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Invited Review

Flow cytometry in immunoglobulin light chain amyloidosis: Short review

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ABSTRACT

Flow cytometry (FCM) has found its application in clinical diagnosis and evaluation of monoclonal gammopathies (MG). Although, research has been mainly focused on multiple myeloma (MM), nowadays FCM becomes to be potential tool in the field of AL amyloidosis. Clonal plasma cells identification and specific phenotype profile detection is important for diagnosis, monitoring and prognosis of AL amyloidosis. Therefore, FCM could be a perspective method for study not only MM but also AL amyloidosis. This review provides an overview and possibilities of FCM application in AL amyloidosis.

Contents

1. Introduction

The systemic amyloidosis are a group of complex diseases caused by tissue deposition of badly folded proteins that result in progressive organ damage [1]. There are different types of amyloidosis, which are classified according to the protein composition of the amyloid deposits, target organs involved in amyloid deposition and their clinical manifestation [2]. The most common form of amyloidosis (70%) is immunoglobulin light chain amyloidosis, which is a clonal plasma cell dyscrasia and an immunoglobulin light chain disease [3–5]. It occurs in approximately 9 cases per 1,000,000 inhabitants per year and the average age of diagnosed patients is 65 years.

AL amyloidosis represents one branch of monoclonal gammapaties evolution. It is proved nowadays that AL amyloidosis may develop from monoclonal gammopathy of undetermined significance (MGUS) [6]. In this type of amyloidosis abnormal plasma cell clones produce immunoglobulin light chains [7], which are stored in the affected organs and lead to their malfunction [8]. The most affected organs resulting in symptoms are the kidneys, heart, skin, peripheral nerves, autonomic nerves and liver [9]. Early symptoms of affected organs are summarized in Table 1.

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Table 1
Early symptoms of affected organs [12].

Affected organ	Present (%)	Early symptoms "red flags"
Heart	70	NT-proBNP > 332 ng/l BNP > 73 ng/l
Kidney	70	Albuminuria > 0.5 g/day eGF < 50 ml/min/1.73 m^2
Liver	22	Elevation of ALP and/or GMT in absence of other causes
Peripheral and autonomic neuropathy	14	Neuropathic pain and loss of sensitivity to temperature Erectile dysfunction
Soft tissues	13	Carpal tunnel syndrome

NT-proBNP – N-terminal pro-natriuretic peptide type B; BNP – brain natriuretic peptide; GF – glomerular filtration; ALP – alkaline phosphatase; GMT – glutamyl transpeptidase.

The detection of free immunoglobulin light chains (FLC) is important for diagnosis of AL amyloidosis. Reference intervals for free FLC in serum are 3.3–19.4 mg/l for κ, and 5.7–26.3 mg/l for λ with the ratio of κ FLC to λ FLC is 0.26–1.65. Increasing these values may indicate MG including AL amyloidosis [10]. Tissue staining by Congo red is used to diagnose amyloid deposits in tissues to confirm amyloidosis while positive materials shows apple-green birefringence under polarized light using microscopy technique [11].

Flow cytometry has many applications in the field of haematology [12]. Due to development of the technology, monoclonal antibodies and progressively broad variety of compatible fluorochromes, FCM has found application in the clinical diagnostic laboratories [13]. A specific group of antigens – CD molecules (Clusters of differentiation) are cell surface/intracellular molecules that provide a targets for immunophenotyping [14]. These molecules can act in numerous ways, often acting as receptors or ligands. CD molecules have been organized through a series of international workshops – the Human Leukocyte differentiation Antigens (HLDA) Workshops and the nomenclature of CD molecules is gradually updated with new markers [15]. Cell identification by FCM is based on the reaction of antigen and fluorescently labelled antibody and can be used for simultaneous detection of intracellular (e.g., cytokines, transcription factors, and phosphoproteins) and cell surface molecules [16]. The EuroFlow Consortium (EFC) offers standardized instrument settings and antibody panels for immunophenotyping of haematological malignancies included plasma cell disorders (PCD) [17,18]. The main objective of EFC is the development and standardization of fast, accurate, and highly sensitive flow cytometric tests for diagnosis and prognostic (sub)classification of haematological malignancies as well as for evaluation of treatment effectiveness during follow-up [19].

FCM becomes a considerably helpful tool in both, diagnosis and definition of prognosis in MM. FCM immunophenotyping is recommended as a part of routine evaluation of MM patients at diagnosis. It was compared with commonly used test by conventional morphology in a large group ($n = 765$) of patients and there was a significant positive correlation between these two methods [20]. Moreover, Ocqueteau et al. has shown that measured by multiparameter FCM the number of residual polyclonal PCs was the most powerful single parameter for the discrimination between MGUS ($n = 76$) and MM ($n = 65$) patients at diagnosis [21]. Paiva et al. has reported that the group of patients ($n = 80$ of 594) with more than 5% residual normal plasma cells from all bone marrow plasma cells (N-PCs/BMPCs) had significantly longer progression-free survival and overall survival than patients with less than or equal to 5% N-PCs/BMPCs [22]. Thus, multiparameter FCM has been proven to have a clinical value in analyses of plasma cell dyscrasias (PCD).

Nevertheless, until now FCM did not cover its potential in the other monoclonal gammapathies, such as immunoglobulin light chain amyloidosis (ALA). It is still to be explored if the detection of abnormal plasma cell clones in bone marrow by FCM could be used as an important diagnostic tool as well as could be helpful for the prognosis assessment and monitoring of the treatment response in ALA cases.

The aim of this work is to provide an overview of studies, focusing on FCM immunophenotyping in AL amyloidosis.

2. Immunophenotype of plasma cells

Plasma cells (PCs) represent less than 0.5% of mononuclear cells in the bone marrow and they are characterized by bright expression of CD38 and CD138 [23]. Protein CD138 (syndecan-1) is a member of the transmembrane heparinsulfate proteoglycan family as an extracellular matrix receptor [24,25]. Expression of CD138 is observed on the surface of mature epithelial cells [26]. Using CD138 we can distinguish plasma cell from other cells in bone marrow [27]. Loss of expression of CD138 leads to induction of apoptosis and inhibition of cell growth [28]. CD38 serves not only as an antigen but has many enzymatic functions: it catalyses the metabolism of cADP-ribose and NAADP, two structurally and functionally distinct Ca(2+) messengers targeting, respectively, the endoplasmic reticulum and lysosomal Ca(2+) stores [29].

PCs heterogeneously express Leucocyte common antigen CD45, requiring for lymphocyte activation and development. PCs lose expression of this marker, depending on their stage of development [23]. CD81, strongly expressed on normal plasma cells, is tetraspanin cell surface protein, is needed for normal expression of CD19 [30,31]. By-turn, CD19 is expressed from early state of B cells development and is essential for their differentiation [32]. Neutral cell adhesion molecule (NCAM) or CD56 primarily mediate adhesion between neural cells and is usually expressed on natural killer cells in bone marrow [33,34]. Both CD19 and CD56 serve as surface markers allowing discrimination of normal and abnormal (aberrant) PCs (Table 2; Fig. 1).

Although positivity for CD19 and negativity for CD56 are considered as normal PC immunophenotype, it was described an aberrant immunophenotype $CD19^{-}CD56^{+}$, which represents normal long lived terminally differentiated plasma cells in healthy people ($n = 10$ of 11). It is worth to be mentioned that $CD19^{-}CD56^{-}$ and $CD19^{+}CD56^{+}$ PCs were also described in healthy donors' samples [35]. This $CD19^{-}CD56^{+}$ aberrant immunophenotype was confirmed by Tembhare et al. study: they analyzed 59 specimens of untreated patients with PCD (14 MGUS, 35 SMM, 10 MM) and 5 normal bone marrow specimens with no evidence of PCD. Loss of CD19 was found in all abnormal plasma cells but it was also found in 50% (17/34) of specimens with normal plasma cells. As well as aberrant CD56 positivity was confirmed in 26% (9/34) of specimens with normal plasma cells. Therefore they recommend combination of CD19 negativity and CD81 dim expression or negativity to distinguish between normal and abnormal plasma cells. This combination was not observed in any of the cases in normal plasma cells [30].

For more accurate distinction between normal and abnormal PCs it is recommended to use κ and λ light chains (LC), because normal plasma cells are able to produce κ and λ LC in physiological ratio. Therefore population of clonal plasma cells can be generally characterized by homogenous expression of only one of these [36].

3. Standardization of flow cytometry analyses in PCDs

There are some significantly important markers, previously described, from which the EuroFlow Consortium (EFC) creates standardized plasma cell disorder (PCD) panel. Using PCD panel we can evaluate MM, MGUS and other less frequent MGs due to the accumulation of the clonal Ig (e.g. AL amyloidosis). In addition, markers

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