Leukemic non-nodal mantle cell lymphoma is a mantle lymphoma in which the patient presents with peripheral blood, bone marrow and splenic involvement without significant adenopathy.

Neoplastic cells may resemble other small B-cell lymphoid neoplasms, but show CCND1 translocation.

CytoMatrix is a synthetic matrix which has the ability to capture and preserve, within its three-dimensional structure, the biological material obtained from fine-needle aspiration.

We report a case of leukemic non-nodal mantle cell lymphoma with an exhaustive immunohistochemical and molecular characterization obtained through trephine biopsy and bone marrow aspiration prepared with CytoMatrix technique.
**INTRODUCTION**

Mantle cell lymphoma (MCL) is a clinically aggressive B-cell lymphoma, usually composed of monomorphic small-to medium-sized cells.

The genetic hallmark t(11;14)(q13;q32), which juxtaposes CCND1 gene on chromosome 11q13 with IGH on chromosome 14q32, resulting in overexpression of CyclinD1 and leading to cell cycle dysregulation, allows the differential diagnosis with other B-cells malignancies, which differ for morphological, immunophenotipical and molecular aspects.

It is considered particularly aggressive in spite of intensive treatment strategies.

A subset of patients, however, presents with indolent disease characterized by bone marrow (BM) and peripheral blood involvement, splenomegaly without adenopathy and correspond to a distinct MCL subtype recognized in the 2017 updated World Health Organization classification of haematopoietic and lymphoid tissues as leukemic non-nodal MCL (LNNMCL) (1).

Trephine biopsy of BM is appropriate for histological examination including morphological and immunohistochemical evaluation, assessment of marrow architecture and the pattern of distribution of any abnormal infiltrate, nevertheless, decalcification achieved with acids could negatively affect the quality and quantity of nucleic acid evaluation, if it is necessary for the diagnosis.

On the other hand, molecular tests, such as real time PCR, even if accurate and reliable, does not allow a simultaneous morphological evaluation of the sample.

CytoMatrix is an innovative support, initially designed to allow the management of the biological material from needle aspirates (2).

In this paper, we report the first use of CytoMatrix for the cytological, immunohistochemical and molecular assessment of BM aspiration.

**CASE REPORT**

**CLINICAL PRESENTATION**

A 50-year-old man presented to the Campus Bio-Medico University Hospital for a specialized hematologic examination with incidental lymphocytosis (13.40 x 103/uL) and splenomegaly.

A CT scan confirmed splenomegaly in the absence of lymphadenomegaly.

The patient underwent BM biopsy for suspected lymphoma.

**PATHOLOGIC FINDINGS**

BM histology showed a cellularity equal to 95% (hypercellular for the patient’s age) and revealed a small lymphoid infiltrate with a diffuse solid pattern of growth equal to about 90% of cellularity with the following immunophenotypic findings: CD20 (Clone L26, Dako)+, Pax5 (Clone DAK-PAX5, Dako)+, CyclinD1 (Clone EP12, Dako)+ (weak and partial expression), BRAF (Clone RM8, Bio-SB) equivocal interpretation due to a non-specific stain of red cells, Annexin A1 (MRQ-3, Cell Marque)-, CD10 (Clone 56C6, Dako)-, bcl6 (Clone PG-B6P, Dako)-, CD5 (Clone 4C7, Dako)-, CD23 (Clone DAK-CD23, Dako)-, CD3 (Policlonal, Dako)-, TdT (Clone EP266, Dako)- and SOX11 (Policlonal, Sigma)-.

There was a small lymphoid component with a diffuse interstitial pattern equal to about 5% of cellularity with the T-cell related phenotypes CD3+ and CD5+. The CD138 (Clone MI15, Dako)+ plasma cell proportion is equal to 3% of cellularity with an IgK (Policlonal, Dako):IgL (Policlonal, Dako) ratio of 1:1 (unbalanced).

Three hematopoietic series in the residual medullary portion were normally represented and in regular ratio.

These findings suggested a CD5- CiclinD1+ peripheral B-cell lymphoproliferative process, with the recommendation to confirm the immunohistochemical results, in particular BRAF expression, through molecular tests.

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Three hematopoietic series in the residual medullary portion were normally represented and in regular ratio.

These findings suggested a CD5- CiclinD1+ peripheral B-cell lymphoproliferative process, with the recommendation to confirm the immunohistochemical results, in particular BRAF expression, through molecular tests.
Molecular analysis

A fresh sample of a repeated BM aspirate was tested for mutations in exon 15 of the BRAF V600E gene (c.1799T>A; p.(Val600Glu)) with pyrosequencing technique and a wild-type BRAF profile was confirmed, excluding the diagnosis of Hairy Cell Leukemia (3).

In addition, part of the blood was sent to pathology laboratory in an EDTA-containing tube and processed using the CytoMatrix method. The sample was centrifuged at 1800 RFM for 10 minutes.

The buffy coat collected was added to the fixative used in the lab in a 1:10 ratio volume (a mixture of 100° alcohol/buffered:formalin in 1:1 ratio) and centrifuged once again at 1800 RFM per 10 minutes. The supernatant was removed and the cell pellet put on CytoMatrix, before the biocassette was immersed in formalin for at least 6-8 hours.

Serial sections from the paraffin embedded material were carried out for morphological, immunohistochemical and molecular testing (fluorescent hybridization in situ/FISH). About 80% of the Hematoxylin/Eosin material appeared to be small lymphoid elements mixed with morphologically regular hematopoietic elements.

The FISH investigation for translocation t(11;14) (q13;q32) using the CCND1/IGH Dual Color Dual Fusion probe (ZytoVision) showed the presence of fusion signals in the cells examined confirming that immunohistochemical CyclinD1 expression was linked to rearrangement. (Figure 1)

Considering the clinical-laboratory picture and the results of the tests performed, the peripheral B-cell lymphoproliferative process was referred to LNNMCL. Considering the CT report that found the lymphoma clinically active, the patient underwent chemo-immunotherapy treatment (R-CHOP).

FIGURE 1

A: Bone marrow cells trapped inside the synthetic matrix (Hematoxylin/Eosin, 10X);
B: immunohistochemical CD20+ stain of neoplastic cells (10X); insert in b: CCND1/IGH translocation indicated by two red/green fusion signals, one separate red signal and one separate green signal (CCND1/IGH Dual Color Dual Fusion probe, 100X).

Case conclusion

Considering the clinical-laboratory picture and the results of the tests performed, the peripheral B-cell lymphoproliferative process was referred to LNNMCL. Considering the CT report that found the lymphoma clinically active, the patient underwent chemo-immunotherapy treatment (R-CHOP).
DISCUSSION

MCL is a distinctive type of B-cell lymphoma that represents 4% to 9% of all cases of non-Hodgkin lymphoma (4).

Furthermore, MCL is considered as an aggressive B-lymphoid neoplasm characterized by extensive dissemination of tumor cells to lymph nodes, BM, peripheral blood and extranodal sites.

Patients have short responses to current therapies and frequent relapses. However, recent studies have identified a subset of MCL with indolent clinical behavior that tends to present with leukemic disease, peripheral blood, BM and splenic involvement without lymphadenopathy (1).

The genetic hallmark of MCL is t(11;14) (q13;q32) which juxtaposes CCND1 gene on chromosome 11q13 with IGH on chromosome 14q32, resulting in overexpression of CyclinD1 and leading to cell cycle dysregulation.

MCL has a distinctive immunophenotype, positive for pan B-cell markers, CD5 and CyclinD1, but usually negative for CD10 and CD23.

Therefore, CD5 expression is useful in distinguishing MCL from CD5 negative (CD5-) small B-cell lymphomas in general.

Nonetheless, rarely, the lack of CD5 may occur in some examples of MCL and pose diagnostic challenges as they can show morphologic overlap with other types of CD5- small B-cell lymphoma. Indeed, cases of MCL that lack CD5 expression have been reported in small numbers or in sporadic case reports in the literature (5) (6) (7) (8).

As a general rule, the diagnosis of CD5- MCL should be based on a combination of the morphologic, immunophenotypic, and cytogenetic features.

By the pathologist point of view, CD5- MCL needs to be distinguished from other CD5- small B-cell lymphomas including Marginal Zone B cell Lymphoma (MZBCL) (nodal, extranodal, splenic), Lymphoplasmacytic Lymphoma (LPL) and Follicular Lymphoma. Of course, lymphoma cells in MZBCL usually show monocytoid differentiation but it may be very challenging to differentiate them when occasional MCL cases display a monocytoid feature. Both MZBCL and LPL are CyclinD1- and lack of t(11;14) CCND1/IGH rearrangement by FISH. What's more, very rarely MCL may shows plasma cell differentiation as well as the expression of germinal-center cell markers such as CD10 and bcl6 (9).

Furthermore, it is also very important to differentiate CD5- MCL from other CD5-lymphoproliferative disorders such as examples of CyclinD1+ hairy cell leukaemia and CyclinD1+ plasma cell myeloma.

Usually, distinctive morphologic and immunophenotypic features readily distinguish these last two tumors from MCL.

Comparing the patients with CD5- MCL to a CD5+ MCL cohort, all clinicopathologic features were similar, except that patients with CD5- MCL showed a higher frequency of BM/splenic non-nodal presentation (10).

Patients with CD5- MCL have a significantly longer Progression Free Survival (PFS) and a tendency for longer Overall Survival (OS) compared with patients with CD5+ MCL.

This finding argue that recognition of the CD5- MCL subgroup not only have diagnostic significance, but also may have prognostic implication, suggesting that patients with CD5- MCL likely have a better survival than patients with CD5+ MCL. In this setting, CD5-leukemic, splenic, BM non-nodal forms of MCL have been correlated with an indolent clinical course (indolent disease) (11) (12).

It is worth mentioning that the frequency of SOX11 expression in MCL is significantly variable in the literature and there are scientific evidences that SOX11-negative MCL is characterized by more frequent leukemic non-nodal disease. [13]

Interestingly, clinical presentation of MCL with leukemic non-nodal disease, could be linked to the role of SOX11, a molecule involved in tumor microenvironment interactions (14).
Although some studies described that lack of SOX11 expression is more commonly seen in CD5- MCL, more recent paper reports that SOX11 expression rate was not significantly different between the CD5-MCL (67%) and CD5+ MCL (69%)\(^{(10)}(11)\).

**CONCLUSIONS**

Our feasibility study demonstrates that CytoMatrix is a powerful and practical method suitable for set up all the techniques necessary for the histological diagnosis on BM sample treated as any other Fixed Formalin and Embedded Tissue (FFPE) sample, avoiding problems linked to decalcification process indispensable for BM trephine biopsies.

The method could integrate conventional available techniques in order to perform an exhaustive morphological and immunophenotipical characterization with molecular tests. It also represent a suitable solution for BM tissue bio-banking.

**BIBLIOGRAFIA**


