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Lymph node core biopsies reliably permit diagnosis of lymphoproliferative diseases. Real-World Experience from 554 sequential core biopsies from a single centre

Running Title: Core lymph node biopsy to diagnose lymphoma

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Summary Statement

1. Advances in radiological and histological techniques make core lymph node biopsy a viable alternative to excision biopsy in diagnosing lymphoma with 93.8% of biopsies diagnostic within this large series.
2. Core lymph node biopsy performed by expert interventional radiologists and analysed by expert haemato-pathologists is an accurate, well-tolerated method for diagnosing lymphoma
3. Core lymph node biopsy can be performed quickly and reliably in the outpatient setting thus reducing a patient's time spent in the department, which is of particular relevance in the Covid-19 era.

Abstract**Introduction**

Whilst excision biopsy is traditionally preferred, advances in radiological and histological techniques warrant a re-look at core biopsy as a viable primary diagnostic method.

Method

Over a 3-year period, all patients who underwent core biopsy to investigate lymphoma at our centre were included.

Results

554 consecutive patients were included (40.1% prior lymphoma and 59.4% new presentations). Three or more cores were taken in 420 (75.8%) cases. Median time from request to biopsy and biopsy to histology report was 2 (0-40) days and 7 (1-24) days respectively. 510/544 (93.8%) biopsies were diagnostic. There was no difference in whether the biopsy was diagnostic based on indication (new vs. relapsed lymphoma) ($p=0.445$), whether biopsy was PET-directed ($p=0.507$), for T-cell lymphoma ($p=0.468$) or nodal vs. extra-nodal ($p=0.693$). Thirty-eight patients (6.9%) required a second biopsy due to inadequate tissue. In a patient experience survey, only 13.9% reported any complications (1 self-limiting minor bleeding, 4 bruising) whilst 16.7% reported any discomfort beyond 12 hours.

Conclusion

Core biopsy performed by experienced radiologists and analysed by expert haemato-pathologists is a reliable, well-tolerated method for diagnosing lymphoma and confirming relapse. Multiple cores can be obtained under local anaesthetic yielding sufficient material in the majority of cases.

Introduction

Implementation of therapy in lymphoma relies upon the attainment of an adequate tissue sample for both histological diagnosis and further specialist assessment (e.g. molecular testing) as required. Lymph node (LN) excision has traditionally been preferred to core biopsy due to concerns regarding tissue yield in the latter ¹. This precept is documented in the internationally accepted lymphoma staging system ² and in international guidelines^{3,4}.

Core LN biopsy is increasingly recognised as a safe, fast, and reliable alternative in the diagnosis of lymphoma ⁵⁻⁹. Using minimally invasive ultrasound-guided core biopsy needles under local anaesthesia, samples can be obtained from accessible anatomical sites. The analysis of core biopsy samples is highly accurate and reproducible amongst haematopathologists ¹⁰.

Furthermore, excision biopsy may require surgical pre-assessment and organisation to perform

the biopsy on an appropriate theatre list. This can result in diagnostic delays and, crucially, delays in initiating management. Moreover, there is morbidity associated with surgery and the use of general anaesthetic. From a financial perspective the costs of an excision biopsy are calculated to be 75% greater ¹¹. Consequently, advances in radiological and histological techniques in extracting and processing material warrants a re-look at the viability of core biopsy as a primary diagnostic procedure in lymphoma.

We carried out a single-centre study over three years of consecutive patients referred for ultrasound-guided core biopsy of an accessible LN or extra-nodal site in order to evaluate this.

Method

All patients who underwent routine core biopsy for suspected lymphoma between 2016 and 2018 at University College London Hospitals NHS Trust were identified from a local database. The main referral sources were haematology and ear, nose and throat (ENT) clinics. Patients underwent baseline blood testing via their respective clinics inclusive of virology to identify common causes of reactive LN enlargement in addition to specific serology based on risk factors, clinical symptoms or travel history. Only patients with suspected nodal pathologies and an absence of a known/suspected alternate solid organ malignancy were included within the study. Baseline data was collected inclusive of biopsy indication, number of cores taken, wait time from referral to histological diagnosis, final diagnosis and any histological requests for extra tissue due to sample inadequacy.

Patients were referred directly to bi-weekly ultrasound-guided walk in clinics run by experienced interventional radiologists. Biopsies were performed under ultrasound guidance using either the Argon medicine Biopince™ needle (in the majority of cases – **Figure 1**) or the Cook Quickcore™. To maximise the diagnostic yield, the minimum target was 3 cores using a 1 centimetre gate where possible. Sixteen gauge needles were used in the majority of cases but 18-gauge needles were employed when the LN was in an anatomically difficult position or small in size. Where available, positive emission tomography-computed tomography (PET-CT) was used to guide choice of biopsy site. Biopsy samples were analysed and reported by experienced haematopathologists on-site. All biopsies underwent hematoxylin and eosin (H&E) staining followed by immunohistochemistry. Biopsies were considered diagnostic based on a multi-disciplinary team (MDT) consensus inclusive of clinical haematologists and expert haematopathologists. Haematopathologists requested extra tissue in cases whereby there was not enough tissue to make a diagnosis or whereby a diagnosis of lymphoma was made but there was insufficient tissue for fluorescence in-situ hybridisation (FISH) or molecular studies specific to a particular lymphoma diagnosis to be performed.

A sample of 40 consecutive patients were invited to participate in a prospective survey within 7 days of core biopsy, prior to reporting of results, in order to document patient experience and record complications.

SPSS version 25 (IBM Corporation, Armonk, NY, USA) was used to perform the analysis. Non-parametric t-tests were used to compare continuous variables. All p values were 2-sided with a significance level of <0.05.

Results

A total of 554 biopsies in 512 patients were included within the study. Indications were: 327 (59.0%) suspected new lymphoma, 191 (34.5%) suspected relapsed lymphoma and 36 (6.5%) suspected transformed lymphoma. Biopsy sites were: cervical lymph node (LN), 326 (58.8%) patients; axillary LN, 71 (12.8%) patients; inguinal LN, 96 (17.3%) patients; extra-nodal, 61 (11.0%). Seventy-eight patients had a recent PET-CT, which was used to guide biopsy site based on the maximum standardised uptake value (SUVmax) of the lymph node to improve diagnostic yield. A median of 3 cores (range 1-4) were taken during the procedure. At least 3 cores were taken in 420 (75.8%) cases.

The median time from biopsy request by the referring clinician to procedure was 2 days (range 1-40) whilst time from biopsy to issuing of the histological report was median 7 days (range 1-24). The full diagnostic pathway is displayed in **Figure 2**. A total of 510/554 (91.9%) initial core biopsies yielded a definitive diagnosis. The haemato-pathologist requested extra material in 64 (11.6%) cases when the biopsy was either non-diagnostic or additional material was required to perform further testing (FISH, molecular). Repeat biopsies were performed in 38/64 (59.4%) of these cases whilst, in the remainder, re-sampling was deemed unnecessary by the clinical team (20/26 patients with resolving symptoms, 3/26 patients with a definitive diagnosis and extra tests not required inclusive of 2 patients entering phase I/II clinical trials, 1/26 multidisciplinary team decision not to repeat as subsequent PET not felt to be consistent with relapsed disease and 2/26 not repeated for unknown reasons). This equated to 6.9% of all patients requiring repeat biopsy within the cohort. The final diagnoses are displayed in **Table 1**. Of 195 patients with a 'reactive' diagnosis, 3 were subsequently diagnosed with lymphoma within one year of core biopsy. In 2 cases, the haemato-pathologist reported that the biopsy samples were inadequate and advised repeats, which were performed. In 1 case, a reactive diagnosis was made; the clinician organised a repeat biopsy due to high clinical suspicion.

In cases where the radiologist was unable to take at least 3 cores, diagnostic sensitivity was reduced (≥ 3 : 392/420 [93.3%] vs. < 3 : 117/134 [87.3%], $p=0.03$). There was no difference in the diagnostic accuracy of core biopsies by lymph node site (Neck: 296/326 [90.8%], Axilla: 64/71 [90.1%], Inguinal: 91/96 [94.8%]; $p=0.26$). There was no difference in diagnostic sensitivity based

on whether the indication for core LN biopsy was to investigate new or relapsed lymphoma ($p=0.445$), whether the biopsy was PET-directed ($p=0.507$), whether the diagnosis was a mature T-cell neoplasm or not ($p=0.468$), and whether the biopsy site was nodal vs. extra nodal ($p=0.693$) (**Table 2**). In cases where the initial biopsy was non-diagnostic, 12/34 (35.3%) were ultimately found to be reactive, 11/34 (32.4%) lymphoma and 11/34 (32.4%) were not repeated. The final lymphoma diagnoses in these cases were: 3/11 (27.3%) classical Hodgkin lymphoma, 2/11 (18.2%) follicular lymphoma, 2/11 (18.2%) marginal zone lymphoma, 2/11 (18.2%) high-grade B-cell lymphoma not otherwise specified (NOS), 1/11 (9.1%) nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), and 1/11 (9.1%) diffuse large B-cell lymphoma (DLBCL).

One hundred and ninety-five cases returned a reactive diagnosis as documented in Table 2.

For the patient experience study, 40 patients were contacted, of whom 36 (90%) agreed to take part. 5 (13.9%) reported minor bleeding or bruising. No other complications were reported. Discomfort was graded on a scale of 1-10, with a median of 1 (range 1-6), and a median duration of discomfort of 30 minutes. Only 16.7% patients reported any discomfort beyond 12 hours. There was no significant difference in discomfort based on needle gauge ($p=0.329$) or diagnosis (benign vs. malignant) ($p=0.2997$). The mean waiting time in the department was 24.3 (range 0-180) minutes and patients were kept for observation for no longer than 20 (range 0-20) minutes. Patients graded their overall impression of the service from 1-5 (1= poor, 5= excellent). 29 (80.6%) graded the service as 'excellent', with the remaining 7 patients grading it as 'very good'.

Discussion

This paper highlights the value of core biopsy in the diagnosis of lymphoma. We demonstrate that, when performed by experienced interventional radiologists and analysed by expert haemato-pathologists, core biopsy is fast (median 9 days from referral to diagnosis), reliable (91.9% diagnostic) and well-tolerated amongst a large cohort of 554 patients. This method allows for multiple cores to be taken to obtain sufficient tissue in the majority of cases inclusive of material for further tests (e.g. FISH, molecular) as required. This is applicable to those lymphomas that are traditionally more difficult to diagnose such as T-cell neoplasms and Hodgkin lymphoma.

There is wide-ranging data comparing core to excision biopsy in diagnosing lymphoma. In Germany, a retrospective study of 1510 consecutive biopsies from patients investigated for lymphoma reported that 91.7% of core needle biopsies resulted in a definitive diagnosis compared to 97.2% of surgical excision biopsies ¹². By contrast, a retrospective UK lymphoma

study of 262 patients showed that only 3% of patients who initially underwent core needle biopsy had non-diagnostic samples ⁶ whilst a Chinese study of 205 patients reported no statistical difference in the diagnostic accuracy between the two groups ⁷. This variability may be attributable to centre-specific patient selection bias if excision biopsy was available as an alternative. Older, co-morbid, or patients in whom the suspicion of lymphoma is higher are more likely to undergo core needle biopsy ¹². In our centre, core biopsy is the first line diagnostic investigation in cases whereby a LN or extra nodal site is amenable to ultrasound-guided sampling thus removing this selection bias. Finally, there are operator-dependent factors including the experience and skill of the interventional radiologist and number of cores they obtain. In our centre, standard practice, where possible, is to obtain a minimum of three cores of tissue for analysis, which may overcome some of the issues with tissue quantity seen with traditional core biopsy.

The utilisation of PET-CT to guide the biopsy also proved to be advantageous contributing to high diagnostic sensitivity rates of 94.9% in patients where PET-CT was available for review by an interventional radiologist prior to biopsy. The majority of these patients had relapsed lymphoma as PET-CT in new cases of lymphoma was not generally performed prior to histological confirmation of diagnosis. Where there are several possible targets, PET-CT imaging is a valuable tool to direct selection and increase the likelihood of confirmatory diagnoses in this setting ¹³.

T-cell neoplasms are traditionally more difficult to diagnose and often require extra tissue for T cell receptor (TCR) assays to provide molecular genetic evidence of clonality ¹⁵. Despite this, the diagnostic sensitivity for cases of T-cell neoplasms in this study was high (94.6%) with 31/35 (88.6%) having enough tissue available for all required analysis. Molecular analysis was conducted in 13/35 (37.1%) cases. Despite the pathologists report giving a definitive diagnosis, five reports stated that extra tissue would be helpful for sub classification of the T-cell neoplasm, leading to three (8.6%) patients undergoing a repeat biopsy. Johl *et al* ¹² had previously reported that molecular testing for clonality was less frequently ordered by pathologists in cases of core, as opposed to excision, biopsy. It remains unclear whether amount of tissue or local practice dictated the number of cases of T-cell lymphoma in which clonality testing was performed, which remains a limitation of this study.

The need for adequate amounts of tissue may potentially impact patient recruitment into clinical trials. However, our site is a lead recruiter to many clinical trials in the United Kingdom and internationally and our preferential use of core biopsies has not been a barrier to trial eligibility or recruitment. In over 75% of cases, at least 3 cores were taken and we did not have any cases where repeat biopsy was required for clinical trial eligibility. We do acknowledge that

extra tissue is important for correlative science and advancing understanding of the pathophysiology of lymphoma. In this paper we have demonstrated the feasibility of obtaining additional cores of tissue (median 3) and in many cases this could be expanded to fulfil requirements of tissue banking and translational research rather than exposing patients to the additional risks associated with excision biopsy when an efficacious, safe and tolerable alternative exists.

This study found that core biopsy was acceptable to patients. Discomfort lasted a median of 30 minutes and only minor complications were seen. Patients waited for a median of 24.3 minutes prior to and 20 minutes after biopsy before discharge. No patient required additional inpatient monitoring. The value of adopting a time-saving procedure, which requires a patient to remain in the department for a shorter time, is especially pertinent in the context of the Covid-19 pandemic. In a randomised trial of core vs. excision biopsy based on 372 patients, core biopsy was shown to be less painful and associated with less inflammation, numbness, lymphorrhoea and wound infections. The same study ran a cost analysis, which found the average cost per patient 4115 euros for excision biopsies in comparison to 171 euros for core-needle biopsies ⁹. Our approach to performing core biopsies as the first diagnostic test may lead to a small over-ordering of immunohistochemistry since immunohistochemistry is performed on all core biopsies whereas it may be omitted from the diagnostic pathway of excisional biopsies that are clearly reactive by morphology on H&E staining. However, any cost saving would be small by comparison to the costs of performing excision biopsy in all cases. A core biopsy approach is cheaper with few complications and is very well tolerated by patients. Whilst patient experience data was collected retrospectively, it was collected within one week of the procedure to minimise recall bias and provide an accurate reflection of patient experience.

Conclusion

We demonstrate that core biopsy can be considered as a first line investigation to provide a histological diagnosis in cases of suspected lymphoma. Our approach to utilise radiology and haemato-pathology expertise, ultrasound guidance and take a minimum of 3 cores where possible yields a diagnostic rate of 91.9% whilst minimising complication rates and providing patients with a fast, acceptable and diagnostic method. In addition, the potential cost-saving nature of this approach warrants reconsideration as a viable alternative to LN excision in all cases of suspected lymphoma whereby tissue is accessible to biopsy under ultrasound guidance.

Author Contributions

OC, IP, WT and SM conceived the study. IP, SP, AR, SO, TB and SJ collected and analysed biopsy samples. OC, MB, AD and NR performed the data collection. OC, MB, IP, KMA, WT and SM analysed the data and wrote the paper. All authors contributed to the manuscript and reviewed the final version prior to submission.

Declaration

KMA and TM are supported by the University College London (UCL)/University College London Hospital (UCLH) Biomedical Research Centre.

There are no additional conflicts of interest to declare

Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure 1: The Argon Biopince™ needle used to obtain core lymph node biopsy. Image courtesy of Argon Medical Devices.

Figure 2: Diagnostic Pathway

Table 1: Final Histological Diagnosis

Final Diagnosis	n	%
Reactive	195	35.2
Not specified	165	29.8
Kikuchi's lymphadenitis	15	2.7
Granulomatous disease	10	1.8
Sarcoidosis	3	0.5
Fibro adenoma	1	0.1
Toxoplasmosis	1	0.1
Lymphoma	346	62.5
Mature B-cell neoplasm	243	43.9
DLBCL	88	15.9
Follicular lymphoma	76	13.7
CLL/SLL	28	5.1
Mantle cell lymphoma	12	2.2
Nodal Marginal Zone lymphoma	12	2.2
Lymphoplasmacytic lymphoma	11	2.0
High-grade B-cell lymphoma, NOS	11	2.0
Primary mediastinal large B-cell lymphoma	2	0.4
T-cell/histiocyte rich large B-cell lymphoma	2	0.4
Burkitt lymphoma	1	0.2
Mature T and NK Neoplasms	37	6.7
Peripheral T-cell lymphoma, NOS	17	3.1
Angio-immunoblastic T-cell lymphoma	10	1.8
Anaplastic large-cell lymphoma	8	1.4
Adult T-cell leukaemia/lymphoma	1	0.2
T-cell prolymphocytic leukaemia	1	0.2
Hodgkin Lymphoma	60	10.8

Classical Hodgkin Lymphoma	56	10.1
NLPHL	4	0.7
PTLD	6	1.1
Other malignancy	12	2.2
Inadequate	1	0.18

Abbreviations: DLBCL: diffuse large B-cell lymphoma; NOS: not otherwise specified; CLL: chronic lymphocytic leukaemia; SLL: small lymphocytic lymphoma; NLPHL: Nodular lymphocyte-predominant Hodgkin lymphoma; PTLD: post-transplant lymphoproliferative disorder.

Table 2: Diagnostic sensitivity based on indication for biopsy

	n (%)	Diagnostic Sensitivity	P value
New lymphoma	207 (59.8)	91.5%	0.445
Relapsed lymphoma	139 (40.2)	92.6%	
PET-directed	78 (14.1)	94.9%	0.507
Non PET-directed	476 (85.9)	91.4%	
T-cell lymphoma	37 (6.7)	94.8%	0.468
Other diagnosis	517 (93.3)	88.0%	
Nodal biopsy	493 (89.0)	91.7%	0.693
Extra-nodal biopsy	61 (11.0)	93.4%	

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