ABSTRACT FOR THE 3rd ITALIAN GROUP OF HEMATOPATHOLOGY WORKSHOP

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THE APPLICATION OF CYTOMATRIX FOR LEUKEMIC NON-NODAL MANTLE CELL LYMPHOMA DIAGNOSIS

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Clinical case 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 10^3/uL) and splenomegaly. A CT scan confirms splenomegaly in the absence of lymphadenomegaly. The patient undergoes bone marrow biopsy for suspected lymphoma.

Bone marrow histology: Excellent bone marrow tissue with cellularity equal to 95% (hypercellular for the patient's age) reveals a small lymphoid infiltrate with a diffuse solid pattern equal to about 90% of cellularity with the following immunophenotypic findings: CD20+, Pax5+, Cyclin D1+ (weak and partial expression), BRAF+ (RM8 BioSB), TdT-, CD10-, bcl6-, CD23-, CD3- and CD5-. There is 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+ plasma cell proportion is equal to 3% of cellularity with an IgK:IgL ratio of 1:1 (unbalanced). Three hematopoietic series in the residual medullary proportion are normally represented and in regular ratios.

These findings suggest an indolent peripheral B-cell lymphoproliferative process with an immunophenotypic profile referable to Hairy Cell Leukemia (HCL). We recommend molecular examination and comparison with smears from bone marrow aspirate to confirm the diagnosis.

Molecular examination on bone marrow biopsy sample:

I. Rearrangement by FISH for t(11;14) (q13;q32) with ZytoLight CCND1/IGH Dual Color Dual Fusion probe not assessable due to insufficient signals;
II. Absence of mutations in exon 15 of the BRAF V600E gene (c.1799T>A; p.(Val600Glu)) with pyrosequencing technique.

If there is any doubt that the data could have been affected by the time of the decalcification process (Osteodec), it is necessary to repeat the bone marrow aspirate examination.

Part of the aspirated, fresh sample is tested for mutations to confirm a wild-type BRAF profile (Figure 3) and part is processed using the CytoMatrix method. Morphological testing (Figure 1), immunohistochemical testing (Figure 2) and FISH (Figure 4) are carried out on the cytoincluse. About 80% of the E/E material appears to be small lymphoid elements mixed with morphologically regular hematopoiesis elements. Immunohistochemical characterization of the above-mentioned lymphoid infiltrate demonstrates positivity for CD20 (Figure 2), weak and partial positivity for Cyclin D1 and weak dot-like expression for BRAF. Given the ambiguous interpretation of the immunohistochemical data, the Annexin A1 staining is performed at the Anatomic Pathology Unit of the Sant'Eugenio Hospital in Rome, both on the bone marrow

aspirate sample and on the previous biopsy. The results were negative for lymphoid cells on both samples and did not confirm the HCL diagnosis.
The FISH investigation for translocation t(11;14) (q13;q32) using the CCND1/IGH Dual Color Dual Fusion probe showed fusion signals in 30% of the cells examined confirming that immunohistochemical expression is linked to rearrangement (Figure 4).
In view of the clinical-laboratory picture and the results of the examinations carried out after the first report was issued, the peripheral B-cell lymphoproliferative process, under the 2017 WHO classification, is referred to as LEUKEMIC NON-NODAL MANTLE CELL LYMPHOMA. Considering the CT report that found the lymphoma clinically active, the patient was started on chemo-immunotherapy treatment (RCHOP).

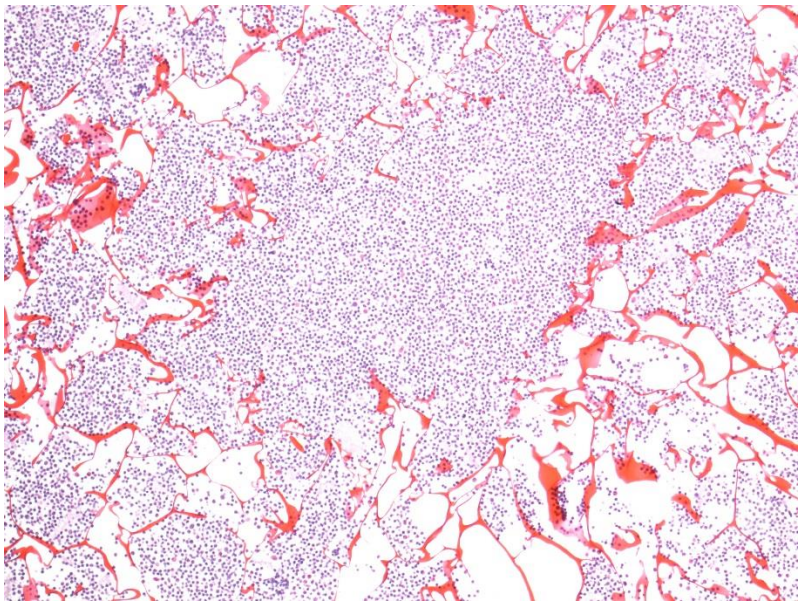

Figure 1: *E/E on CytoMatrix (10x)*

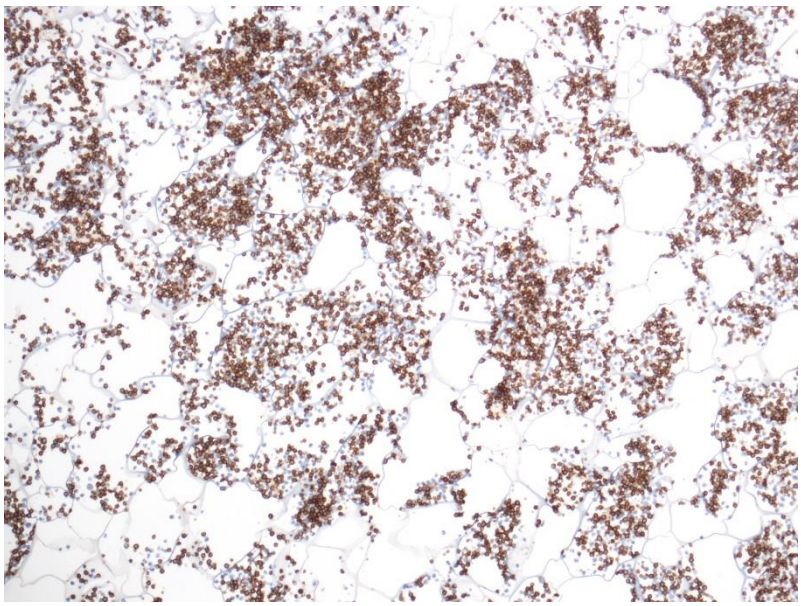

Figure 2: *CD20+ on CytoMatrix (10x)*

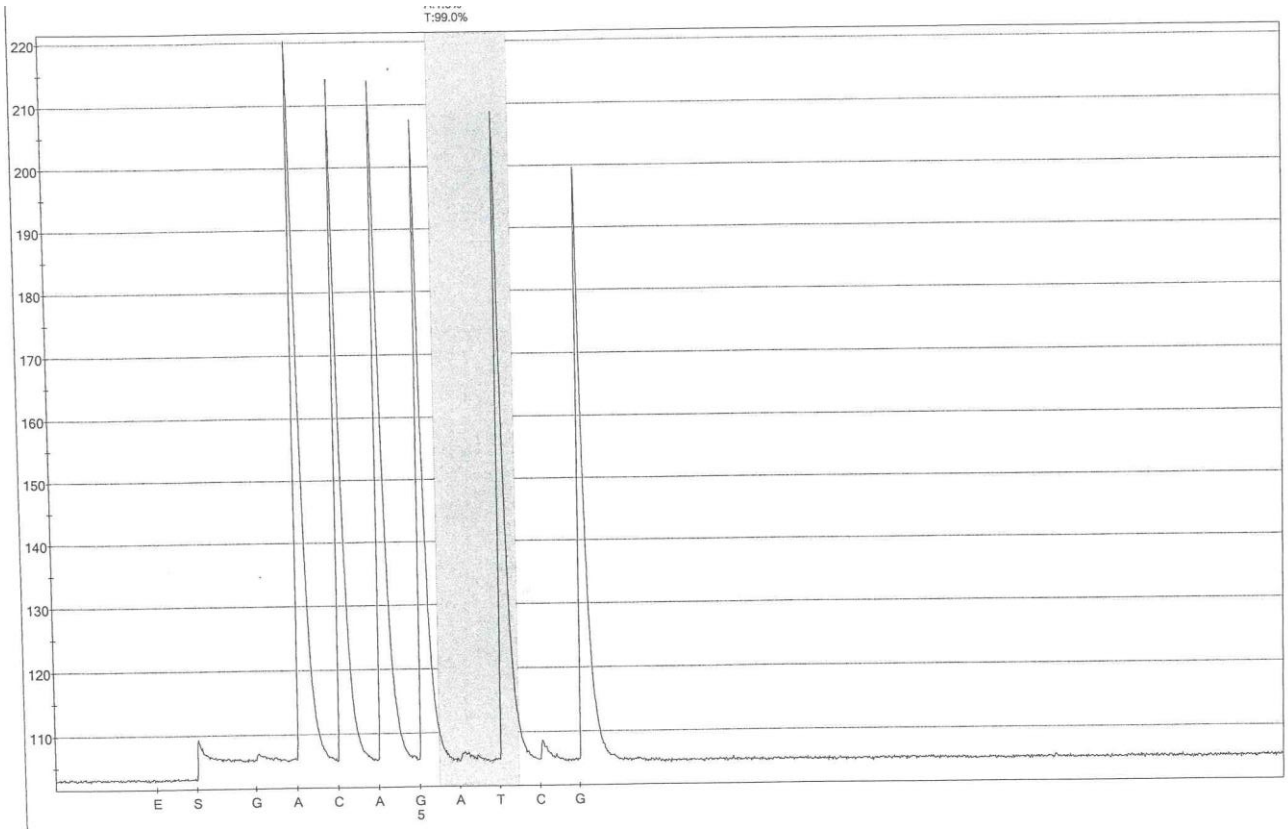

Figure 3: *BRAFwt on medullary blood*

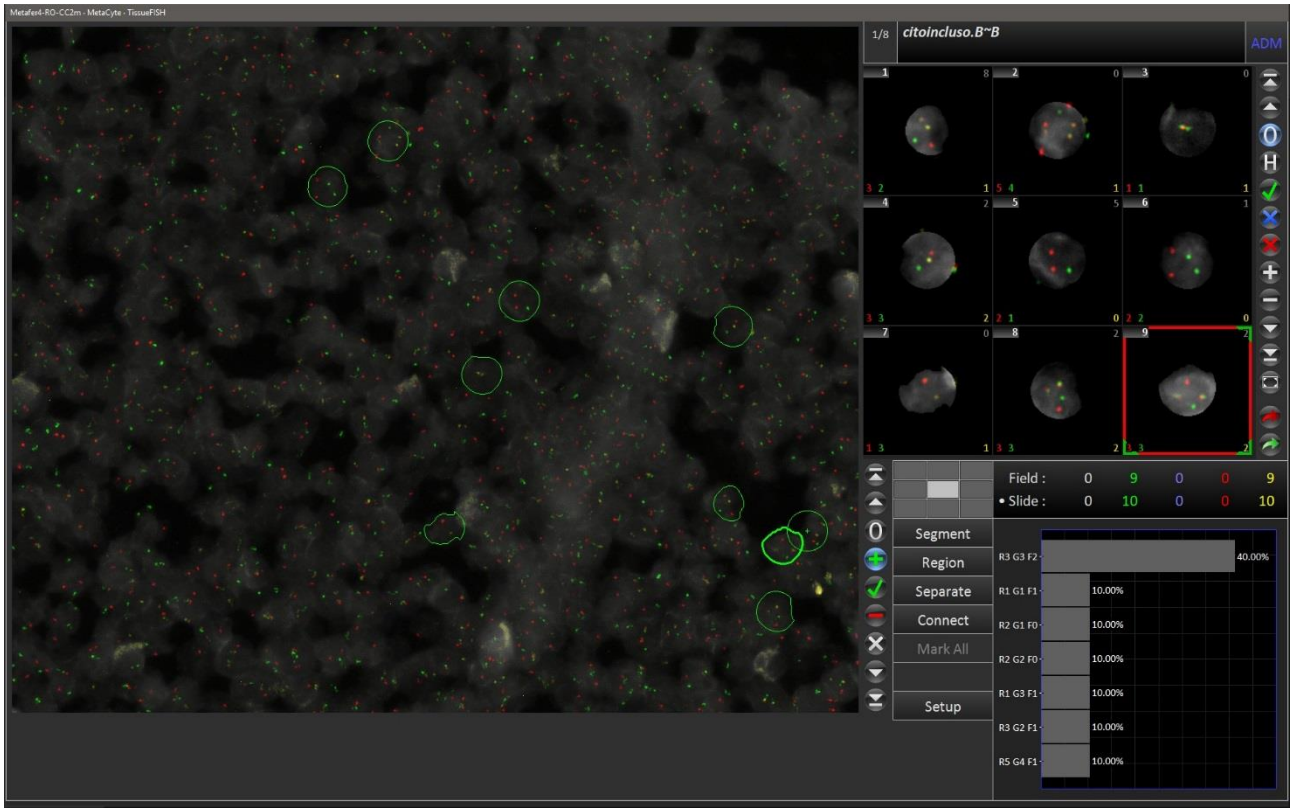

Figure 4: *FISH CCND1/IGH on CytoMatrix with Zeiss-MetaSystems*