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Mini Review

Introduction of the WHO Reporting System for Lymph Node Cytopathology

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Abstract

Lymph node fine needle aspiration biopsy (FNAB) is a useful diagnostic tool in the initial evaluation of lymphadenopathy of unknown etiology. The World Health Organization (WHO) Reporting System for lymph node cytopathology comprises five categories: insufficient/inadequate/nondiagnostic, benign, atypical, suspicious for malignancy, and malignant. This review focuses on the diagnostic criteria for each category, including cytomorphology, ancillary studies, differential diagnosis, and associated risk of malignancy. Its primary goal is to standardize the reporting and interpretation of lymph node samples, minimizing interobserver variability among pathologists. By establishing clear guidelines and standardized terminology, this system improves communication between pathologists and clinicians, leading to enhanced consistency, accuracy, and patient care in lymph node specimen evaluation. The WHO Reporting System serves as a unified and reproducible framework for the precise categorization of lymph node aspirates, enabling better communication between cytopathologists and clinicians and ultimately facilitating more effective patient management.

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Introduction

Lymph node fine needle aspiration biopsy (FNAB) is widely used in current daily practice in the initial evaluation of lymphadenopathy, particularly in lymph nodes that are difficult to access with larger biopsy needles. The advantages of FNAB are rapid turnaround time, low cost, ability to easily perform morphological assessment and triage, and ease of providing cells for immunophenotyping and molecular tests with less morbidity. Cytology specimens are known to be particularly advantageous in providing intact well-preserved cells for FISH, flow cytometry, and other biomarker studies.[1] The quality of DNA and RNA may even surpass that derived from cells obtained by extraction from formalin-fixed, paraffin-embedded tissue biopsies.

Despite the advantages of FNAB in lymphoproliferative lesions, there was historically no formal classification system dedicated to lymph node cytopathology to improve standardization until a categorical system for performance, classification, and reporting of lymph node cytopathology was proposed at the 20th International Congress of Cytology held in Sydney in 2019.[2] Recently, the WHO, the International Agency for Research on Cancer, and the International Academy of Cytology have joined forces to create a series of International Reporting Systems for Cytology, including lymph node cytology by an expert editorial board of cytopathologists. The WHO Reporting System for lymph node cytology has established five categories based on clinical, radiological, and key cytopathological features with ancillary studies for optimal diagnosis. This reporting system may lead to a greater acceptance and utilization of FNAB, a better interdisciplinary understanding of the results, and eventually benefit patients.

The WHO reporting system

Insufficient/Inadequate/Nondiagnostic

Diagnostic criteria

- No material for assessment (Fig. 1);
- Technical problems, which prevent assessment and diagnosis of the material on the slides.

Differential diagnosis and potential pitfalls

- Currently, there are limited publications defining the criteria for adequacy on lymph nodes. This category includes cases that cannot permit a reliable interpretation due to qualitative and/or quantitative reasons, such as scant cellularity, extensive necrosis, or technical limitations that cannot be overcome such as air-drying artefacts related to fixation and/or obscuring material and poor-quality smearing. Repeat FNAB or core needle biopsy (CNB) or excision biopsy should be requested based

Keywords: Lymph node; Cytology; Fine needle aspiration; Reporting system.
Abbreviations: CNB, core needle biopsy; FC, flow cytometry; FNAB, fine needle aspiration biopsy; IHC, Immunohistochemical stains; NHL, non-Hodgkin lymphoma.; ROM, risk of malignancy; ROSE, rapid on-site evaluation; WHO, the world health organization.
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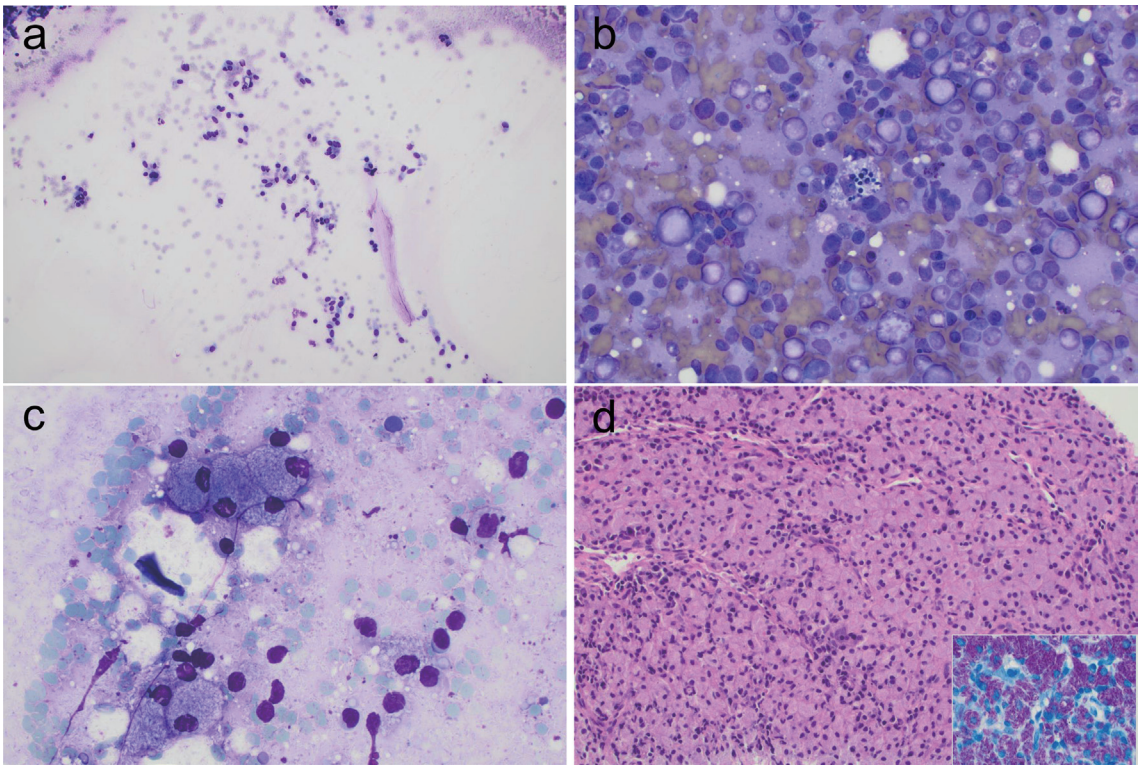

Fig. 1. Non-diagnostic (a). The fine-needle aspiration of the subcarinal lymph node shows abundant bronchial epithelial cells only (DQ, 100×). Benign (b–d). b. Polymorphous lymphocytes and tingible body macrophages of a reactive axillary lymph node (DQ, 100×). c–d. Foamy histiocytes with abundant intra-/extracellular acid-fast bacilli and lymphocytes of a cervical lymph node with Mycobacterium avium complex infection (c. DQ, 400×; d. Needle core biopsy: H&E, 200×; insert, acid-fast stain, 500×).

on the specific clinical context, particularly with rapid on-site evaluation (ROSE) (Fig. 2). Some studies defined an adequate specimen to have lymphocytes beyond what would be expected in normal blood.[3] The amount of at least 40 lymphocytes per high-power field in the most cellular areas of air-dried DQ smear was recommended in some of the literature to be adequate. A rare study considered the presence of clusters of anthracotic pigment-laden macrophages on EBUS-FNA of mediastinal lymph nodes as indicators of adequacy.[4] However, most studies focused on EBUS-FNA of mediastinal lymph nodes suggested that the presence of histocytes alone does not

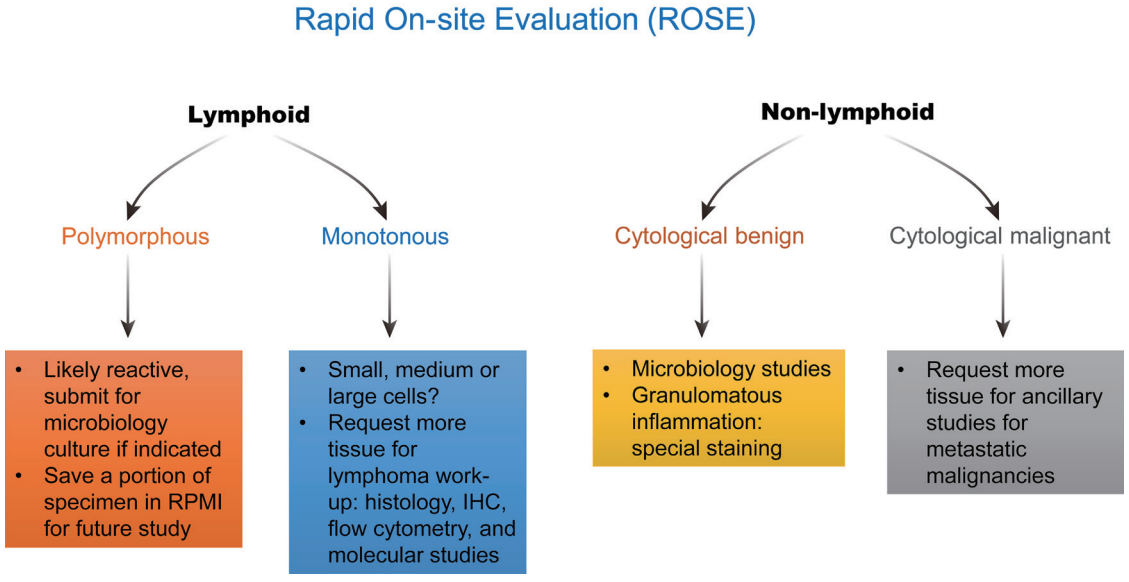

Fig. 2. Rapid on-site evaluation (ROSE). RPMI: Roswell Park Memorial Institute (RPMI) medium or RPMI 1640; IHC: immunohistochemistry.

Table 1. Summary of risk of malignancy studies

Studies	Total Cases	Inadequate	Benign	Atypical	Suspicious	Malignant
		Case Number/ROM*	Case Number/ROM	Case Number/ROM	Case Number/ROM	Case Number/ROM
Caputo et al.,[6] 2021	1,458	8/66.7%	716/9.38%	23/28.6%	58/100%	653/99.8%
Gupta et al.,[7] 2021	6,983	289/27.5%	3,397/11.5%	33/66.7%	96/88%	3,168/99.6%
Vigliar et al.,[8] 2021	300	20/50%	104/1.92%	25/58.3%	13/100%	138/100%
Torres Rivas et al.,[9] 2021	363	13/27%	208/3%	7/50%	21/100%	114/100%
Makarenko et al.,[10] 2022	349	24/58.3%	109/6.4%	52/69.2%	30/96.7%	134/99.3%
Uzun et al.,[11] 2022	504	24/16.6%	283/0.7%	36/88.8%	48/100%	113/100%
Ahuja et al.,[12] 2022	1,205	53/9.1%	488/1.5%	10/37.5%	275/96.9%	379/98.2%

*Abbreviation: ROM: risk of malignancy, based on histopathologic correlation and malignant outcomes.

necessarily determine the adequacy of the sample, but they can be contributory when found in conjunction with other cell types.[5]
- In some cases, with insufficient cytopathological lymphoid material, needle rinsing may subsequently provide diagnostic information from flow cytometry (FC) or cell blocks or other ancillary testing such as cytogenetics, due to improved sensitivity in hypocellular samples or due to sample heterogeneity with passes obtained for ancillary studies containing more adequate lymphoid tissue than seen on the aspirate smears.
- Correlation with imaging and clinical findings is always important. For example, in this category for cases where there is good lymphoid material, but it does not explain the imaging/clinical findings, either "nondiagnostic" or "benign" can be used with the caveat that "the material may not represent the targeted lesion" and emphasizing the need for clinical and radiological correlation.
- In practice, one term from insufficient/inadequate/nondiagnostic needs to be chosen and used consistently.

Risk of malignancy (ROM) and clinical management recommendation

Based on limited published studies, the prevalence of this category is 0.55–6.9%, and the ROM ranges from 9.1% to 66.7% (Table 1[6–12]). Thus, a repeat FNAB with ROSE for adequacy assessment and triage is recommended. Additionally, if feasible, a CNB with touch preparation and ROSE may be considered.

Benign

Diagnostic criteria

- Unequivocal benign features that may or may not be specific for a particular infection or non-infectious process;
- Normal or reactive lymphoid components and inflammatory processes.

Entities and cytomorphologic features

- Inflammatory/Infectious processes:
  - Acute inflammation;
  - Granulomatous inflammation;
- Benign reactive lymphadenopathy:
  - Follicular hyperplasia (Fig. 1);
  - Immunoblastic reactions;
  - Prominent histiocytosis (Fig. 1);
  - Prominent plasmacytosis;
  - Prominent necrosis.

Differential diagnosis and potential pitfalls

- A wide range of lymphoid patterns can be seen in viral infections, autoimmune processes, and other lesions. These patterns include predominantly follicular hyperplasia, immunoblastic reactions, prominent histiocytosis, prominent plasmacytosis and necrosis, or mixed findings;
- If the features are benign but a precise diagnosis cannot be made, the features should be described, and a differential diagnosis should be provided;
- Distinguishing between follicular hyperplasia and follicular lymphoma, or between a viral immunoblastic reaction and lymphoma can be difficult in cytology specimens;
- If the features raise a differential diagnosis that includes lymphoma, then the case is placed in the 'Atypical' category;
- As with all categories, correlation with clinical and imaging findings, in addition to any pertinent serological tests that are available, is mandatory. The ability to confidently provide a diagnosis based on the cytomorphology of lymph nodes is variable and can be misleading without the use of additional information and ancillary studies. Distinguishing between benign and atypical inflammatory reactive patterns can also be challenging and is influenced by many factors such as local practices, expertise, as well as the expectations and support of clinical colleagues;
- A relatively low to intermediate ROM will allow for a high negative predictive value (NPV) for a 'Benign' diagnosis:
  1. One example: a young patient with a clinical presentation of infectious mononucleosis, who has FNAB showing a prominent immunoblastic component, could be watched to see if the lymphadenopathy recedes and if serology and clinical outcomes correlate:
  2. Another example: a polymorphous lymphoid population with tingible-body macrophages and dendritic cells in recognizable germinal centers on smears suggests follicular hyperplasia. A provisional or preliminary diagnosis of follicular hyperplasia (Fig. 1) can be made, with a comment of "Correlation with FC is recommended. Repeat FNAB if lymphadenopathy persists". FC is very helpful to exclude a monoclonal B-cell population, and CNB may be considered. If ancillary tests are not available, the patient may be watched for a 2-to-4-week period. If lymphadenopathy persists, repeat FNAB or excision biopsy is recommended.

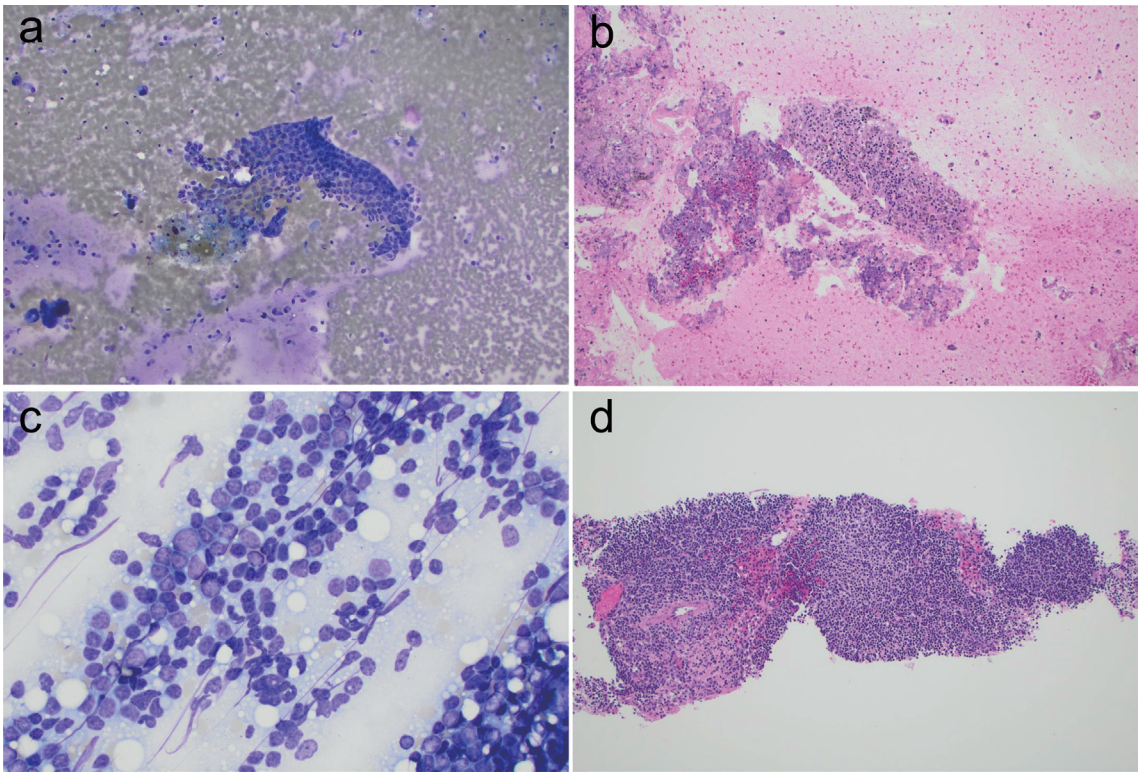

Fig. 3. Atypical (a–b). Rare clusters of atypical cells and lymphocytes on smear slide but absent on the cell block slide from a pre-carinal lymph node (a. DQ, 200×; b. H&E 100×). Suspicious for malignancy (c–d). Monomorphic atypical lymphocytes on FNA smear and core biopsy of a para-aortic lymph node, which proved to be a low-grade follicular lymphoma (c. DQ, 400×; d. H&E, 100×).

Ancillary testing

- Special Stains: FNAB may demonstrate or suggest the presence of infectious organisms in routine stains, prompting the need for additional special stains. For instance, the Ziehl-Neelsen stain for acid-fast bacilli, consistent with tuberculosis, may aid in making the diagnosis;
- Additionally, in plasma cell-rich lesions with an amorphous background material, a Congo Red stain may be helpful in detecting amyloid deposition;
- Immunohistochemical stains (IHC): In situations where clinical and radiological findings suggest reactive lymph nodes, lymphoid proliferation may be diagnosed as reactive without FC or IHC, and patients may be referred for clinical follow-up. However, when clinical and/or imaging findings are discrepant or suspicious, FNAB with immunophenotyping, preferably by FC or alternatively by IHC, is recommended if material is available;
- Molecular and FC: Ancillary testing, such as PCR and microbiological culture for organisms, or a cell block for organisms, enhances the diagnosis of a reactive lymphoid population.

Risk of malignancy (ROM) and clinical management recommendation

Based on limited published studies, the prevalence of this category is 31.2–56.1%, and the ROM ranges from 0.7% to 11.5% (see Table 1). In certain cases, additional ancillary studies, such as special stains for tuberculosis, PCR, and microbial culture for organisms, or flow cytometry to exclude lymphoma, might be required. When an exact diagnosis cannot be established, it is essential to describe the observed features and provide a differential diagnosis in the report.

Atypical

Diagnostic criteria

Scant or poorly prepared cellular material demonstrates predominantly benign cytological features, while a few cells may show minimal features of atypia, raising the possibility of a malignant lesion (Fig. 3). Insufficient features either in number or quality to diagnose a benign or malignant process or lesion. The "atypical" category helps maintain a high NPV for the benign category.

Entities and cytomorphologic features

Atypia of uncertain significance (AUS) includes possible epithelial inclusions and non-lymphoid lesions such as histiocytic proliferations.

- Atypical lymphoid cells of uncertain significance (ALUS) include any case in which the lymphoid material suggests a benign process but cannot entirely exclude lymphoma. For example, a mixture of lymphoid cell types with a relative lack of tingible body macrophages and small lymphocytes raises the differential diagnosis of follicular hyperplasia and follicular lymphoma.

Differential diagnosis and potential pitfalls

This category includes cases demonstrating features predominantly seen in benign lesions and minimal features that may raise the possibility of a malignant lesion, but with insufficient features either in quantity or quality to diagnose a benign or malignant process or lesion.

Ancillary testing

Cytopathological features that make the smear atypical should always be stated in the report, and the possible diagnosis or differential diagnosis should be stated whenever possible. Repeat FNAB, preferably with FC and cytogenetics, or CNB is required regardless of clinical and US findings. ROSE should be considered, if available, to ensure adequate material and to triage appropriately.

Risk of malignancy (ROM) and clinical management recommendation

Based on limited published studies, the prevalence of this category is 0.5–14.9%, and the ROM ranges from 28.6% to 88.8% (Table 1). Samples falling into this category require repeat sampling for more material and ancillary studies, such as flow cytometry and cell block, CNB, or excisional biopsy. Sometimes, a "wait and watch" approach may be applicable.

Suspicious for malignancy

Diagnostic criteria

- Some cytopathological features suggestive of malignancy but with insufficient features either in quantity or quality to make an unequivocal diagnosis of malignancy;
- This category supports a high positive predictive value (PPV) for the "malignant" category.

Entities and cytomorphologic features

- Suspicious for lymphoma: Small and/or medium-sized, monomorphic atypical lymphoid cells (Fig. 3); polymorphous lymphoid smears with rare Hodgkin or Reed-Sternberg-like cells; large cell or Burkitt lymphomas with scant cellularity;
- Suspicious for metastatic carcinoma: atypical epithelioid cells suspicious for metastasis.

Differential diagnosis and potential pitfalls

- Small and/or medium-sized, monomorphic atypical lymphoid cells suspicious for lymphoma, but the cytomorphology alone is not sufficient for diagnosis and FC or IHC results are not available or do not demonstrate B-cell monoclonality;
- Polymorphous lymphoid smears in which few Hodgkin or Reed-Sternberg-like cells are detected, and IHC is not performable or has not been diagnostic;
- Large cells or Burkitt lymphomas with scant cellularity, and ancillary studies are not available;
- Smears in which atypical cells suspicious for metastasis are detected but are too scant to be diagnostic and there is no CNB material available to perform IHC.

Ancillary testing

- Ancillary testing, including FC for non-Hodgkin lymphoma (NHL) or cell block with IHC for Hodgkin lymphoma or carcinoma, may be definitive and can "upgrade" the category.

Risk of malignancy (ROM) and clinical management recommendation

Based on limited published studies, the prevalence of this category is 1.4–22.8%, and the ROM ranges from 88% to 100% (Table 1). This category supports a high PPV for the "malignant" category. Whenever possible, provide differential diagnoses. Ancillary studies, including flow cytometry, cell block with IHC, or IHC performed on FNAB smears, may be definitive and upgrade the category. Repeat FNAB, core needle biopsy, or excisional biopsy is typically recommended.

Malignant

Diagnostic criteria

- Unequivocal cytopathological features of malignancy.

Entities and cytomorphologic features

- Mixed lymphoid cell pattern:
  - Follicular lymphoma;
  - Marginal zone lymphoma;
  - Angioimmunoblastic T-cell lymphoma;
- Predominantly small/intermediate cell pattern:
  - Chronic lymphocytic leukemia;
  - Mantle cell lymphoma;
  - Lymphoplasmacytic lymphoma;
  - Plasma cell neoplasms;
  - Mastocytosis;
- Predominantly intermediate/pleomorphic/blastic cell pattern:
  - Lymphoblastic lymphomas;
  - Large/Aggressive B cell lymphomas (Fig. 4);
  - Burkitt lymphoma;
  - Blastoid mantle cell lymphoma;
  - Anaplastic large cell lymphoma;
  - Breast implant-associated anaplastic large cell lymphoma;
  - Primary effusion lymphoma;
  - Peripheral T-cell lymphoma, NOS;
  - Myeloid sarcoma;
- Single very large, atypical cell pattern:
  - Classic Hodgkin lymphoma;
  - Nodular lymphocyte predominant Hodgkin lymphoma;
  - T-cell/histiocyte rich large B-cell lymphoma;
- Histiocytic and dendritic cell neoplasms:
  - Langerhans cell histiocytosis;
  - Histiocytic sarcoma;
  - Interdigitating dendritic cell sarcoma;
  - Follicular dendritic cell sarcoma;
- Metastases:
  - Metastases (Fig. 5).

Differential diagnosis and potential pitfalls

- Due to similarity in morphology on FNAB smears, it is difficult to make a precise diagnosis of a specific carcinoma, lymphoma, or other malignancy. The precise diagnosis of carcinoma and lymphoma subtypes usually requires ancillary testing;
- The presentation of tumors in this WHO Reporting System follows the order found in the 5th edition WHO Classification of Hematopoietic and Lymphoid Tissues, but not all tumors are included because they have not been described in the cytopathology literature.

Ancillary testing

- This category includes small to medium-sized cells of NHL supported by evidence of clonality shown by FC or molecular studies showing clonal immunoglobulin or T-cell receptor gene rearrangements, and all the entities in which cytopathological features alone are sufficient to identify malignancy as large cell NHL. Most B-cell lymphomas have characteristic immunophenotypic profiles (Table 2). However, no single marker is specific, thus a panel of immunophenotypic markers is necessary;
- This category also includes Hodgkin lymphoma (HL) in

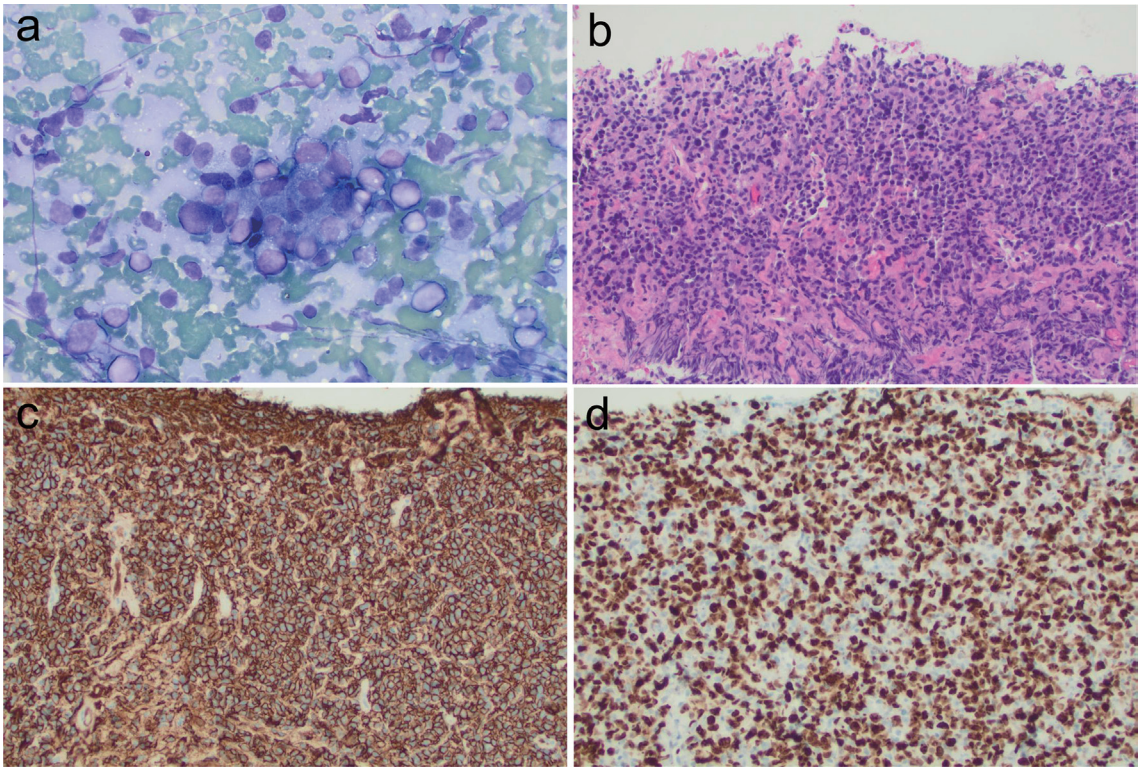

Fig. 4. Malignant. Diffuse large B cell lymphoma, high grade, in a cervical lymph node: Isolated and loosely clustered large lymphoma cells on smears. The tumor cells are positive for CD20 with a high Ki-67 proliferative index of >90% (a. DQ, 400×; b. H&E, 200×; c. CD20, 200×; d. Ki-67, 200×).

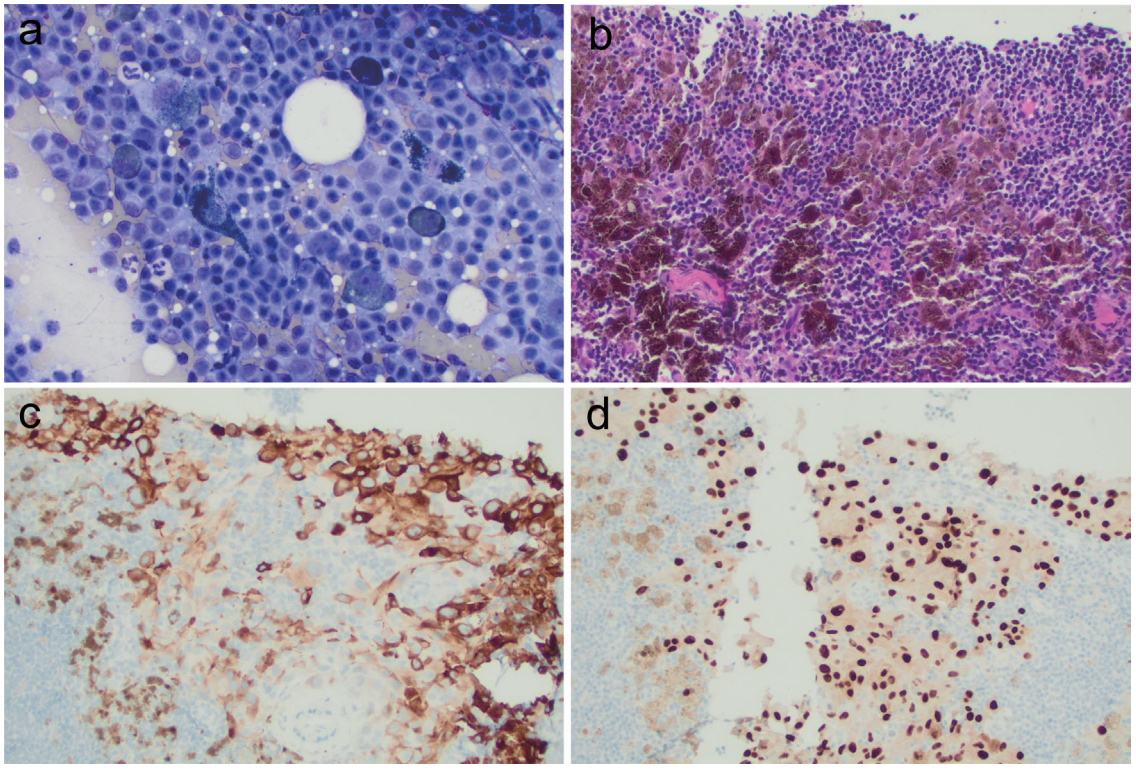

Fig. 5. Malignant Metastatic melanoma of right inguinal lymph node: atypical epithelioid cells with enlarged nuclei and prominent nucleoli and scattered dark granular pigment consistent with melanin. The tumor cells are positive for Mart-1 and SOX10 (a. DQ, 400×; b–d. Needle core biopsy: b. H&E, 200×; c. Mart-1, 200×; d. SOX10, 200×).

Table 2. Immunophenotypic and genetic/molecular characteristics of common small B cell lymphomas

	CD19	CD20	CD5	CD10/ BCL6	LEF1	CD23	CD200	Cyclin D1/ SOX11	Molecular Genetics
CLL	+	+ dim	+	–	+	+	+	–	del (13q); del (11q); del (17p); trisomy 12
MCL	+	+	+	–	–	–	–	+	*IGH::CCND1*
FL	+	+	–	+	–	–	–	–	*IGH::BCL2*
HCL	+	+	–	–	–	–	+	+weak	*BRAF p.V600E* mutation
LPL	+	+	–/+	–/+	–	–/+	–	–	*MYD88* p.L265P mutation
MZL	+	+	–/+	–	–	–/+	–	–	*BIRC3::MALT1*; *IGH::BCL10*; *IGH::MALT1*; *IGH::FOXP1*

Abbreviations: CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; HCL, hairy cell leukemia; LPL, lymphoplasmacytic lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma.

which there is an appropriate cellular background and diagnostic Hodgkin and Reed-Stern-berg cells as well as metastatic neoplasms. The second diagnostic level of the WHO System provides additional information and identification of specific entities by utilizing ancillary testing;

- Ancillary testing makes the diagnosis more specific and may change the category, as with all FNAB cytopathology (e.g., "suspicious for malignancy" to "malignancy"):
  - For example, large cell lymphoma can be diagnosed on direct smears, confirmed on FC as a B-cell lymphoma, and FISH for c-Myc and IGH-Bcl2 on the cell block material, can confirm a "double-hit" high-grade lymphoma;
  - Metastatic carcinoma in a mediastinal hilar lymph node at Endobronchial Ultrasound Bronchoscopy (EBUS) can be diagnosed by cytopathology, and in many cases the diagnosis can be made, followed by limited IHC on the cell block for definitive characterization in order to save material for potential molecular testing on the cell block or slide scraping to diagnose *EGFR1, KRAS, ROS1* and other biomarkers that correlate with theragnostic information.

Risk of malignancy (ROM) and clinical management recommendation

Based on limited published studies, the prevalence of this category is 22.5–46%, and the ROM ranges from 98.2% to 100% (Table 1). This category demonstrates unequivocal cytological evidence of malignancy. Ancillary studies such as IHC, FC, and molecular tests are often required for a specific diagnosis.

Conclusions

The WHO Reporting System for Lymph Node Cytopathology plays a crucial role in standardizing nomenclature and reporting systems in cytopathology.[13] It facilitates the integration of diagnostic and management algorithms, assisting clinicians in effectively managing patients. Through international consensus, it establishes key diagnostic criteria in cytopathology, improving the quality of diagnostic assessment and reporting in lymph node cases. The system also establishes a dynamic practical link between cytopathology and surgical pathology through its direct connection to the 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors "blue book" on the website. By raising awareness of the current diagnostic role of FNAB cytopathology and its potential in personalized medicine with ancillary testing, including molecular pathology, the WHO Reporting System promotes the use of FNAB internationally in both developed and low-middle-income countries, ultimately enhancing patient care and outcomes.

Overall, FNAB demonstrates high diagnostic accuracy in various lymph node disorders. It has many benefits such as minimal invasiveness, rapid turnaround time, cost-effectiveness, and easy provision of cells for a variety of studies either for diagnostic or therapeutic purposes. Implementing the proposed WHO system contributes to achieving uniformity and reproducibility in cytologic diagnoses and facilitates risk stratification based on cytopathology. Optimal patient management can be guided by understanding the risks of malignancy associated with FNAB diagnostic categories. However, the interpretation of FNAB requires awareness of certain inherent pitfalls, such as sampling error or misinterpretation, especially in lymphoma diagnosis. A critical element that might significantly improve the adoption and refinement of the WHO system is to promote the involvement of hematopathologists, hematologists, and oncologists. Further studies from multiple different centers with various epidemiologic settings and larger sample sizes are necessary to assess the reliability and validity of this system.

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Conflict of interest

The manuscript was submitted during Dr. Y. Helen Zhang's term as an editorial board member of *Journal of Clinical and Translational Pathology*. The authors have no other conflict of interests to declare.

Author contributions

Study concept and design, data collection, analysis and interpretation of data, literature review, and drafting of the manuscript (YHZ, YG, MS, KR). All authors have made a significant contribution to this study and have approved the final manuscript.

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