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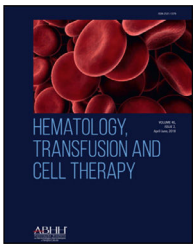

Letter to the Editor

Fluorescence in situ hybridization (FISH) in imprint of biopsies suspected of lymphoproliferative neoplasms: report on 17 cases

Dear Editor,

The diagnosis of lymphoproliferative neoplasms is mainly established by morphological and immunophenotypic aspects of Formalin-Fixed Paraffin Embedded (FFPE) tissue.[1] However, distinct genetic alterations have been specifically associated with some lymphoproliferative neoplasms, and its identification may be crucial for a complete diagnosis.[2,3] Fluorescence In Situ Hybridization (FISH) technique can be applied to different types of sample preparation, such as methanol–acetic acid fixed cells suspensions, tissue imprints and FFPE.[4,5] The FISH technique in dewaxed tissue sections is widely used and very challenging, depending on pre-analytical processes such as fixation and paraffinization; cell overlap is also an obstacle.[6,7] Due to these difficulties, we propose to validate the FISH technique in imprint of biopsies from several materials suspected of having lymphoproliferative neoplasms.

Imprint slides from 17 tissue samples: lymph nodes ($n = 12$), muscle lesion, mediastinal mass, chest wall lesion, thyroid and lung ($n = 1$, each) with suspected lymphomas ($n = 15$) or myeloid sarcoma ($n = 2$) were performed by pathologists during excisional or core biopsy. Four to 5 imprint slides were prepared for each fragment, dried at room temperature and sent to the cytogenetic laboratory. One slide per case was stained with Rosenfeld dye and the others were stored at room temperature; FISH technique was performed within 90 days. The same probes already used for routine laboratory FISH in cell suspension were validated for this type of sample. Break apart *IGH* probe was performed in all cases and *CMYC*, *BCL2* and *BCL6* (break apart probes: Cytocell®, Cambridge, UK) were also used in two samples. Interphase FISH analysis was performed under a fluorescence microscope by two analysts with a total score of 100 nuclei, reference values previously established in bone marrow cell suspensions; *IGH*, *CMYC*, *BCL2*, *BCL6* rearrangements cut-offs were 8.5%, 11.9%, 4.6% and 6.0%, respectively.[5,8]

Hybridization was successful in all samples. Four of the six samples diagnosed with non- Hodgkin B cell lymphoma (NHL-B) presented *IGH* rearrangement, two also presented with *CMYC* and *BCL2* rearrangements (Diffuse B cell lymphoma double hit) (Figure 1). In one case of T cell NHL and one case of undifferentiated neoplasia, a gain in the *IGH* signal was observed. Two cases of Hodgkin Lymphoma, two thymomas, three reactive lymphoid proliferations and two myeloid sarcomas showed normal FISH IGH study (supplementary Table 1).

FISH in FFPE material is a useful technique, but dependent on pre-analytical factors and is limited by the smaller variety of commercial probes. This study validated the FISH technique in imprint of various tissues, showing to be easy to perform, score and interpret, being a quick and useful option for diagnostic purposes.

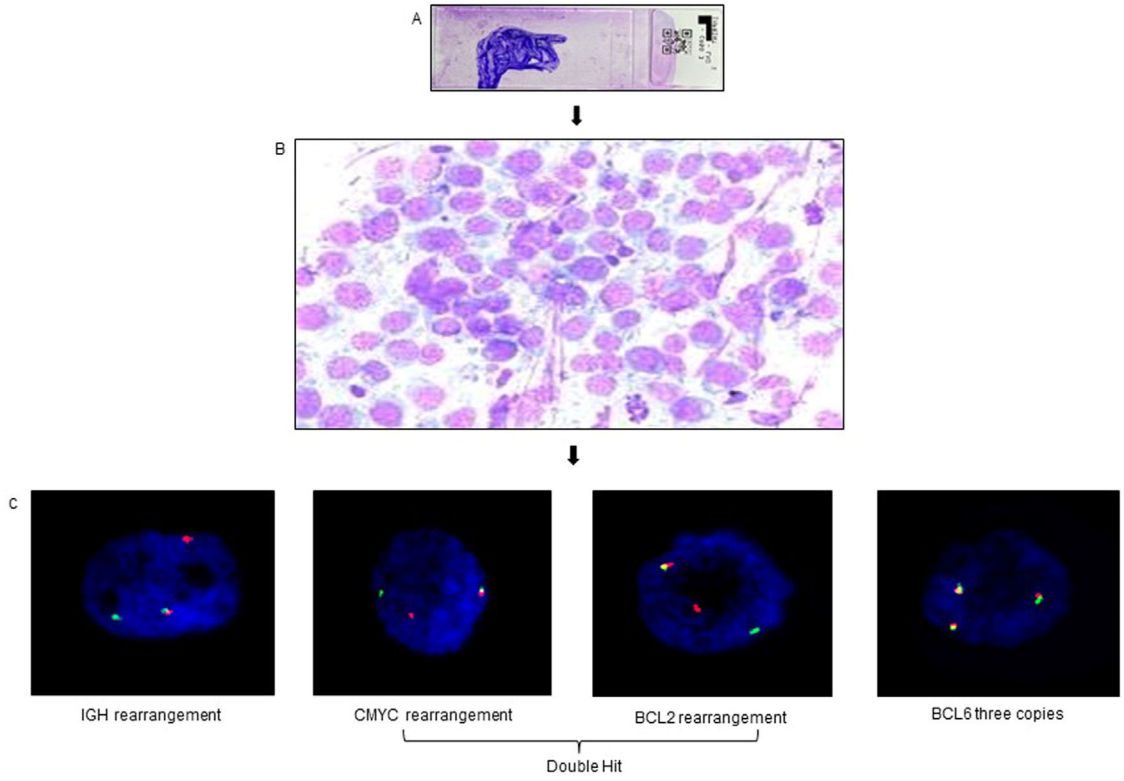

Figure 1 – Diffuse large B-cell lymphoma, double hit (lymph nodes biopsy). (A) Imprint slide stained with Rosenfeld. (B) Lymph node shows neoplastic lymphoid cell (Rosenfeld, ×500). (C) Interphase FISH analyses using probes dual fusion break apart showing IGH, CMYC and BCL2 rearrangements (one normal fusion signal and one red and one green signals) and three copies of BCL6 gene (three fusion signals).

Conflicts of interest

We have no conflict of interest to declare associated with this publication and as corresponding author, I confirm that the manuscript has been read and approved for submission by all the authors.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.htct.2023.01.008.

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