Flow Cytometry in Mastocytosis

Utility as a Diagnostic and Prognostic Tool

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KEYWORDS

• Mast cells • Mastocytosis • Flow cytometry • Bone marrow • Cell purification • Diagnosis • Classification • Prognosis

KEY POINTS

- Multiparametric flow cytometry is an essential technique applied in the diagnosis of systemic mastocytosis (SM).
- The application of specific protocols for the analysis of rare samples is mandatory when studying bone marrow (BM) aspirates to establish a correct diagnosis.
- To establish the prognosis of each patient, BM cell populations should be purified to study the degree of BM hematopoiesis involvement by the *KIT* mutation.

INTRODUCTION

Mastocytosis is a heterogeneous disease characterized by the accumulation of pathological mast cells (MCs) in different tissues.[1,2] Because the prevalence of Mastocytosis disease is low and, consequently, knowledge regarding the management of the disease is restricted to few professionals, patients are usually referred to specialized

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reference centers, where their diagnosis and follow-up are performed using the most sensitive and specific techniques.

In practice, diagnosis of SM is typically suspected in patients having mastocytosis in the skin[1,3] or in patients without skin lesions presenting with systemic symptoms of MC activation (systemic MC activation syndrome)[3,4] and it is currently based on clinical, biochemical, morphologic, histopathologic, immunophenotypical, and molecular data. With the exception of the more aggressive categories of SM (eg, aggressive SM [ASM] and MC leukemia [MCL]), MCs represent only a small proportion of all nucleated BM cells. Such a low MC burden raises the need for applying highly sensitive and specific methodological approaches for the study of MCs in routine laboratory diagnosis.[5,6]

In the following sections, readers are provided with information about the methodological approaches that should be applied to perform an objective and reproducible diagnosis and classification of SM, with particular emphasis on BM studies. Additionally, the importance of flow cytometry for the establishment of long-term prognosis for patients individually is explored.

METHODOLOGICAL APPROACH FOR THE IDENTIFICATION, ENUMERATION, AND CHARACTERIZATION OF BONE MARROW MAST CELLS

Although other techniques, such as immunohistochemistry and immunocytochemistry, can be used for analysis of the expression of specific antigens on MCs, flow cytometry is currently the recommended method for this analysis, because it allows sensitive detection and quantitative evaluation of the expression of multiple antigens simultaneously in large numbers of MCs, even when they are present in a sample at low frequencies.[5] To accomplish optimal results, however, several issues need to be taken into account. Among these issues, several critical parameters have been identified regarding the immunophenotypical analysis of BM MCs (reviewed by Escribano and colleagues[5] and Sanchez-Munoz colleagues[6] and in **Box 1**).

First, the BM aspirate should contain sufficient numbers of BM particles; because MCs are firmly attached to the stromal cells, BM samples should be executed firmly and quickly in the posterior iliac crest by a 14G to 8G biopsy needle. Samples obtained without BM particles could be adequate for other immunophenotypical studies but not for MC enumeration. In addition, only fresh samples with high cell viability (>95%) should be used for the enumeration of BM MCs; this parameter is especially critical for the enumeration of MCs from ascitic fluid and other body fluids, where MC viability rapidly decreases with time (Sanchez-Muñoz, personal communication).

After the collection, BM samples should be disaggregated through 25G needles to separate MCs from BM stroma for obtaining a homogeneous cell suspension.

A critical parameter when a low BM MC burden is expected, and preferably always used as a routine technique, is performing a 0.5% toluidine blue (pH 0.5 or 3) stain of a BM smear prior to immunophenotyping, to get an overall impression on the percentage of MCs in the sample. This stain provide a chance to stain duplicates or even triplicates of each tube to get enough number of MCs to analyze (**Fig. 1**).

Another technical factor that may have an impact on the results relates to the specific monoclonal antibody (mAb) clones, fluorochrome reagents, and combinations used, which should be carefully selected for optimal performance.[5–8] Human MCs strongly express CD117, CD203c, and the high-affinity receptor for immunoglobulin E (FcεRI) (reviewed in Refs.[5,9–11]). Nevertheless, none of these proteins is specific for MCs and, therefore, at least 2 antigens are needed for the accurate identification