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WALDENSTROM MACROGLOBULINEMIA'S IMMUNOPHENOTYPES AND ITS RELATION WITH OTHERS HEMATOPATHIES

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Abstract: Waldenstrom's Macroglobulinemia (MW) is characterized by a type of mature B-cell non-Hodking lymphoma with proliferation of lymphoplasmocyte elements in the bone marrow and presence of monoclonal immunoglobulin M gamopathy. Given the rarity of the disease, the wide spectrum of hematopathies can mask the diagnosis of this disease. Therefore, the identification of immunophenotypes is one of the main medical challenges for making an early diagnosis. The present study brings a retrospective and descriptive essay, based on systematic reviews around the main biological markers used to make the diagnosis of patients with Waldenstrom's Macroglobulinemia, through the active search for original articles in the Pubmed, Science direct, Scielo, UpToDate and Portal Capes databases. Six articles with a specific theme were selected, categorized according to the differential diagnoses of Waldenstrom's Macroglobulinemia. Among the articles analyzed, it is known that monoclonal IgM, detected by immunofixation electrophoresis, has a higher diagnostic value in MW compared to other hematopathies. In addition, genetic mutations in MYD88 and in the CXCR4 receptor are frequently found in this pathology, corroborating the possible specificity of these findings in the diagnosis of affected patients. The identification of other specific immunophenotypes, according to the literature, is verified through flow cytometry. Current data point to the MYD88L265P mutation and monoclonal IgM as the main biomarkers, accompanied by the CD19, CD20, CD22 and CD79a immunophenotypes. The CXCR4 mutation is still uncertain, so it needs long-term studies to assess its predictive value in MW.

Keywords: Lymphoma, Non-Hodgkin, Immunoglobulin M, Immunophenotyping.

INTRODUCTION

Lymphomas are a group of disease characterized by the presence by malignant cells lymphoids that accumulate in the lymphodones and could be divided in Hodgkin and non-Hodgkin lymphons[13]. Among the non-Hodgkin has a group of neoplasms of cells mature T and the neoplasms group of cell mature B, being the Waldenstrom's Macroglobulinemia (WM) one of their examples.

The bone marrow has a physic microenviroment consisting of a range of different cells, including hematopoietics, blood, osteoblasts, osteoclasts, endothelial cells, besides chemokines, growth factors, extracellular matrix and mesenchymal cells[15]. These, characterized by a heterogenous population of auto-renewable cells established by different markers, such as Nestine, neural-glial antigen and leptin receptor[17]. Mesenchymal and hematopoietic cells' association leads to secretion of support factors and chemokine binding 12 (CXCL 12), angiopoetin and stem cell factor (binding SCF). In addition, endothelial cells also provide support and maintenance to hematopoietic cells, through secretion of the same factors mentioned above, as well as fibroblast growth factor (FGF2) and Delta-like 1, encouraging the process of supporting medullar microenviroments[36].

This medullar microenviroment are divided into endosteal niche and vascular niche[7]. First is localized in the interface between bone marrow cells and osteoblasts and they stimulate and regulate the function of hematopoietic cells through a direct connection between the two cells or by via paracrine, where there is cytokines production by the osteoblasts which will act on their cognate receptor in the target cell. Notwithstanding, vascular niche is composed of the sinusoidal capillaries and surrounding hematopoietic cells, facilitating their dissemination into the vascular system. This characteristic is considered important in the study of infiltrative hematopoietic, because the physiological conditions of this niche facilitate the development of the pathological mechanism[6,31].

In view of the medical difficulty of performing a consistent diagnostic confirmation of this pathology, as it is the same to several other modular neoplasms, this research's objective was to investigate the pathogenesis of Waldenstrom's Macrogobulinemia and the typical immunophenotypes involved, relating the markers expressed in this pathology and their early diagnosis.

MATERIALS AND METHODS

This research is about a retrospective and descriptive trial based on systematic reviews around the main biological markers used to make the diagnosis of patients with Waldenstrom's Macroglobulinemia.

The research planning and development took place between October 2020 and December 2020, through the active search for original articles in the databases Pubmed, Science direct, Scielo, UpToDate and Portal Capes, using the descriptor "Lymphoma", "immunoglobulin", "diagnosis", separated by semicolons, in Portuguese and English.

Articles published between 2000 and 2020 were selected to address similar topics and explore the differential diagnoses of several etiologies of non-Hodking lymphoma and other bone marrow hematopathies to perform comparative analysis between the different markers and diagnostic methods through review, clinical trials or case studies. The articles were evaluated according to the updates on the subject, predominantly their year of publication, whether they were in Portuguese or English and the quality of the indexed database. The researches that explored similarity with the proposed theme,

as well as the pathogenesis and diagnostic criteria of the different gammopathies were included. Articles that did not correspond to the mentioned factors were excluded from the study.

RESULTS

Six articles were found with the specific theme, categorized according to the several etiologies that permeate the bone marrow hematopathies that resemble Waldenstrom's Macroglobulinemia.

The articles were summarized according to the author, year of publication, the hematopathy and the biomarkers used for the diagnosis and will be presented in table 1.

DISCUSSION

MW is a rare condition, representing approximately 2% of cases of Non-Hodgkin's Lymphoma[28], with a higher prevalence in adult Caucasian male patients, around the seventh decade of life and has an incidence of 3-4/1,000,000 cases per year[36,16].

Histologically, it is characterized by proliferation of lymphoplasmocyte elements in the bone marrow and the presence of monoclonal immunoglobulin M (IgM) gamopathy[13, 15]. Although the presence of this serum paraprotein is related to lymphoplasmocyte lymphoma (LPL), it is not a typical marker of this pathology. Based on the bone marrow involvement status, LPL is categorized into the subtypes: Waldenstrom's Macroglobulinemia and non-MW LPL[36].

Normally, MW shows itself as an indolent disease, although there is considerable heterogeneity in its clinical manifestations when present. In about 25% of the patients are asymptomatic and with almost 40 to 70% develop symptoms within 3 and 10 years after diagnosis, respectively[4]. Among the main signs

Author	Year of publication	Hematopathy	Biomarkers and diagnostic technique
Andrade[1]	2009	Monoclonal Gamma disease of Undetermined Significance	IgG or IgA serum levels>3mg/dL or monoclonal proliferation <10% of plasma cells in the bone marrow; differs from multiple myeloma by the absence of lesions in peripheral organs.
Calheiros et al[6]	2010	Multiple Myeloma	Expression of myeloid markers (CD117++, CD33++, CD28++, CD56++, CD13++) on the surface of myelomaplasmocytes by immunohistochemistry or flow cytometry methodology.
Rajkumar et al[30]	2014	Multiple Myeloma	Monoclonal IgM plasmocytes presence in 10 to 60% and/ or serum monoclonal protein (IgG or IgA) >30g/L by immunohistochemical biopsy analysis.
Treon et al[32]	2014	Waldenstrom's Macroglobulinemia	Mutation in the MYD88L265P gene, identified by the allele-specific polymerase chain reaction technique (PCR-AE); mutation in the CXCR4 terminal in DNA analysis of bone marrow aspirate and sequencing by the Sanger method.
Rodrigues et al[24]	2016	Chronic Lymphocytic Leukemia	Presence of $5x10^9$/L monoclonal $CD5^+/CD23^+$ B lymphocytes in peripheral blood, using the flow cytometry technique.
Dimopoulos; Kastritis[7]	2019	Waldenstrom's Macroglobulinemia	Biopsy shows medullary infiltrate with >10% monoclonal IgM and infiltration by clonal lymphoplasmocyte cells, detected by immunoassay electrophoresis.

Table 1 - Waldenstrom's macroglobulinemia and its main differential diagnoses.

and symptoms, anemia is prevalent in most patients due to insufficient erythropoiesis due to infiltration of the medulla and decreased erythrocyte survival related to IgM hemolysis. 25% of patients have lymphadenopathy and/or hepatosplenomegaly[4]. Another recurrent manifestation in patients with MW is the hyperviscosity syndrome, due to the involvement of peripheral blood, which leads to dizziness, pain, ataxia, visual disorders, deafness, nystagmus, mucocutaneous bleeding and, in some cases, damage to cognitive function and alteration of mental status[4, 36].

In MW there is a molecular control with the malignant cells that internalize in the bone marrow. It is known that CXCL12 (stromal-derived factor) is highly expressed in the bone marrow of patients with MW and its action is aggravated by the mutation in the CXCR4 chemokine receptor. Increased CXCR4 and CXCL12 interaction promotes a significant homing of malignant cells from MW to bone marrow[25], as is the case with Chronic Lymphocytic Leukemia (CLL). Alsagaby and Alhumaydhi, 2019, cited in their studies that the relationship between CXCR4 and CXCL12 expresses CLL identifying factors in marrow cells, such as prognostic markers CD38 and CD49d, produced by the malignant cells in CLL[3] and other types of leukemia, ensuring their survival in the spinal cord environment. No retrospective study reported the presence of similar markers in Waldenstrom's Macroglobulinemia.

The migration of malignant cells in the stroma of bone marrow promotes the secretion of a number of monoclonal immunoglobulins. The MW studies with a typical finding of monoclonal IgM secretion by B lymphocytes, through the activation factor of B cells (BAFF)[27] present in lymphoplasmocytic cells, which bind to the receptors present in the lymphocytes (BAFF-R), inducing its proliferation, in addition to the action of the chemokine ligand 5 (CCL-5), very much expressed in patients with MW, which stimulates the release of IL-6 by the malignant cells, which will act on the B lymphocytes in the secretion of IgM[12].

The monoclonal immunoglobulin M detection in MW is performed by means of the immunoassay electrophoresis technique from bone marrow biopsy[4, 12]. The accuracy of the diagnosed is limited by the presence of spinal cord infiltrate with monoclonal IgM protein, associated with >10% of lymphoplasmocytic cells[11, 12], demonstrating, in retrospective studies, sensitivity and specificity of 80.6% and 89.2%[39], respectively. Furthermore, a monoclonal IgM-free LPL, as well as the presence of IgM without histopathological findings of LPL in medullary biopsy, does not give parameters for MW as the main diagnostic assumption[9], running with differential diagnosis for 377 monoclonal gammopathies, such as nodal lymphoma and Gamopathy of undetermined meaning (MGUS)[23], due to its histological characteristics similar to the findings mentioned above.

A similar case of this mechanism was studied in a work on Multiple Myeloma (MM) by Rajkumar et. al in 2014. In it, the author addresses monoclonal IgM secretion as low diagnostic value, since its sensitivity to monoclonal IgA and IgG is minimal and therefore of little value[27]. Dauen Ryu and collaborators, 2016, also stated that IgM secretion in the MM is a rare subtype of condition that presents a low prognosis (IgM-MM)[28]. In addition, myeloma cells express aberrant phenotypes such as CD56+++, CD117++, CD33++, CD28++[7], documented by the incubation of bone marrow samples with monoclonal antibodies and immunophenotypic analysis in flow cytometry[15], representing great value in the diagnostic identification of the MM.

Gammapathy of Undetermined Significance (MGUS) has high monoclonal sensitivity in IgG, found in approximately 70% of patients, followed by IgM (15%) and IgA (12%)[21]. Andrade, 2009, addresses in his scientific study a pathological condition in which a MGUS subtype has serum IgM peaks and medullary findings very similar to MW and other lymphoplasmocytic lymphomas[27]. In this case, the differentiation occurs by the clinical history of the patient, showing absence of hyperviscosity in peripheral blood, hepatosplenomegaly and lymphadenopathy[1, 4].

Studies have shown that monoclonal IgM secretion is not characteristic of Chronic Lymphocytic Leukemia (CLL)[12, 36]. Its gene expression is much greater in $CD5^+$ B cells[38], leading to clonal expansion in the peripheral blood of adult patients. The differentiation between CLL and MW, besides the absence of monoclonal IgM, is given by clinical and laboratory variants, through the peripheral blood smear with visualization of small mature lymphocytes, increased nuclear density with aggregate chromatin, absence of visible nucleoli[22] and presence of at least $5x10^9$/L of B cells with $CD5^+$ phenotype in the absence of splenomegaly, hepatomegaly and lymphadenopathy[22]. The negativation of the FMC7, CD79b and CD22 fractions in leukemic lymphocytes allows their differential diagnosis with other monoclonal B-cell gamopathies[17]. This finding is ratified by the study developed by EuroFlow group, through a cytochemical analysis with the combination of several appropriate monoclonal antibody markers, that identify the main markers expressed in CLL cells, such as CD5 +, CD23 + and the absence of FMC7 and CD22 verified by flow cytometry[8].

It is noticeable that the flow cytometry techniques for the various neoplastic hematopathies of the bone marrow show a great advance in the confirmation of early diagnosis, compared to MW[22]. The best accepted hypothesis for diagnostic differentiation today is the presence of a population of clonal lymphocytic and plasmocytic cells in the marrow in patients with MW, evidenced by the expression of CD19, CD20, CD22 and CD79a biomarkers, identified by immunohistochemistry or flow cytometry[37]. As previously mentioned, the presence of a CD22 positive helps in the diagnostic exclusion of other gamopathies, especially CLL, which does not present such a marker in laboratory tests.

This finding complements the analysis performed by B Paiva et. al, 2014, with 244 patients diagnosed with monoclonal IgM, 100 of them with symptomatic MW[22]. Laboratory studies with malignant MW cells documented higher positivation in light chain B cells and a characteristic phenotyping in these patients (CD19 / CD20/ CD22 [+dim]/ $CD25^+$ / IgM+) besides differing from other lymphomas by negativating the expressions CD5, CD10, CD11c or CD103c[22].

However, the great value findings in the identification of Macroglobulinemia are by genomic sequencing and identification of somatic mutations in the myeloid differentiation factor (MYD88)[33], due to the L265P mutation, which changes the position 265 of leucine in proline in MYD88[38]. This mutation activates the kinase associated with IL-1 receptor (IRAK) and Bruton's tyrosine kinase (BRK) promoting the translocation of the nuclear factor kB-p65 guaranteeing the development and growth of malignant cells[33]. The studies conducted by Xinfang Yu and collaborators, 2013, demonstrated a low spectrum of this mutational change in different cancers, once ratified by Treon et. al, in 2012, which identified the presence of $MYD88^{L265P}$ in 90% of patients diagnosed with MW included in the study[38]. The detection

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of mutations in the LPL MW performed by Vinarkar et. al, 2018, showed a rate of 84.8% of MYD88-L265P patients positive by conventional PCR-AE[34] technique, Ondrejka et al and Maria et al, 2013, claimed 100% of MYD88-L265P mutational positivity using the same technique[21, 35], corroborating the high specificity of this finding in the diagnosis of these patients.

At the same time, the MYD88-L265P mutation is accompanied by $CXCR4^{MUT}$, a genetic alteration in the chemokine receptor CXCR4[38], ensuring the migration of malignant lymphoid cells in the stroma of bone marrow[11].

Two classes of mutations are found in CXCR4: $CXCR4^{NS}$ and $CXCR4^{FS}$, both equally distributed among patients with MW[23]. Bone marrow and peripheral blood aspiration and analysis by the Sanger method performed by Treon et. al, 2014, in lymphoplasmocyte cells with $CD19^{+}$ markers was the most reliable method for CXCR4 mutational identification[32]. Another large-scale study presented by Ballester et. al, 2016, reported a high correlation MYD88-L265P and $CXCR4^{MUT}$, where a clinical trial was conducted with 8 patients with CXCR4 mutation, among which 7 had the diagnosis of MW confirmed by laboratory methods[4]. Recently, an experimental study by Bárbara Muz and collaborators, 2019, demonstrated the identification of $CXCR4^{MUT}$ through a ^{64}Cu (copper) radiomarker, associated with a CXCR4 inhibitor (AMD3100)[20]. The detection of mutation in this gene by in vivo radiolabeling with PET/TC was effective, besides identifying high potential metastatic in patients diagnosed with MW. However, the CXCR4 mutation, although rarely, has also been found in patients with the congenital immunodeficiency syndrome associated with chronic leukopenia (WHIM)[18], given its pleiotropic properties. Thus, reducing the specificity of the mutation of this gene in MW.

CONCLUSION

The Waldenstrom Macroglobulinemia diagnosis is one of the most current medical challenges of modernity, given the rarity of the disease. Laboratory and clinical findings show a potential path for specific diagnosis of this pathology, even though there is a broad spectrum of hematopathies triggered by bone marrow dysfunction that, in certain cases, can mask this path.

Immunophenotypes, in general, are the main markers for the differentiation between medullary neoplasms. According to the analysis of the subject, it is evident that monoclonal IgM still shows itself as the biomarker of great accuracy in the diagnosis of MW, associated with greater expression of CD19, CD20, CD22 and CD79a, resulting from lymphoplasmatic infiltration. Together with these findings, the gene mutation MYD88L265P complements the diagnosis, due to the great specificity of the disease in question, obtained through gene sequencing.

Another mutation under study is the one in the CXCR4 gene. Although the above findings ratify the mutation hypothesis in this specific receptor, few studies have brought significant results correlated with its presence in Waldenstrom's Macroglobulinemia, emphasizing the importance of long-term research in this area to reach a concrete conclusion on the predictive value of the CXCR4 mutation in this pathology.

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