DETECTION OF EBV ANTIGENS BY UNCONVENTIONAL METHODS IN DLBCL FROM ARGENTINA DIAGNOSED AS EBV NEGATIVE.

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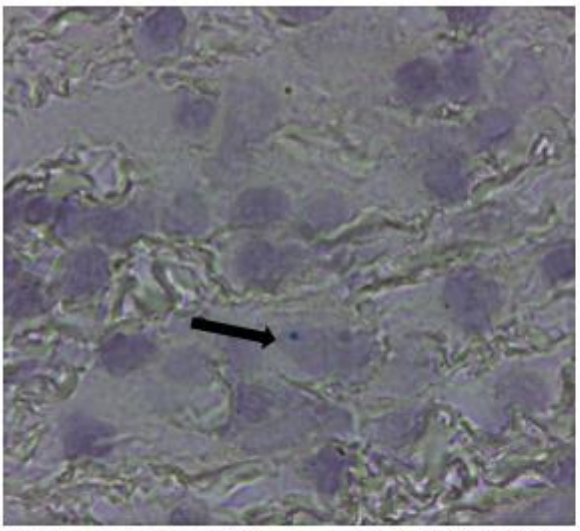
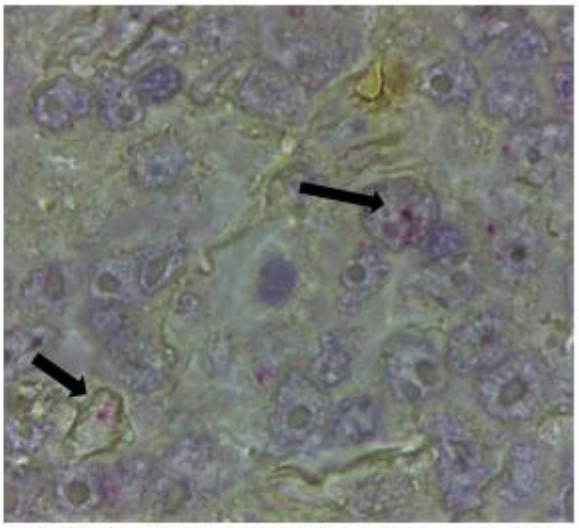
Introduction: Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma (NHL) representing approximately 30% to 40% of all newly diagnosed lymphomas. In 2017, the WHO confirmed a previous provisional entity, EBV+DLBCL, NOS [1]. The prevalence of EBV + DLBCL varies from 3 to 14%, depending on the geographic area. EBV-associated lymphomas show a differential expression pattern of latent genes with oncogenic abilities, such as LMP1 and EBNA2. The detection of traces of EBV was previously reported by sensitive methods [2], thus, the use of unconventional methods could be useful to describe cases originally considered as negative by conventional techniques. Therefore, the aim of this study was to detect viral LMP1 and EBNA2 transcripts with sensitive methods and compare the expression with EBERs in situ hybridization (ISH). Methods: 45 DLBCL cases (19 pediatric and 26 adult) were included in this study. The median age was 27.2 (range 2 to 73 years). EBERs ISH was performed in formalin fixed paraffin embedded (FFPE) DLBCL biopsies. In addition, LMP1 and EBNA2 RNAs were detected by double ISH with ViewRNA ISH Tissue 2-Plex Assay (Affymetrix). LMP1+, EBNA2+ and double LMP1+/EBNA2+ cells were counted in tumor cells and at the microenvironment, and expressed as +cells/mm2 and +cells%. Results: EBV was detected by EBERs ISH in 6.7% (3/45) cases, 1 pediatric and 2 adult cases. By double ViewRNA ISH, the expression of LMP1 and EBNA2 in single cells was observed in 1/3 EBERs+ cases (mean of 66.7 cells/mm2 and 1.86% cells for LMP1, and 11.1 cells/mm2 and 0.31% cells for EBNA2). Remarkably, LMP1 expression was also found at the microenvironment in a few cells at the microenvironment. In addition, within the 42 EBERs- cases, LMP1 expression was detected in 13/42 (31%) cases (mean 17.5 cells/mm2 and 0.42% cells), whereas EBNA2 expression was observed in 7% (3/42) cases (mean 1.6 cells/mm2 and 0.05% cells). In 10/13 and 2/13 cases LMP1 and EBNA2 were expressed in the microenvironment, respectively. Unexpectedly in 6 cases LPM1 expression was observed exclusively at the microenvironment. Conclusion: this study provides further evidence that EBV could be detected in tumor cells in DLBCL by unconventional methods, in a cohort from a population with high incidence of EBV infection in children, and that the virus could be implicated in DLBCL pathogenesis in more cases than originally considered.

1. Steven H. Swerdlow et al. 2016. The 2016 revision of The World Health Organization classification of lymphoide neoplams. Blood Journal.127:2375-2388.Doi: 10.1182/blood-2016- 01-643569
2. Mundo Lucia et al. 2020. Frequent traces of EBV infection in Hodgkin and non-Hodgkinlymphomas classified as EBV-negative by routine methods expanding the landscape of EBV-related lymphomas.Modern Pathology. 33: 2407-2421.https://doi.org/10.1038/s41379-020-0575-3



EBNA2 transcript in tumor cell by double ISH with ViewRNA ISH Tissue 2-Plex Assay (Affymetrix)



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