Accuracy of percutaneous ultrasound-guided needle biopsy of abdominal lymph nodes for the diagnosis of malignant disease

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Received January 22, 2026; Accepted May 11, 2026

DOI: 10.3892/ol.2026.15794

Abstract. The differential diagnosis of suspected neoplastic disease involving deep lymph nodes remains challenging, particularly in the abdomen, where biopsies are technically more complex and associated with higher procedural risks. The present retrospective single-center study aimed to evaluate the feasibility, safety and diagnostic accuracy of ultrasound-guided needle biopsy in patients with neoplastic disease of deep abdominal lymph nodes. A total of 77 abdominal lymph node biopsies were analyzed. Among them, 15 (19.5%) were non-diagnostic. Of the remaining 62 diagnostic biopsies, 40 (64.5%) were lymphomas, 17 (27.4%) were solid tumors, and 5 (8.1%) were non-neoplastic conditions. Among the 57 biopsies positive for neoplastic disease, 32 of 40 (80.0%) lymphomas and six of 17 (35.3%) solid tumors represented new diagnoses, while the remaining cases were histologically confirmed. No procedure-related adverse events were observed. The sensitivity, specificity, positive predictive value and negative predictive value were 100, 83.3, 98.2 and 100%, respectively. The overall diagnostic yield was 80.5%, and the diagnostic accuracy reached 98.4%. US-NB could represent an accurate and reliable diagnostic tool for evaluating suspected neoplastic disease of deep abdominal lymph nodes, both at initial diagnosis and at relapse, offering a favorable safety profile and high diagnostic performance.

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Key words: lymph-node malignancies, ultrasound-guided needle biopsy, diagnosis, accuracy

Introduction

Despite significant advances in radiological and molecular diagnostics, histological confirmation remains the most common approach for the diagnosis of neoplastic diseases, including both solid tumors and hematologic malignancies. In lymphoma, histological sampling of the affected tissue is required for comprehensive evaluation, including morphological assessment, immunohistochemistry and genetic profiling. Excisional biopsy remains the gold standard, allowing full evaluation of the nodal architecture and identification of histologic features unique to specific types (for example, grading in follicular lymphoma) (1). Repeat biopsy is often necessary to confirm disease recurrence or detect histologic transformation, particularly in the context of indolent lymphoma, where progression to high-grade histology is well recognized. By contrast, for the diagnosis of nodal metastatic disease in solid tumors, current guidelines recommend that the choice of biopsy technique should depend on the disease site, with lymph node evaluation performed using either non-invasive or invasive staging methods at the clinician's discretion (2,3).

In deep abdominal lymph nodes, a biopsy can be challenging due to limited accessibility, particularly in the retroperitoneum or sites close to major vessels and vital organs. In addition, surgical excisional biopsy of abdominal disease can be excessively invasive, commonly requiring hospitalization, general anesthesia, and carrying risks of infectious complications and delayed diagnosis. However, it may not be feasible in patients with high operative risk (4,5).

Ultrasound-guided needle biopsy (US-NB) represents a valuable diagnostic tool employed to diagnose abdominal masses, thus enabling preservation of tissue architecture and allowing multiple tissue sections for immunocytochemical or histochemical analyses (6,7). However, its application in assessing deeply located abdominal lymph nodes remains poorly investigated, with only a limited number of case studies reported (8-12).

Although US-NB is established for superficial or easily accessible lymph nodes, its role in deep abdominal lymph nodes

remains poorly documented (13). It was hypothesized that US-NB could be considered a feasible and effective approach for diagnosing malignancies involving deep abdominal lymph nodes. Thus, the present study aimed to report a single-center, real-world experience with percutaneous US-NB for the diagnosis of abdominal lymph node malignancies.

Materials and methods

Study design and patients. In the present single-center, observational, retrospective study, adult patients who underwent a percutaneous US-NB for suspected malignancy of the abdominal lymph nodes between October 2013 and September 2023 were included. All procedures were performed as part of routine clinical care, and patient data were recorded in an institutional database. The study population included 77 patients, of whom 33 were female (42.8%) and 44 were male (57.1%). The median age was 65 years (range, 26-87 years). Institutional ethics committee approval was obtained in 2021, prior to the initiation of the retrospective data analysis. Patients were eligible if they met predefined safety criteria to minimize the risk of procedure-related bleeding. These included a platelet count of >50,000/mm^3 and adequate coagulation parameters, defined as an international normalized ratio (INR) of <1.5. Temporary discontinuation of antithrombotic therapy prior to the procedure was also required, as follows: At least 4 days for antiplatelet agents, 12 h for low-molecular-weight heparin, and 24 h for activated Factor Xa inhibitors. In patients receiving warfarin, normalization of INR prior to the procedure was required. All patients were instructed to fast for ≥12 h before the intervention. The exclusion criteria were as follows: Platelet count of ≤50,000/mm^3, INR ≥1.5, failure to discontinue antiplatelet or anticoagulant therapy within the specified time intervals or inability to comply with pre-procedural fasting requirements. Patients were enrolled consecutively to avoid selection bias after providing written informed consent. For patients who were lost to follow-up or deceased, authorization was obtained from the institutional privacy guarantor. The study was approved by the Ethical Committee AVEC of Bologna, the competent ethics committee for the IRCCS Azienda Ospedaliero-Universitaria di Bologna 'Policlinico di Sant'Orsola-Malpighi', with initial approval granted in 2021 (approval no. 1043/2021/Oss/AOUBo) and a subsequent extension approved in 2024 to allow continued data collection (approval no. EM189-2024_1043/2021/Oss/AOUBo). Due to the retrospective nature of the study, clinical data and biological samples collected from 2013 onward were analyzed. At the time of hospital admission and biopsy procedures, all patients had provided written informed consent for the diagnostic procedures and for the use of anonymized clinical data and biological samples for research purposes.

US-NB procedure. All biopsies were performed under real-time US guidance after selecting the most appropriate approach based on lymph node size and anatomical disposition. A biopsy was considered successful if sufficient tissue was obtained for a histopathological diagnosis. A medium-frequency (6-9 MHz) linear transducer was used for superficial lymph nodes, while a low-frequency (1-6 MHz) convex probe was employed for deeper lymph nodes. A needle guide with a five-angle trajectory system was utilized to enable accurate needle placement under on-screen US guidance. As conventional grayscale US could not reliably differentiate necrotic lymph nodes from viable ones, contrast-enhanced US (CEUS) using an intravenous contrast agent (SonoVue; Bracco) was carried out to detect the vascularized regions and avoid necrotic areas. The biopsy path was planned to ensure the safest access (anterior, lateral, or posterior), while avoiding bowel and major vessels. Following skin sterilization, local anesthesia with 2% lidocaine hydrochloride was administered. A modified Menghini 16-gauge needle was used, and one (in most cases) to three needle passes were performed depending on sample size. All procedures were conducted on an outpatient basis. Following biopsy, patients were required to remain in the supine position for 2 h to monitor for potential complications. Follow-up US examination was performed only in the presence of symptoms, such as pain, to assess for post-procedural complications, particularly bleeding.

Study endpoints. The primary endpoint of the study was the detection rate of US-NB in establishing a diagnosis of malignant disease in the abdominal lymph nodes. This was defined as the ratio of biopsy procedures that yielded a specific diagnosis relative to the total number of completed procedures. Diagnostic performance was also assessed by calculating sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV) and overall diagnostic accuracy. Detection rate assessment included all biopsies, both diagnostic and non-diagnostic, while diagnostic accuracy was calculated only among biopsies that successfully yielded a definitive diagnosis, in order to distinguish intrinsic diagnostic performance from overall procedural effectiveness. This approach was chosen to avoid conflating technical or sampling failures with the intrinsic diagnostic capability of the technique, thereby providing a more precise estimate of its performance once adequate tissue is obtained. Exploratory endpoints included the association of needle size and biopsy targeting the region with the highest standardized uptake value (SUVmax) on positron emission tomography (PET) imaging with diagnostic outcome. True positive patients were those diagnosed with malignancy by histology, while true negative patients were those histologically confirmed with non-malignant disease. Additionally, false positive and false negative patients were those who were incorrectly diagnosed with malignancy or with non-malignant disease, respectively. The secondary endpoint was procedural safety, defined by recording any adverse events occurring during or immediately after the procedure. The adverse events were graded according to the NCI Common Terminology Criteria for AEs v5.0 (/ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm), where applicable.

Statistical analysis. Descriptive statistics were used to summarize patient characteristics and safety outcomes. Diagnostic performance was assessed in terms of sensitivity, specificity, PPV, NPV and overall accuracy. Confidence interval (CI) were calculated using the exact binomial method at 95%. The association between needle size and biopsy targeting the region with the highest SUVmax on PET scan, and diagnostic outcome was assessed using Pearson's correlation coefficient (r). A multivariable

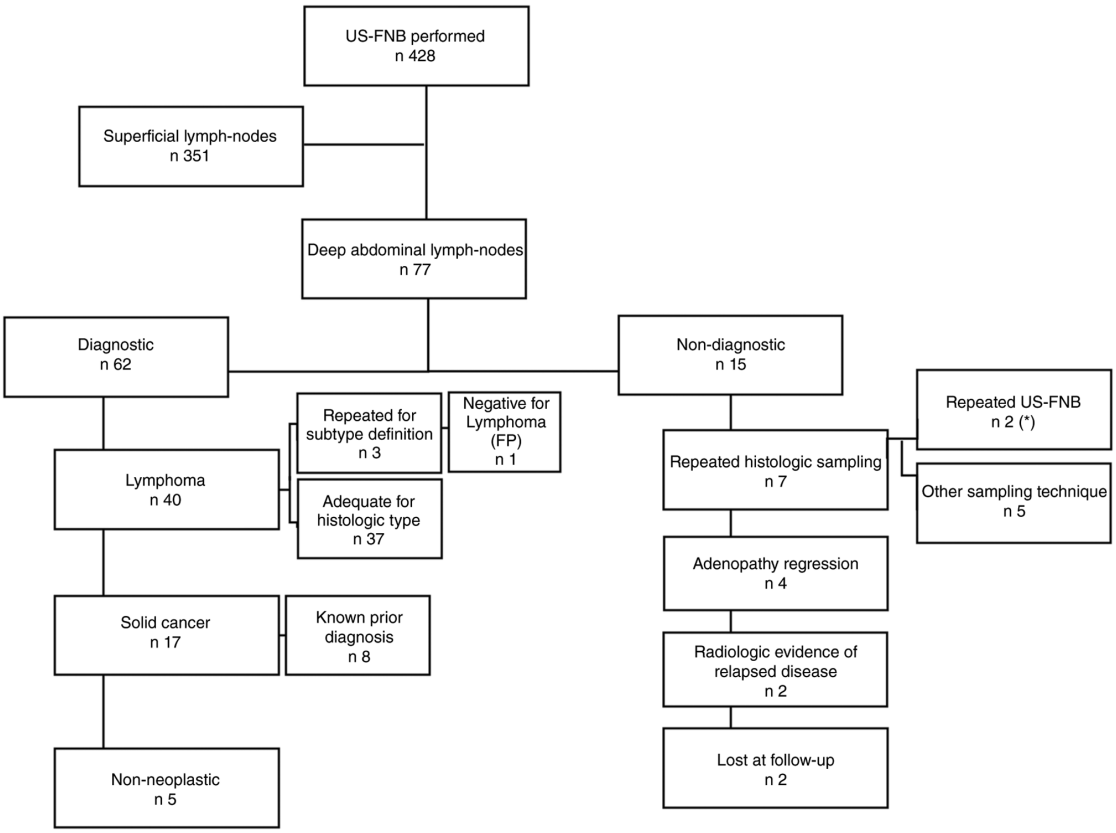

Figure 1. Flow diagram of patients undergoing a percutaneous US-NB between October 2013 and September 2023. *One performed on a superficial node. FP, false positive; US-NB, ultrasound-guided needle biopsy.

logistic regression model was used to identify predictors of diagnostic biopsy outcome. Odds ratios (OR) with 95% confidence intervals (CI) were reported, with $P<0.05$ considered to indicate a statistically significant difference. All statistical analyses were performed using Stata 17 (StataCorp LP).

Results

Patient characteristics and histological findings. Between 27 March 2013 and 9 January 2024, a total of 428 US-NB was performed at the Ultrasound Unit of the IRCCS Sant'Orsola Malpighi Hospital. Among them, 77 targeted abdominal lymph nodes (Fig. 1). Baseline patient characteristics are listed in Table I. All patients underwent CEUS as part of the biopsy procedure.

Overall, 62 of 77 abdominal lymph node US-NBs (80.5%) yielded a definitive histological diagnosis. Particularly, 40 patients were diagnosed with lymphoma (64.5%), 17 with metastatic cancer (27.4%), and five with non-neoplastic disease (8.1%; Table II). The remaining 15 procedures (19.5%) were non-diagnostic (Table III). The median lymph node diameter, available for 70 biopsies, was 47.4 mm.

Lymphoma diagnosis. Among the 40 biopsies positive for lymphoma, 32 (80.0%) were newly diagnosed, while eight (20.0%) were performed to histologically confirm disease recurrence in patients with prior lymphoma diagnosis. In total, three patients (7.5%) were diagnosed with lymphoma, not otherwise specified, and required additional sampling for further characterization. Two of these patients were subsequently diagnosed with Hodgkin lymphoma (HL) based on bone marrow biopsy and repeat NB of a superficial lymph node, respectively. The third patient remained under follow-up, with subsequent imaging being negative for pathological lymph nodes, representing the only false-positive result. Overall, sample adequacy for clinical decision-making in patients with lymphoma was 37/40 (92.5%).

Diagnosis of solid tumors and non-neoplastic diseases. Among the 17 biopsies positive for solid tumors, the majority (9 cases, 52.9%) represented histological confirmation of metastatic lymph node in patients with a known malignancy, while six cases (35.3%) corresponded to new diagnoses. Sample adequacy for clinical decision-making in this setting was 100%, and no repeat biopsies were required. Finally, five of the 62 diagnostic biopsies did not show any neoplastic disease, including two cases of reactive nodal tissue, two cases of normal lymph nodal tissue, and one case of disseminated tuberculosis. No evidence of malignancy was recorded during follow-up in patients diagnosed with reactive or normal lymph nodes (true negative cases).

Non-diagnostic biopsies and follow-up. A total of 15 of 77 US-NBs (19.5%) were non-diagnostic due to missed targets or insufficient tissue sampling. The median lymph node diameter, available for 15 biopsies, was 28 mm. Among the aforementioned patients, four experienced spontaneous regression of lymph nodes without further intervention; two had known

Table I. Characteristics of patients undergoing ultrasound-guided needle biopsy (n=77).

Variable	N (%)
Sex, n (%)	
Female	33 (42.8)
Male	44 (57.1)
Site, n (%)	
Abdominal, not specified	33 (42.8)
Para-aortic	12 (15.6)
Retroperitoneal	11 (14.3)
Iliac	6 (7.8)
Epigastric	4 (5.2)
Mesogastric	3 (3.9)
Hypogastric	1 (1.3)
Mesenteric	6 (7.8)
Hepatic hilum	1 (1.3)
Outcome, n (%)	
Diagnostic	62 (80.5)
Non-diagnostic	15 (19.5)
Median diameter, mm (range)	
All biopsies	47.4 (9-200)
Diagnostic	36.5 (9-150)
Non-diagnostic	28 (15-200)
Calibre of needle used for biopsy, n (%)	
14 G	2 (2.6)
16 G	23 (29.9)
18 G	52 (67.5)

G, gauge.

aggressive lymphoma treated with CAR-T cell therapy, and biopsies were not repeated due to radiological and clinical disease progression; and two were lost to follow-up. New histological sampling was performed in seven patients. Particularly, one patient underwent repeat US-NB of abdominal nodes, resulting in a diagnosis of follicular lymphoma; one patient underwent US-NB of a superficial inguinal lymph node, confirming diffuse large B-cell lymphoma (DLBCL) recurrence; three patients were diagnosed with lymphoma following excisional biopsy of superficial lymph nodes (two DLBCL and one HL); one patient was diagnosed with HL following bone marrow biopsy; and two patients underwent open abdominal surgery for resection of a suspected large nodal mass, resulting in a final histological diagnosis of lipoma and nodal metastasis from leiomyosarcoma, respectively.

Diagnostic performance of US-NB. Of the 77 biopsies performed, 62 provided sufficient material for a definitive diagnosis, resulting in a diagnostic yield of 80.5%. Among all diagnostic samples, sensitivity was 100% (95% CI, 0.936-1.000), specificity was 83.3% (95% CI, 0.359-0.996), PPV was 98.2% (95% CI, 0.906-0.999) and NPV was 100% (95% CI, 0.936-1.000). The overall diagnostic accuracy was 98.4%.

Table II. Diagnostic biopsies.

A, Lymphomas (n=40)

Variable	Value
Histological type, n (%)	
DLBCL	9 (22.5)
FL	16 (40)
HL	7 (17.5)
HGL	2 (5)
MCL	1 (2.5)
CLL	2 (5)
Lymphoma, NOS	3 (7.5)
Calibre of needle used for biopsy, n (%)	
14 G	0 (0)
16 G	14 (35)
18 G	26 (75)
Median age, years (range)	64.5 (26-87)

B, Solid cancers (n=17)

Variable	Value
Histological type, n (%)	
GU tract	5 (29.4)
NET	4 (23.5)
GI tract	3 (17.6)
HCC	1 (5.9)
Breast	1 (5.9)
Lung	1 (5.9)
Melanoma	1 (5.9)
Sarcoma	1 (5.9)
Calibre of needle used for biopsy, n (%)	
14 G	1 (5.9)
16 G	3 (17.6)
18 G	13 (76.5)
Median age, years (range)	64 (35-83)

C, Non-neoplastic (n=5)

Variable	Value
Histological type, n (%)	
Reactive tissue	2 (40)
Normal tissue	2 (40)
TB	1 (20)
Calibre of needle used for biopsy, n (%)	
14 G	0 (0)
16 G	1 (20)
18 G	4 (80)
Median age, years (range)	52 (40-77)

CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; G, gauge; GI, gastro-intestinal; GU, genitourinary; HCC, hepatocellular carcinoma; HGL, high grade lymphoma; HL, Hodgkin lymphoma; MCL, mantle cell lymphoma; NET, neuroendocrine tumor; NOS, not otherwise specified; TB, tuberculosis.

Table III. Study outcomes and diagnostic reliability.

Outcome/parameter	N (%)
Failure rate (interrupted procedure)[a], n (%)	0/77 (0.0)
Rate of non-diagnostic sampling[a], n (%)	15/77 (19.5)
Chance to repeat any non-diagnostic procedure[b], n (%)	7/15 (46.7)
Chance to repeat any diagnostic procedure[c], n (%)	3/62 (4.8)
Diagnostic yield[a], n (%)	62/77 (80.5)
Sample adequacy for clinical decisions[c], n (%)	59/62 (95.2)

[a]Calculated on all completed procedures. [b]Calculated on all non-diagnostic biopsies. [c]Calculated on all diagnostic biopsies.

Table IV. Published experience with percutaneous US-NB of abdominal lymph nodes.

First author/s, year	US-NB, n	Abdominal deep site, n (%)	Diagnostic performance	Notes	(Refs.)
Kälkner *et al*, 1994	129	62 (48)	89% detection rate	No abdominal biopsy sub analysis	(24)
Zinzani *et al*, 1998	55	55 (100)	87% diagnostic accuracy	Only abdominal biopsies analyzed	(9)
Demharter *et al*, 2001	128	12 (9.4)	93% diagnostic accuracy	No abdominal biopsy sub analysis	(19)
De Larrinoa AF *et al*, 2007	102	11 (10.8)	88.2% diagnostic accuracy	No abdominal biopsy sub analysis	(22)
Yuan *et al*, 2010	1,119	197 (17.5)	73% detection rate	No abdominal biopsy sub analysis	(20)
He *et al*, 2015	110	48 (43.6)	97.8 % detection rate	No abdominal biopsy sub analysis	(11)
Pugliese *et al*, 2017	185	23 (12.4)	98.8% sensitivity	No abdominal biopsy sub analysis	(21)
Wilczynski *et al*, 2020	793	256 (32)	95% diagnostic accuracy	No statistically significant difference in the diagnostic accuracy between peripheral and abdominal lymph-node biopsies	(23)
Zadeh *et al*, 2025	87	87 (100)	94.2% diagnostic accuracy		(12)
Present study	428	77 (5.5)	98.4% diagnostic accuracy	-	

Needle size, PET and safety outcomes. An 18-G, 16-G and 14-G needle was used in 69.4, 29.0 and 1.6% of diagnostic biopsies, respectively, and in 60.0, 33.3 and 6.7% of non-diagnostic biopsies. No significant association between needle size (larger needles, 16-G and 14-G, vs. smaller needles, 18-G) and diagnostic outcomes was recorded (P=0.53). A total of 38 patients underwent PET scan, including 32 who had biopsy targeting the lymph node with the highest SUVmax (range, 2.5-47). Among these, 28 of 32 biopsies yielded a diagnosis of neoplastic disease. Among the 32 patients with available PET imaging, 6 non-diagnostic cases were observed, 5 of which underwent biopsy of the area with highest tracer uptake. No significant association was found between biopsy at the SUVmax site and diagnostic outcome. A multivariable logistic regression model was performed to identify factors associated with a diagnostic biopsy outcome. The model included age, needle gauge, retroperitoneal location and lesion size, the latter categorized into tertiles (T1-T3) based on the distribution of the study population. In the adjusted analysis, only the largest lesion category (T3) was significantly associated with diagnostic yield (OR 10.74, p=0.038), whereas age, needle gauge, and retroperitoneal location were not independently associated with the outcome. Finally, among the 77 deep lymph node biopsies performed, no procedure-related adverse events were observed, either during or after the procedure. Particularly, no cases of bleeding, swelling, or significant pain were reported.

Discussion

The literature on tissue sampling of suspected malignant abdominal lymph nodes primarily focuses on the diagnosis of lymphoma, due to both the anatomical site involved and the management of solid tumors, in which lymph nodes are commonly resected for histopathological analysis during surgery or are not biopsied in case of metastatic disease. The high diagnostic accuracy, safety, and cost-effectiveness of NB in the diagnosis of lymphoma have been widely reported, yielding a definitive diagnosis in >90% of cases (14). However, the majority of these studies mainly focus on specific imaging-guided techniques, including computed tomography (CT)-, PET/CT-, or US-guided NB), without taking into account whether the disease site is superficial or deep (4,5,10,14-17).

Evidence on percutaneous US-NB in deep abdominal lymph nodes remains limited (9,15,18-23), thus reflecting the

need for experienced operators and a preference for CT-guided or echo-endoscopic approaches, which can provide improved lesion visualization (16,17). Needle selection is affected by lesion size and location. Therefore, larger core needles (14 G) are typically used for superficial lesions, while 16-18 G needles are preferred for deeper lesions. Although smaller needles are associated with lower complication rate, they can yield less informative samples. For example, small tissue samples could fail to detect histological transformation in lymphoma or could limit accurate assessment of features, such as the centroblast/centrocyte ratio in follicular lymphoma.

To the best of our knowledge, the current study represented one of the largest series evaluating the diagnostic yield and accuracy of percutaneous US-NB for suspected malignant disease, including both lymphomatous and solid, in deep abdominal lymph nodes (Table IV). The study demonstrated a high diagnostic yield (80.5%) and excellent diagnostic accuracy (98.4%), consistent with previous studies (9,11,12,16,19). Malignancy-negative results accounted for 8.1% of diagnostic samples and were all confirmed as true negatives on follow-up.

Despite the aforementioned encouraging results, 19.5% of biopsies were non-diagnostic, thus highlighting the challenges associated with sampling deep lymph nodes, particularly when lesions are small or necrotic. Although diagnostic biopsies showed a NPV of 100%, sensitivity could be overestimated due to the retrospective design and the exclusion of non-diagnostic cases from accuracy calculations.

As expected, the majority of biopsies were positive for lymphoma, with histological subtypes reflecting disease epidemiology. The most common diagnoses were follicular lymphoma, DLBCL and HL. Notably, no T-cell lymphomas were identified, which could be explained by their relative rarity and frequent extra-nodal involvement. Overall sample adequacy for clinical decision-making was 92.5%. However, in three cases the histological category could not be reliably established. Following repeat sampling, one patient was found to be free of lymphoma, while the remaining two were diagnosed with HL. Occasionally, distinguishing reactive lymphoid changes from low-grade non-HL on core biopsy could be challenging and could have contributed to the false positive result observed. Additionally, percutaneous biopsy yielding limited material could also lead to misclassification of lymphoma subtypes, particularly those with low tumor cell content or areas of sclerosis, such as HL. Notably, HL was subsequently diagnosed in the two cases with initially uncertain subtype. The vast majority of cases were newly diagnosed with lymphomas, while ~20% were repeat biopsies performed to confirm previous diagnoses. In this context, repeat biopsy at relapse is clinically valuable for excluding disease transformation and for evaluating antigen expression to guide targeted antibody-based salvage therapy (24,25). Notably, two non-diagnostic biopsies were carried out in patients with rapidly progressive disease following CAR-T cell therapy; both specimens were histologically characterized by extensive necrosis, suggesting that alternative or extensive tissue sampling strategies could be required in this setting.

Solid tumor diagnoses were less frequent, accounting for ~25% of all informative samples, with ~65% representing metastatic disease. Importantly, six of seven patients who underwent biopsy for suspected metastatic solid tumors were initially assessed for possible lymphoproliferative disease, underscoring the role of biopsy in excluding lymphoma. The most common solid tumor diagnoses were malignancies prone to abdominal nodal metastasis, particularly genitourinary, gastrointestinal, and neuroendocrine tumors.

In total, ~50% of the patients had available PET imaging to guide US biopsy, and 28 of 32 biopsies performed at sites of maximal PET uptake were diagnostic. Although no statistically significant association with biopsy outcome was obtained, likely due to the limited sample size, functional imaging could increase the likelihood of sampling representative malignant tissue, supporting an integrated approach to optimize sampling success (26).

Furthermore, the absence of procedure-related complications and the feasibility of performing US-NB in an outpatient setting further supported its safety, even among patients with significant comorbidities who were not candidates for more invasive approaches, such as surgery. These findings were consistent with previous oncologic reports.

However, the present study has certain limitations, including its retrospective design and relatively small sample size, which could introduce selection bias and limit generalizability. Additionally, the lack of procedural standardization, including variability in needle size and number of passes, could also affect outcomes. The risk of verification bias was considered low, as all five negative biopsies were closely monitored and no malignancies were identified. Finally, as diagnostic accuracy was calculated conditionally on diagnostic samples, the reported estimates may be subject to selection bias and could appear higher than those obtained with an intention-to-diagnose approach. The monocentric design of the present study represented a major strength, as procedures were performed by the same operators using consistent equipment and standardized techniques. Nevertheless, larger prospective studies are needed to confirm these findings.

In conclusion, our experience suggested that US-guided percutaneous NB could be a reliable, rapid and safe diagnostic tool for evaluating suspected malignant lymph node disease in the deep abdomen, applicable to both initial diagnosis and disease recurrence, with high diagnostic accuracy. However, caution is warranted in particular scenarios. In low-grade lymphoma, limited tissue samples could hinder differentiation between reactive and pathological tissues. In cases of suspected histological transformation or heterogeneous disease, NB could not fully capture full disease complexity. Furthermore, in rapidly proliferating lesions, extensive necrosis could reduce diagnostic yield, thus indicating that alternative sampling approaches capable of providing larger histological specimens should be considered.

Acknowledgements

The authors would like to thank Mr. Massimo Agostini (Associazione Italiana Contro Leucemia, Linfomi e Mieloma; Bologna, Italy) for collecting patient data.

Funding

No funding was received.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CM, LA, CS and PLZ designed and conceived the study and drafted and revised the manuscript. LA and CM conducted all data analyses. CM, DDB, SMB, LDM and MM collected the data. DDB, SMB, BC, CP, LDM, MM and AB provided study materials and interpreted data. All authors read and approved the final manuscript. CM and LA confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was conducted in accordance with The Declaration of Helsinki and was approved by the Ethical Committee AVEC of Bologna (approval nos. 1043/2021/Oss/AOUBo and EM189-2024_1043/2021/Oss/AOUBo; Bologna, Italy).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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