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BRIEF COMMUNICATION

Clonal karyotypic abnormalities associated with reactive lymphoid hyperplasia

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Cytogenetic abnormalities are important in the diagnosis and prognosis of hematolymphoid neoplasms. Although many recurrent karyotypic abnormalities are well-defined and known to underlie pathophysiologic processes contributing to malignancy, the significance of other cytogenetic changes is less clear. This uncertainty reflects an incomplete understanding of the frequency with which karyotypic abnormalities arise in benign processes. Numerous case reports and a small number of retrospective series have noted clonal cytogenetic changes in association with reactive-appearing lymph nodes. However, the incidence of such abnormalities has varied widely in published series. Here, we report the largest retrospective series of karyotypic abnormalities in association with reactive lymphoid hyperplasia published to date. Clonal karyotypic abnormalities were present in 6.3% of reactive lymph nodes with informative karyotypes and 5.1% of all reactive lymphoid tissues. These data suggest that karyotypic abnormalities are less frequently found in association with reactive lymphoid tissue than previously reported and provide a clearer picture of the baseline incidence of cytogenetic changes in benign lymphoid processes.

Keywords Reactive, lymphoid, benign, cytogenetics, karyotype

Cytogenetic abnormalities are frequently found in association with hematolymphoid neoplasms, serving as defining criteria in certain entities and informing prognoses in others. The importance of cytogenetic features is particularly emphasized in the evaluation of myeloid lesions (1). In addition, cytogenetic abnormalities are also an important adjunct in the evaluation of lymphoid neoplasms, including non-Hodgkin lymphomas (NHLs). For instance, balanced translocations involving *BCL2* and *MYC* are identified in approximately 90% of cases of low grade follicular lymphoma and Burkitt lymphoma, respectively (2–4). These rearrangements are both diagnostically and mechanistically relevant, as they juxtapose oncogenes with immunoglobulin loci promoters, driving the ectopic expression of anti-apoptotic and pro-mitotic proteins.

The interpretation of karyotypic abnormalities is complicated by the observation that cytogenetic abnormalities classically associated with NHL have been reported at low levels in presumably healthy individuals in the general population (5,6). Likewise, several case reports and small series have reported clonal karyotypic changes in morphologically reactive lymphoid tissue (7–11). Generally, these clonally expanded populations do not herald future malignancy but are instead clinically benign (9,11).

Three retrospective series have attempted to address the frequency of clonal karyotypic abnormalities associated with reactive lymphoid hyperplasia (RLH), but the incidence reported in these studies has varied from 12% to 45% (9–11). In general, estimates have trended downward with time. This trend may reflect the increasing sensitivity of ancillary tools, such as flow cytometry and immunohistochemistry, which facilitate the identification of smaller populations of malignant cells that were missed in earlier studies. The extent to which published data can be extrapolated to current practice is unclear, and the wide variability of published estimates has generated

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Table 1 Summary of cytogenetic results from cases of reactive lymphoid hyperplasia

	n	Cytogenetics performed	Uninformative	Abnormal cytogenetics	Normal cytogenetics
Lymph nodes	433	322 [74%]	69 {21%}	16 (6.3%)	237
Tonsils and adenoids	110	64 [58%]	10 {16%}	2 (3.7%)	52
Nontraumatic spleen	175	49 [28%]	11 {22%}	1 (2.6%)	37
Other sites	139	62 [45%]	36 {58%}	0	26
Total	857	497 [58%]	126 {25%}	19 (5.1%)	351

Summary of cytogenetic results from excisional biopsies diagnosed as RLH in the University of North Carolina Division of Hematopathology between 2004 and 2012. The values in brackets correspond to the percentage of cases in which G-banded karyotypic analysis was attempted. The values in braces correspond to the percentage of cases with uninformative karyotypes; "uninformative" indicates that no clonal cytogenetic abnormality and fewer than 10 interpretable metaphases were identified. The values in parentheses correspond to the percentage of cases with informative karyotypes that were found to have a clonal cytogenetic abnormality.

considerable uncertainty about the true incidence of karyotypic abnormalities in reactive lymphoid tissues.

At our institution, morphologic, flow cytometric, and routine karyotypic analyses are attempted on nearly all excisional biopsies submitted for lymphoma evaluation, provided that adequate tissue is available and that specimens are received fresh. Immunohistochemical studies are also performed when indicated. Due to this protocol, a large and relatively unbiased evaluation of cytogenetic changes in morphologically and immunophenotypically reactive lymphoid tissue is possible. As a result, we were able to retrospectively review cytogenetic changes associated with RLH. Herein, we report the largest such series reported to date and further revise downward the likely incidence of karyotypic abnormalities in morphologically reactive lymph nodes.

Materials and methods

Cytogenetic studies

Lymph node samples were set in culture as soon as possible after receipt. Cytogenetic results were typically generated from one 5–10 ml overnight, unstimulated suspension culture, except in rare cases when a large amount of tissue was received. Culture volume was adjusted for the size of the cell pellet. When the cell pellet was large, two 5 ml cultures were initiated. Prior to harvest, 100 μg of ethidium bromide in solution and 0.5 μg of colcemid in solution were added per 10 ml of culture for 90 minutes. The harvest was performed according to standard cytogenetic protocols using 0.075M KCl hypotonic solution and a single application of Carnoy's fixative. Pellets were stored at 4°C until slides were prepared for chromosome or FISH analyses.

Reactive lymphoid hyperplasia analysis

We reviewed all cases submitted between July 2004 and June 2012 to the University of North Carolina Department of Pathology for evaluation of possible lymphoid malignancy. Spleens removed for nontraumatic reasons were also included in our review, as routine karyotypes are frequently requested on these samples. Conversely, cases of splenectomy performed for traumatic injury were excluded from the evaluation. We identified 857 cases of benign lymphoid tissue. Samples were categorized by tissue of origin: lymph nodes, tonsils and adenoids, nontraumatic spleens, or all other sites.

Morphologic, immunohistochemical, flow cytometric, and clinical data from these cases were reviewed and correlated with cytogenetic findings from conventional Giemsa-banded (G-banded) karyotypes.

Follicular lymphoma analysis

As a quality assurance measure to evaluate the rate of detection and reporting of cytogenetic abnormalities at our institution, we identified all cases of low grade (World Health Organization [WHO] grades 1–2) follicular lymphoma evaluated by routine cytogenetics at the University of North Carolina Hospitals between July 2004 and June 2012. Results of G-banded karyotypes were correlated with the results of fluorescence in situ hybridization (FISH) performed with the IGH/BCL2 fusion and/or BCL2 break-apart probes (Abbott Molecular, Abbott Park, IL).

Results

Incidence of cytogenetic abnormalities in association with RLH

Between July 2004 and June 2012, 857 excisional biopsy specimens submitted to the University of North Carolina Division of Hematopathology were diagnosed as RLH. Approximately half of these samples were excisional lymph node biopsies (50.5%), with smaller numbers representing tonsils and adenoids (12.8%), nontraumatic spleens (20.4%), and all other sites (16.2%) (Table 1). G-banded karyotypic analysis was attempted on 58% of these specimens, including 74% of the lymph node samples (Table 1). The most common reasons for not attempting routine cytogenetics included inadequate tissue sample and formalin fixation prior to hematopathology evaluation.

A karyotypic analysis was considered to be informative either if a clonal cytogenetic abnormality was identified (defined as three metaphase spreads with loss of the same chromosome or two or more metaphase spreads with any other karyotypic abnormality) or if 10 or more normal metaphases were evaluated. Using these criteria, informative karyotypes were produced in 75% of samples undergoing G-banded karyotypic analysis. Lymph nodes, tonsils and adenoids, and spleens had a comparable failure