

## Histopathology and immunohistochemistry of lymph node biopsies: A prospective study from a tertiary care hospital in Kashmir

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### Abstract

**Objective:** To determine the histopathological spectrum of lymphadenopathy by evaluation of the biopsy specimen and relevant immunohistochemistry of accessible lymph nodes.

**Materials and Methods:** A total of 196 cases of lymph node biopsies were analysed. En bloc lymph node dissection in known cases of primary malignancy or associated with evidence of primaries elsewhere in the body are excluded from the study.

**Results:** A total of 196 cases of lymph node biopsies analysed included 102(52.04%) males and 94(47.96%) females. The mean age for males was  $35.2 \pm 19.9$  years and that for females was  $36.4 \pm 19.7$  years. Maximum numbers of cases were seen in the age group 11-30 years (76 cases, 38.76%). The most common site for nodal biopsy was cervical (60.2%), followed by axillary (18.9%), and inguinal (7.1%). Cervical region was the commonest site (15.72% males and 14.9% females) of biopsy in the age group 11-20 years. Benign lesions (52.1%), were more common than Malignancies (47.9%). The most common benign diagnosis was Reactive LAP in 77(39.3%), followed by tubercular lymphadenitis accounting for 24 cases (12.2%).

Among the malignancies, lymphomas were predominating accounting for 85 (43.3%) cases. Among the lymphomas, non-Hodgkin lymphomas (NHLs) were more common accounting for 63 (32.2%) than the Hodgkin's disease (HD) which constituted 22 (11.2%) of lymphadenopathies with mixed cellularity as the commonest form. Metastases from unknown primary were seen in 9 (4.6%) cases of palpable enlarged peripheral nodes. Among the patients with reactive LAP, the commonest age group involved was 21-30 years in males and 11-20 years in females, while that for NHL (DLBCL) it was 41-50 years and 51-60 years respectively. Tuberculosis was most commonly seen in the age group of 21-30 years in both sexes. Hodgkin's disease was common in age group >61 years and metastasis in 31-40 years age group.

**Conclusion:** In our study among the biopsied nodes, lymphomas were the most common (43.3%) followed by reactive LAP (39.3%), tuberculous lymphadenitis (12.2%) and metastasis (4.6%). Lymph node biopsy is gold standard investigation in establishing the cause of lymphadenopathy. Though lymphomas can be confidently diagnosed on morphology, application of monoclonal antibodies and identification of immunophenotypic profile has enhanced diagnostic accuracy and has prognostic implications.

### Introduction

Lymph nodes (LNs) are group of specialized cells that represent a division of the defence system in the human body. The body has approximately 600 LNs, and their locations are scattered around ports of entry as well as the exit of major vessels.<sup>1</sup> Lymphadenopathy can be defined as LNs that are abnormal in size, consistency or number. Fortunately, the majority of patients presenting with peripheral LAP have easily identifiable causes that are benign or self-limiting.<sup>2</sup> Localized LAP is more common and it has been reported to affect nearly 75 percent of patients presenting with LAP, whereas 25 percent of those patients had generalized LAP.<sup>3</sup> The causes of lymphadenopathy include infections, neoplasms, lymphoproliferative diseases, connective tissue

disorders, immunologic disorders, endocrine diseases and other miscellaneous conditions.<sup>4</sup>

Reaching an accurate diagnosis for a patient presenting with LAP needs methodological approach guided by educated evidence-based evaluation including a careful history and physical examination. Where the cause and course of LAP is not obvious, further assessment is necessary.<sup>4</sup> Palpable lymph nodes offer an important diagnostic clue to the aetiology of the underlying condition. For assessment of lymphadenopathy, different modalities are used which include fine needle aspiration cytology (FNAC), automatic core needle biopsy, flow cytometry, radiological guided core needle biopsy and open biopsy.<sup>5,6</sup> Though fine needle aspiration cytology is commonly used to establish the

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etiologically diagnosis, excision biopsy of the lymph node remains the “gold standard” for diagnosis.<sup>7-10</sup> Ideally, the most accessible node is selected for biopsy. Although less commonly used because of the advent of new immunohistochemical analytic techniques that have increased the sensitivity and specificity of FNA, excisional biopsy with immunohistochemistry of lymph nodes remains the diagnostic procedure of choice in delivery of timely and appropriate medical care to patients presenting with LAP.<sup>4</sup>

This study aims to determine the histopathological spectrum of lymphadenopathy by evaluation of the biopsy specimen and relevant immunohistochemistry of accessible lymph nodes.

## Materials and Methods

All cases of lymph node biopsies received at Department of Pathology, Government Medical College Srinagar, J &K, India, from Aug 2013 – July 2015 were analysed. En bloc lymph node dissection in known cases of primary malignancy or associated with evidence of primaries elsewhere in the body are excluded from the study.

## Method

Clinic demographic data was obtained. The tissue was processed as per standard procedure. 4-5 micron sections were cut on microtome and stained by Haematoxylin and eosin stain and special stain like AFB, Reticulin, PAS with and without diastase were done where required. Immunohistochemistry (IHC) was performed using relevant antibodies according to the histomorphological features. The panel of monoclonal antibodies routinely used included CD45, CD20, CD3, CD15 and CD30. In specific cases antibodies against epithelial membrane antigen (EMA), and cytokeratin were used. All lymphoma cases will be classified according to standard World Health Organization classification of hemato-lymphoid malignancies. All the cases diagnosed as Hodgkin’s Lymphoma morphologically were further subjected for immunophenotyping using CD15 and CD30 to study immunoreactivity of Reed-Sternberg cells. Likewise all cases of NHL that were divided into B or T cell type employing pan B cell marker CD20 and pan T cell marker CD3 respectively. Immunohistochemical studies were carried out using 5-micron paraffin sections. The

sections were stained for CD3/CD 20/CD5/CD15/CD30/ /CYTOKERATIN using DAKO LSAB-2® system HRP.

## Results

A total of 196 cases of lymph node biopsies were analysed. Table 1 depicts the age and sex distribution of patients. Mean age of males was  $35.248 \pm 19.9451$  years, and that of females  $36.470 \pm 19.7346$  years, (P value = 0.668).

Site of nodal biopsy in males (n=102) included cervical in 59.8%, axillary in 18.6%, inguinal in 8.8%, sub-mandibular in 5.9%, epitrochlear in 3.9%, mesenteric in 2% and supraclavicular in 1% of the cases. [Table 2].

Site of nodal biopsy in females (n=94) included cervical in 60.6%, axillary in 19.1%, inguinal in 5.3%, supraclavicular in 5.3%, mesenteric in 4.3%, sub-mandibular in 3.2%, and submental in 2.1% of the cases. [Table 3]

The most common site of biopsy was cervical (60.2%) followed by axillary (18.9%) and inguinal nodes (7.1%).

Table 4 and Table 5 depicts histopathology and age relation in males and females respectively.

In the present study benign lesions constituted a total of 102 (52.1%) cases, while malignancies comprised 94 (47.9%) of enlarged peripheral lymph nodes. Among the malignancies, lymphomas predominated accounting for 85 (43.3%) of cases. Non-Hodgkin lymphomas (NHLs) were more common accounting for 63 (32.1%) cases than the Hodgkin’s disease (HD) which constituted 22 (11.2%) cases of lymphadenopathies with mixed cellularity as the commonest form (Table 6).

Metastases constituted the remaining malignancies representing 9 (4.59%) cases of palpably enlarged peripheral nodes. These included metastasis from SCC in two, poorly differentiated carcinoma in five, papillary carcinoma thyroid in one, and adeno-carcinoma in one of the patients.

On immunohistochemistry, all the cases of NHL were positive for CD20 except two cases who had inconclusive IHC results. In the patients with Hodgkin’s disease, CD15 and CD30 positivity was noted. Cases of NPLHL were positive for CD20.

**Table 1:** Age and sex distribution of patients

Age Group (Years)	Male	Female	Total
0-10	12 (11.8%)	4 (4.3%)	16 (8.16%)
11-20	18 (17.6%)	21 (22.3%)	39 (19.88%)
21-30	18 (17.6%)	19 (20.2%)	37 (18.88%)
31-40	15 (14.7%)	20 (21.3%)	35 (17.86%)
41-50	14 (13.7%)	8 (8.5%)	22 (11.22%)
51-60	15 (14.7%)	11 (11.7%)	26 (13.27%)
61 or More	10 (9.8%)	11 (11.7%)	21 (10.71%)
<b>Total</b>	<b>102 (52.04%)</b>	<b>94 (47.96%)</b>	<b>196 (100%)</b>

**Table 2:** Age wise distribution of site of biopsy in males

Age Group	Site of Biopsy (n= 102)						
	Axillary	Cervical	Epitrochlear	Inguinal	Mesenteric	Submandibular	Supraclavicular
0-10	1 (1%)	10 (9.8%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)
11-20	2 (2%)	16 (15.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

21-30	0 (0%)	12 (11.8%)	2 (2%)	4 (3.9%)	0 (0%)	0 (0%)	0 (0%)
31-40	6 (5.9%)	6 (5.9%)	2 (2%)	0 (0%)	1 (1%)	0 (0%)	0 (0%)
41-50	0 (0%)	9 (8.8%)	0 (0%)	3 (2.9%)	0 (0%)	2 (2%)	0 (0%)
51-60	5 (4.9%)	6 (5.9%)	0 (0%)	2 (2%)	0 (0%)	2 (2%)	0 (0%)
61 or More	5 (4.9%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	2 (2%)	1 (1%)
<b>Total</b>	<b>19 (18.6%)</b>	<b>61 (59.8%)</b>	<b>4 (3.9%)</b>	<b>9 (8.8%)</b>	<b>2 (2%)</b>	<b>6 (5.9%)</b>	<b>1 (1%)</b>

P value < 0.0001

**Table 3:** Age wise distribution of site of biopsy in females

Age Group	Site of Biopsy (n = 94)						
	Axillary	Cervical	Inguinal	Mesenteric	Submandibular	Submental	Supraclavicular
0-10	1 (1.1%)	3 (3.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
11-20	1 (1.1%)	14 (14.9%)	2 (2.1%)	3 (3.2%)	0 (0%)	1 (1.1%)	0 (0%)
21-30	3 (3.2%)	11 (11.7%)	2 (2.1%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	0 (0%)
31-40	5 (5.3%)	11 (11.7%)	0 (0%)	0 (0%)	2 (2.1%)	0 (0%)	2 (2.1%)
41-50	3 (3.2%)	2 (2.1%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)
51-60	1 (1.1%)	10 (10.6%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
61 or More	4 (4.3%)	6 (6.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.1%)
<b>Total</b>	<b>18 (19.1%)</b>	<b>57 (60.6%)</b>	<b>5 (5.3%)</b>	<b>4 (4.3%)</b>	<b>3 (3.2%)</b>	<b>2 (2.1%)</b>	<b>5 (5.3%)</b>

P value < 0.0001

**Table 4:** Histopathology and Age relation in Males

Microscopy (n=102)	Age Group (Years)							Total (n=102)
	0-10	11-20	21-30	31-40	41-50	51-60	61 or More	
Nonspecific reactive LAP	2 (2%)	8 (7.8%)	10 (9.8%)	1 (1%)	1 (1%)	0 (0%)	0 (0%)	<b>22 (21.5%)</b>
Reactive follicular Hyperplasia	1 (1%)	2 (2%)	4 (3.9%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	<b>9 (8.8%)</b>
Sinus Histiocytosis	2 (2%)	3 (2.9%)	0 (0%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)	<b>7 (6.9%)</b>
Dermatopathic Lymphadenitis	0(0%)	0(0%)	0(0%)	1(1%)	0(0%)	0(0%)	0(0%)	<b>1(1%)</b>
Granulomatous Lymphadenitis	1 (1%)	0 (0%)	3 (2.9%)	2 (2%)	0 (0%)	1 (1%)	0 (0%)	<b>7 (6.9%)</b>
Castleman's Disease	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	<b>1 (1%)</b>
NHL (SLL)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	5 (4.9%)	1 (1%)	<b>7 (6.9%)</b>
NHL (DLBCL)	0 (0%)	5 (4.9%)	0 (0%)	3 (2.9%)	8 (7.8%)	7 (6.9%)	7 (6.9%)	<b>30 (29.4%)</b>
Hodgkin's Lymphoma	6 (5.9%)	0 (0%)	0 (0%)	5 (4.9%)	0 (0%)	0 (0%)	0 (0%)	<b>11 (10.8%)</b>
NLPHL	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (2%)	0 (0%)	0 (0%)	<b>2 (2%)</b>
Metastasis	0 (0%)	0 (0%)	1 (1%)	0 (0%)	2 (2%)	1 (1%)	1 (1%)	<b>5 (4.9%)</b>

P value < 0.0001

**Table 5:** Histopathology and Age relation in females

Microscopy (n=94)	Age Group (Years)							Total (n=94)
	0-10	11-20	21-30	31-40	41-50	51-60	61 or More	
Nonspecific reactive LAP	3 (3.2%)	10 (10.6%)	7 (7.4%)	4 (4.3%)	1 (1.1%)	2 (2.1%)	1 (1.1%)	<b>28 (29.8%)</b>
Reactive follicular Hyperplasia	0 (0%)	4 (4.3%)	0 (0%)	2 (2.1%)	0 (0%)	0 (0%)	0 (0%)	<b>6 (6.4%)</b>
Sinus Histiocytosis	0 (0%)	0 (0%)	1 (1.1%)	2 (2.1%)	0 (0%)	0 (0%)	0 (0%)	<b>3 (3.2%)</b>
RosaiDorfmann	0(0%)	1(1.1%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	<b>1(1.1%)</b>
Granulomatous Lymphadenitis	1 (1.1%)	1 (1.1%)	10(10.6%)	1 (1.1%)	1 (1.1%)	1 (1.1%)	2 (2.1%)	<b>17 (18.1%)</b>
NHL (SLL)	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)	1 (1.1%)	0 (0%)	0 (0%)	<b>3 (3.2%)</b>
NHL (DLBCL)	0 (0%)	1 (1.1%)	0 (0%)	7 (7.4%)	3 (3.2%)	8 (8.5%)	2 (2.1%)	<b>21 (22.3%)</b>
NHL (Burkitt's)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<b>1 (1.1%)</b>
NHL (Follicular)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1(1.1%)	0 (0%)	<b>1(1.1%)</b>
Hodgkin's Disease	0 (0%)	0 (0%)	0 (0%)	2 (2.1%)	0 (0%)	0 (0%)	6 (6.4%)	<b>8 (8.5%)</b>
NLPHL	0 (0%)	0 (0%)	1 (1.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	<b>1 (1.1%)</b>
Metastasis	0 (0%)	1 (1.1%)	0 (0%)	2 (2.1%)	1 (1.1%)	0 (0%)	0 (0%)	<b>4 (4.3%)</b>

P value < 0.0001

**Table 6:** Histopathology of malignant lesions

Microscopy	Female	Male	Total (n=196)
NHL (SLL)	3	7	<b>10 (5.1%)</b>
NHL (DLBCL)	21	24	<b>45 (22.9%)</b>
Immunoblastic DLBCL	0	6	<b>6 (3.06%)</b>
NHL (Burkitt's)	1	0	<b>1 (0.51%)</b>
NHL(Follicular)	1	0	<b>1(0.51%)</b>
Hodgkin's Disease (Mixed Cellularity)	7	10	<b>17(8.67%)</b>
Hodgkin's Disease (Lymphocyte Rich)	1	0	<b>1(0.51%)</b>
Hodgkin's Disease (Nodular Sclerosis)	1	0	<b>1(0.51%)</b>
NLPHL	1	2	<b>3 (1.53%)</b>
Metastasis	4	5	<b>9 (4.59%)</b>
<b>Total</b>	<b>39(41.48%)</b>	<b>55(58.51%)</b>	<b>94 (47.9%)</b>

## Discussion

Palpable lymph nodes offer an important diagnostic clue to the etiology of the underlying condition. Though fine needle aspiration cytology is commonly used to establish the etiological diagnosis, excision biopsy of the lymph node remains the "gold standard" for diagnosis.<sup>7-10</sup>

Adeniji KA et al,<sup>9</sup> Adesuwa N et al<sup>11</sup> and Roy A et al<sup>12</sup> in their studies reported a male: female ratio of 1.6:1, 1.3:1 and 1.7:1 respectively indicating male preponderance, whereas, Mbata GC et al<sup>13</sup> reported a male: female ratio of 1:1.3 showing female preponderance. In the present study, male: female ratio is almost equal (1.08:1) which is in discordance with most of the above studies.

In the present study, age ranged between 14 months to 80 years. Maximum number of cases were seen in the age group 11-30 years (76 cases, 38.76%).

Roy A et al<sup>12</sup> also reported the age range between 1-87 years and most cases were seen in the age group of 11-30 years (354 cases, 35%). The least number of cases were seen in the age group above 70 years (30 cases, 3%) which was in discordance with our study, where the least number of cases (16 cases, 8.16%) were in the age group of 0-10 years.

The most common site for lymph node biopsy in our study was cervical (60.2%) followed by axillary nodes. Similar findings have been reported by number of different studies.<sup>11,13,14</sup> The preponderance of cervical lymphadenopathy may be related to its location near a common primary site of infections and malignancy that are drained through this single channel (the cervical lymph nodes).<sup>15</sup>

In the present study, benign lesions constituted a total of 102 (52.1%) cases. The most common benign diagnosis on histopathology was reactive LAP in 77(39.3%) patients. Among the latter, majority, i.e.25.5% (50 cases) were non-specific reactive lymphadenitis. Malignancies comprised 94 (47.9%) of cases, with lymphomas predominating accounting for 85 (43.3%) cases. Among the lymphomas, non-hodgkin's lymphomas (NHLs) were more common accounting for 63 (32.2%) cases of lymphadenopathies. Hodgkin's disease (HD) constituted 22 (11.2%) with mixed cellularity as the commonest form. Metastases constituted the remaining malignancies representing 9 (4.6%) cases of palpable enlarged peripheral nodes.

Generally speaking lymphadenopathy is common in the first three decades of life; with reactive lymphadenopathy more common in the early years of life, TB common in the young adult and malignancy seen more in the adult and elderly people. Analysis of lymphadenopathy in the clinical practice in the developing nations of the world has shown that infection remains an important cause with TB as the most common aetiology in most areas. Nonspecific causes (reactive hyperplasia) and upper respiratory tract infections due to bacterial and viral agents are also important cause of lymphadenopathy in the developing world, while malignancy and reactive hyperplasia are more commonly implicated in the developed world.<sup>13</sup>

Lymphomas were the most common cause of malignancies in our study accounting for 43.4% of lymphadenopathy. This is lower than the findings in the studies by Ali K Ageep et al<sup>15</sup> (16.6%), O Ochicha et al<sup>14</sup> (24%), and Mbata GC et al<sup>13</sup> (17.1%). In our study non Hodgkin's lymphoma is higher (32.2%) than Hodgkin's (11.2%). Most other studies by Roy A et al<sup>12</sup>, Ali K Ageep et al,<sup>15</sup> O Ochicha et al,<sup>14</sup> and Mbata GC et al<sup>13</sup> also gave a higher preponderance of non-Hodgkin's lymphoma. Also in the western world non Hodgkin's lymphoma is reported to be three to four times more common than Hodgkin's disease.<sup>16,17</sup>

In the present study, out of 196 patients nonspecific cause (reactive hyperplasia) was found in 77 (39.3%) making it the second commonest etiologic factor after lymphomas (43.4%) in our study. Granulomatous lymphadenitis which is the most common etiologic factor in many studies done within our environment was found to be the third etiologic factor in our study with 24(12.2%) cases. The percentage of granulomatous lymphadenitis found here was also smaller than that found in some series including those by Roy A et al,<sup>12</sup> Ali K Ageep et al,<sup>15</sup> O Ochicha et al,<sup>14</sup> and Mbata GC et al.<sup>13</sup> Higher prevalence has been quoted in Nigeria, India, Pakistan and Bangladesh.<sup>13</sup> Reactive lymphadenopathy has been seen more commonly reactive in children and has been related to a reaction to minor stimuli because of the yet developing immune system.<sup>10,14</sup> In the United States of America reactive hyperplasia is more common cause of lymphadenopathy. The lower prevalence of tuberculosis and earlier detection of malignancies before the onset of nodal metastasis may be the explanation for the prominence of

reactive hyperplasia as a more common cause in the western world. Lymph node hyperplasia was also found to be common in studies done in India, South Africa and Zimbabwe.<sup>10</sup> The hyperplasia appears to be a consequence of variety of pathological processes; an important factor being that of HIV infection. The trend of changes in primary HIV lymphadenopathy has been reported to range from mild follicular hyperplasia through diffuse follicular hyperplasia to "burnt out lymph node."<sup>18</sup>

In the present study (n=196), malignancies comprised 94 (47.9%) of enlarged peripheral lymph nodes, with lymphomas predominating accounting for 85 (43.3%) cases. Metastases constituted the remaining malignancies representing only 9 (4.6%) cases of palpably enlarged peripheral nodes. Number of studies revealed metastasis as the most common cause (19.1 - 43%) for malignant enlargement of the examined nodes.<sup>12-15</sup>

The incidence of HL is about three per 100 000 in Western Europe and the United States, consistently lower than that of NHL and has remained stable over the last 25 years. Lower incidence rates have been reported for Asia, especially Japan and China, suggesting genetic resistance to disease development, possibly associated with human leukocyte antigen type, as well as environmental influences in the aetiology of HL. Hodgkin's disease can occur in both children and adults. It is more common, however, in two age groups: early adulthood (age 15–40, usually 25–30) and late adulthood (after age 55). About 10% of cases are diagnosed in young boys before 15 years of age but the disease is very rare before 5 years of age.

The overall incidence of NHL has steadily risen in most developed areas of the World. Some of the rise may be related to acquired immunodeficiency syndrome (AIDS) and some may be the results of better diagnosis but the causes of this long-term increase are largely unknown, though, age-related immunodeficiency is likely involved. In our study, although NHL was the most common malignant lesion noticed, we did not have any case related to AIDS. In the early 2000s, NHL in Western countries has become in general the sixth most common cancer in males and the fifth most common cancer in females. It represents the third most common neoplasm group in children (after ALL and central nervous system tumors). The incidence of NHL is usually slightly higher in men than in women and this difference is more marked in younger than older individuals. The most common histological subtype, diffuse large B-cell lymphoma (DLBCL), accounts for about 40% of NHL and in our study it represented even higher (71.4%) cases of NHL. It occurs more frequently among males than females at middle age (as noticed in our study) and among whites than blacks at older ages. Distribution of the most common subtypes of NHL (diffuse large B cell and follicular) appears to differ by geographic region, suggesting differences in etiologic or host factors. The difference is particularly striking for follicular NHL, which is most common in North America and Western Europe, and for Burkitt's lymphoma, which is endemic in equatorial Africa, but constitutes only 1%–2% of lymphomas in the United States and Western Europe. In Western

countries, NHL is more commonly of B-cell origin; a higher frequency of T-cell diseases is seen in the Far East. In the majority of NHL patients the disease arises in lymph nodes, but primary extra nodal disease accounts for 30% of new lymphoma patients and often present as localized disease. The most frequent primary extra nodal sites are the stomach, small intestine, skin, and brain. Incidence rates increased 3.0%– 6.9% per year for extra nodal cases, compared to 1.7%–2.5% per year for nodal cases.<sup>19</sup>

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