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ORIGINAL ARTICLE

Ultrasonography-guided core-needle biopsy of lymphadenopathies suspected of lymphoma: Analysis on diagnostic efficacy and safety of 1000 front-line biopsies in a multicenter Italian study

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Abstract

The reliability and safety of front-line ultrasonography guided core needle biopsy (UG-CNB) performed with specific uniform approach have never been evaluated in a large series of patients with lymphadenopathies suspected of lymphoma. The aim of this study was to assess the overall accuracy of UG-CNB in the lymph node histological diagnosis, using a standard reference based on pathologist consensus, molecular biology, and/or surgery. We retrospectively checked the findings concerning the application of lymph node UG-CNB from four Italian clinical units that routinely utilized 16-gauge diameter modified Menghini needle under power-Doppler ultrasonographic guidance. A data schedule was sent to all centers to investigate the information regarding techniques, results, and complications of lymph node UG-CNB in untreated patients over a 12-year period. Overall, 1000 (superficial target, $n = 750$; deep-seated target, $n = 250$) biopsies have been evaluated in 1000 patients; other 48 biopsies (4.5%), screened in the same period, were excluded because inadequate for a confident histological diagnosis. Most patients were suffering from lymphomas (aggressive B-cell non-Hodgkin lymphoma [aBc-NHL], 309 cases; indolent B-cell [iBc]-NHL, 279 cases; Hodgkin lymphoma [HL], 212 cases; and nodal peripheral T-cell [NPTC]-NHL, 30 cases) and 100 cases from metastatic carcinoma; 70 patients had non-malignant disorders. The majority of CNB results met at least one criterion of the composite reference standard. The overall accuracy of the micro-histological sampling was 97% (95% confidence interval: 95%–98%) for the series. The sensitivity of UG-CNB for the detection of aBc-NHL was 100%, for iBc-NHL 95%, for HL 93%, and for NPTC-NHL 90%, with an

overall false negative rate of 3.3%. The complication rate was low (6% for all complications); no patient suffered from biopsy-related complications of grade >2 according to the Common Terminology Criteria for Adverse Events. Lymph node UG-CNB as mini-invasive diagnostic procedure is effective with minimal risk for the patient.

KEYWORDS
core-needle biopsy, lymphoma diagnosis, ultrasonography

1 | INTRODUCTION

Patients with clinical suspect of lymphoma require a prompt and correct histological diagnosis in order to plan the most accurate therapy scheme.[1] The combination of new generation ultrasonographic and biopsy needle devices as diagnostic procedure for patients with lymphadenopathies has provided the opportunity to improve tissue characterization in several cancer centers.[2–8] Nonetheless, a reluctance to adopt this mini-invasive interventional approach for the diagnosis still exists among many practitioners. This reluctance reflects the mistrust on the ability of ultrasonography guided core needle biopsy (UG-CNB) to fulfill all the required criteria according to the World Health Organization (WHO) of Tumors of Haematopoietic and Lymphoid Tissues for several lymphoproliferative disorders (LPDs).[1] This seems particularly challenging for entities whose recognition relies on peculiar tissue architecture and/or growth patterns or cell types that are hardly identifiable in exiguous tissue sample yielded by UG-CNB biopsy, such as the nodal peripheral T cell (NPTC) lymphoma, and classic or nodular lymphocyte predominant (NLP) Hodgkin lymphoma (HL), which at times are misinterpreted as reactive adenitis,[9] or reactive atypical lymphadenopathies mimicking lymphoma (ALML), such as florid follicular hyperplasia, Castleman disease and/or Kikuchi-Fujimoto adenitis.[9,10]

According to the Lugano classification, CNB under imaging guidance is recommended only when surgical intervention is impracticable and/or in the cases of relapse of lymphomas.[11] However, the existing guidelines do not fit all cases with the same completeness, and do not show the same level of evidence.[11,12] On the other hand, the reported series on the use of UG-CNB for diagnostic purposes involved a small number of patients and even many single-case reports. Nonetheless, information regarding the characteristics of biopsy needle, that is, gauge, length, tip configurations, and sampling mechanisms, are still a matter of opinion among experts.[11,12] Finally, the results of front-line UG-CNB for the lymph nodes suspected of lymphoma were never analyzed in a large population, with respect to the type of sampling, underlying diseases, and complication rate. Thus, such diagnostic approach requires validation in trials based on the above reported characteristics.[13]

Therefore, we undertook a multicenter retrospective study involving some large southern Italy centers with a long-standing specific experience in interventional procedures performed under imaging guidance particularly in the lymphadenopathies suspected of lymphomas. The aim of the study was obtaining definite information on the efficacy and safety of UG-CNB carried out with the top-of-the range tools as the first invasive diagnostic approach in a large cohort of patients. The primary endpoint was the overall accuracy of UG-CNB in the histological diagnosis of lymph nodes, adopting as standard reference a 3-point work-up: (1) consensus among pathologists on samples obtained by UG-CNB, (2) molecular biology tests including polymerase chain reaction [PCR] and/or fluorescence in situ hybridization [FISH] techniques for specific lymphoma subsets, and/or (3) follow-up surgical biopsy.

2 | PATIENTS AND METHODS

2.1 | Study design and oversight

In this retrospective study, a predefined adequate series of 1000 (one in each patient) CNBs had to be reached in order to be representative of the main lymphoma subsets. The period of time used in the study was adjusted to provide this result. We consequently acquired in a 12-year period, from July 2009 to January 2022, consistent detailed information on a large sample size of lymph node UG-CNBs.

This study was conducted in hospital units of tertiary centers in Italy: the Hematology Unit of the Federico II University Medical School of Naples, Pathology Unit of the Federico II University Medical School of Naples, Pathology Unit of the Luigi Vanvitelli University Medical School of Campania, and Pathology Unit of the Salerno University Medical School of Baronissi. In the four Units, UG-CNB was used as a routine mini-invasive practice for lymphadenopathies suspected of harboring a lymphoma.[2–7,14] Data collection forms were sent to all centers requesting several information (Supplemental data S1).[15–17]

2.2 | Participants

All the following criteria had to be fulfilled: (i) de novo superficial or deep-seated lymphadenopathies scanned with power Doppler ultrasonography by using the top-of-the-range equipment, and assessed

retrospectively by investigators on recorded video clips and/or images (see below); (ii) lymph node samples gained by 16-gauge CNB with powered automatic suction and 1.6 mm needle diameter with an ultra-thin tip and wall, under ultrasonographic guidance; (iii) morphological and/or immunohistochemical and/or PCR and/or FISH information (as assessed retrospectively by expert haematopathologists) of the core biopsy (see below); and (iv) information of an accepted diagnostic reference standard. We adopted a standard reference including a 3-point work-up (see above). The CNB histological diagnosis was confirmed on the basis of at least one criterion fulfilled of the 3-point work-up, as already reported (8).

2.3 | Ultrasonographic examinations and CNB procedures

A blind independent review committee (constituted by three expert operators in the field of diagnostic ultrasound: C. G., N. P., R. D. P.)[2–6,14] checked the recorded video clips and/or images, unaware of the results of the other operators, to assess the selected lymph node as biopsy target (interobserver reproducibility of ultrasonographic scans).

All patients were scanned using the same instruments (Supplemental data, 2). CNBs were performed by M. P., E. V., I. C. and/or P. Z. with 16-gauge diameter modified Menghini needle (Supplemental data, 3).[2–7,14]

2.4 | CNB histological sections staining and diagnosis

A blind independent review committee (constituted by three operators with >10 years of experience in haematopathological analysis: G. T., D. R., M. M.)[2–6] checked the lymph node sections of CNB samples, unaware of the results of the other operators (interobserver reproducibility of histologic results).

All lymph node samples obtained by UG-CNB were routinely fixed in formalin and embedded in paraffin (FFEP) and the histologic sections stained with hematoxylin and eosin, and Giemsa. The FFEP slides were then assessed with several tests (Supplemental data, 4). Overall, if CNB samples contained an adequate number of cells with morphologic atypia and evidence of monoclonality they were finally categorized as positive for malignancy while negative for malignancy if the samples contained an adequate number of cells but no evidence of malignancy. All cases of lymphoma were classified according to the current WHO criteria of Tumors of Haematopoietic and Lymphoid Tissues based on the combination of morphologic, immunohistochemical and/or FISH and/or molecular analyses.[1] We explored the histological yield of sampling obtained by UG-CNB in a broad-spectrum of LPDs, including aggressive B-cell non-Hodgkin lymphoma (aBc-NHL), indolent B-cell NHL (iBc-NHL), NPTC-NHL, classic or NLP-HL and, the clinical category of ALML.[1,10] CNB samples were considered inconclusive when morphologic, immunohistochemical, and/or FISH and/or molecular analyses did not allow a final diagnosis.

2.5 | Statistical analyses

The sensitivity, specificity and accuracy of the mini-invasive biopsy for diagnosis were evaluated using standard methods (Supplemental data, 5).

The complications due to UG-CNB were reported according to the Common Terminology Criteria for Adverse Events (CTCAE).

A univariate and multivariate logistic regression analysis was used to examine the factors affecting the histological yield of sampling.

Differences between proportions were compared using the chi-square test. Numerical data are expressed as means ± SD. All statistical analyses were performed using SPSS software (version 18.0 for Windows; SPSS, Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Participants and recruitment

We retrospectively collected data from the clinical charts of 1048 patients who underwent UG-CNB between 1 July 2009 and 1 January 2022 for suspected pathological lymphadenopathies. In total, 1000 cases qualified for the study corresponding to 1000 completed procedures (one for each patient) yielding adequate lymph nodal tissue sampling while 48 cases failed during the screening phase and were excluded (Supplemental data, 6). A consolidated standard for reporting of diagnostic accuracy study diagram summarizes the flow of UG-CNBs and patients through the study in Figure 1. The demographics and clinical characteristics of 1000 analyzed patients, and the details of the procedures are shown in Table 1.

3.2 | Power Doppler ultrasonography findings and core-needle characteristics

Interobserver reproducibility of ultrasonographic and intranodal vascular mapping assessments, and thus selection of biopsy targets was excellent. Of the 1000 videoclips and/or images tested for reproducibility, 950 (95%) were classified identically by the three observers ($r = 0.9$).

Power Doppler ultrasonographic examination and CNB procedure average time was 40 min (range, 30–50 min). Seventy-five percent of patients underwent biopsy in the superficial compartment, and 25% of patients in the deep-seated regions. The lymph node, procedure modality, and sample characteristics are depicted in Table 1.

3.3 | Lymph node etiology results according to the standard reference

According to the standard reference, a total of 830 of 1000 lymphadenopathies were classified as lymphomas (Table 2): aBc-NHL

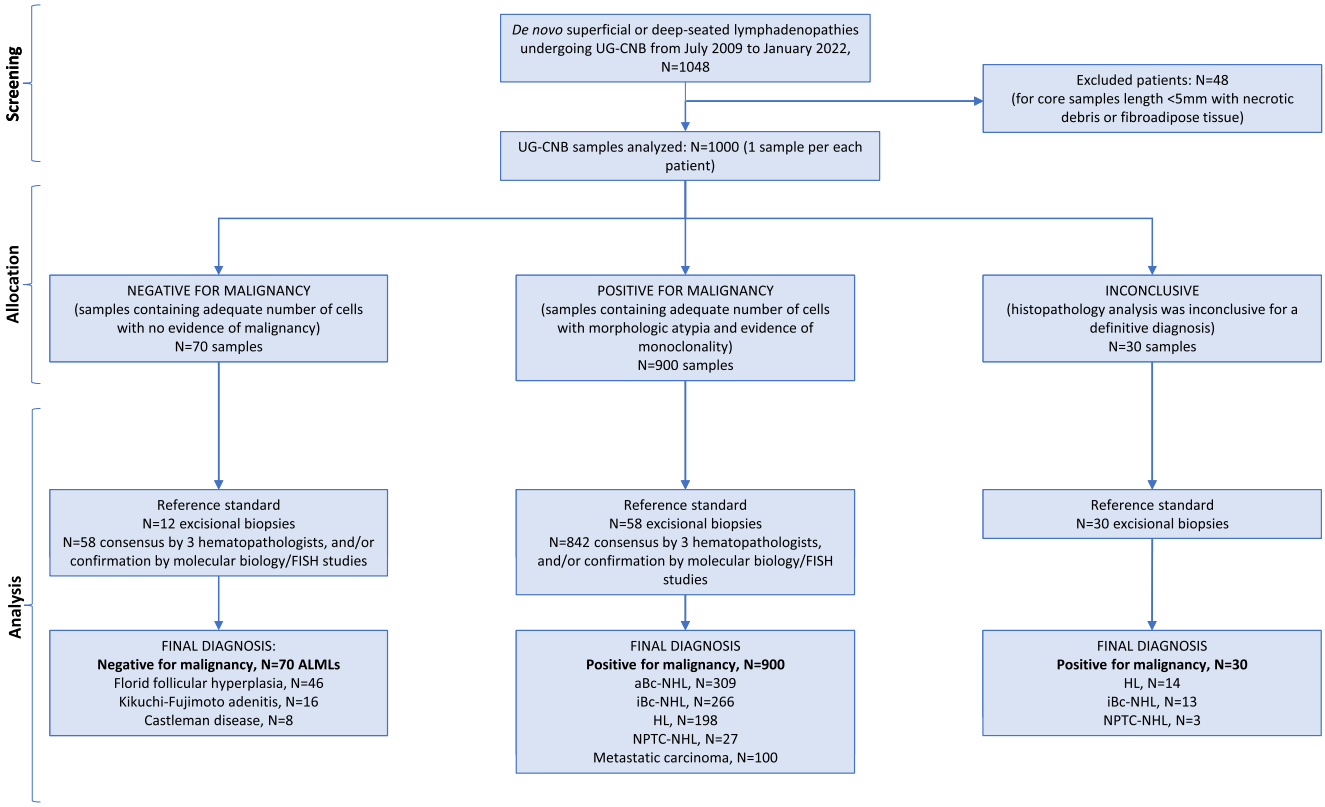

FIGURE 1 Consort diagram. The flow of UG-CNB samples through the study. aBc-NHL, aggressive B-cell non-Hodgkin lymphoma; ALML, atypical lymphadenopathies mimicking lymphoma; CNB, core needle biopsy; FISH, fluorescence in situ hybridization; HL, Hodgkin lymphoma; iBc-NHL, indolent B cell non-Hodgkin lymphoma; NPTC-NHL, nodal peripheral T-cell non-Hodgkin lymphoma; UG, ultrasound-guided.

for 309 lymph nodes (n = 220, diffuse large B cell lymphoma with germinal center B-cell subtype in 60% of cases, activated B-cell subtype in 40%, over-expression [>40% tumor cells] of MYC, BCL-2 and/or BCL-6 in 30%, and MYC, BCL-2, and/or BCL-6 rearrangements in 10%; n = 49, mantle cell lymphoma with classic subtype in 80% of cases and blastoid subtype in 20%; and n = 40, primary mediastinal large B cell lymphoma); iBc-NHL for 279 lymph nodes (n = 189 grade 1–2 follicular lymphoma [FL]; n = 60, small lymphocytic lymphoma; and n = 30, nodal marginal zone lymphoma [MZL]); HL for 212 lymph nodes (n = 201, classic subtypes; and n = 11, NLP-subtypes); and NPTC-NHL for 30 lymph nodes (n = 12 not otherwise specified [NOS], n = 10 angioimmunoblastic T-cell lymphoma [AITL], n = 4 anaplastic lymphoma kinase (ALK)+ anaplastic large cell lymphoma [ALCL], and n = 4 ALK- ALCL).

The remaining 170 lymph nodes were found with metastatic carcinoma (n = 100) or benign etiology according to the standard reference (Table 2).

In total, out of the 1000 UG-CNBs yielding macroscopically adequate material for histological diagnosis, 90% (n = 900) resulted in lymph-nodes positive for malignancy. Most core biopsy samples resulted in lymphomas (aBc-NHL, 309 cases [Figure 2]; iBc-NHL, 266 cases [Figure 3]; HL, 198 cases [Figure 4]; and NPTC-NHL, 27 cases [Figure 5]) while in 100 cases in metastatic carcinoma; 70 CNBs resulted in non-malignant etiology. The remaining 30 samples (3%) were classified as inconclusive according to the CNB results: samples containing adequate number of cells without morphologic atypia and evidence of monoclonality, thus the morphologic, immunohistochemical and/or molecular investigations did not allow for a final diagnosis. Figure 1 and Table 2 show the final diagnoses of UG-CNB inconclusive samples according to the standard reference (excisional biopsy in each patient): all cases were positive for malignancy (classic HL [nodular sclerosis subtype] in 11 cases and NLP-HL in 3 cases, iBc-NHL in 13 cases [n = 8, grade 1–2 FLs; n = 5, MZLs]; and NPTC-NHL in 3 cases [n = 2, AITL; n = 1, NOS]).

3.4 | Lymph node histological results according to UG-CNB

Among the three pathologists, interobserver reproducibility for the histological assessment of CNB samples had a median kappa score of 0.916 (range, 0.90–0.95): of the 1000 samples tested for reproducibility, about 95% were classified identically by the observers.

3.5 | Diagnostic accuracy of UG-CNB

The standard reference confirmed the final histological diagnosis of lymphadenopathies on the basis of at least one out of the 3 criteria of the composite work-up, with the following distribution: agreement of the three blinded hematopathologists for 940 CNB samples (94%), molecular biology results for specific lymphoma subsets (e.g.,

TABLE 1 Baseline characteristics of patients and ultrasonography guided core needle biopsy (UG-CNB) procedures.

Characteristics	*N*
Total patients	1000
Male	460
Female	540
Age, median (range), years	65 (18–91)
B symptoms	260
Fever	156
Weight loss	70
Sweat	34
Superficial lymphonodal sites as target lesion	750
Cervical	200
Clavicular	200
Axillary	250
Inguinal	100
Deep-seated lymphonodal sites as target lesion	250
Antero-superior mediastinum	70
Abdomen-pelvic	180
Biopsed lymph node size at imaging scans	
Long axis, mean (range), cm	3.0 (2.0–7.0)
Abnormal vascularization behavior at power Doppler US	1000
Hypervascularization	480
Anarchic	370
Hilar	150
Imaging guidance, US	1000
UG-CNB	1000
Gauge 16	1000
Needle passes, median (range)	2 (1–4)
Length of core tissue, mean (range), mm	32 (8–70)
Volume of core tissue, mean (range), mm^3	185 (92–430)

Note: Hypervascularization behavior: generalized increased vascularization of the lymph node, that is, intranodal arterial vessels with high-resistive index value (>0.6). Anarchic vascularization behavior: hilus absent and intranodal vascularization with chaotic feature. Hilar vascularization behavior: increased vascularization of the lymph node at hilus.

Abbreviations: CNB, core needle biopsy; cm, centimeter; mm, millimeter; UG, ultrasonography-guided; US, ultrasonography.

immunoglobulin gene rearrangements and T-cell receptor gene rearrangements) for 200 CNB samples (20%), and/or follow-up surgical biopsy for 100 CNB samples (10%). Thus, there was excellent concordance in classifying positive findings for malignancy between the reference standard and UG-CNB. The sensitivity rate was 96.7% (95% confidence interval [CI]: 95%–98%), that is, 900 of 930 sample positive for malignancy were identified by UG-CNB, with a false negative rate of 3.3% (30 [those with inconclusive results] of 930 patients with lymph nodes positive for malignancy at the reference standard were not identified). Notably, the specificity rate was 100% (95% CI: 95%–100%), PPV rate was 100% (95% CI: 99%–100%), NPV rate was 70% (95% CI: 62%–77%), and the negative likelihood ratio was 0.03 (95% CI: 0.02–0.05) confirming the value of UG-CNB in diagnosing lymph adenomegaly suspected for malignancy. Finally, the overall diagnostic accuracy rate was 97% (95% CI: 96%–98%), that is, results accurate in 970 of 1000 samples.

3.6 | Sensitivity rate of UG-CNB within the five clinical categories of lymphoproliferative disorders

The sensitivity rate of UG-CNB procedures was also explored according to the five LPD subtypes (aBc-NHL, iBc-NHL, HL, NPTC-NHL, and ALML) mentioned above (Figure 6).

For aBc-NHL neoplasms, the sensitivity rate was 100% (95% CI: 98%–100%), that is, 309 of 309 samples positive for aBc-NHL according to the reference standard were correctly identified by UG-CNB; for iBc-NHL, the sensitivity rate was 95% (95% CI: 92%–98%) with a false negative rate of 5% (13 [those with inconclusive results] of 279 cases with lymph nodes positive for iBc-NHL according to the reference standard were not identified by UG-CNB); for HL, the sensitivity rate was 93% (95% CI: 89%–96%) with a false negative rate of 7% (14 [those with inconclusive results] of 212 samples positive for HL according to the reference standard were not identified by UG-CNB); and for NPTC-NHL, the sensitivity rate was 90% (95% CI: 73%–98%) with a false negative rate of 10% (3 [those with inconclusive results] of 30 cases with lymph nodes positive for NPTC-NHL according to the reference standard were not identified by UG-CNB). Finally, the sensitivity rate for ALML was 100% (95% CI: 95%–100%), that is, 70 of 70 samples positive for ALML according to the reference standard were correctly identified by UG-CNB.

3.7 | Factors affecting UG-CNB diagnostic accuracy

Among the possible factors affecting the histological yield of sampling, that is, lymph node location, and size (long axis in cm), penetration distance, dimension of tissue cores obtained, and nature (benign vs. malignant, according to the reference standard), and final diagnosis of lymphoma subtype (according to the reference standard), multivariate logistic regression analysis showed that the location of HL in sub-clavicular area yielded diagnostic tissue samples by UG-CNB with worse accuracy ($p = 0.032$).

3.8 | Procedure-related complications

All patients, except for those biopsied in the mediastinum compartment ($n = 70$) who were hospitalized for a median of 2 days, underwent UG-CNB in a day hospital regimen under local anesthesia. No patients suffered from biopsy related complications of grade >2 according to the CTCAE (Table 3; Supplemental data, 7).

TABLE 2 Histological results according to ultrasonography guided core needle biopsys (UG-CNBs) and according to the reference standard.

Histological results		CNB *n*, (%)		Reference standard *n*[a,b,c]
Total samples	**Histological features**	**1000**		**1000**
B-cell				
Non-Hodgkin lymphomas		575	(58)	575 (58)
	Diffuse large B cell	220	(38)	220 (38)
	Follicular	181	(31)	181 (31)
	CLL/SLL	60	(10)	60 (10)
	Mantle cell	49	(8)	49 (8)
	Primary mediastinal large B cell	40	(8)	40 (8)
	Nodal marginal zone	25	(5)	25 (5)
Hodgkin lymphoma		198	(20)	198 (20)
	Classic	190	(96)	190 (96)
	Nodular lymphocyte predominant	8	(4)	8 (4)
Nodal perpheral *T* cell lymhomas		27	(3)	27 (3)
	Not otherwise specified	11	(40)	11 (40)
	Angioimmunoblastic	8	(30)	8 (30)
	Anaplastic large cell, ALK-positive	4	(15)	4 (15)
	Anaplastic large cell, ALK-negative	4	(15)	4 (15)
Metastatic carcinoma		100	(10)	100 (10)
Non-malignant findings		70	(7)	70 (7)
	Florid follicular hyperplasia	46	(66)	46 (66)
	Kikuchi-Fujimoto adenitis	16	(23)	16 (23)
	Castelman disease	8	(11)	8 (11)
Inconclusive samples				
	Samples containing adequate number of cells without morphologic atypia and evidence of monoclonality, thus the morphologic, immunohistochemical and/or molecular investigations did not allow for final diagnosis	30	(3)	Positive for malignancy, *N* = 30: c-HL, *N* = 11; NLP-HL, *N* = 3; FL, *N* = 8; MZL, *N* = 5; AITL, *N* = 2; NPTC-NOS, *N* = 1

Abbreviations: AITL, angioimmunoblastic lymphoma; ALK, anaplastic lymphoma kinase; c-HL, classic-Hodgkin lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; CNB, core needle biopsy; FL, follicular lymphoma; MZL, marginal zone lymphoma; NLP-HL, nodular lymphocyte predominant Hodgkin lymphoma; NOS, not otherwise specified; NPTC, nodal peripheral T-cell; UG, ultrasonography-guided.

[a]The confirmed diagnoses of CNB samples positive for malignancy were established on the basis of the reference standard, that is, histologic results of the complete surgical resection of suspected lymphadenopathy for 58 patients, or, for the remaining 842 patients, the consensus by 3 blinded hematopathologists on reviewed CNB samples, and/or confirmation by molecular biology/FISH studies on CNB samples.

[b]The definitive etiology of CNB benign lesions was obtained by performing lymphadenopathy excisional biopsy in 12 cases and for the remaining 58 cases by consensus by 3 blinded hematopathologists on reviewed CNB samples, and/or confirmation by molecular biology/FISH studies on CNB samples.

[c]All patients with inconclusive samples at UG-CNB received an excisional biopsy resulting in a diagnosis of c-HL in 11 cases, NLP-HL in 3 cases, indolent B-cell non-Hodgkin in 13 cases, and nodal peripheral T-cell NHL in 3 cases.

4 | DISCUSSION

To the best of our knowledge, this is the first study aimed to assess the accuracy of UG-CNB as primary invasive tool to establish the diagnosis in a homogeneous large cohort of patients affected by lymphadenopathies suspected of malignancy. We showed that CNB under ultrasonographic guidance can achieve the etiological diagnosis of lymphadenopathies with excellent accuracy based on our wide sample size of 1000 lymph nodal samples. As reported in a recent very large French lymphopathy network survey, CNB diagnostic performance may be improved by increasing the needle caliber and the number of cores.[18] However, a limitation of the study

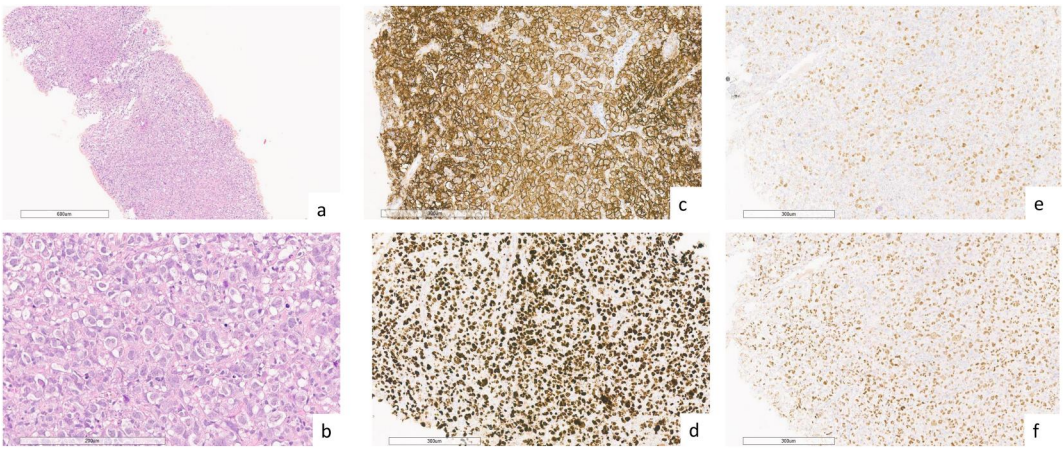

FIGURE 2 Core Needle Biopsy histological features of aggressive B cell (aBc)-non-Hodgkin lymphoma (NHL) sample, Diffuse Large B Cell Lymphoma (DLBCL). (A, B) Neoplastic lymphoid infiltrates of large-sized B cells with a diffuse pattern (hematoxylin and eosin, original magnification, x4, x20); (C) neoplastic cells stained for CD20 (CD20, original magnification, x10); (D) positive staining for MUM-1 (MUM-1, original magnification, x10); (E) positive staining for Bcl-2 (Bcl-2, original magnification, x10); (F) positive staining for Bcl-6 (Bcl-6, original magnification, x10). (The slides were digitized with an Aperio AT2 scanner with 40x optics).

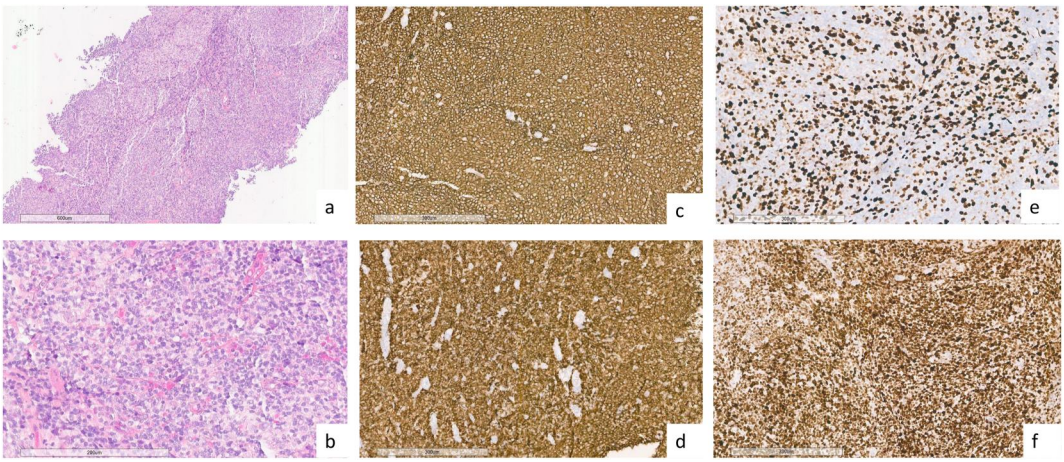

FIGURE 3 Core Needle Biopsy histological features of indolent B cell (iBc)-non-Hodgkin lymphoma (NHL) sample, Follicular Lymphoma (FL). (A) Inset: low-power image (H&E, ×1) of a core-needle biopsy specimen obtained from a right iliac lymph node: the core-needles reveal large follicular nodules closely packed with a back-to-back arrangement (H&E, ×20). (B) The neoplastic lymphoid follicles are composed of a uniform, small size, cell population (H&E, ×40). (C, D, E) The immunohistochemical stain strongly highlights CD20 (C), CD10 (D), and BCL-2 (E) (ABC, ×40). These samples are large enough to preserve tissue architecture and to assess the diagnosis of follicular lymphoma.

described by the same authors was the lack of information regarding the needle gauge.[18] Registry reports and clinical guidelines recommend the use of excisional biopsy for the initial diagnosis of lymphoma due to the inability of CNB to provide information on the histological architecture of lymph node[12,18–21]. However, in recent years, the development of new generation ultrasonographic machines and biopsy needle devices have provided the opportunity to develop mini-invasive and effective combined diagnostic strategy, avoiding psychological and physical pain of surgical intervention, and increasing consent by patients and treating physicians to histologically characterize suspected lesions by using this approach.[2–8,13,14]

In the Tertiary Centers involved in the study, modern power Doppler ultrasonographic technology allowed a clear examination of nodal lesions, an accurate selection of the most suspected target, and a simultaneous monitoring of the entire puncture process (in both superficial and deep-seated regions). The selected lymph nodes had a long axis of at least 2 cm, with abnormal vascular mapping at intranodal power Doppler assessment. The needles had a diameter of 1.6 mm with ultra-thinner tip and wall and powered automatic suction. A median of two needle passes was performed. Although the mean tissue volume obtained by CNB was 185 mm^3 (range, 92–430 mm^3), it provided enough tissue for architectural morphologic pattern assessment, immunohistochemical staining, and/or molecular testing in most patients.

To provide a solid basis for nonconventional histological diagnosis of lymphoma, we assumed that the findings of malignant lymph nodes should be confirmed by at least another criterion in the cases not directly proven by surgical biopsy as already described.[8] In our

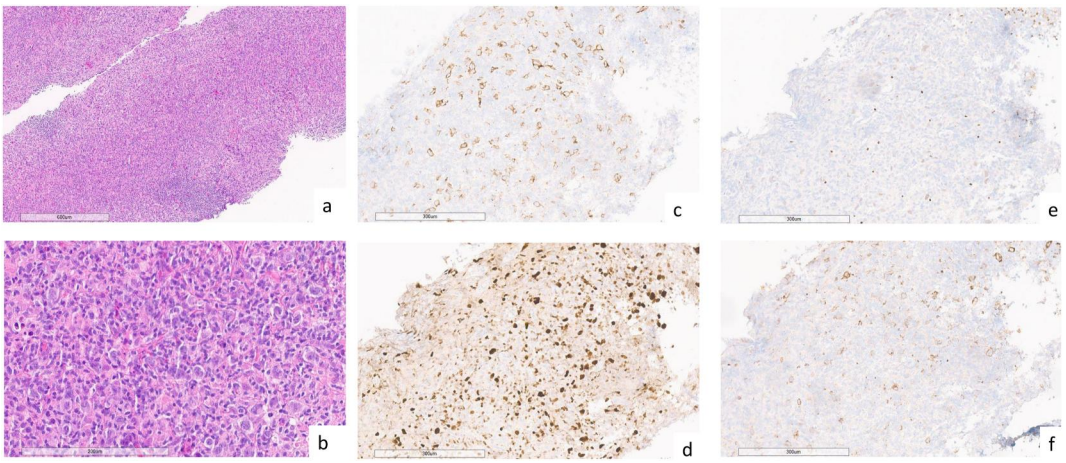

FIGURE 4 Core Needle Biopsy histological features of classic Hodgkin lymphoma (c-HL) sample. (A) Inset: low-power image (H&E, ×1) of a core-needle biopsy specimen obtained from a right latero-cervical lymph node: the core-needle appears fragmented due to an obvious fibrosis (H&E, ×5). (B) Higher power views show several Reed-Sternberg cells (H&E, ×40). The Reed-Sternberg cells are CD30 (C), CD15 (D), and fascin (E) positive (ABC, ×40). These samples are large enough to preserve tissue architecture and to assess the diagnosis of nodular sclerosis classical Hodgkin lymphoma.

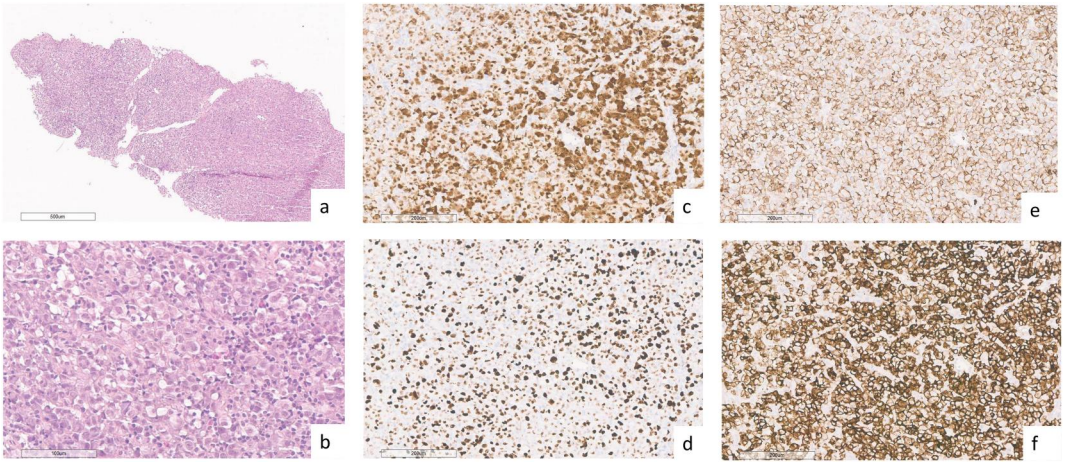

FIGURE 5 Core Needle Biopsy histological features of nodal peripheral T cell (NPTC) lymphoma, Anaplastic Large Cell Lymphoma (ALCL) sample. (A,B) Anaplastic lymphoma ALK positive (hematoxylin and eosin, original magnification, x4, x20); (C) neoplastic cells stained for ALK (ALK, original magnification, x10); (D) cells stained for CD43 (CD43, original magnification, x10); (F) neoplastic cells stained for CD30 (CD30, original magnification, x10); *(The slides were digitized with an Aperio A T2 scanner with 40x optics).*

study, within the standard reference, the final diagnoses were uniformly controlled by the consensus made by 3 blinded hematopathologists on reviewed CNB samples (94% of cases) and/or confirmation by molecular biology tests including PCR and/or FISH techniques for specific lymphoma subsets (20% of cases). Hu Q. et al in a study on CNB diagnostic accuracy of suspected lymphadenopathies published the validity of a composite reference standard (the same used by us) for CNB result confirmation, with excellent concordance.[8] The diagnostic efficacy rate of UG-CNB procedures was also explored in the sub-classification of LPDs according to the five clinical categories (reported above) thus resulting in a concordance (between CNB and reference standard) which was complete for aBc-NHL (diagnostic accuracy rate of 100%) and excellent for iBc-NHL (diagnostic accuracy rate of 95%), HL (diagnostic accuracy rate of 93%) and NPTC-NHL (diagnostic accuracy rate of 90%). Lastly, the concordance between the CNB and reference standard was complete for ALML classification also (diagnostic accuracy rate of 100%).

An important point in this study is the balance between the potential harm done to the 3% of patients with lymphomas missed by UG-CNBs and the beneficial effect on the 7% of patients with benign findings at UG-CNBs. A 10% false-negative rate for NPTCL-NHLs is likely not acceptable, given the aggressive nature of such lymphomas. However, the patients with inconclusive results at CNBs promptly underwent complete surgical resection of the suspected lymphadenopathies without significantly delaying the definitive histological diagnosis of lymphoma. Whereas, the cases with benign findings can presumably be spared from the surgical intervention, that is, lymph node excision (certainly more invasive and unnecessary procedure).

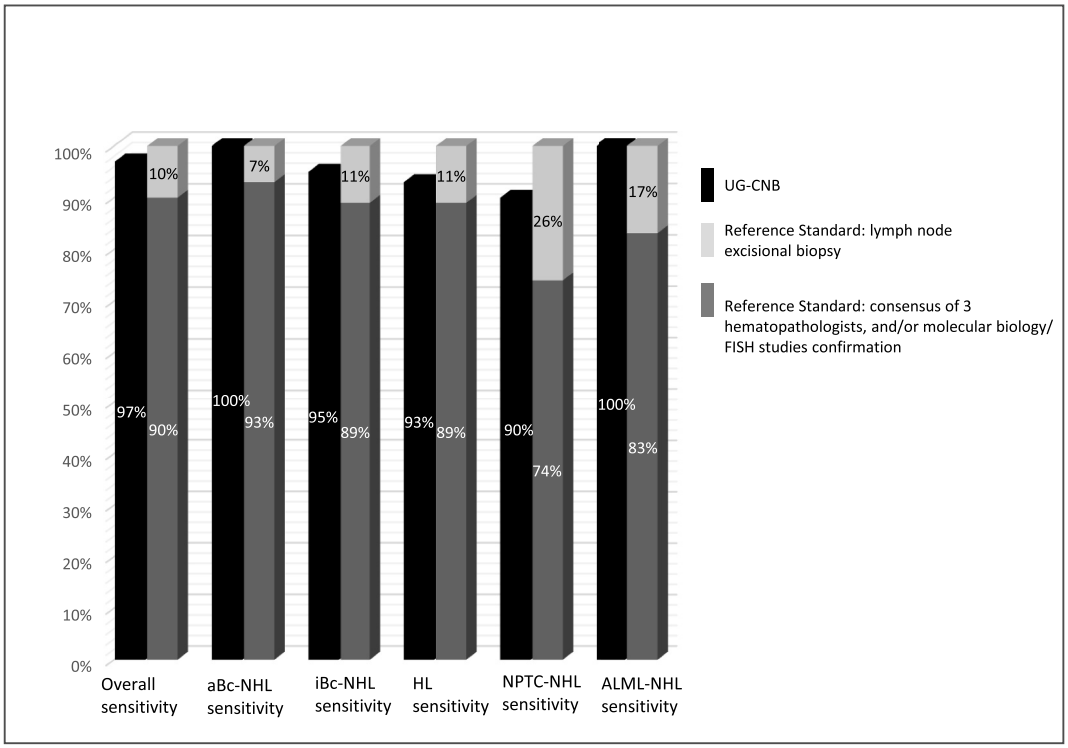

FIGURE 6 Histogram of sensitivity rate for all 1000 CNB procedures and of sensitivity rate within the clinical categories of lymphomas (aBc-NHL, iBc-NHL, HL, NPTC-NHL, ALML). aBc-NHL, aggressive B-cell non-Hodgkin lymphoma; ALML, atypical lymphadenopathies mimicking lymphoma; FISH, fluorescent in situ hybridization; H, Hodgkin lymphoma; iBc-NHL, indolent B cell non-Hodgkin lymphoma; NPTC-NHL, nodal peripheral T-cell Non Hodgkin Lymphoma; UG-CNB, ultrasound-guided core needle biopsy.

TABLE 3 Compliance of ultrasonography guided core needle biopsy (UG-CNB).

Complications	*n* (%)
Pain on biopsy site[a]	
No	770 (77)
Yes, mild and transient	150 (15)
Yes, continuous	80 (8)
Numbness on biopsy site	
No	730 (73)
Yes	270 (27)
Swelling on biopsy site	
No	870 (87)
Yes	130 (13)
Hematoma on biopsy site	
No	970 (97)
Yes	30 (3)

Abbreviations: CNB, core needle biopsy; UG, ultrasonography-guided.
[a]Post-operative pain was evaluated as absent, mild (not requiring analgesia), or continuous (requiring analgesia).

Few studies have analyzed the factors influencing UG-CNB sampling.[22–24] We analyzed these factors as secondary endpoint. Our results revealed that some malignant lymphadenopathies, and neck location tended to be associated with worse diagnostic yields. Stiffened tissue of nodular sclerosis sub-type HL that were seated in hindered regions of neck led to more failures with UG-CNB suggesting that, in some instances, there is a need of a large amount of lymph node tissue sampled with traditional surgical approach for correct histological assessment.

Overall, the procedure was well tolerated and for 93% it was performed in outpatient regimen. Most patients reported mild adverse events and since no patients suffered from biopsy related complications of grade >2 according to the CTCAE, no patients required hospitalized treatment.

Our study suffers from some limitations. First, the most relevant is the result of the surrogate reference standard, with the 3-point composite work-up; among these items, the surgical biopsy confirmation was obtained in a very minority of patients. Overall, 88 out 930 patients (9.4%) with malignant findings underwent follow-up excisional biopsy with complete surgical resection of the suspected lymphadenopathy (Figure 1). Although the histological evidence from the lymphadenopathy complete resection is the standard reference for the diagnosis of involvement by lymphoma, for obvious practical and

ethical reasons, it is not possible to perform surgical biopsies in all lymph nodes already undergoing CNBs. In the study, only selected patients underwent re-biopsy with excision by the surgeon: for inconclusive samples at CNB or for specific physician's or patient's choice. Second, this is a retrospective analysis; therefore, data from prospective studies are required. Third, in 4.5% of all 1048 procedures, screened in a 12-year period, a sampling error was performed, and insufficient nodal tissue was obtained, decreasing the diagnostic yield of UG-CNB. Thus, 48 UG-CNBs were excluded for this study with a resulting rate of adequate samples of about 95%, but still consistently higher than in literature.[12,18–21] The reason for sampling error may lie in the failure of target (patients with obesity and/or very deep-seated lymph nodes). These CNB-related limitations increase for widely fibrotic samples or technical artifacts that reduce tissue evaluability or induce immunohistochemical over-stains. Such instances can also occur when a steroids treatment is administered in the preceding days of CNB. Fourth, no cases of follicular helper T-cell related lymphoma, T-cell/histiocyte-rich large B-cell lymphoma or G3 follicular lymphoma or high-grade transformation of paraimmunoblast-rich chronic lymphocytic leukemia, or, as reactive lesion, progressive transformation of germinal centers, whose diagnosis are reported difficult on CNB sampling, was present in our series.[9,10] Finally, while UG-CNB certainly allows for considerable savings when compared to surgical resection, our study does not imply per se greater cost-effectiveness as this was not an endpoint of the study.[25]

Core needle samples allow the diagnosis of most LPDs according to the current WHO classification of Tumors of Haematopoietic and Lymphoid Tissues since histological patterns of the lymphoma are easily recognizable in this material.[1] Evidently, in our hands, the rate of failure with core biopsy was very low, and UG-CNB resulted to be (compared to excisional biopsy) well tolerated by patients. A needle with powered automatic suction and 1.6 mm diameter with an ultra-thin tip and wall is recommended, and at least two passes yielding two tissue cores, with a total length of 30–60 mm, should be sampled. CNB is a reliable front-line mini-invasive diagnostic procedure if a modern ultrasonographic equipment and biopsy needle device and an experienced operator are employed, but patients with obesity, pre-treated with steroids, and suspected lymph nodes with long axis <2 cm are not good candidates for this type of procedure.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are available by the author upon request.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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