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# Lymph node hyperplasia: clonal chromosomal and genomic rearrangements. Report of two new cases and literature review

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Cytogenetic analysis is not routinely performed on lymph node hyperplasia (LH). We describe clonal chromosomal rearrangements in two unrelated cases of LH. Lymph nodes of both patients showed typical morphologic features of benign follicular hyperplasia. Cytogenetic analysis revealed clonal chromosomal rearrangements in both cases. Patient 1 showed interstitial 14q and 6q mosaic deletions, whereas patient 2 showed a terminal 14q mosaic deletion. Fluorescence in situ hybridization with *IGH* break-apart probes identified a partial deletion of *IGH* in both cases, but the loss of the LSI IGH in patient 2 and loss of the LSI *IGHV* in patient 1 were observed on the morphologically normal chromosome 14. In the latter case, the finding of two morphologically normal chromosomes 14 with the *IGHV* deletion in one of the chromosomes suggested that the first mutational event was the *IGH* deletion and the second event was the interstitial deletion of chromosome 14 with the *IGH* intact. Array comparative genomic hybridization performed on both biopsies confirmed the *IGH* deletion at mosaic, but not the chromosomal deletion. Patient 1 was re-biopsied after 9 months and a marginal zone lymphoma was diagnosed. The finding of clonal cytogenetic abnormalities in LH highlighted the difficulties in interpretation of results and clinical follow-up.

**Keywords** Array CGH, clonal chromosomal rearrangements, immunoglobulin heavy chain, lymph node hyperplasia, fluorescence in situ hybridization



Lymphoproliferative disorders are characterized by specific chromosomal rearrangements that could have diagnostic and prognostic significance and are an important contribution to the morphological and clinical evaluations.

Non-Hodgkin lymphomas show a large number of recurrent chromosomal rearrangements: t(14;18) in follicular lymphoma, t(8;14) in Burkitt lymphoma, t(11;14) in mantle cell lymphoma, and t(2;5) in anaplastic large cell lymphoma. Deletion of the long arm of chromosome 6 is observed in hematological malignancies, but is more frequently described in lymphoproliferative disorders. Rarely reported as the only cytogenetic abnormality, the 6q deletion is usually associated with other cytogenetic abnormalities, suggesting a role in disease progression. Deletion of band 6q21q23 is the most recurrent cytogenetic abnormality in B cell lymphoma. Taborelli et al. (1) found 6q deletions by fluorescence in situ hybridization (FISH) in 94.2% of malignant lymphomas (Hodgkin and non-Hodgkin) and suggested a correlation between chromosome band deletion and lymphoma subtypes, as previously reported by Offit et al. (2). Thelander et al. (3) confirmed the 6q deletion in lymphoma and restricted the common deleted region in 6q21 to 3 Mb, where three candidate genes are mapped (*FOXO3A*, *PRDM1*, and *HACE1*). On the contrary, lymph node hyperplasia (LH) is

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a benign lymphadenopathy of unexplained origin without morphological or immunophenotypic characteristics of malignancy. To our knowledge, in the last 10 years, only 3 reports (261 total cases) in the literature focused on the cytogenetic analysis of LH, but without a complete molecular characterization.

Here we describe two cases with histological diagnoses of reactive LH and clonal chromosomal rearrangements. The findings of clonal chromosomal rearrangements in LH, which have been linked to subsequent development of malignancy, should prompt a thorough morphologic, phenotypic, and clinical examination as well as a careful follow-up observation.

## Case reports

Patient 1 was a 64-year-old woman with an unremarkable previous personal history. After a persistent lymphoadenomegaly, she underwent a first biopsy of a left supraclavicular lymph node that was 2 cm in diameter. A diagnosis of reactive LH was rendered. Nine months later, a second biopsy on persistent lymph nodes was performed at the same site. At this time, a diagnosis of marginal zone lymphoma (MZL) was made on the basis of morphological and immunophenotypical results, which was confirmed by a clonal rearrangement of the immunoglobulin heavy chain gene by PCR and FISH analyses. Based on the results of histopathological and genetic reports, the patient underwent chemotherapy (6 cycles of the CHOP-cyclophosphamide, doxorubicin, vincristine, and prednisone-scheme) and has been in complete remission for 4 years.

Patient 2 was a 55-year-old man who developed a follicular lymphoma when he was 39. During a programmed checkup, a 1.5-cm laterocervical lymph node was removed. After 5 years, the patient is alive and well.

In both cases, lymph nodes showed typical morphologic and phenotypic features of benign follicular hyperplasia (Figure 1A). The germinal centers (GCs) were polymorphous in shape, with a follicular appearance, expansion of the cortical zone, and an attenuated but preserved mantle zone. The interfollicular zone was well evident. In particular, in patient 1, the paracortex displayed a moderate proliferation of the high endothelial venules. Peripheral sinuses were patent.

At high power, tingible body that contained macrophages were easily identified in the GC. The immunophenotypic study confirmed the topographic preservation of the lymph node cytoarchitecture.

The GCs demonstrated a reactive immunoprofile: CD10/Bcl6+; Bcl2 negative (Figure 1B,C). A homogeneous high proliferation rate was evident with the Ki67 antibody (data not shown). Occasional CD30-positive immunoblastic-like cells were noted mostly in perigerminal areas and, more rarely, inside the GC (Figure 1D).

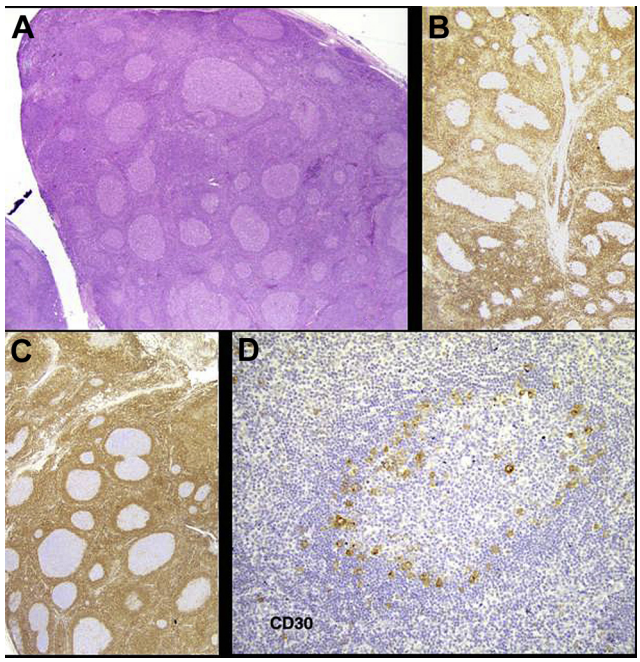


**Figure 1** (A) Lymph node histology after H&E stain (100x, patient 1 case); (B) immunohistochemical assay with BCL2 negative in germinal centers (100x, patient 1 case); (C) immunohistochemical assay with BCL2 antibody negative in germinal centers (100x, patient 2 case); (D) immunohistochemical assay with CD30 antibody (100x, patient 2 case) showing occasional CD30 positive immunoblastic-like cells mostly perigerminal areas.

## Materials and methods

### Cytogenetic analysis

Lymph node biopsies were mechanically disaggregated for cytogenetic analyses. Representative parts of the biopsies were fixed in buffered formalin and paraffin, and were routinely processed. Slides were stained with H&E. Immunohistochemical analysis was performed by a panel of the following primary antibodies: CD20, CD3, CD5, CD23, CD43, CD30, BCL2, BCL6, CD10, cyclin D1, and Ki67.

Following the protocol suggested by The AGT Cytogenetics Laboratory Manual (7), the cell suspensions were spun, and the pellets were washed with Hank's balanced solution and cultured in RPMI1640 medium supplemented with 20% fetal calf serum. The cell suspensions were subsequently processed after a 24-hour culture (37°C) and 30 minutes of colcemid (N-Methyl-N-deacetyl-cholchicine, Roche Diagnostics). The karyotypes were described following the International System of Cytogenetic Nomenclature 2013 (ISCN 2013).

### PCR analysis

PCR was performed for the immunoglobulin heavy chain (*IGH*) and T cell receptor rearrangement analyses (8,9).

### FISH analysis

FISH experiments were performed according to the manufacturer's protocol. FISH was performed on the cell suspension and/or on paraffin embedded tissue. The probes used were: 14q telomere (Cytocell Ltd., Cambridge, UK) (locus D14S1420, 200 kb from the telomere); LSI IGH dual-color break-apart (14q32.1) (LSI IGHV 900-kb spectrum