A Proposal for the Performance, Classification, and Reporting of Lymph Node Fine-Needle Aspiration Cytopathology: The Sydney System

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Keywords

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Abstract

Background: The evaluation of lymph nodes (LN) by fine-needle aspiration cytology (FNAC) is routinely used in many institutions but it is not uniformly accepted mainly because

of the lack of guidelines and a cytopathological diagnostic classification. A committee of cytopathologists has developed a system of performance, classification, and reporting for LN-FNAC. **Methods:** The committee members prepared a document that has circulated among them five times; the final text has been approved by all the participants. It is based on a review of the international literature and on the expertise of the members. The system integrates clinical and imaging data with cytopathological features and ancillary

techniques. The project has received the endorsement and patronage of the International Academy of Cytology and the European Federation of the Cytology Societies. **Results:** Clinical, imaging, and serological data of lymphadenopathies, indications for LN-FNAC, technical procedures, and ancillary techniques are evaluated with specific recommendations. The reporting system includes two diagnostic levels. The first should provide basic diagnostic information and includes five categories: inadequate/insufficient, benign, atypical lymphoid cells of undetermined/uncertain significance, suspicious, and malignant. For each category, specific recommendations are provided. The second diagnostic level, when achievable, should produce the identification of specific benign or malignant entities and additional information by utilizing ancillary testing. **Conclusion:** The authors believe that the introduction of this system for performing and reporting LN-FNAC may improve the quality of the procedure, the report, and the communication between cytopathologists and the clinicians. This system may lead to a greater acceptance and utilization of LN-FNAC and to a better interdisciplinary understanding of the results of this procedure.

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Introduction

The evaluation of lymph nodes (LN) by fine-needle aspiration cytology (FNAC) has been routinely used as an initial diagnostic tool for many years at many institutions. LN are among the most common sites targeted by FNAC. In both the adult and pediatric setting, LN-FNAC can assess whether lymphadenopathy (LAP) is benign or malignant and provide staging information in patients with an established diagnosis of malignancy. LN-FNAC can be performed to relieve anxiety and provide material for microbial cultures and reduce unnecessary surgery.

The current WHO classification of lymphoproliferative disorders [1] incorporates clinical, morphological, and ancillary data that are required for specific diagnoses. LN-FNAC can provide cytomorphological information and material for ancillary testing that is diagnostic, highlighting the key role of FNAC and other small biopsies in the evaluation of LAP. Despite the tremendous progress made in performing and interpreting LN-FNAC and its correlation with ancillary tests [2–8], the technique is not uniformly accepted by clinicians and pathologists [9–11]. This is mainly due to the lack of widely shared and accepted guidelines and a cytopathological classification that directly relates to management. A consensus on a diagnostic classification system is required to improve

LN-FNAC reliability, efficiency, reproducibility of diagnoses, and acceptance by clinicians and pathologists.

However, taking into account the wide spectrum and complexity of pathology presenting in LN [1], the resulting cytopathology, and required ancillary techniques, a simple classification system similar to those used for the thyroid gland, urine, or salivary gland FNAC is not adequate for LN-FNAC. At the same time, the clinical contexts and indications to perform LN-FNAC vary greatly and the technical procedures and testing are not equally available in different institutions and different countries.

In this setting, after an intense online exchange of views and opinions, a steering committee of international cytopathologists involved in LN-FNAC met at the International Cytology Congress in May 2019 in Sydney, Australia, and decided to develop a system for reporting LN-FNAC. This proposed system, based on a review of the international literature and on the expertise of the committee members, integrates clinical and imaging information with key diagnostic cytopathological features and ancillary techniques, and is linked to a management algorithm, including options, which reflects the varying medical infrastructure available internationally. The steering committee will be expanded to include other cytopathologists as a writing team, and the proposal will be further assessed through a web-based survey to incorporate an international perspective. In 2019, the present project received the endorsement and patronage of the International Academy of Cytology during the IAC Sydney Congress and the European Federation of the Cytology Societies during the EFCS Congress in Malmo, Sweden. Further patronages and endorsements will be requested to other international and continental representative scientific cytology societies. A survey on the questions that deal with the proposed classification system and interpretative criteria for LN-FNAC will be proposed by an online survey in the next months.

Aims of the Proposed LN Consensus System

The proposed system for reporting LN-FNAC cytopathology has the following aims.

- Provide consensus guidelines and a framework of reference to facilitate communication among cytopathologists, hematopathologists, clinicians, surgeons, and other healthcare providers.
- Define and identify LN-FNAC indications, preferred operators, recommended performance, analytical and preanalytical issues, technical and diagnostic limita-

Table 1. LN-FNAC: clinical assessment and indications

LN-FNAC issues	Significant data	Course of action
Clinical context for requesting LN-FNAC	Single or multiple LN with no relevant history Single or multiple LN in known pathology	Mandatory Mandatory
Clinical data to review when interpreting LN-FNAC	Age, symptoms, site, size, time of onset, imaging (US) Remote and current medical history Basic serology (ESR, LDH, ToRCH complex, ANA, others) Specific serology (known or suspected disease)	Mandatory Mandatory Recommended Recommended
Goals of performing LN-FNAC	LN identification: LN or not-LN (H&N area), atypical sites (intercostal, intra-mammary, epitrochlear, intra-parotid, retroperitoneal), others	Recommended
	LN diagnosis to prove or exclude malignancy, and avoid LN excision for benign/reactive processes	Recommended
	Diagnosis and staging of lymphoma or metastasis	Recommended
	Diagnosis and microbial culture material for infectious etiologies	Suggested
	Relieve anxiety for benign/reactive processes	Recommended
	Cell collection for diagnostic and predictive tests Cell collection for clinical trials or other research tests	Recommended Suggested

LN, lymph node; US, ultrasound; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; ToRCH, toxoplasmosis rubella cytomegalovirus herpes; ANA, antinuclear antibodies.

tions, as well as the basic diagnostic reporting categories and additional diagnostic information that can produce specific disease subtyping when possible.

- Provide the key diagnostic cytopathological features of lesions that occur commonly in the various categories.
- Provide recommendations on the components of standardized diagnostic reports with the aim to improve reporting and communication between cytopathologists and clinicians.
- Provide management recommendations linked to the reporting categories with possible options that include the use of clinical and imaging follow-up, ancillary testing, and possible need of LN excision.
- Foster cytohistopathological correlations, cell storage, and research on neoplastic and non-neoplastic LN specimens.
- Increase LN-FNAC reliability and clinician awareness of its diagnostic potential.

LN-FNAC Request

The initial request for LN-FNAC typically originates from the clinician after they have examined the patient. The type of request can vary depending on the specialty of the referring clinician and also the ancillary techniques available at various institutions. In addition to an accurate diagnosis, general practitioners may be interested in

the confirmation that the nodule and/or swelling are actually LN. Head and neck surgeons and otorhinolaryngologists may similarly be interested in this anatomical confirmation and in a report that directly addresses specific regional lesions. Interventional endoscopists may require specific answers for a potential pathology in mediastinal LN. Hematologists are usually interested in the diagnosis of a suspected lymphoproliferative process and posttherapy changes. Pediatricians and general practitioners often require a minimally invasive diagnosis of expected reactive processes, while avoiding an open biopsy, and this diagnosis will relieve the concerns of patients and parents. Therefore, cytopathologists dealing with LN-FNAC should be aware of the specific clinical contexts affecting LN-FNAC performance and understand the questions that the FNAC is expected to answer. The clinical data, goals, and indications for LN-FNAC are summarized in Table 1.

Clinical, Imaging, and Serological Evaluation of Lymphadenopathy

Clinical evaluation of patients with LAP may be a complex task for clinicians. Medical history and physical examination often suggest the cause of LAP and, in most cases with a clear clinical context, the diagnosis and management of reactive LAP is quite straightforward. In

Table 2. LN US features

Criteria	Inflammation/infection	Metastases	Lymphoma
Size	Variable	Large	Large
Shape	Oval	Round/oval	Round/oval
Border	Regular/sharp	Irregular, ill-defined, blurred, angular, capsule infiltration	Regular/sharp
Architecture	Preserved, homogeneous; thin or variable thickened cortex	Destroyed, hypoechoic cortical infiltration inhomogeneous internal structure, no hilum, surrounding edema	Hypoechoic cortical thickening, no echogenic hilum; perinodular edema
Structural changes: cortical nodules, necrosis (coagulative, cystic), reticulation, calcification, matting	Generally absent (cystic necrosis, calcification and matting may occur in bacterial, fungal or TBC)	May be present	May be present
Soft tissue edema	May be present	Generally absent	Generally absent
Echogenicity/echotexture	Hypoechoic, heterogeneous	Hypoechoic, variable	Hypoechoic, homogeneous
Color Doppler US	Low density, normal vascular-hilar pattern, no neoangiogenesis Resistive index (RI) <0.8 Pulsatility index (PI) <1.6	High density, mixed vascular pattern, split, tortuous arteries RI >0.8 PI >1.6	High density High vascular pattern no preserved hilar and peripheral vessels RI >0.75 PI >0.75
Elastography	Normal architecture	Focal infiltration, relatively harder	Normal but asymmetric architecture
Contrast enhanced US	Homogeneous enhancement from the hilum, centrifugal enhancement	Centripetal, different intranodal enhancement levels Inhomogeneous washout, perfusion defects	Intense homogeneous enhancement, diffuse bright spots, peripheral hypo- or no enhancement

other cases, the cause of LAP is unclear because the clinical presentation and the response to therapy are non-specific.

The etiology of LAP correlates with patient age and clinical history, and the clinical relevance of LAP varies between adults and children [12]. LAP malignancy rates increase with age and the size of the LN. Metastatic cancers are diagnosed in 4% of patients with unexplained LAP aged >40 years versus 0.4% of those <40 years [13].

Abnormal LN are typically defined by size, consistency, and/or imaging findings. For example, LAP typically involves enlarged LN defined as greater than 1 cm in the largest dimension, but at specific sites, such as the supraclavicular, popliteal, iliac, and epitrochlear region, LN greater than 0.5 cm are considered abnormal (Table 1). Palpation of enlarged LN may suggest a pathologic process, such as soft fluctuant LN suggesting infection or firm to hard LN suggesting a malignant neoplasm. The

etiologies of LAP can be easily memorized by means of the MIAMI acronym: malignancies, infections, autoimmune disorders, miscellaneous and unusual conditions, and iatrogenic causes [14, 15].

Imaging evaluation, particularly by ultrasound (US), is a key tool for the initial evaluation of LN (Table 2), as well as to guide FNAC of non-palpable or challenging lesions. US devices have become less expensive and portable, thus more available in hospitals and used more often in pathologist-performed FNACs. Conventional B-mode US (transcutaneous and endoscopic) provides key information on the size and site of LN, and other size-independent criteria, including loss of the fatty hilum, the presence of posterior acoustic shadowing or enhancement, and the Solbiati index. Other key US information concern the shape, border, architecture, echogenicity, echotexture, focal infiltration, bulky mass, blood vessel density, vascular pattern, resistance index, and elastography of the

LN [16, 17]. Additional information on the LN vascular pattern can be provided by the power Doppler; this pattern differs in normal, inflammatory, and neoplastic LN, and may be perinodal, intranodal, diffuse, or localized (Table 2). US provides useful information on the approach to the target LN, avoiding adjacent vascular structures and the LN hilum. In the case of multiple enlarged LN, US helps in selecting the most significant or the most approachable LN to be targeted, where additional passes can be performed.

The serologic evaluation of a patient with LAP should consider the history and the clinical evaluation of the LN and is usually requested after 2 weeks of persistent LAP. Basic laboratory tests as part of the workup include a full or complete blood count (FBC or CBC) and the review of the peripheral blood smear and chemical serum analytes, such as lactate dehydrogenase, beta-2-microglobulin, creatinine, and serum immunoglobulins. The evaluation of hepatic and renal function and urine analysis are useful in identifying underlying systemic disorders that may be associated with LAP. High serum levels of lactate dehydrogenase suggest neoplasia or lymphoma.

Titers for specific infections or antibodies may also be useful. Heterophile antibody tests, FBC, and peripheral blood smear review (atypical lymphocytes) are indicated for suspected infectious mononucleosis. *Cytomegalovirus* (CMV) infection can be identified by CMV IgM and IgG antibody titers, CMV polymerase chain reaction (PCR), and CMV antigenemia test. *Toxoplasma gondii* and *Bartonella henselae* infections can be identified by elevated titers of IgM and IgG antibodies as well as PCR for specific DNA sequences. The Sabin-Feldman test is most used for suspected toxoplasmosis. The standard testing for Lyme disease is 2-tiered serology: the first-tier EIA quantifies potential antibodies against *Borrelia burgdorferi*; if this test is positive or equivocal, second-tier immunoblotting can detect antibodies against *B. burgdorferi* surface proteins. In suspected cases of lymphogranuloma venereum, anti-chlamydia IgA and a complement fixation test are used. Serum VDRL (venereal disease research laboratory test), rapid plasma reagin, fluorescence treponemal antibody absorption, and microhemagglutination assay are indicated for suspected *Treponema pallidum* infection. In patients with suspected human immunodeficiency virus (HIV) infection, HIV antibodies (ELISA), HIV viral RNA quantitative assay, rapid HIV test, and Western blot are used. Acid-fast bacilli stain, PCR, culture, and skin tuberculin test are used for suspected mycobacterial infection [18–20], and the Ziehl-Neelsen stain for *Mycobacterium tuberculosis* and Wade-Fite stain for

M. leprae. Serum latex agglutination and ELISA for fungal antigens may be used for cryptococcus infections [21]. Rheumatoid factor, other autoantibodies, pharyngeal swab, and Mantoux intradermal reaction are other tests that can be used in appropriate clinical situations.

LN-FNAC Indications

When the clinical and US presentation is less clear and serological data do not explain or do not match the clinical context, diagnostic imaging and/or pathological evaluation are required. Computed tomography (CT) with or without positron emission tomography and other non-invasive procedures are used to identify the nature of the LAP. However, these non-invasive procedures lead infrequently to a definitive diagnosis and, if LAP persists, pathological assessment with FNAC, core-needle biopsy (CNB), and/or surgical excision and histopathological evaluation are required. Criteria for LN-FNAC operators and preanalytical issues are provided in Table 3.

Traditionally, excisional biopsy of an enlarged LN has been the standard approach to establish a diagnosis, and histopathological examination has formed the basis for therapy and prognostic evaluation in patients with metastatic carcinomas and lymphomas. However, excisional biopsy is a demanding procedure for the patient, the physician, and the healthcare system in general. It usually requires hospitalization, a surgical procedure with at least local anesthetic and sedation, and has a greater risk of complications. CNB has been increasingly used to obtain material for histopathology but is a sampling technique with its own limitations and complication rate [22]. For the most frequent causes of LAP, such as benign reactive hyperplasia, specific infections or a metastasis from a known or unknown primary tumor, LN-FNAC is an accurate, quick, and cost-effective procedure, often making LN excisional biopsy an unnecessary and costly alternative. FNAC can determine whether a palpable or impalpable mass is actually an LN and distinguish a benign from a malignant entity, or a hematology process from a non-hematology process. LN-FNAC can be the first-choice procedure for patients who are poor candidates for surgical biopsy or with abnormal LN in deep or inaccessible locations. However, without widely accepted guidelines on LN-FNAC technical procedures and diagnostic criteria, the use of FNAC and the value of FNAC diagnoses varies between countries and institutions.

The authors of this consensus agree that an LN-FNAC diagnosis made in conjunction with appropriate ancillary

Table 3. LN-FNAC: operator, preanalytical procedures, ancillary techniques

LN-FNAC issues	Significant data	Course of action
Operator	Cytopathologist (with US guidance)	Recommended
	Cytopathologists (palpable LN, without US guidance)	Acceptable
	Radiologists, endoscopists, and clinicians able to perform FNAC	Acceptable with ROSE
	Operators untrained in FNAC	Acceptable with ROSE
Preanalytical procedures	Patient informed consent (parents or legal guardian for children and dependents) and procedure timeout to confirm patient identifiers	Recommended
	Manual (superficial, small-mobile, specific sites: axillary, retroauricular cervical, groin, supraclavicular) guide	Acceptable
	US, CT, EUS, EBUS guide	Recommended
	Number of passes (2 or more)	Recommended
	Needle length, gauge (23–27 G)	Recommended
	FNAC standard performance	Recommended
	ROSE	Recommended
	Accurate triage of the material	Recommended
Use of ancillary techniques	Flow cytometry for NHL	Recommended
	Flow cytometry for classical HL	Acceptable (to exclude NHL)
	Flow cytometry for metastases	Avoid
	Immunocytochemistry for NHL, HL, metastases	Recommended
	Special stains for infections	If necessary/available
	Microbial cultures for infections	If necessary/available
	FISH/CISH (translocations, amplifications, deletions)	If necessary/available
	Molecular tests (clonality assessment, predictive tests)	If necessary/available

techniques, when necessary, and in a proper clinical context does not require histopathological confirmation in cases of benign reactive lymphoid hyperplasia, specific infections, recurrent lymphoproliferative disorders, and metastases. In these patients, a CNB may add value and be suggested if it can be performed safely but is not routinely required. If there is uncertainty regarding the FNAC diagnosis or there is discrepancy between the clinical, imaging, or serological findings and the FNAC diagnosis, CNB or excision biopsy is recommended.

Furthermore, histological confirmation of LN-FNAC findings is recommended for the primary diagnosis of Hodgkin lymphoma (HL) and non-HL (NHL), except for specific clinical situations or in certain anatomical locations in which biopsies or surgery are contraindicated or not possible. This recommendation, however, can be tailored to the type of lymphoma suspected. For instance, in

a patient with chronic lymphocytic leukemia/small lymphocytic lymphoma, LN-FNAC with flow cytometry (FC) immunophenotyping is usually sufficient without histopathological confirmation. In certain specific clinical situations or in certain anatomical locations, CNB or excision biopsy are contraindicated or not possible, but histopathological examination may be required as part of some clinical treatment protocols.

LN-FNAC can be particularly useful in staging and follow up, including the response to treatment, in patients with known malignant processes. LN-FNAC can obtain tissue for immunophenotypic and molecular studies and obtain cellular and genetic material for storage. Furthermore, LN-FNAC can be used to determine eligibility for clinical trials and research protocols, since many patients with widespread metastatic disease have easily accessible LN that can be safely and repeatedly targeted with FNAC [2–8, 23].

The performance of LN-FNAC involves a number of considerations, including clinical evaluation, US examination, the performance by palpation or US guidance, the making of direct smears, triaging the case for ancillary tests, and optimal management of the material and cytopathological assessment. All these steps are optimized if the procedure is performed by cytopathologists or by other well-trained physicians, including radiologists, endoscopists, or other clinicians, in the presence of a cytopathologist for rapid on-site evaluation (ROSE) and immediate triage of the diagnostic material (Table 1) [24, 25].

Patient Informed Consent

The informed consent of patients is required, based on the guidelines of the Internal Review Board or Ethics Committee of the involved institution. Consent should emphasize the importance of providing patients with adequate information about the LN-FNAC procedure, the accuracy of the LN-FNAC diagnosis and its limitations, enabling them to make informed decisions. Parents or legal guardians are responsible for the informed consent of pediatric or incompetent patients (Table 1).

LN-FNAC Techniques and Procedural Considerations

FNAC of both palpable and impalpable LN can be guided by US, CT, and transesophageal or transbronchial endoscopic US (EUS-EBUS) where available. For palpable, superficial, and bulky LN, and/or in specific anatomical sites, such as axillary LNs, the needle can be guided by palpation. US guidance for FNAC is recommended for palpable LN in other sites to improve the placement of the needle and accuracy of sampling but is not essential [26].

FNAC requires labelled glass slides, 23-, 25-, or 27-G needles of different lengths, preferably with a flanged tip, disinfectant, gauze, and band aid or simple adhesive dressing. Ten- or twenty-milliliter syringes attached to the needle provide a closed system and where necessary negative pressure for aspiration and can be used with a mechanical syringe holder. Alternatively, a non-aspiration technique using the needle-only technique is well suited to LN-FNAC. The needle-only technique is useful for cases requiring superior fine motor control (e.g., pediatric biopsies, small mobile LNs), for anxious patients fearful of the large syringe holder and syringe, or in LN that have increased vascularity to decrease peripheral blood dilution of the specimen.

LN-FNAC material should be expelled gently so that it can be divided into small drops to prepare multiple conventional smears or used for ancillary techniques. The number of prepared slides depends on the quantity of material, the diagnostic needs, and clinical situation. Expelling all the aspirated material on one single slide minimizes the ability to have material for additional testing and might also lead to thick suboptimal smearing. A specimen-splitting technique which divides the material for routine Romanowsky-type staining and alcohol-fixed Papanicolaou staining and other special stains is recommended by some authors [27]. The smearing technique may be chosen based on the quality of the material: a hematologic technique (smearing with the edge of another slide) is used in the case of fluid material, whereas a direct smear (smearing between two slides or “pull-apart” smearing) is advisable in cases with bloody or dense material. Slide preparation should be rapid to prevent coagulation and drying artifact. The pressure exerted on the slides should be gentle, but firm and uniform in order to avoid mechanical trauma, provide a uniform thin smear, and to preserve the morphology of lymphoid cells that are fragile and easily crushed. Two conventional smears, one air dried for the Diff-Quik type stain and one immediately alcohol fixed for the Papanicolaou stain, are recommended in all cases. Hematoxylin and eosin can also be helpful in some cases.

Residual material in the needle and in some cases the syringe, and the material from additional passes is managed according to the clinical and imaging information, greatly assisted by the provisional ROSE. Multiple passes may be required to achieve sufficient tissue for ancillary studies, including cytospins, liquid-based cytology, cell blocks (CB), and additional smears for special stains according to their specific performance on the different supports in different pathological processes and according to their availability in different laboratories (Table 3) [28]. The preparation of a CB by various methods, including rapid fixation in formalin to be followed by centrifugation, is always recommended and may obviate the need for histopathological examination if a generous sample is available.

When LN-FNAC is not performed by cytopathologists or well-trained radiologists or endoscopists and no ROSE is available for triage, liquid-based cytology may be acceptable but air-dried stains cannot be used, pattern recognition of slides is reduced, cytomorphology is limited to alcohol-fixed Papanicolaou stains, and FC immunophenotyping cannot be performed. If it is not possible for a cytopathologist to perform ROSE, the opera-

Table 4. LN-FNAC: first and second diagnostic levels and post-LN-FNAC management

LN-FNAC issues	Diagnostic reporting categories	Course of action
1st diagnostic level	Inadequate/non-diagnostic	Mandatory
	Benign	Mandatory
	Atypical undetermined significance/atypical lymphoid uncertain significance (AUS/ALUS): possibly benign, not fully supported by cytology and ancillary techniques	Mandatory
	Suspicious: probably malignant, not fully supported by cytology and ancillary techniques	Mandatory
	Malignant (NHL, HL, metastases)	Mandatory
2nd diagnostic level (additional diagnostic information)	Provide specific etiology in reactive processes	Recommended if available
	NHL subtyping and specific diagnoses	Recommended if available
	HL	Recommended if available
	Specific primary tumor in metastases	Recommended if available
Post-LN-FNAC management recommendations	Inadequate/non-diagnostic	LN-FNAC repetition and/or CNB or excision
	Benign	Clinical follow-up/specific treatment (depending on diagnosis, e.g., antibiotics for bacterial infection)
	Atypical undetermined significance/atypical lymphoid uncertain significance (AUS/ALUS)	LN-FNAC repetition with acquisition of material for ancillary studies and/or CNB or excision
	Suspicious	LN-FNAC repetition with acquisition of material for appropriate ancillary studies and/or CNB or excision
	Malignant	Histological biopsy requested (note: histological biopsy not requested for HL and NHL relapses or metastases from known or clearly indicated primary tumor, LN located at particular sites, or in case of debilitated patients, or specific clinical settings)

tor performing the FNAC should be provided a protocol for making appropriate direct smears, and rinse each needle and syringe in buffered saline or other appropriate medium suitable for either FC or CB preparation (Table 1).

Ancillary techniques including immunocytochemistry (ICC), FC, fluorescence or colorimetric in situ hybridization (FISH or CISH), and molecular procedures are required for an accurate LN-FNAC diagnosis in many patients, especially in cases of NHL (Table 3). Ancillary techniques are generally used to distinguish NHL from benign reactive lymphoid hyperplasia, to classify NHL, and to identify diagnostic cells in HL and metastases. FNAC material is often scant and the choice of a specific technique is aimed at the most effective use of the material, as suggested by several algorithms (Table 4) [29–42].

Proposed Diagnostic Reporting Categories for LN-FNAC: Recommendations and Indications

A proposal for a diagnostic system for reporting LN-FNAC has been developed by the above-reported panel of international cytopathologists. This system has two diagnostic levels; the first diagnostic level contains basic diagnostic information and includes the following five categories.

- *Inadequate/Insufficient.* This category includes cases that cannot be diagnosed due to scant cellularity, extensive necrosis, or technical limitations that cannot be overcome; repeat FNAC or CNB or excision biopsy should be requested based on the specific clinical context.
- *Benign.* This category includes cases with suppurative and granulomatous inflammation and specific infections, and other cases with a heterogeneous lymphoid

population with small lymphocytes predominating, and often germinal centers with dendritic cells and tingible body macrophages. The lymphoid proliferations can be diagnosed as reactive with or without FC or ICC and referred for clinical follow-up when the FNAC findings agree with the clinical presentation and US features. When the clinical or US features are discrepant or suspicious, repeat LN-FNAC with immunophenotyping, preferably by FC, is required.

- *Atypical (Cells) Undetermined Significance/Atypical Lymphoid (Cells) of Uncertain Significance (ALUS/AUS).* This category includes cases with a heterogeneous lymphoid population where the features suggest a reactive process, but a follicular lymphoma cannot be excluded or where there is an excess of large cells (centroblasts or immunoblasts) or immature small lymphoid cells or cases where the atypical cells are not lymphoid cells. For these last cases, AUS should be used. Repeat FNAC, preferably with FC and cytogenetics, or CNB or excisional biopsy is required regardless of clinical and US findings.
- *Suspicious.* This category includes cases with small and/or medium-sized, monomorphic atypical lymphoid cells suspicious of lymphoma, but the cytomorphology alone is not sufficient and FC or ICC 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 ICC is not performable or has not been diagnostic; large cell or Burkitt lymphomas scanty cellular in which ancillary techniques are not available; smears in which atypical cells suspicious for metastasis are detected, but are too scant to be diagnostic and there is no CB material available to perform ICC. Repeating FNAC to obtain a diagnostic CB may be one of the management options, or CNB or excisional biopsy is required.
- *Malignant.* This category includes small to medium-sized cells of NHL supported by evidence of clonality shown by FC or molecular studies showing clonal immunoglobulin (*IGH* or *IGK*) or T-cell receptor (*TRG*, *TRB*) gene rearrangements and all the entities in which cytopathological features alone are sufficient to identify malignancy as large cell NHL. This category also includes HL in which there is an appropriate cellular background and diagnostic Hodgkin and Reed-Sternberg cells as well as metastatic neoplasms.

After categorization each case should have a specific diagnosis established, or if not possible, a preferred diagnosis should be made with a discussion of the possible

differential diagnoses. For example, a benign categorization should be followed by a diagnostic statement that granulomatous inflammation is present, and acid-fast bacilli are seen on the Ziehl-Neelsen stain. Other examples include a large cell lymphoma present and the case is categorized as malignant and correlation with FC is required, or metastatic carcinoma is present, and the case is categorized as malignant.

The second diagnostic level, if achievable, provides additional information and identification of specific entities by utilizing ancillary testing. The goal of the second diagnostic level is to:

- *identify* specific benign entities, such as specific infections for example, using PCR, culture, and drug sensitivity results to confirm a mycobacterial infection or by ICC or ISH for EBV infection
- *diagnose* specific NHL entities as listed in the current WHO hematopathology classification using FC for B-cell monoclonality and other specific markers; for example, the diagnosis of mantle cell lymphoma is supported by CD5+ B-cell lineage, best shown by FC, combined with cyclin D1 and/or SOX11 positivity shown by ICC
- *diagnose* the site of origin of a primary tumor in LN metastases and any possible prognostic or treatment markers; for example, the estrogen and progesterone receptors and HER2 status in metastatic breast carcinoma

When the combination of the cytomorphology and ancillary testing findings allows the cytopathologist to achieve the second diagnostic level, corresponding findings should be reported in one final integrated cytopathology report with the diagnosed specific entity. The criteria for the first and second diagnostic levels and management recommendations for LN-FNAC are summarized in Table 5 [38, 41, 43–174].

LN-FNAC Report

The LN-FNAC report should be composed of different sections that summarize the entire procedure. The report should include: patient demographics, referring doctor, the target site, clinical and US features, a description of the LN-FNAC procedure (the needle gauge, guidance-type, number of passes, number of prepared smears, fixation and staining, and the management and allotment of material for ancillary tests). The report should provide one of the five first-level diagnostic categories followed by a clear cytomorphological description focusing on key cy-

Table 5. 2017 WHO classification of mature lymphoid, histiocytic and dendritic neoplasms identifiable by LN-FNAC

Mature B-cell neoplasms [38, 43–50]
Chronic lymphocytic leukemia/small lymphocytic lymphoma [38, 43–51]
Monoclonal B-cell lymphocytosis, new entity
B-cell prolymphocytic leukemia
Splenic marginal zone lymphoma
Hairy cell leukemia [52–55]
Splenic B-cell lymphoma/leukemia, unclassifiable, provisional entity
Splenic diffuse red pulp small B-cell lymphoma, provisional entity
Hairy cell leukemia-variant, provisional entity
Lymphoplasmacytic lymphoma [38, 43–50]
Waldenström macroglobulinemia
Monoclonal gammopathy of undetermined significance (MGUS), immunoglobulin, new entity
μ heavy-chain disease, γ heavy-chain disease, α heavy-chain disease
MGUS, immunoglobulin G/A, provisional/new entity
Plasma cell myeloma [56]
Extrasseous plasmacytoma [56, 60–68]
Monoclonal immunoglobulin deposition diseases, new entity
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue [69–72]
Nodal marginal zone lymphoma [38, 43–50, 73–75]
Pediatric nodal marginal zone lymphoma, provisional entity
Follicular lymphoma [38, 43–50, 76–78]
In situ follicular neoplasia
Duodenal-type follicular lymphoma, new entity
Pediatric-type follicular lymphoma, new entity
Large B-cell lymphoma with IRF4 rearrangement, provisional/new entity
Primary cutaneous follicle center lymphoma
Mantle cell lymphoma [38, 43–50, 79, 80]
In situ mantle cell neoplasia, new entity
Diffuse large B-cell lymphoma (DLBCL), NOS [38, 43–50, 81]
Germinal center B-cell type, new entity [81]
Activated B-cell type, new entity [81]
T-cell/histiocyte-rich large B-cell lymphoma [82]
Primary cutaneous DLBCL, leg type [83]
EBV+ DLBCL, NOS, new entity
EBV+ mucocutaneous ulcer, provisional/new entity
DLBCL associated with chronic inflammation, new entity
Lymphomatoid granulomatosis [84, 85]
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK+ large B-cell lymphoma [86, 87]
Plasmablastic lymphoma [88–91]
Primary effusion lymphoma [92–94]
HHV8+ DLBCL, NOS, provisional/new entity
Burkitt lymphoma [38, 43–50, 95–97]
Burkitt-like lymphoma with 11q aberration, provisional/new entity
High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 rearrangements
High-grade B-cell lymphoma, NOS
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma [102]
Mature T and NK neoplasms [99–104]
T-cell prolymphocytic leukemia
T-cell large granular lymphocytic leukemia
Chronic lymphoproliferative disorder of NK cells, provisional/new entity
Aggressive NK-cell leukemia
Systemic EBV+ T-cell lymphoma of childhood – new entity
Hydroa vacciniforme-like lymphoproliferative disorder, new entity
Adult T-cell leukemia/lymphoma [99–104]
Extranodal NK-/T-cell lymphoma, nasal type [99–104]

Table 5 (continued)**Enteropathy-associated T-cell lymphoma [105]**

Monomorphic epitheliotropic intestinal T-cell lymphoma

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract, provisional/new entity

Hepatosplenic T-cell lymphoma [106]**Subcutaneous panniculitis-like T-cell lymphoma [107, 108]****Mycosis fungoides, Sézary syndrome [109, 110]**

Primary cutaneous CD30+ T-cell lymphoproliferative disorders

Lymphomatoid papulosis

Primary cutaneous anaplastic large cell lymphoma

Primary cutaneous $\gamma\delta$ T-cell lymphoma

Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T cell

lymphoma provisional/new entity

Primary cutaneous acral CD8+ T-cell lymphoma, provisional/new entity

Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder, provisional/new entity

Peripheral T-cell lymphoma, NOS [111–113]**Angioimmunoblastic T-cell lymphoma [114, 115]**

Follicular T-cell lymphoma, provisional/new entity

Nodal peripheral T-cell lymphoma with TFH phenotype, provisional/new entity

Anaplastic large-cell lymphoma, ALK+ [116–129]**Anaplastic large-cell lymphoma, ALK– [116–129]****Breast implant-associated anaplastic large-cell lymphoma, provisional/new entity [130–138]****Hodgkin lymphoma [116, 139–152]****Nodular lymphocyte predominant Hodgkin lymphoma [139, 140]****Classical Hodgkin lymphoma [116, 141–149]****Nodular sclerosis classical Hodgkin lymphoma [150–152]**

Lymphocyte-rich classical Hodgkin lymphoma

Mixed cellularity classical Hodgkin lymphoma

Lymphocyte-depleted classical Hodgkin lymphoma

Posttransplant lymphoproliferative disorders (PTLD) [153, 154]

Plasmacytic hyperplasia PTLD

Infectious mononucleosis PTLD

Florid follicular hyperplasia PTLD, new entity

Polymorphic PTLD

Monomorphic PTLD (B- and T-/NK-cell types)

Classical Hodgkin lymphoma PTLD

Histiocytic and dendritic cell neoplasms

Histiocytic sarcoma [155–157]**Langerhans cell histiocytosis [148, 158–164]****Langerhans cell sarcoma [165, 166]**

Indeterminate dendritic cell tumor

Interdigitating dendritic cell sarcoma [166–169]**Follicular dendritic cell sarcoma [170–173]**

Disseminated juvenile xanthogranuloma

Adapted from Swerdlow et al. [1]. Entities identifiable by FNAC are reported in bold with corresponding references.

topathological components that are diagnostic for specific lesions, if present, the ancillary tests that are underway, if any, or required to enhance the diagnosis, and a summary conclusion. When cytomorphological features correlated with subsequent ancillary test results are diagnostic of a specific pathological entity, including specific types of reactive LAP and lymphadenitis, specific infec-

tion, NHL, HL, and metastases, a final report and conclusion can be issued as a second diagnostic level in one all-encompassing report. This report may include prognostic and therapy-related predictive markers, for example, the EGFR status for an LN metastatic lung adenocarcinoma. The ancillary test results should be presented in the final report even if they are non-contributory and do not

Table 6. LN-FNAC: report

LN-FNAC issues	Procedures	Course of action
LN-FNAC report elements	Clinical data, site, imaging (US/CT) features	Recommended
	Procedure description: G-needle, guide, number of passes, ROSE, method, sample type(s), processing, staining	Suggested
	Basic diagnostic class (L1-L5)	Recommended
	Microscopic description, ancillary technique/s used	Suggested
	Secondary diagnosis or specific subtyping (if any)	Suggested
	Sample suitable (or not) for further studies (ICC, molecular) for predictive markers (possibly % content of the tumor)	Recommended
	Recommendations (follow-up for reactive, repetition-biopsy for undetermined/suspicious, biopsy for first diagnosis HL-NHL, and undetermined metastases)	Suggested
	Notes	If necessary

advance the cytomorphological diagnosis. Further recommendations based on the final report also can be made for management options. This information is summarized in Table 6.

Conclusion

The authors believe that the introduction of this standardized system for reporting for LN-FNAC may improve the quality of the procedure, the handling of material for diagnostic ancillary testing, and the understanding of the report and communication between the cytopathologist and the clinician, thereby improving patient care. This system may lead to a greater acceptance and utilization of LN-FNAC as a valuable, minimally invasive diagnostic method by all clinical disciplines, and will enable, in analogy to the Bethesda System for Reporting Thyroid FNAB Cytopathology [174], a better interdisciplinary understanding of the results of this procedure.

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Statement of Ethics

This study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

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