/lambda) in APCs and NPCs in patients with MM, SMM and MGUS to determine their sensitivity and specificity in detecting PCD. Furthermore, we evaluated the role of these markers in an attempt to differentiate the early stage disease of MGUS from SMM and MM.

2. Materials and methods

2.1. Patients

Bone marrow aspirates from 59 untreated patients with PCD (14 MGUS, 35 SMM, and 10 MM) were submitted for diagnostic FC evaluation as part of screening for a prospective clinical natural history study (NCT01109407) of myeloma precursor disease and in some cases to determine eligibility for several research protocols. In addition bone marrow was evaluated in 5 patients referred to our institution to rule out MGUS, primarily due to anemia and reported mildly increased plasma cells in bone marrow evaluations performed at other institutions. These 5 patients were determined to have no evidence of a PCD (including absence of M spike, less than 5% PC on BM biopsy, polyclonal PCs by IHC and FC) or other neoplastic process and normal bone marrow specimen. All patients signed institutional review board approved informed consent to be screened for eligibility. The diagnosis of MGUS, SMM and MM was based on bone marrow PC infiltration, serum M-protein levels and radiological and clinical findings as per the criteria defined by International Myeloma Working Group [20]. The patterns of antigen expression of all markers were evaluated in the APCs (as defined by monoclonal light chain expression) in 59 cases and in NPCs from 5 normal bone marrows. 29 cases with PCD had greater than 5% NPCs (as defined by polyclonal intracellular light chain expression) (14 MGUS, 14 SMM, and 1 MM) and the patterns of antigen expression were evaluated in these normal polyclonal plasma cells. Therefore normal plasma cells were evaluated in 34 bone marrows (5 normal and 29 cases with co-existent PCD).

2.2. Flow cytometric immunophenotyping

Specimens were processed within 12 h of collection by washing twice with phosphate buffered saline (PBS) to remove cytophilic antibodies and staining for 30 min at room temperature with a panel of antibodies against surface and intracellular kappa and lambda (Polyclonal Rabbit Anti-Human, $F(ab')_2$, (Dako) and surface CD19APC (clone SJ25C1), CD20APC-H7 (clone L27), CD38v450 (clone HB7), CD45v500 (clone HI30), CD56PE-Cy7 (clone NCAM16.2), CD81FITC (clone JS-81), and CD138 PerCP-Cy5.5 (clone MI15) (BD Biosciences, San Jose, CA) as previously described [21]. For intracellular light chain evaluation, cells were stained with antibodies against surface antigens and then permeabilized with Fix and Perm (Invitrogen, Frederick, MD) followed by incubation with anti-kappa and anti-lambda antibodies or isotype controls. All cells were fixed in 1.0% paraformaldehyde and stored at 4 °C for up to 12 h before acquisition. Specimens were acquired using an 8-color multiparametric approach on a 3-laser FACS Canto II (BD Biosciences, San Jose, CA) with DiVa 6.1.1 software and analyzed by FCS Express 3 software (DeNovo Software, Los Angeles, CA). Approximately 2–5 million cells were acquired for each cocktail.

2.3. Definition of APC and NPC

PCs were identified by gating on cells with CD138 positivity and strong CD38 expression. CD45 and light scatter properties were also examined to exclude debris, doublets and lymphocytes. NPCs were defined based upon polyclonal intracellular light chain expression. APCs were defined based upon a cluster (antigen expression profile) of plasma cells with monoclonal intracellular light chain expression. The pattern of CD81 and CD19, CD20, CD45, CD56 and intracellular light chain expression was studied in NPCs and APCs. The levels of antigen expression were categorized as follows: negative (N) when there was no staining demonstrated; partial positive (PP) when there was partial expression; weak positive (WP) when all the cells had dim expression; moderate positive (MP) when the cells had moderate expression; and strong positive (SP) when the cells had strong expression.

2.4. Bone marrow biopsy

Sections from decalcified B5 fixed core biopsies were stained with CD138 antibody using a Dako (Carpinteria, CA) or a Ventana (Tucson, AZ) autostaining system. The percent plasma cells were estimated based upon CD138 expression by immunohistochemistry.

2.5. Biochemistry

Serum M-protein quantitation was performed by electrophoresis with the Sebia Hydrasys® (Norcross, GA 30071) automated system utilizing protein Hydragels™. The protein gels were then scanned using a HYRYS™ densitometer and the serum proteins including M-proteins calculated using Sebia Phoresis™ software. Identification of the M-proteins was carried out on a Sebia Hydrasys® system utilizing IF Hydragels™. Serum free light chain (sFLC) analysis was performed with a Siemens BN™ II nephelometric system (Siemens, USA) coupled with Freelite® reagents from The Binding Site Group Ltd. (Birmingham, UK).

3. Results

3.1. Patient characteristics

We studied 59 specimens of PCD (14 MGUS, 35 SMM, and 10 MM) and 5 normal bone marrow specimens with no evidence of PCD. Clinical and laboratory details are listed in Table 1. The mean age of patient groups were as follows: MGUS 59.4 years; SMM 59.6 years; and MM 57.9 years). The male to female ratio differed little between the MGUS, SMM and MM categories, except for a slight male predominance (20/15) in the SMM category only. The mean percent of BM plasma cells and M protein were lowest in MGUS (7.8%, 0.6 g/dL), intermediate in SMM (20.9%, 1.95 g/dL) and highest in MM (40.8%, 2.7 g/dL).

3.2. Immunophenotypic profile of NPC versus APC

The antigenic expression patterns for CD19, CD20, CD45, CD56 and CD81 were studied in APCs (59 BM specimens) and NPCs (34 BM specimens) (Tables 2 and 3 and Fig. 1). This combination of antibodies allowed ready identification of APCs in all cases based on an aberrant pattern of expression of CD19, CD20, CD45, CD56 and CD81. Loss of CD19 was found to be a highly sensitive marker in detecting APCs; it was negative in all APCs (100%) but was also negative in a small subset of polyclonal NPCs in 17 of 34 specimens (50%) (Table 3). In these 17 cases a mean 24% of normal plasma cells were CD19 negative (S.D.: 15, range 7–50) (Table 3 and Fig. 1). Aberrant expression of CD56 was present in the APCs in 41 of 59 specimens (69%) but also found to be expressed by a small subset of polyclonal NPCs in 9 of 34 NPC specimens (mean 10% of NPCs CD56+, S.D.: 5, range: 5–22%). CD45 expression was abnormal (negative, partial or aberrantly weak positive) in 53 (90%) of 59 APC cases. Intermediate (normal) CD45 expression was observed in the APCs in only 6 of 59 cases (10%). Interestingly, there was dim CD45 expression in a subset of polyclonal NPCs in 14 of 34 NPC specimens (mean 86% of NPCs CD45 dim, S.D.: 15, range: 63–100%). Aberrant CD20 expression was found in APCs in 20 of 59 specimens (34%) but was also found to be positive in a subset of polyclonal NPCs in 3 of 34 NPC specimens (mean 21% of NPCs CD20+, S.D.: 14, range: 13–37%). On evaluation of CD81 expression, we found that it was homogeneously brightly expressed by NPCs in all specimens (100%) (Table 3 and Fig. 1). However CD81 was negative or dim in the APCs in 56 of 59 cases (95%). No NPC populations with dim or negative CD81 were identified.

Since exact enumeration of APCs is utilized in determining risk in plasma cell dyscrasias, the goal of FC is highly sensitive detection