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Institutional Experience of Lymphoproliferative Disorders with Initial Diagnosis Made via Fine Needle Aspiration at Otolaryngology Clinic

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Abstract

Background. This study characterizes lymphomas presenting as palpable head and neck masses and evaluates the role of fine needle aspiration (FNA) and flow cytometry (FC) in diagnosis. **Design.** A 5-year retrospective review of FNAs performed by pathologists in an ENT clinic identified cases with a predominant lymphoid population that lacked an epithelial component. Cytology, FC, and subsequent surgical pathology diagnoses were correlated. **Results.** Of 821 FNAs, 154 (19%) met selection criteria. Reactive lymph nodes accounted for 43% (67/154), with most diagnosed as 'negative for malignancy,' except one 'atypical' (ATY) case. Lymphoma was confirmed in 57% (87/154) of cases, categorized as ATY (55%), suspicious for lymphoma (SFM) (36%), or positive for lymphoma (PFM) (9%). Lymphoma patients were older (median 66 vs. 46 years). Thyroid and salivary gland lymphomas typically indicated systemic involvement, except for two cases of marginal zone lymphoma (MZL) in patients with Sjögren syndrome. FC alone had a sensitivity of 67.5% for detecting lymphoma, but when combined with cytology, the sensitivity increased to 100%. The combined approach maintained a specificity of 98%. More abnormal clonal cells were identified by FC in PFM cases compared to SFM or ATY cases (P=0.004). Cytologic atypia with negative FC occurred in 29% of lymphomas, including Hodgkin and diffuse large B-cell lymphoma (DLBCL). **Conclusion.** Lymphomas presenting as neck masses are diverse, with FNA playing a key diagnostic role. Cytologic atypia and FC complement each other, as some cases show minimal atypia but positive FC, while others show significant atypia with negative FC.

Key Words: Lymphoproliferative ▪ FNA ▪ Otolaryngology.

Introduction

Fine needle aspiration (FNA) represents a cornerstone in diagnostic pathology, valued for its reliability, minimal invasiveness, cost-effectiveness and adaptability across diverse clinical settings. In an otolaryngology outpatient clinic, patients presenting with neck masses often undergo this procedure as an initial step in the diagnostic workup. FNA samples are suitable for morphologic evaluation as well as ancillary studies such as immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), molecular analysis and flow cytometry (FC), which are especially important for diagnosing and subtyping lymphoma (1). Lymphomas comprise 12% of all malignant tumors in the head and neck, ranking as the third most common malignancy after squamous cell carcinoma (46%) and thyroid carcinoma (33%) (2-4).

Hence, a meticulous evaluation for lymphoma is imperative in patients presenting with a new mass in the head and neck region. Within the context of salivary gland lesions, the diagnostic spectrum for lymphoid cell-rich FNAs extends beyond lymphoma to include a range of non-neoplastic conditions such as lymphoepithelial cyst, reactive lymph nodes and chronic sialadenitis, as well as lymphoid-rich neoplasms such as Warthin tumor (5). Despite the frequent occurrence of lymphomas in the head and neck region, the existing literature offers limited insights into their initial

presentation as palpable masses in patients seeking care at an otolaryngology clinic.

Therefore, our study endeavors to fill this knowledge gap by sharing our institutional experience of patients who presented to our outpatient otolaryngology clinic with a primary concern of mass or swelling and underwent FNA revealing a lymphoid lesion. Through the analysis of these cases, we provide a comprehensive characterization of the frequency and types of lymphoma encountered in this clinical context. Furthermore, our investigation highlights the pivotal roles played by FNA and FC in achieving accurate and precise diagnoses of lymphoproliferative disorders.

Method

A five-year retrospective search was conducted within our institutional database to identify eligible cases. Cytopathology reports from patients who underwent pathologist performed FNA of a neck mass (including cervical lymph nodes, thyroid and salivary sites) at our institution's Ear, Nose, and Throat (ENT) clinic were reviewed to identify cases which contained a lymphoid component; cases which also contained an epithelial component were excluded in order to capture pure lymphoid lesions. If applicable, FC results and subsequent surgical pathology diagnoses were recorded.

The FNA procedure adhered to established institutional protocols and was guided by palpation for superficial masses or ultrasound for deep masses. Rapid on-site assessment using air-dried, Diff-Quik-stained smears was performed by the performing pathologist at ENT clinic, and additional needle passes were taken until adequate diagnostic material was obtained. Alcohol-fixed smears were also prepared for subsequent staining with Papanicolaou stain. Needles were rinsed in separate vials containing formalin and RPMI medium, for cell block preparation and flow cytometry analysis, respectively.

For flow cytometry, fresh tissue samples underwent analysis within 24 hours using a 6-color flow cytometry approach. Neoplastic cells were initially identified through a CD45/forward scatter gating strategy, with abnormal B-cells further characterized by the expression of pan B-cell antigens and monotypic immunoglobulin light chain expression. Stained cells were acquired using a benchtop flow cytometer (FACS Canto, Becton Dickinson) and analyzed with Kaluza software (Beckman Coulter, Fullerton, CA), with fluorescence intensity measured on a logarithmic scale ranging from 100 to 104.

Ethics Statement

This study was approved by the Institutional Review Board of Thomas Jefferson University (iRISID-2023-2047).

Statistical Analysis

Data pertaining to clinical presentation, FNA cytology results, FC findings, and final surgical excision outcomes, if applicable, were collected and organized using Microsoft® Excel® for Microsoft 365 MSO (Version 2403). Descriptive analysis and one-way analysis of variance (ANOVA) were conducted using IBM SPSS Statistics 28.0 to assess the relationship between cytology diagnosis categories and the proportion of the clonal population. A chi-square test of independence was conducted to assess the association between the performance of IHC and the categorization of cytology diagnoses (atypical, positive for lymphoma, and suspicious for lymphoma).

Results

In our 5-year search of 821 cases of pathologist-performed FNA at ENT clinic, 155 (19%) fulfilled our selection criteria. Among these, 43% (67/155) were samples from benign/reactive lymph nodes; one case was characterized as "atypical cytology" (ATY) and the remainder received a "negative for malignancy" (NFM) cytologic diagnosis. The remaining 55% (86/155) of cases represented sampled lesions that were ultimately proven to be lymphoma; these were categorized on FNA as

ATY (54%, 47/87), "suspicious for malignancy" (SFM) (36%,31/86), or "positive for malignancy" (PFM) (9%,8/86). Notably, 1% (2/154) of cases exhibited marked lymphoid proliferation on cytology but were later identified as nasopharyngeal carcinoma and SMARCA4 (BRG1)-deficient high-grade tumors upon surgical resection.

The median age among patients with reactive lymph nodes was 46 years (interquartile range: 33-57), with a male-to-female ratio of 1.4:1. In contrast, lymphoma patients exhibited a median age of 66 years (interquartile range: 51-73), with a male-to-female ratio of 0.7:1. Notably, 88% (76/86) of lymphoma cases presented with a *de novo* mass, lacking any prior history of lymphoma, and two patients had a history of laryngeal squamous cell carcinoma.

FC analysis was conducted on samples from 80/86 (93%) lymphoma cases and 51/67 (76%) reactive cases. In comparative terms, the PFM category exhibited a notably higher proportion of abnormal clonal cells on FC analysis compared to both the SFM and ATY categories, as shown by ANOVA analysis ($F(2, 48) = 6.340$, $P=0.004$). Specifically, the ATY category had a mean proportion of 0.22 (SD=0.18), the PFM category had a mean proportion of 0.53 (SD=0.22), and the SFM category had a mean proportion of 0.27 (SD=0.19). However, no statistically significant difference was observed between the SFM and ATY categories ($P=0.972$, Bonferroni correction applied, with alpha adjusted to 0.017 to uphold significance levels).

When performed, IHC is typically ordered upon receipt of the cell block, with results generally available concurrently with the flow cytometry findings. The final cytologic diagnosis is rendered by integrating the cytomorphology with the results from both flow cytometry and IHC. In cases of reactive masses, predominantly lymph nodes, IHC was performed in 11 out of 67 cases (16.4%). The IHC panel for these cases primarily included lymphoid markers (CD3 and CD20), but occasionally, non-lymphoid markers (cytokeratin, TTF-1, and thyroglobulin) were performed to exclude the possibility of occult metastatic carcinoma, particularly in patients with a prior cancer history or current evidence of a mass in another anatomic location. For lymphoma cases, IHC was performed in 32 out of 87 cases (36.8%). The IHC panel for these cases typically included lymphoid markers (CD3, CD20, CD30, CD10, CD23, BCL1) and cytokeratin, especially when large atypical cells were observed in the FNA sample.

IHC was performed in 14 out of 48 ATY cases (29.2%), 9 out of 31 SFM cases (29.0%), and 5 out of 8 PFM cases (62.5%). A chi-square test of independence was conducted to evaluate the association between the performance of IHC ("performed" vs. "not performed") and the categorization of these cytology diagnoses, which revealed no statistically significant association between the two variables (Pearson Chi-Square = 3.087, df = 2, $P=0.214$). In cases of lymphoma, patients typically presented with a unilateral mass, and the duration of enlargement extended over a span of weeks to up to one year. Longer duration was notably more frequent in indolent types of lymphoma, aligning with expectations. Cervical lymphadenopathy emerged as the predominant site of lymphoma manifestation within the head and neck region, accounting for 62% of cases (53/86). Other notable sites of involvement included the parotid gland (25%), submandibular region (6%), thyroid (5%), nasal cavity (1%) and submental region (1%).

In addition to a mass lesion, 7% of patients complained of dysphagia. Remarkably, two patients presented with orbital masses concomitant with a parotid mass, with one experiencing diplopia and the other reporting painless gradual vision decline over a few days. A minority of cases, comprising only 14%, exhibited B symptoms such as weight loss, fever, night sweats or chills.

Surgical resection was performed in 73/86 (85%) lymphoma cases, facilitating histologic diagnosis, as depicted in Figure 1. Non-Hodgkin lymphoma (NHL) constituted 78% (57/73), of cases, followed by Hodgkin lymphoma (HL) at 21% (15/73). Additionally, one case of chronic myeloid leukemia (CML) involving a lymph node was observed. HL represented 25% (13/53) of lymphomas involving cervical lymph nodes, while it

occurred much less commonly in extranodal head and neck sites (5% (2/34) cases affecting the parotid and thyroid glands). Marginal zone lymphoma (MZL) was the predominant type observed in the parotid gland, with two cases identified as primary lymphomas confined to the gland and associated with Sjögren syndrome. All other lymphomas in the head and neck region were associated with systemic disease.

There were 5 cases of T-cell lymphoma, including two cases of Anaplastic Large Cell Lymphoma, ALK-negative (AN-ALCL) and one case each of angioimmunoblastic T-cell lymphoma, follicular helper T-cell lymphoma and peripheral T-cell lymphoma, not otherwise specified (NOS). For these cases, abnormal FC results were observed in all but one case, which was AN-ALCL.

In terms of diagnostic performance, the sensitivity of FC alone in identifying lymphoma stood at 67.5%, a figure that rose to 100% when combined with cytomorphologic diagnosis. Meanwhile, FC alone demonstrated a specificity of 98%, which remained consistent when combined with cytomorphology results. The positive predictive value (PPV) was 98% and the negative predictive value (NPV) was 66%. The combined sensitivity, defined as the sum of SFM cases plus PFM cases divided by biopsy-proven lymphomas, was 45% across all lymphoma subtypes. When stratified by lymphoma subtype, the combined sensitivity was 55% for non-Hodgkin B-cell lymphomas and 36% for other lymphomas (primarily HL and T-cell lymphoma). It is noteworthy that 25 cases exhibited significant cytologic atypia but concurrently showed negative FC results. These cases encompassed various lymphoma subtypes, including HL (N=14), diffuse large B-cell lymphoma (DLBCL) (N=4), large B-cell NHL (N=3), MZL (N=1), AN-ALCL (N=1) and mycosis fungoides (N=1). Examples of these lymphoma cases with false-negative FC results are illustrated in Figure 2.

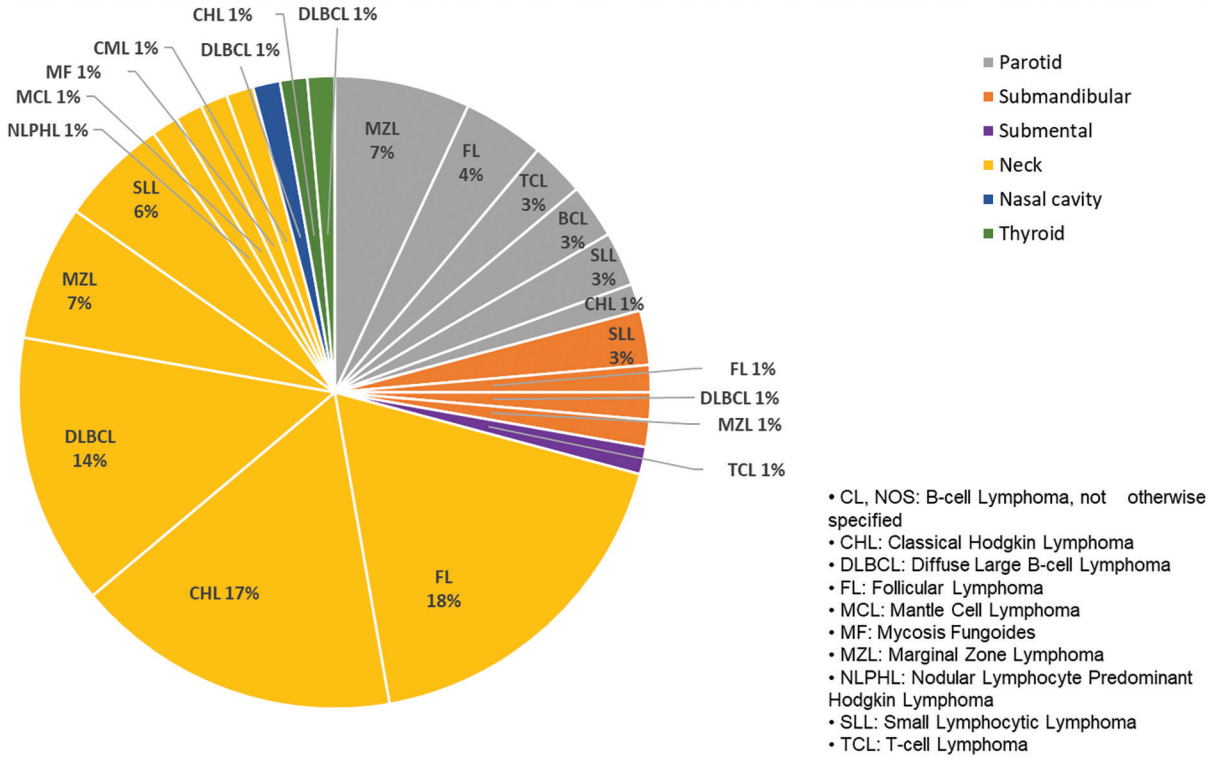

Figure 1. Final histopathologic classification of lymphomas sampled by FNA.

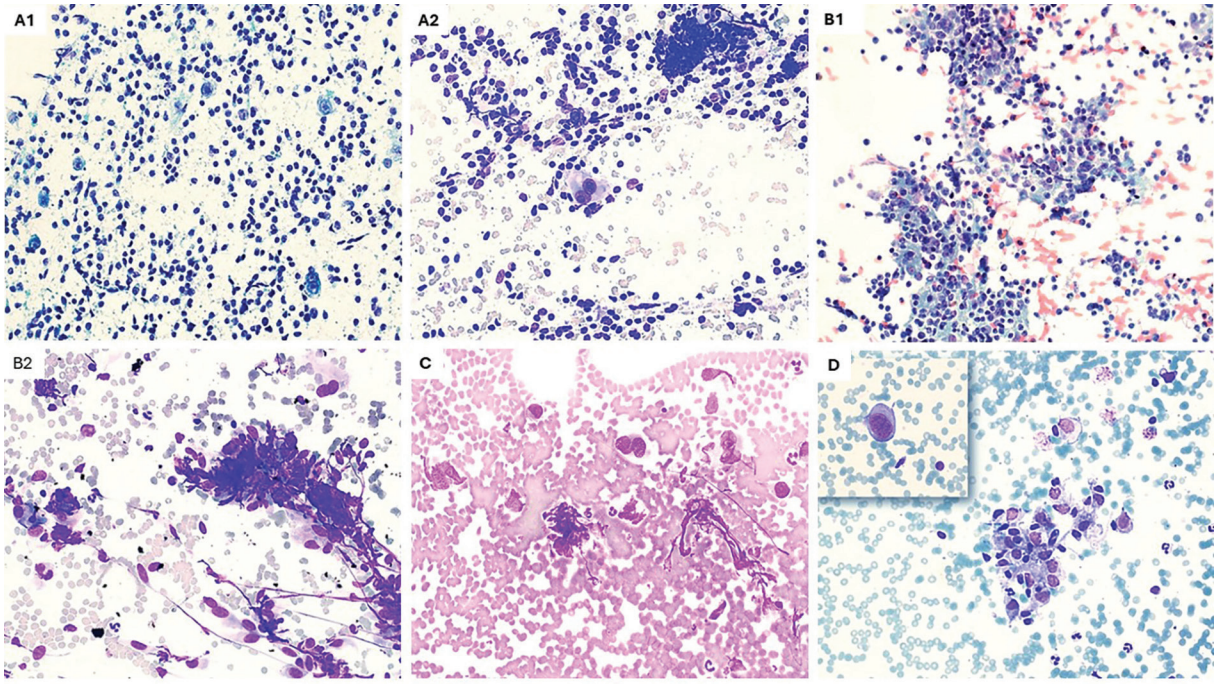

Figure 2. Examples of lymphomas exhibiting significant cytologic atypia but negative flow cytometry results. A. Classical Hodgkin lymphoma (A1. Pap stain, 400×; A2. Diff-quik, 400×). B. Diffuse large B-cell lymphoma (B1. Pap stain, 400×; B2. Diff-quik, 400×). C. Mycosis fungoides (Diff-quik, 400×). D. Nodular lymphocyte-predominant Hodgkin lymphoma [inset: large atypical lymphoid cell] (Diff-quik, 400×).

Discussion

Our study selected for patients who presented with a unilateral mass or swelling, recognized as one of the most common presenting symptoms for head and neck lymphoma (7). Notably, two patients had a previous history of laryngeal squamous cell carcinoma, which initially raised the possibility of metastasis. However, the subsequent diagnosis of lymphoma in these cases underscores the importance of keeping lymphoma on the list of differential diagnoses, regardless of previous history, and collecting additional material for ancillary study purposes.

Only 14% of our patient cohort exhibited B symptoms. This finding is consistent with existing literature indicating that only a small percentage of head and neck lymphoma patients present with constitutional or specific B symptoms (2, 6). Therefore, physicians should remain vigilant to the possibility of lymphoma when encountering a patient with a unilateral neck mass, even in the absence of B symptoms. The majority of lymphoma cases in our study were NHL, which is in line with previous findings (8). Notably, HL represented 25% of all lymphomas in lymph nodes. However, its occurrence in extranodal sites within the head and neck was relatively rare. This trend corresponds with existing literature, which indicates that HL predominantly affects lymph nodes, with only a small proportion involving extranodal areas. Conversely, NHL is known to more commonly involve extranodal sites (2, 9-11).

In our study, MZL emerged as the predominant type of extranodal NHL in the head and neck region. This finding contrasts with existing literature, which typically identifies DLBCL as the most common type (2, 12-15). However, our observation is consistent with the fact that MZL of the mucosa-associated lymphoid tissue (MALT) is the most frequent NHL affecting the parotid gland (16), which harbors the majority of extranodal lymphomas in our study. Notably, two of these cases were primary lymphomas confined

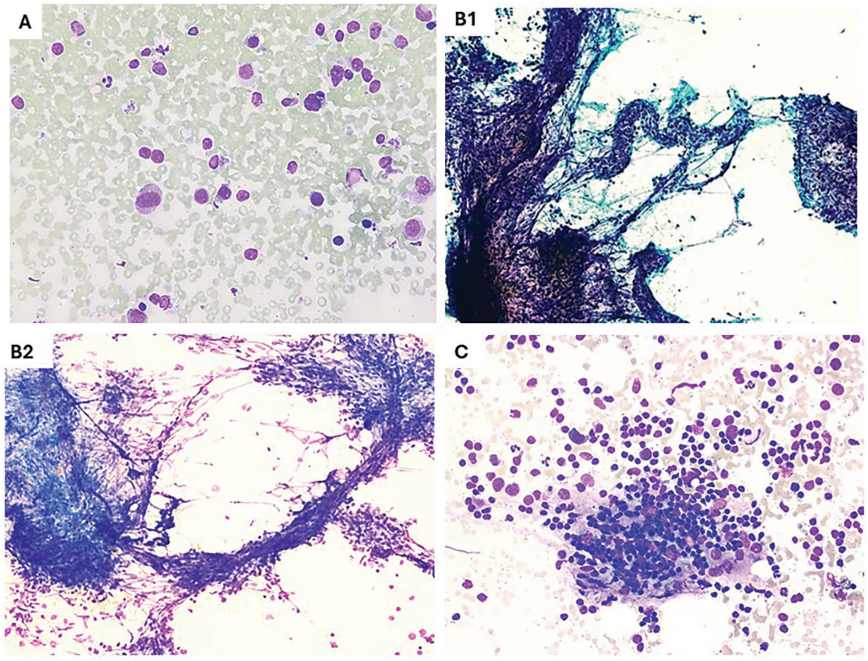

Figure 3. T-cell lymphoma examples. A: Diff-quik stained smear revealing anaplastic large T-cell lymphoma with prominent large atypical lymphoid cells (400×); B: Angioimmunoblastic T-cell lymphoma displaying atypical lymphoid tissue aggregates containing a scaffold of arborizing small vessels (B1. Pap stain, 400×; B2. Diff-quik, 400×); C: Peripheral T-cell lymphoma, not otherwise specified, showing a mixture of small, medium, and large lymphoid cells on a Diff-quik stained slide (400×).

to the parotid gland and associated with Sjögren syndrome. It is well recognized that patients with Sjögren syndrome have a significantly increased risk of lymphoma, approximately 40 times the relative risk compared to the general population (17).

FNA demonstrates variable diagnostic accuracy contingent upon the specific type of neoplasm, exhibiting higher efficacy for recurrent disease and certain aggressive primary lymphomas (18, 19). Conversely, its diagnostic accuracy for T-cell lymphomas tends to be lower. However, in our study all cases of T-cell lymphoma were successfully identified based on cytomorphology and FC and IHC results. FC analysis detected abnormal populations in all cases except for one instance of AN-ALCL, where atypia was observed via cytomorphologic evaluation (Figure 3).

Reactive follicular hyperplasia is a known diagnostic pitfall in cytopathology, as germinal center cells may be easily misinterpreted as atypical lymphoid cells (20-22). In our study, we encountered two cases of reactive follicular hyperplasia mimicking lymphoma based on limited cytology interpretation. In the first case (Figure 4A), initial suspicion of a lymphoproliferative disorder arose due to an abundance of large lymphocytes and IHC showing a predominance of CD20 over CD3-positive cells. However, final resection revealed reactive follicular hyperplasia. Similarly, in the second case (Figure 4B), FC suggested a large B-cell lymphoma based on an abnormal CD10+ B-cell population with no light chain expression. Yet, final resection unveiled a benign lymph node with follicular hyperplasia. The anomalous cellular population that lacked surface kappa or lambda expression was retrospectively identified as immunoblasts, which are commonly seen in viral infections (23), during which intense immune responses may lead to reduced or absent expression of immunoglobulin light chains (24). Upon correlating final histopathologic diagnoses with initial cytologic diagnoses, additional potential pitfalls

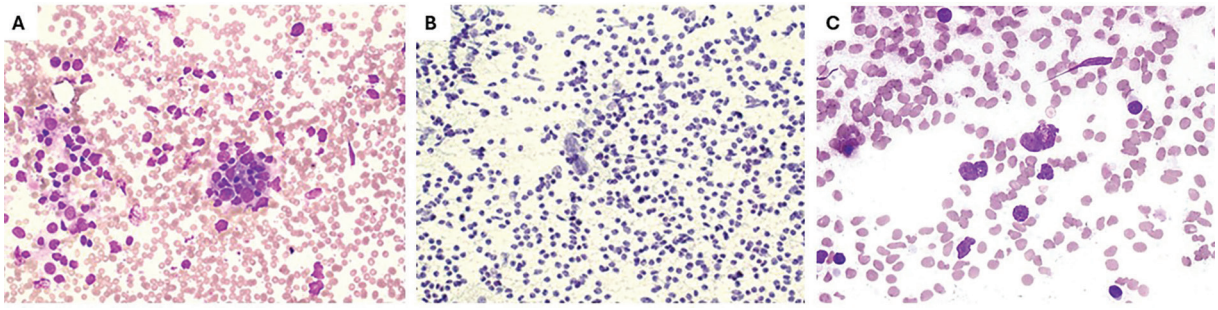

Figure 4. Entities that mimic lymphoma. A: Large atypical lymphocytes (400×); B: Abnormal B-cell population with absent light chain expression. Both cases exhibited follicular hyperplasia upon surgical excision (400×); C: Diff-quik stained smear displaying two large binucleated cells with stripped cytoplasm, resembling Reed-Sternberg (RS) cells, in a mixed inflammatory background, ultimately diagnosed as nasopharyngeal carcinoma (600×).

emerge. In one case (Figure 4C), a submandibular lymph node FNA revealed polymorphous lymphocytic population alongside rare benign salivary gland elements.

Although rare large, atypical lymphocytes resembling a Reed-Sternberg cell were identified on one smear, FC analysis showed no abnormal population, which is not unexpected given the possibility of HL. Surprisingly, the final diagnosis revealed metastatic nasopharyngeal carcinoma. The undifferentiated type of nasopharyngeal carcinoma is a well-known mimic of lymphoma, alongside malignant small round cell tumors and poorly differentiated squamous cell carcinomas (25). This case underscores the necessity of comprehensive evaluation, clinical correlation, and utilization of ancillary testing, to ensure accurate diagnosis and appropriate management. Additionally, it highlights the potential benefit of performing core needle biopsy (CNB) in addition to FNA. CNB is not typically conducted at our ENT clinic as part of the primary workup, which could be a limitation at times. At our institution, the standard approach involves performing FNA to obtain material for preparing Diff-Quik stained slides, Papanicolaou-stained slides and cell blocks. Additionally, if rapid onsite assessment reveals a lymphocyte-rich lesion, an attempt is made to collect additional material for FC analysis. This comprehensive approach has proven valuable in diagnosing and even subtyping lymphomas in certain cases.

Our analysis indicates a positive correlation between the degree of suspicion and the proportion of the clonal cell population as detected by FC. Specifically, the PFM category displayed a significantly higher proportion of abnormal clonal cells on FC analysis compared to both the SFM and ATY categories. However, no statistically significant difference was observed between the SFM and ATY categories. It remains unclear whether this observation may be related to a potential bias where cytopathologists may upgrade the diagnostic category if the percentage of clonal cells is high upon review of the concurrent FC results. Alternatively, it could suggest that a higher percentage of clonal population manifests as greater quantity and/or quality of atypia on cytomorphologic evaluation.

In lymphoma cases, IHC is particularly valuable when FC results are normal, providing critical insights when cytomorphology shows mild atypia. For instance, IHC can reveal a predominance of CD20-positive B cells or CD3-positive T cells in cases of B cell lymphoma or T cell lymphoma, respectively. CD30 is especially useful in cases where FC is negative but large, atypical cells suggest HL. Additionally, cytokeratin is often utilized to rule out metastatic carcinoma when large atypical cells are observed.

Although IHC is more frequently performed in cases ultimately categorized as “positive for lymphoma” (PFM) compared to other diagnoses, this difference was not statistically significant, indicating no association between the use of IHC and the final cytology categorization (P=0.214). This indicates that IHC does not appear to play a decisive

role in upgrading a diagnosis to PFM or SFM. Our review suggests that cases receiving these upgraded diagnoses are primarily those with significant cytologic atypia combined with positive FC results. The high incidence of ATY diagnoses is likely due to cases with positive flow results but only mild cytologic atypia. Additionally, it is important to recognize that the assessment of atypia in cytomorphology can be subjective and heavily reliant on the cytopathologist's level of expertise, which contributes to the higher rates of atypical diagnoses. IHC is more frequently ordered when FC results are negative, yet there is a presence of cytological atypia (particularly an abundance of intermediate-sized lymphocytes) or a significant clinical history that prompts the pathologist to rule out occult metastatic carcinoma. This suggests that, in reactive cases, IHC is used as an additional diagnostic tool when cytomorphological features alone are insufficient to rule out a malignant process.

To ensure standardized and replicable cytologic diagnoses of lymphoma in lymph nodes, the Sydney System offers a structured framework comprising five categories. It aims to provide essential diagnostic information and, when possible, identifies specific benign or malignant entities through ancillary testing (26). Conversely, Chong et al. proposed a stepwise approach primarily focused on morphological features, without placing significant emphasis on ancillary studies (25). In our investigation, among lesions ultimately diagnosed as lymphoma, the majority (57%; 87/154) were cytologically categorized as ATY (55%), SFM (36%) and PFM (9%). Diagnosis in most cases relied on a combination of cytomorphology, IHC on cell block, and FC results. Although ancillary studies are suggested to refine atypical and suspicious cases into definite categories (benign or malignant) (27), our cohort still observed a notable proportion of such cases. The discordance observed may stem from multiple factors, such as variability in cytopathologist experience with diagnosing lymphoid lesions. Additionally, the higher prevalence of ATY cases could be linked, at least in part, to the outcomes of concurrent FC. Specifically, in our study, 37% of cases labeled as ATY yielded normal results, 4% were deemed non-contributory due to insufficient lymphocytes, and FC was not performed in 10% of cases.

Among the lymphoma cases, 74 out of 87 underwent surgical resection, allowing for histologic diagnosis. For the remaining patients, clinical information regarding whether they underwent surgical resection with histologic diagnosis was unavailable in their electronic medical records. FNA rendered specific diagnosis in only two patients, identifying chronic lymphocytic leukemia (CLL) and DLBCL based on cytomorphology, IHC and FC. All the remaining lymphoma diagnoses were made after surgical excisional biopsy. This highlights the role of surgical biopsy as the "gold standard" for diagnosis owing to the larger quantity of tissue obtained, with preserved architectural features and increased overall diagnostic sensitivity (28-30). Based on the available evidence, there is consensus that the moderate to large benefits of employing excisional biopsies outweigh the moderate to trivial potential harms associated with using a more invasive procedure than FNA or CNB (31). However, CNB and FNA with ancillary studies can be a successful substitute for excisional biopsy in cases where excisional biopsy is not feasible (1, 32-34).

The sensitivity of FC alone in identifying lymphoma in our study was 67.5%. However, when combined with cytomorphologic evaluation, sensitivity increased to 100% in our study, which is higher than what has been reported in other studies (32, 35, 36). The overall combined sensitivity, defined as the proportion of cases with either positive or suspicious cytology among the biopsy-confirmed lymphoma cases (37), was 45%. When stratified by subtype, the combined sensitivity was 55% for non-Hodgkin B-cell lymphomas and 36% for other lymphomas, primarily HL and T-cell lymphoma. This observed difference may be influenced by the role of FC, which is often available at the time of cytology sign-out at our institution. For B-cell lymphomas, FC frequently detects clonal proliferation, providing critical evidence that supports upgrading the diagnosis from ATY to SFM

or PFM. In contrast, non-B-cell lymphomas, particularly HL, typically yield negative FC results. As a result, when cytology does not reveal a significant number of atypical cells, the diagnosis is more likely to remain classified as ATY. It is noteworthy that evaluating the performance of FNA cytomorphology alone, without the aid of flow cytometry, proved challenging in our study, as turnaround time for FC at our institution is 1-2 days, providing cytologists with concurrent FC results by the time of slide review.

In our study, 26 cases exhibited significant cytomorphologic atypia (characterized by large cell size, hyperchromatic nuclei, and irregular nuclear contours) despite concurrent negative FC results. The majority of these cases were HL (54%; 14/26) and large B-cell lymphoma (27%; 7/26). This finding is consistent with the notion in the literature that large cells are often underrepresented in FC analysis due to their low viability. Consequently, a significant proportion of large B-cell lymphomas may yield false-negative or nondiagnostic FC results (38, 39). The low sensitivity of FC can also be attributed to limitations associated with the FNA procedure itself and the histologic characteristics of lymphoma. The adequacy of diagnostic material may be influenced by the number of needle passes during the procedure, the size of the needle used, and the experience level of the aspirator (40). Additionally, features intrinsic to the lesion itself, such as sclerosis, necrosis, obscuring inflammation, or partial involvement of the lymph (41) node, may reduce the number of lesional cells available for both cytomorphologic assessment and FC analysis (29, 41, 42). To address these challenges, strategies such as careful morphological assessment and the use of IHC on cell blocks are crucial to avoid downgrading a diagnosis based solely on negative FC results.

In our study, all cases of HL were identified through cytomorphology, with each case being diagnosed at least as ATY. This contrasts with findings from other studies where HL was identified in less than half of the cases (43, 44). Notably, all HL cases in our sample yielded negative results on FC, which often struggles to detect neoplastic Hodgkin and Reed-Sternberg (HRS) cells in lymph nodes (41). This challenge has been attributed to cell lysis during preparations or cell acquisition (45). However, one study examined 53 cases of classical Hodgkin lymphoma (CHL) defined morphologically and found that HRS cells, often forming T-cell-HRS-cell rosettes, could be identified by FC with a sensitivity of 88.7% and specificity of 100% (46). Another study investigated the FC immunophenotype of T cells infiltrating HL for diagnostic assistance. The findings revealed an elevated CD4:CD8 ratio and increased CD7 expression in CD4(+) T cells, distinguishing HL from reactive lymphadenopathy. Using a CD7 mean fluorescence intensity (MFI) cutoff value from the data, this approach achieved a sensitivity of 69% and specificity of 90% for diagnosing classic HL (47).

Limitations of Study

One limitation of our study stems from our specific study design, which focused on identifying lymphocyte-rich FNA samples and then distinguishing between lymphoma and reactive cases. Consequently, we did not include "non-diagnostic" specimens that were ultimately diagnosed as lymphoma, potentially resulting in an overestimation of sensitivity for FNA performance in our analysis. The lack of CNB utilization in our practice is another potential limitation. Its adoption could have reduced the dependence on excisional biopsy and provided subtype-specific diagnoses of lymphoma in a significant number of cases, as evidenced by a median rate of 74% in a systematic review (34).

Conclusion

Our study highlights the diverse range of lymphomas that can initially present a palpable mass in the head and neck region and thus be amenable to FNA sampling. Recognizing cytologic atypia in lymphoid cells, combined with FC analysis, is crucial for early diagnosis and treatment, particularly in cases with minimal cytologic atypia but positive FC (e.g., SLL) and cases with significant cytologic atypia but negative FC (e.g., HL).

What Is Already Known on This Topic:

Fine needle aspiration (FNA) is widely recognized as a reliable, minimally invasive, and cost-effective diagnostic tool, especially for evaluating palpable masses in the head and neck region. FNA, in conjunction with flow cytometry (FC), plays a critical role in the diagnosis and subtyping of lymphomas, which account for approximately 12% of all malignant head and neck tumors. Lymphomas in this region are often secondary to systemic involvement, and while histologic examination remains the gold standard, FNA is frequently used in otolaryngology clinics as a first-line diagnostic method. Existing literature primarily focuses on the diagnostic utility of FNA in lymphomas, but the initial presentation of lymphoma as a palpable mass in patients seeking care at otolaryngology clinics remains underreported. This gap underscores the importance of institutional studies that evaluate the effectiveness of FNA and FC in diagnosing lymphoproliferative disorders in this clinical setting.

What This Study Adds:

This study provides a comprehensive evaluation of lymphomas presenting as palpable neck masses in an otolaryngology clinic. It highlights the diagnostic utility of combining FNA with flow cytometry, showing that their combined use significantly increases diagnostic sensitivity (100%) for lymphoma, with high specificity (98%). The study also emphasizes the diversity of lymphomas diagnosed, and the importance of recognizing cases where cytologic atypia may be present despite negative flow cytometry, especially in Hodgkin lymphoma.

Authors' Contributions: Conception and design: SMG and ASH; Acquisition, analysis and interpretation of data: ASH, JG and SMG; Drafting the article: ASH, JG and SMG; Revising it critically for important intellectual content: ASH, JG and SMG; Approved final version of the manuscript: ASH, JG and SMG.

Conflict of Interest: The authors declare that they have no conflict of interest.

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