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Received: 5 February 2023 | Revised: 20 March 2023 | Accepted: 10 April 2023

DOI: 10.1002/cncy.22741

ORIGINAL ARTICLE

Digital Examination of LYmph node CYtopathology Using the Sydney system (DELYCYUS). An international, multi-institutional study

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Abstract

Background: After a series of standardized reporting systems in cytopathology, the Sydney system was recently introduced to address the need for reproducibility and standardization in lymph node cytopathology. Since then, the risk of malignancy for the categories of the Sydney system has been explored by several studies, but no studies have yet examined the interobserver reproducibility of the Sydney system.

Methods: The authors assessed interobserver reproducibility of the Sydney system on 85 lymph node fine-needle aspiration cytology cases reviewed by 15 cytopathologists from 12 institutions in eight different countries, resulting in 1275 diagnoses. In total, 186 slides stained with Diff-Quik, Papanicolaou, and immunocytochemistry were scanned. A subset of the cases included clinical data and results from ultrasound examinations, flow cytometry immunophenotyping, and fluorescence in situ hybridization analysis. The study participants assessed the cases digitally using whole-slide images.

Results: Overall, the authors observed an almost perfect agreement of cytopathologists with the ground truth (median weighted Cohen κ = 0.887; interquartile range, κ = 0.210) and moderate overall interobserver concordance (Fleiss κ = 0.476). There was substantial agreement for the *inadequate* and *malignant* categories (κ = 0.794 and κ = 0.729, respectively), moderate agreement for the *benign* category (κ = 0.490), and very slight agreement for the *suspicious* (κ = 0.104) and *atypical* (κ = 0.075) categories.

Conclusions: The Sydney system for reporting lymph node cytopathology shows adequate interobserver concordance. Digital microscopy is an adequate means to assess lymph node cytopathology specimens.

KEYWORDS
digital cytopathology, lymph node, reproducibility, virtual microscopy, whole-slide imaging

INTRODUCTION

Lymph node fine-needle aspiration cytology (LN-FNAC) is an inexpensive, effective, and safe technique for the assessment of lymphadenopathy.[1–4] However, LN-FNAC has not yet been widely accepted, and its adoption is still controversial.[5,6] Recently, a proposal for the performance, classification, and reporting of LN-FNAC was published: the Sydney system.[7] Since its publication, several studies have applied the Sydney system to retrospective case series[8–13] calculating the risk of malignancy of the proposed categories. To date, however, no studies have assessed the reproducibility of the Sydney system. This knowledge gap is explained, in part, by the fact that large-scale interobserver reproducibility studies are hard to realize and because, traditionally, large numbers of glass slides have to be circulated among a large group of pathologists. Alternatively, the pathologists participating in the study need to move to analyze the slides. By using either approach, there are appreciable costs and risks (e.g., slide damage) proportional to the size of the group.

Telepathology, a subset of digital pathology (DP), is a technique that leverages whole-slide images (WSIs) to overcome these problems. As noted by others, moving an image around is often cheaper and easier than moving a patient or a pathologist.[14] However, no studies have explored the applicability of DP or telepathology to LN-FNAC to date, with the exception of some anecdotal evidence in veterinary pathology.[15,16]

The aim of this study was to evaluate the reproducibility of diagnoses rendered according to the Sydney system on WSIs of LN-FNAC by a large international group of cytopathologists. At the same time, the applicability of DP to LN-FNAC could be evaluated. This study was approved by the Comitato Etico Campania Sud (determination number 2022-156).

MATERIALS AND METHODS

Case selection

The overall structure of the current study is illustrated in Figure 1. Eighty-five LN-FNAC cases were extracted from the archives of the Department of Pathology of the University Hospital of Salerno. All LN-FNACs were performed using a 23-gauge needle attached to a 10-mL syringe that is used to apply negative pressure. The material was smeared directly onto glass slides, which were air-dried, stained with a modified Romanowsky-type stain (Diff-Quik; Bio-Optica), and subjected to rapid on-site evaluation (ROSE). Depending on the ROSE results, more passes were performed to obtain material for additional smears or for ancillary techniques. Immunocytochemistry (ICC) was performed in most cases on alcohol-fixed, direct smears (Figure 2) using an automated platform (Ventana Benchmark Ultra; Ventana Medical Systems), manufacturer-provided reagents, and dedicated protocols for cytology slides. In a minority of cases from the current series, ICC was performed on a cell block (Figure 3). Cell blocks are prepared by fixing aspirated material at the time of LN-FNAC in formalin. After centrifugation, the pellet is processed using Cytoblock (Thermo Fisher Scientific) reagents and instruments according to the manufacturer's instructions. Immunohistochemistry on cell-block sections is performed on the same instrument (Ventana Benchmark Ultra) using standard protocols for formalin-fixed, paraffin-embedded tissue.[17]

The slides of the selected cases, together with the associated clinical data, were retrieved and reviewed by two experienced cytopathologists (A.C., P.Z.). Diagnoses were formulated according to the Sydney system. Disagreements were resolved either by a discussion between the two cytopathologists or by means of a consultation with a third experienced cytopathologist (I.C.).

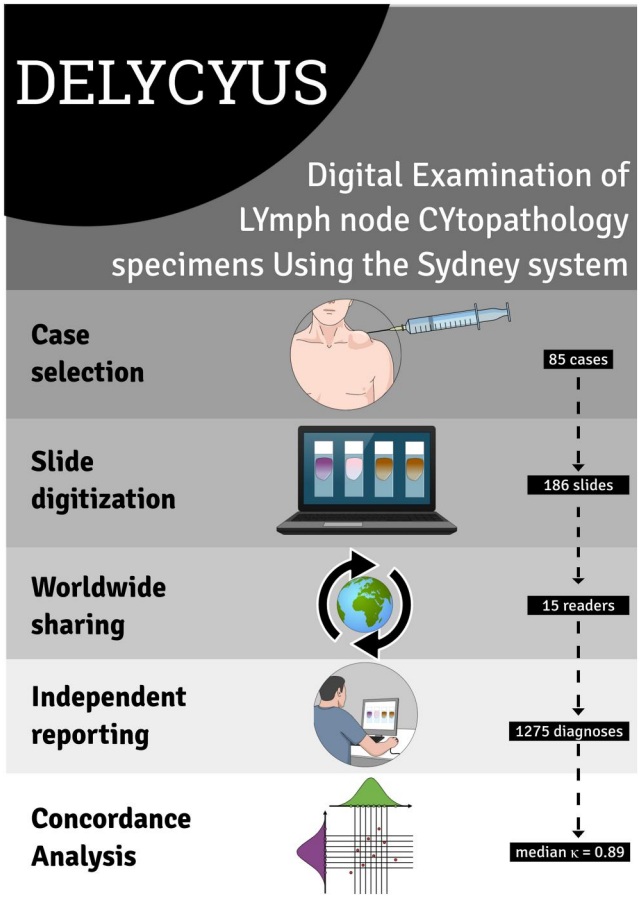

FIGURE 1 Graphical abstract summarizing the current work.

One representative Diff-Quik–stained slide and one Papanicolaou-stained slide, when available, were selected for scanning from the multiple direct smears from each case. All ICC slides both on smears and cell-block sections, when available, were scanned. Hematoxylin and eosin-stained sections from cell blocks were not scanned.

Case assembly

Each selected study case was assigned a random numerical pseudonym used only for the purposes of the current study. Selected slides were gently wiped with a gauze dampened with alcohol to remove any dust, fingerprints, and stains; then, they were scanned using a Ventana DP200 slide scanner (Ventana Medical Systems) at ×40 magnification (final resolution, 0.23 µm/pixel) on a single-focus plane using extended focus.[18] Before scanning, the area of interest was manually adjusted when necessary to include all of the smeared area. In all cases, slide labels were not included in the final image. After scanning, the quality of the WSI was assessed to ensure that most of the slide was in focus and that diagnostic details were evident. When the quality was deemed unsatisfactory, an attempt was made to re-scan with the same settings but changing the focus point. For rare cases in which this effort was insufficient, multiple focal planes (z-stacking) were used.

The slides were uploaded to the uPath image management system (Ventana Medical Systems) for sharing and examination by the

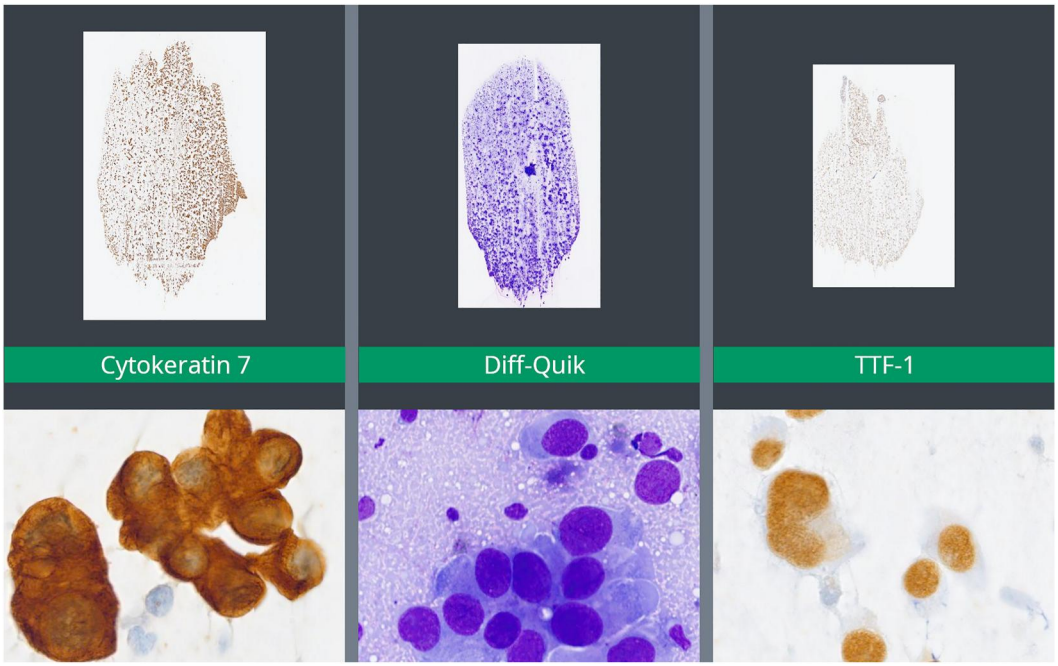

FIGURE 2 Lymph node fine-needle aspiration cytology specimen of a metastatic lung adenocarcinoma demonstrating immunocytochemistry on direct smears. Top: overview of the three digitized smears: one stained with Diff-Quik and two stained with immunocytochemistry on alcohol-fixed, direct smears. Bottom: representative areas from each slide showing typical morphology [center] accompanied by strong cytoplasmic positivity for cytokeratin 7 [left] and nuclear positivity for TTF-1 [right]. Internal negative controls are present in both cases(original magnification: ×0.50 [top] and ×400 [bottom]).

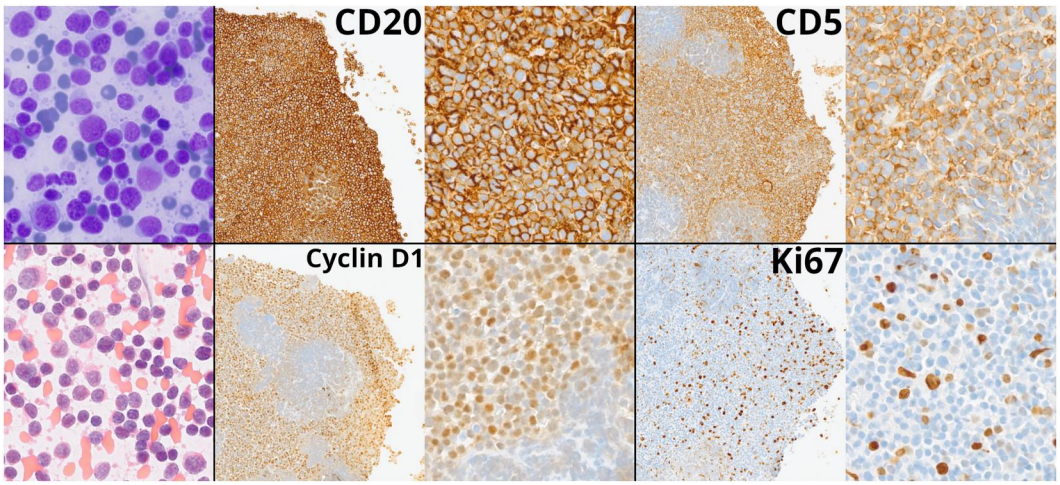

FIGURE 3 Lymph node fine-needle aspiration cytology specimen of a mantle cell lymphoma demonstrating immunocytochemistry on cell-block sections. (*Left*) Both images show typical mantle cell lymphoma morphology with small to medium-sized cells that have round/oval nuclei and a rim of cytoplasm. (*Middle and Right*) Immunocytochemistry on cell-block sections reveals positivity for CD20, CD5, and cyclin D1 and a relatively low proliferation index assessed by Ki67 (left: Diff-Quik stain [top] and Papanicolaou stain [bottom]; original magnification ×400; middle and right: diaminobenzidine immunocytochemistry; original magnification ×100 [middle] and ×400 [right]).

cytopathologists. Cases were identified using their random numerical pseudonym. A spreadsheet was compiled to link each case with the respective clinical data (age, sex, site and size of the lymph node, relevant clinical history, and ancillary techniques, such as flow cytometry and fluorescence in-situ hybridization, if performed).

Because the main focus of the current study was the assessment of diagnostic concordance of the Sydney system, only clinical history up to the point of LN-FNAC was shared with the readers. Hence, the readers were blinded to information obtained from clinical follow-up or subsequent histology that happened after the LN-FNAC. However, all cases in the current series that represented first diagnoses of lymphoma as well as some lymphoma relapses and metastases were subsequently histologically confirmed. Other cases for which the cytologic diagnosis was considered sufficient by the treating clinician were clinically followed and confirmed. These included most benign cases as well as recurrences of known lymphoma and metastases of known primary tumors.

Readers

Fifteen cytopathologists from 12 different institutions in eight different countries analyzed and scored all the 85 LN-FNAC cases, totaling 1275 diagnoses. Each cytopathologist diagnosed the cases independently and blindly, with only the clinical data shared in the spreadsheet and using their own personal computers. Each case was assigned to one of the five categories of the Sydney system (*inadequate*, *benign*, *atypical*, *suspicious*, or *malignant*), and authors were offered a field to report an optional second-level diagnosis, which could include the specific nosological entity, such as tuberculous lymphadenitis, mantle cell lymphoma, or breast cancer metastasis. To avoid recall by the pathologists who were involved in the case assembly, who had examined of at least some of the original glass slides (A.C., P.Z., I.C.), a washout period of 3 months was enforced between the assembly of the case series and the evaluation of WSIs by these pathologists. The printed labels of the glass slides were not visible in the WSIs, which were identified by means of a random numerical pseudonym.

Statistical analysis

Concordance of each cytopathologist with the ground-truth diagnosis was calculated using the Cohen κ with squared weights. The Fleiss κ was used to measure the interobserver concordance and the category-wise κ.[19] All statistical analyses were performed using R version 4.2.2 (R Foundation for Statistical Computing). Statistical significance was set at the $p = .05$ threshold.

RESULTS

Case series

The final case set included 186 slides from 85 LN-FNAC cases. Of these slides, 87 were stained with Diff-Quik, 53 were stained with Papanicolaou, and 46 were ICC slides. The vast majority of the slides (n = 185; 99.5%) produced WSIs of satisfactory quality without z-stacking; one slide (0.54%) required z-stacking. Forty patients (47%) were male and 45 (53%) were female, and their ages at the time of FNAC ranged from 12 to 88 years (average ± standard deviation, 52.0 ± 17.9 years). The ground-truth diagnoses included insufficient/inadequate ($n = 1$), benign ($n = 41$), atypical ($n = 2$), suspicious ($n = 1$), and malignant ($n = 40$). Overall, at least one ancillary technique was available in 70 cases (82%); in detail, ancillary techniques included ICC ($n = 23$ cases; 27%), flow cytometry ($n = 58$; 68%), and

fluorescence in-situ hybridization (n = 3; 3.5%). Of the 23 cases in which ICC was performed, 17 (74%) were hematolymphoid malignancies, and six (26%) were metastases of carcinoma (n = 5) or melanoma (n = 1). Some cases were assessed using multiple ancillary techniques. Relevant clinical data, such as history of neoplasia or presence of symptoms, were available in 35 cases (41%). In particular, 18 cases (21.2%) had a history of neoplasia, and nine of these were then identified as recurrences of the previous neoplasm (four follicular lymphomas, one diffuse large B-cell lymphoma, one multiple myeloma, one Burkitt lymphoma, 1 mantle cell lymphoma, and one Merkel cell carcinoma). The other nine cases, despite a history of neoplasia, were identified as benign (reactive) in six cases, inadequate in one case, or the site of a new neoplasm unrelated to the previous neoplasm in two cases (a lung adenocarcinoma in a patient with a history of breast cancer and a follicular lymphoma in a patient with a history of small lymphocytic lymphoma/chronic lymphocytic leukemia. The sex and age were known in all cases.

The site of the biopsied lymph node was known in all cases: cervical (n = 25; 29%), axillary (n = 17; 20%), submandibular (n = 14; 16%), inguinal (n = 13; 15%), and supraclavicular (n = 12; 14%) lymph nodes were aspirated as well as intraparotid nodes (n = 2; 2.3%), a soft tissue swelling of the thigh (n = 1; 1.1%), and a peribronchial lymph node (n = 1; 1.1%). The lymph node size (greatest dimension) was known in 67 cases (78%) and ranged from 8 to 60 mm (average ± standard deviation, 24.69 ± 8.17 mm). The ultrasound features of the biopsied lymph node, including shape, echogenicity, structure, and hilum status, were available in 53 case (62%). The ground-truth, first-level diagnoses are shown in Table 1, and the second-level diagnoses are shown in Table 2.

Interobserver agreement

The chance-corrected agreement for each cytopathologist against the ground truth (weighted Cohen κ), on average, was almost perfect (median, κ = 0.887; interquartile range, κ = 0.210).

The overall interobserver concordance was moderate (Fleiss κ = 0.476). Category-wise analysis (Fleiss κ; Table 3) revealed higher agreement for the inadequate, benign, and malignant categories and much less agreement for the atypical and suspicious categories (p < .0001 for all).

TABLE 1 The distribution of first-level diagnoses (ground-truth).

First-level diagnosis	No.
Inadequate	1
Benign	41
Atypical	2
Suspicious	1
Malignant	40
Total	85

Discordances

The overall magnitude of observed discordances is shown in Table 4. Most diagnoses were fully concordant (967 of 1275 cases; 75.8%). The magnitude of discordance was one category in 174 cases (13.6%), two categories in 90 cases (7.06%), and three categories in 44 cases (3.45%).

The distribution of diagnosis for each ground-truth category is illustrated in Figure 4. The single inadequate case was diagnosed by all cytopathologists as such. Most benign cases were so diagnosed (n =417 of 615; 67.8%) but showed a relatively high rate of discordance, with 198 (32.1%) discordant diagnoses overall, of which 98 (15.9%) were diagnosed as suspicious or malignant (Figure 4). Table 5 summarizes the benign cases with the lowest and highest discordance. The few atypical (n =2) and suspicious (n = 1) cases showed a

TABLE 2 The distribution of second-level diagnoses.

Second-level diagnosis	No.
Benign	32
Follicular lymphoma	10
Benign, granulomatous	6
Mantle cell lymphoma	4
Burkitt lymphoma	3
Hodgkin lymphoma	3
Diffuse large B-cell lymphoma	3
Small lymphocytic lymphoma/CLL	4
Multiple myeloma	2
Metastasis, breast cancer	2
Metastasis, lung adenocarcinoma	2
Suspicious for non-Hodgkin lymphoma	1
Anaplastic non-Hodgkin lymphoma, ALK-negative	1
T-cell acute lymphoblastic leukemia/LBL	1
Inadequate	1
Benign, suppurative	1
Metastasis, small cell lung cancer	1
Metastasis, melanoma	1
Mantle-zone lymphoma	1
Peripheral T-cell lymphoma	1
Benign, granulomatous, necrotizing	1
Metastasis, Merkel cell carcinoma	1
Atypical, polymorphous, excess of large cells	1
Atypical, polymorphous, excess of small cells	1
Benign, granulomatous, and suppurative	1
Total	85

Abbreviations: ALK, anaplastic lymphoma kinase; CLL, chronic lymphocytic leukemia; LBL, lymphoblastic leukemia.

relatively wide distribution. Finally, malignant cases were diagnosed as such in 517 of 600 cases (86.2%) and as either suspicious or malignant in 567 of 600 cases (94.5%). In 21 cases (3.50%), malignant cases were diagnosed as atypical; and, in 12 cases (2.00%), malignant cases were diagnosed as benign. Table 6 summarizes the malignant cases with the lowest and highest discordance.

TABLE 3 Category-wise interobserver concordance for each of the five categories of the Sydney system.

Sydney category	Fleiss κ
Inadequate	0.794
Benign	0.490
Atypical	0.075
Suspicious	0.104
Malignant	0.729

TABLE 4 Distribution by magnitude of the diagnostic discordances.

Delta	No.	Percentage	Cumulative percentage
0	967	75.8	75.8
1	174	13.6	89.4
2	90	7.06	96.5
3	44	3.45	100.0

Note: Delta indicates the difference between each of the 1275 diagnoses and the ground truth (e.g., 0 indicates perfect concordance, and 1 indicates a one-step disagreement, such as benign vs. atypical).

DISCUSSION

Reproducibility is an essential virtue of any classification system. Perfect intraobserver and interobserver reproducibility are seldom achieved, but assessment is instrumental to set a baseline and to identify and resolve systematic problems.[20,21]

In this study, we observed almost perfect concordance (median, κ = 0.887, interquartile range, κ = 0.210) among a group of 15 cytopathologists examining 85 LN-FNAC cases. Literature is scarce on interobserver agreement in LN-FNACs, but other studies have observed comparable or slightly worse values.[22–24] It is conceivable that use of the Sydney system enhances interobserver agreement by providing a uniform terminology and clear criteria for classification. The rates of agreement observed are in line with those reported using other classification systems.[21,25]

We observed substantial agreement for the inadequate and malignant categories (κ = 0.794 and κ = 0.729, respectively), moderate agreement for the benign category (κ = 0.490), and very slight agreement for the suspicious (κ = 0.104) and atypical (κ = 0.075) categories. The lower agreement for atypical and suspicious categories was expected because these cases represent a gray zone in which the diagnostic confidence is insufficient to render a clear-cut diagnosis of benign or malignant. The Sydney system suggests using these categories when morphology is ambiguous and ancillary

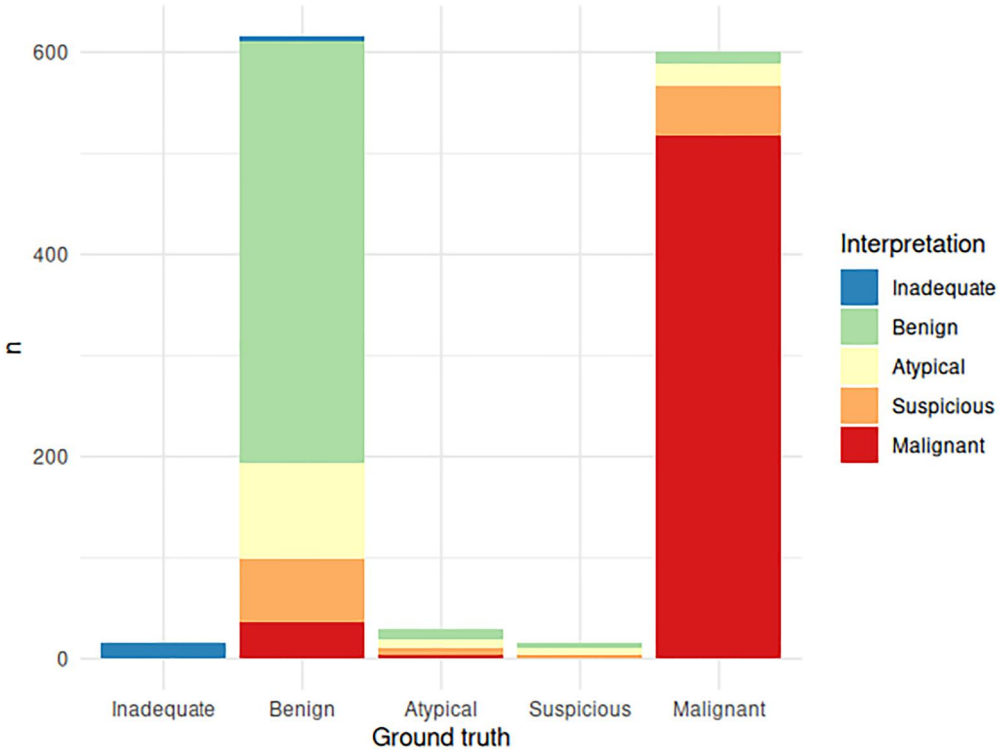

FIGURE 4 Bar plot showing the cumulative distribution of diagnoses rendered by all readers for each ground-truth category.

TABLE 5 Details of a sample of benign cases with the lowest (cases 1–5) and highest (cases 6–10) discordance.

			Distribution of diagnoses, No. (%)				
Case	Ground truth	Follow-up	Benign	Atypical	Suspicious	Malignant	Helping factors
1	Benign	Clinical	15 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	History of cat bite
2	Benign, granulomatous	Clinical, tuberculosis	14 (93.0)	1 (7.0)	0 (0.0)	0 (0.0)	History of tuberculosis
3	Benign, granulomatous	Clinical	13 (87.0)	2 (13.0)	0 (0.0)	0 (0.0)	
4	Benign, granulomatous	Clinical, tuberculosis	13 (87.0)	2 (13.0)	0 (0.0)	0 (0.0)	Sub-Saharan immigrant
5	Benign, granulomatous	Clinical	13 (87.0)	2 (13.0)	0 (0.0)	0 (0.0)	
			Distribution of diagnoses, No. (%)				
Case	Ground truth	Follow-up	Benign	Atypical	Suspicious	Malignant	Misleading factors
6	Benign	Clinical, regressed	10 (67.0)	0 (0.0)	3 (20.0)	2 (13.0)	Florid follicular hyperplasia
7	Benign	Clinical, regressed	9 (60.0)	2 (13.0)	1 (7.0)	3 (20.0)	Florid follicular hyperplasia
8	Benign	Clinical, regressed	9 (60.0)	2 (13.0)	2 (13.0)	2 (13.0)	Polyadenopathy
9	Benign	Clinical, regressed	7 (47.0)	1 (7.0)	3 (20.0)	4 (27.0)	Clinical concern for metastasis
10	Benign	Mononucleosis	6 (40.0)	4 (27.0)	3 (20.0)	2 (13.0)	Excess immunoblasts

TABLE 6 Details of a sample of malignant cases with the lowest (cases 1–5) and highest (cases 6–10) discordance.

			Distribution of diagnoses, No. (%)				
Case	Ground truth	Follow-up	Benign	Atypical	Suspicious	Malignant	Helping factors
1	Malignant, Burkitt lymphoma	Confirmed	0 (0.0)	0 (0.0)	0 (0.0)	15 (100.0)	ICC, FC, FISH, history of BL
2	Malignant, mantle cell lymphoma	Confirmed	0 (0.0)	0 (0.0)	0 (0.0)	15 (100.0)	ICC, FC
3	Malignant, SLL/CLL	Confirmed	0 (0.0)	0 (0.0)	0 (0.0)	15 (100.0)	FC
4	Malignant; metastasis, melanoma	Confirmed	0 (0.0)	0 (0.0)	0 (0.0)	15 (100.0)	ICC, no history
5	Malignant; metastasis, lung adenocarcinoma	Confirmed	0 (0.0)	0 (0.0)	0 (0.0)	15 (100.0)	Supraclavicular LN, ICC; no history
			Distribution of diagnoses, No. (%)				
Case	Ground truth	Follow-up	Benign	Atypical	Suspicious	Malignant	Misleading factors
6	Malignant; metastasis, breast	Clinical	0 (0.0)	1 (7.0)	0 (0.0)	14 (93.0)	No history, limited ICC
7	Malignant, DLBCL	Clinical	0 (0.0)	1 (7.0)	5 (33.0)	9 (60.0)	FC, noncontributory
8	Malignant, HL	cHL	0 (0.0)	4 (27.0)	3 (20.0)	8 (53.0)	No ancillary techniques
9	Malignant, HL	NLPHL	3 (20.0)	3 (20.0)	5 (33.0)	4 (27.0)	Intrinsic difficulty, no ancillary techniques
10	Malignant, FL	FL	2 (13.0)	3 (20.0)	6 (40.0)	4 (27.0)	FC misleading because of partial involvement

Abbreviations: BL, Burkitt lymphoma; cHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; FC, flow cytometry; FISH, fluorescence in-situ hybridization; FL, follicular lymphoma; HL, Hodgkin lymphoma; ICC, immunocytochemistry; LN, lymph node; NLPHL, nodular lymphocyte-predominant Hodgkin lymphoma; SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic leukemia.

techniques are unavailable or noncontributory.[7] In addition, in the current study, we observed that cytopathologists tend to use these categories for subjective reasons because of a lack of confidence when one is not performing ROSE and handling the tissue according to one's own usual practices. Examples include when only a Diff-Quik–stained slide was available and the cytopathologist preferred Papanicolaou staining or when ancillary techniques were performed in a manner different from their routine practice. This parallels what is observed with other cytopathology reporting systems and ultimately reflects the difficulty of assigning discrete categories to cases that often lie on a continuum.[26] Nonetheless, efforts should be made to use the recommendations of the Sydney system to minimize the number of cases diagnosed as atypical or suspicious.

Interestingly, the benign category showed only moderate agreement ($\kappa = 0.490$), whereas the malignant category showed substantial agreement ($\kappa = 0.729$). Some benign cases posed no

difficulty to the readers (Table 5; cases 1–5), who showed nearly perfect concordance. These were mostly cases in which clinical history suggested a specific etiology that matched the observed morphology and ancillary technique results, if any (such as a history of cat bite or of tuberculosis). Instead, when benign cases were misclassified (Figure 4), they were diagnosed as atypical in approximately one half of cases ($n = 96$) and as either suspicious or malignant in the other one half ($n = 62$ and $n = 36$, respectively). Among the five benign cases with the highest discordance (Table 5; cases 6–10), cases 6 and 7 showed florid follicular hyperplasia, cases 8 and 9 had a clinical concern for lymphoma and metastasis (respectively), and case 10 showed an excess of immunoblasts in a large cervical lymph node from a young patient (this case was later diagnosed clinically as infectious mononucleosis).

When benign cases were overdiagnosed, common confounders included overinterpretation of epithelioid histiocytes (both dispersed and in granulomas) as metastatic carcinoma cells and misinterpretation of reactive lymphoid patterns, such as florid follicular hyperplasia and excess centroblasts or immunoblasts, as malignant lymphomas. These discordances occurred especially in cases for which the clinical setting was concerning or unknown, when only one slide was available, and/or if ancillary techniques were not performed. Furthermore, a subset of readers was mostly responsible for these overinterpretations, suggesting a role for reader-related factors.

Distinguishing florid follicular hyperplasia from follicular lymphoma is a known pitfall in the assessment of LN-FNAC specimens because the cytopathologic aspects of these two entities overlap considerably. Hence, flow cytometry is an indispensable tool for reaching the correct diagnosis in such cases (Figures 5 and 6).[7,27] When ROSE is performed, sufficient material in an adequate medium can be harvested to increase the diagnostic yield of ancillary techniques. Similar issues were encountered when morphologically benign cases that included ancillary techniques consistent with benignancy were misclassified as atypical based on clinical features that were equivocal for malignancy, such as a very large lymph node, an elderly patient, or the presence of abnormal clinical symptoms or of a history of malignancy. In these examples, should the correct diagnostic category be benign or atypical? In other words, should the assigned Sydney category reflect exclusively the morphologic and ancillary data, or should clinical features be taken into account? The possibility of LN-FNAC sampling error, partial lymph node involvement, and other explanations for false-negative results should be carefully considered when there is a high clinical (pretest) probability of malignancy. In the current study, some cytopathologists did indeed classify these cases as atypical, often capturing and summarizing the problem by mentioning something along the lines of *cannot exclude malignancy*. Conversely, the Sydney proposal[7] suggests downgrading morphologically atypical cases to benign if the results of ancillary techniques support a benign diagnosis. Ultimately, the Sydney system, like all other reporting systems in cytopathology, is an attempt to improve communication between the cytopathologist and clinician; and, in these cases, regardless of the cytopathologic diagnostic category, the clinician needs to recognize the importance of clinical and imaging findings that may increase the risk of malignancy in an individual patient and manage the patient accordingly with repeat FNAC or other biopsy modalities.

In contrast with these more difficult scenarios, LN-FNAC provides an immediate diagnosis in the majority of cases (Figures 7 and 8). For example, LN-FNAC can quickly diagnose metastases with high specificity and positive predictive value.[28–30] In the current series, the seven metastatic cases were all diagnosed correctly by all cytopathologists (100%), who, in most cases, were able to use the provided ICC slides to identify the primary tumor ($n = 2$ breast adenocarcinomas, $n = 2$ lung adenocarcinomas, $n = 1$ melanoma, $n = 1$ Merkel cell carcinoma, $n = 1$ small cell lung cancer). It should be

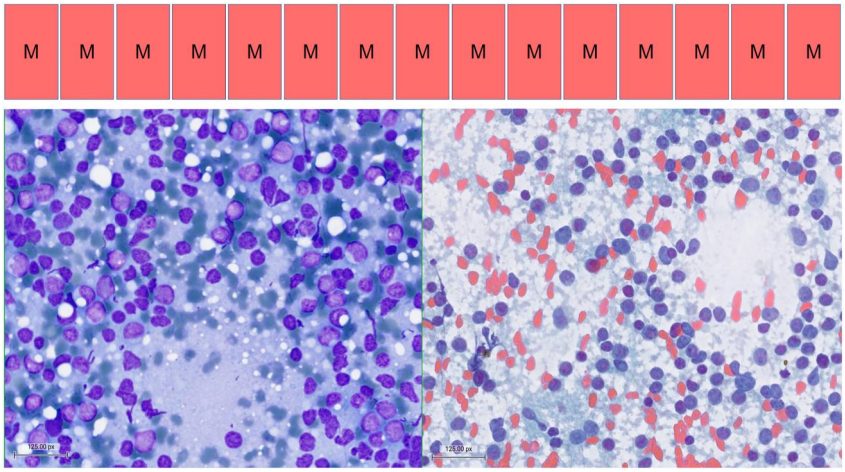

FIGURE 5 A case of follicular lymphoma that was diagnosed as malignant by all 15 readers. (*Bottom*) Cytomorphology shows a prevalence of centrofollicular cells, and flow cytometry confirmed CD10–CD19 coexpression and light chain restriction (Diff-Quik stain [left] and Papanicolaou stain [right]; original magnification ×400 [left and right]). M indicates malignant.

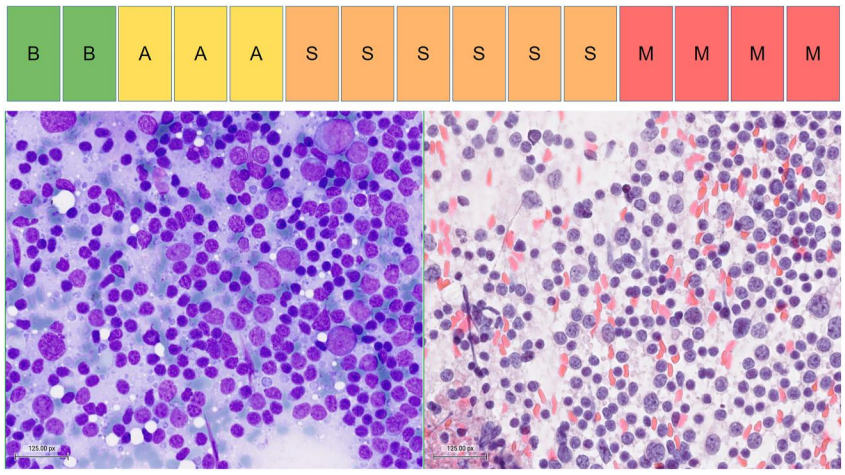

FIGURE 6 A case of follicular lymphoma that showed very discordant interpretations among the readers. (*Bottom*) Cytomorphology shows an excess of centrofollicular cells, but flow cytometry was consistent with a benign reactive process. (Top) This particular case was diagnosed as benign by two readers, as atypical by three readers, as suspicious by six readers, and as malignant by four readers. Histology confirmed the diagnosis of follicular lymphoma (Diff-Quik stain [left] and Papanicolaou stain [right]; original magnification ×400 [left and right]). A indicates atypical; B, benign; M, malignant; S, suspicious.

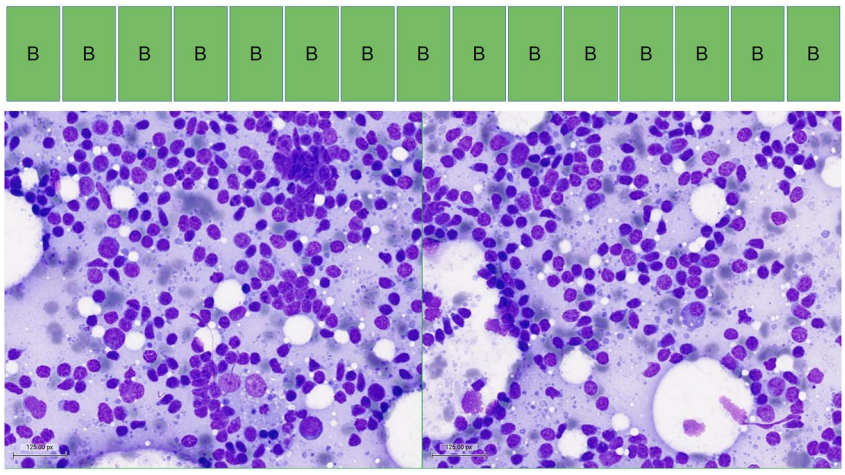

FIGURE 7 A case of reactive hyperplasia that was diagnosed as benign by all 15 readers. Cytomorphology shows a polymorphous lymphoid population with small lymphocytes, centrofollicular elements, plasma cells, and granulocytes. Flow cytometry was consistent with a reactive process (Diff-Quik stain, original magnification ×400 [left and right]). B indicates benign.

noted that LN-FNAC in this setting is also a highly effective tool to harvest material for prognostic and predictive biomarkers.[31,32] The high positive predictive value can be explained by the low risk of false-positive results because of the relative paucity of differential diagnoses in such cases. Conversely, sensitivity and negative predictive value can be improved by using ultrasound guidance and expert cytopathologists, but some inherent pitfalls that preclude perfect concordance must be considered, such as the difficulties encountered by LN-FNAC and core-needle biopsy in the detection of micrometastases. Similarly, when dealing with lymphoproliferative disorders (n = 33), most cases (n = 23; 69.6%) were diagnosed as malignant or suspicious by all 15 readers (100%), and discordances clustered around a minority of cases, such as two classic Hodgkin lymphomas without any ancillary techniques, and a case of follicular lymphoma with ambiguous flow cytometry results (Figure 6). This particular case illustrates the difficulties with confidently diagnosing follicular lymphoma on LN-FNAC alone. These findings are further shown in Table 6, where only a minority of the malignant cases with fully concordant diagnosis by all 15 readers are shown (cases 1–5). Importantly, a clear and positive diagnosis can be rendered even in the absence of clinical history if ancillary techniques are performed and yield unambiguous results. In both cases of metastasis (cases 4 and 5), a positive diagnosis was obtained without any clinical history, prompting the treating physician to search (and find) the malignancy

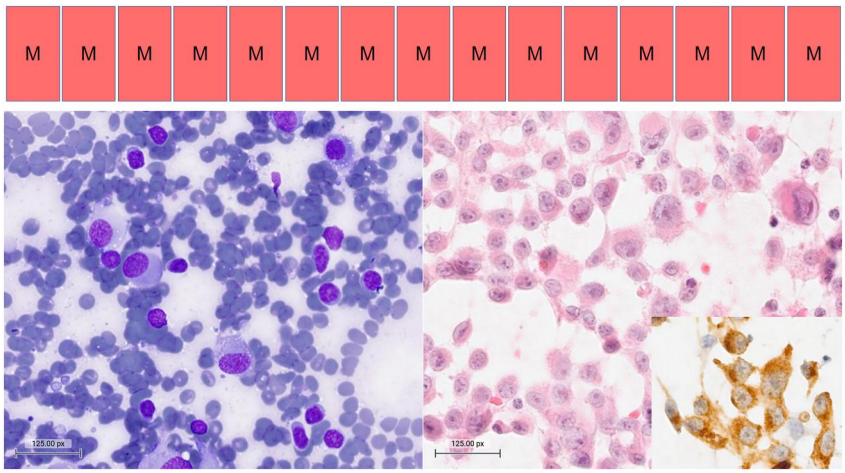

FIGURE 8 A case of metastatic malignant melanoma that was diagnosed as malignant by all 15 readers. Cytomorphology shows large, discohesive cells that have dense cytoplasm and eccentric nuclei with prominent nucleoli. *Inset:* Immunocytochemical positivity for HMB45 confirms the diagnosis (Diff-Quik stain [left], Papanicolaou stain [right], and HMB-45 immunocytochemistry [inset]; original magnification ×400 [left, right, and inset]). M indicates malignant.

reported by the cytopathologist, which was a lung adenocarcinoma in case 4 (also shown in Figure 2) and a melanoma in case 5 (also shown in Figure 8).

Regarding the digital approach based on telecytopathology and WSI, at the scanning phase, only one slide was consistently out of focus and required multiple focus levels (z-stacking) to be diagnostically adequate. This was a Diff-Quik–stained, very thick smear in a case of classic Hodgkin lymphoma. Even in the z-stacked WSI, there were some artifacts, but it was diagnostically adequate. All other slides were adequately represented by a single-level, extended-focus WSI at ×40 magnification (0.23 μm/pixel). After a review of all 1275 diagnoses, in only six (0.47%) did a cytopathologist mention that one of the slides had areas out of focus. It should be noted that, among cytopathologic preparations, direct smears are the hardest to digitize, whereas cell-block sections are easiest thanks to their uniform 4–6 μm thickness, and liquid-based cytopathology slides sit somewhere in between.[18,33] In a systematic review of concordance between WSI and light microscopy across many organ systems, Girolami et al. reported that, in nongynecologic, nonliquid-based cytopathology, as used in the current study, the intraobserver and interobserver agreements were lower than the average of all cytopathology studies.[34] This may be explained by the fact that scanners suffer an inherent difficulty in capturing in-focus details in the z-axis, especially when the thickness of the material on the direct smear increases, for example, in tridimensional tissue fragments or aggregates of cells. Smears from LN-FNAC specimens may be easier to digitize than other smears thanks in part to the dispersed nature of the major cell population of lymphoid cells, which helps limit the overall thickness.[35]

Embracing WSI and digital cytopathology has some important and far-reaching consequences.[36–38] First, telecytology allows pathologists to send a digital slide for a second opinion in a few seconds without risks of damaging or losing the glass slide, which remains safely archived.[34,39,40] It is worth noting that the same digital slides can be sent simultaneously to different consultants. Furthermore, pathologists are not limited to using telecytology solely for diagnostic purposes but can leverage this technology for research, quality assurance (both internal and external), and teaching purposes.

Several advantages of WSI compared with traditional light microscopy are not quantifiable because they represent quantum leaps. In other words, WSIs enable tasks that are otherwise extremely slow or impossible with conventional methods, such as remote reporting and integration with artificial intelligence.[34,41–43] WSI is the gateway to computer-aided diagnosis tools. It is already possible to identify atypical cells automatically and present them to the user separately, before looking at the whole slide.[44] The potential of this tool for speeding up routine workflow is significant,[45] for example, when looking for rare metastatic cells or Reed–Sternberg cells in multiple smears.

Strengths of the current study include the large number of cytopathologists who read the slides and came from different institutions worldwide with different amounts of experience in performing and analyzing LN-FNAC specimens. Furthermore, our case series comes from a real-world setting, is moderately large, and includes numerous instances of common diagnoses. All cases in this study were performed under ultrasound guidance with ROSE. This approach minimizes the rate of inadequate specimens because further passes are immediately performed. In addition, ROSE lowers the rate of atypical and suspicious diagnoses because the need for ancillary techniques is immediately recognized, facilitating the harvesting of additional material with immediate further passes.[27,46,47] Finally, in addition to Diff-Quik–stained and Papanicolaou-stained smears, digitized ICC smears were also provided in many cases to aid interpretation.

Limitations of the current study include that our case series was composed mostly of benign and malignant cases, with only a few inadequate, atypical, and suspicious cases. We believe this did not bias

the participating cytopathologists because they were unaware of the distribution of cases. The readers could always attribute any of the five diagnostic categories to each case. However, larger numbers of inadequate, atypical, and suspicious cases would be required to draw confident conclusions about the performance of cytopathologists when dealing with such cases. In addition, all cases came from a single institution and thus reflect a particular practice pattern. For example, a Diff-Quik smear was always available, whereas a Papanicolaou stain was not always prepared if material was scant. An additional potential limitation is that all patients in the cohort came from the same hospital and the same geographic area, and this might represent a bias or difficulty for cytopathologists who are used to different epidemiologic settings. It is of note that published case series on LN-FNAC using the Sydney system have shown widely varying proportions of malignant cases.[8–11] Furthermore, the numbers of cases and readers were not large enough to adequately assess whether concordance is influenced by case-related variables (such as the presence of clinical information, ultrasound features, or ancillary techniques) or reader-related variables (such as years of experience with LN-FNAC).

In conclusion, readers using the Sydney system for reporting lymph node cytopathology, on average, show high concordance compared with a consensus ground truth. Interobserver concordance is moderate, and intermediate categories (atypical, suspicious) show less concordance than benign and malignant categories. Virtual microscopy (WSI) proved to be an adequate means with which to assess LN-FNAC specimens.

The interpretation of LN-FNAC requires awareness about certain inherent pitfalls that are present in light microscopy and virtual microscopy alike. These pitfalls must be addressed in a systematic manner and require specific training for cytopathologists and effective communication with clinicians.

A final crucial point that might significantly improve the adoption and refinement of the Sydney system is to promote the involvement of hematopathologists, hematologists, and oncologists. Close collaboration with clinicians, surgeons, oncologists, and surgical pathologists was the key to enhancing the adoption of other cytopathology reporting systems, such as The Milan System[48] and The Paris System.[49] Ultimately, consensus should be reached on which diagnostic entities can be routinely diagnosed by LN-FNAC and under what conditions. The integration of LN-FNAC into clinical practice guidelines would finally allow clinicians to treat patients based on the results of LN-FNAC *when it is prudent to do so*.[2,4] This will free LN-FNAC of the burden of being considered a screening procedure to be confirmed by other tests, giving it the role of a diagnostic procedure, which it has earned through decades of experience.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from Roche Diagnostics SpA and valuable help and inputs from Pasquale Alfano, Filippo Masperi, Salvatore Pugliese, and Maria Teresa Majorini. The authors are also indebted to the anonymous reviewers for their precious comments.

CONFLICT OF INTEREST STATEMENT

Alessandro Caputo reports institutional grants/contracts from Roche Diagnostics SpA; personal fees from Roche Diagnostics SpA; and other support from AEQUIP Srl outside the submitted work. Albino Eccher reports personal fees from 3B Medical outside the submitted work. Ilaria Girolami reports support from Roche Diagnostics SpA outside the submitted work. Catarina Eloy reports personal fees from Leica Microsystems Inc. outside the submitted work. Nalini Gupta reports personal fees from PGIMER outside the submitted work. Tala Arar reports support from Alcon Laboratories Inc. outside the submitted work. Oscar Lin reports personal fees from Hologic Inc. and Janssen Biotech and other support from the American Society of Cytopathology outside the submitted work. The remaining authors disclosed no conflicts of interest.

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How to cite this article: Caputo A, Fraggetta F, Cretella P, et al. Digital Examination of LYmph node CYtopathology Using the Sydney system (DELYCYUS). An international, multi-institutional study. *Cancer Cytopathol*. 2023;1-14. doi:10.1002/cncy.22741