Use of Core-Needle Biopsy for the Diagnosis of Malignant Lymphomas in Clinical Practice

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Abstract
Introduction: Excisional biopsy (EB) is considered the gold standard for lymphoma diagnosis. Although recent advances in interventional radiology enable sampling with core-needle biopsy (CNB), only few studies evaluated the utility of CNB compared to that of EB. Methods: We analyzed patients with lymphoma who had a diagnostic biopsy at the National Cancer Center Hospital during 2002–2017. We investigated the clinical and pathological characteristics of CNB in 2017. Results: The proportion of CNB utility in total biopsy procedures had increased from 11 to 48% during the 15 years. In 2017, CNB was opted more frequently than EB for a biopsy of superficial, abdominal, or anterior mediastinal lesions. Only one out of 72 patients who had CNB required re-biopsy with EB because of insufficiency. The incidence of complications was comparable between CNB and EB: 2 (4%) cases of minor bleeding with CNB and 1 (8%) case of minor bleeding with EB. The median time from the first visit to biopsy was significantly shorter with CNB (5.5 days) than with EB (15 days). Conclusion: There is an increasing trend in the utility of CNB. CNB is a less invasive method with shorter time to biopsy and can be considered an alternative to EB.

Introduction
Traditionally, excisional biopsy (EB) has been considered the gold standard method for the diagnosis of malignant lymphomas [1]. This is partly because evaluation of the whole histological architecture of the lymph nodes is important to distinguish lymphoma from other conditions including reactive lymphadenopathy or to determine the histological subtype of lymphoma. Furthermore, EB enables to obtain sufficient tissue samples for various ancillary tests including flow cytometry, cytogenetic analysis with fluorescence in situ hybridization (FISH), and molecular genetic tests. Currently, the World Health Organization (WHO) diagnostic schemes of malignant lymphomas and major clinical practice guidelines recommend EB as the primary method for lymphoma diagnosis [2–9]. EB might be a reasonable option for patients with superficial lymphadenopathy because superfici-
Obtaining biopsy specimens for lymphoma can be challenging. Here, we have described the characteristics of core-needle biopsy (CNB) compared to endobronchial ultrasound transbronchial needle aspiration (EB) and other procedures. In this retrospective study, we evaluated the trend in the use of CNB and EB as diagnostic procedures for lymphoma over 15 years. We compared 2002, 2007, 2012, and 2017 data for a total of 151 patients who underwent primary diagnostic biopsy, regardless of biopsy sites and diagnostic results. The trend significantly increased from 11% in 2002 to 48% in 2017, as seen in Figure 1. The authors concluded that CNB is considered to be a first-line diagnostic approach in clinical practice, demonstrating a high diagnostic rate (79–97%), high reproducibility, and safety. Compared to EB, CNB results in shorter time to recovery and a lower risk of complications such as bleeding or infection. Additionally, the recently published literature review article confirmed the usefulness of CNB because of its diagnostic efficacy, reproducibility, and safety. Therefore, the authors concluded that CNB is considered to be a first-line diagnostic approach in clinical practice.

Materials and Methods

Patients and Outcomes

In this retrospective study, we evaluated the trend of the frequency of CNB and EB in patients who were diagnosed with malignant lymphoma. We analyzed consecutive 73, 78, 87, and 151 patients who underwent primary diagnostic biopsy, regardless of biopsy sites and diagnostic results. The trend significantly increased from 43 to 9%, the proportion of CNB utility significantly increased from 11% in 2002 to 48% in 2017, as shown in Figure 1. The present study was approved by the Ethics Committee of the National Cancer Center (Approval number: 2017-161). Informed consent was waived because of the retrospective nature of the study.

Biopsy Procedures

In terms of CNB, all procedures were performed under computed tomography or ultrasound guidance by expert physicians. An 18-gauge semi-automatic core needle was used in almost all cases with at least 3 passes for each biopsy under local anesthesia. As needed, some of them were analyzed by flow cytometry and G-banding or FISH and/or cryopreserved for additional future examinations. Indication for these ancillary tests using fresh specimen was decided at the discretion of hematologists who ordered the biopsy. Complications were described based on the Clavien-Dindo classification of the Japan Clinical Oncology Group.

Pathologic Diagnosis

All tissues for histology were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4-μm-thick sections, and stained with hematoxylin and eosin for histological evaluation. All specimens were diagnosed and further evaluated by immunohistochemistry and other ancillary studies including FISH on formalin-fixed paraffin-embedded tissue, as required. The diagnosis was made according to the latest lymphoma classification system available at the time of diagnosis.

Statistical Analysis

Categorical variables in the 2 groups were compared by Fisher’s exact test. Continuous variables such as age, lesion size, number of slides for immunohistochecmical assessment, time from the first visit to biopsy, and diagnosis were compared using the Mann-Whitney U test. All tests were 2-sided, and a p value <0.05 was considered statistically significant. All calculations were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria). More precisely, EZR is a modified version of R commander (version 1.6-3) designed to add statistical functions frequently used in biostatistics.

Results

Trend in Increased Frequency of CNB from 2002 to 2017

Out of 73, 78, 87, and 151 patients who underwent primary diagnostic biopsy regardless of biopsy sites and were diagnosed with lymphoma in 2002, 2007, 2012, and 2017, respectively, the trend in the frequency of CNB and EB was analyzed. While the proportion of EB utility decreased from 43 to 9%, the proportion of CNB utility significantly increased from 11% in 2002 to 48% in 2017, as shown in Figure 1.
Patients’ Characteristics and Proportion of Each Biopsy Method in 2017

For the 151 patients who received diagnostic biopsy at our institution and were diagnosed with malignant lymphoma, the distribution of the histologic subtype is summarized in Table 1. There were 4 cases with low-grade B-cell lymphoma which could not be subclassified according to the World Health Organization (WHO) classification in the CNB cohort. In those 4 cases, follicular lymphoma, chronic lymphocytic leukemia/small lymphocytic leukemia, or mantle cell lymphoma was ruled out based on immunostaining studies (CD5, CD10, BCL2, and cyclin D1) and FISH (IGH/BCL2). The median patient age was 64 (range 18–89) years, and 87 patients (57.6%) were male. Regarding biopsy methods other than CNB and EB, endoscopy was the second most frequent procedure at 19% (29 patients). The remaining procedures including punch biopsy, endobronchial ultrasound transbronchial needle aspiration, surgery except for lymph node biopsy, bone marrow biopsy, and centesis of body cavity fluid accounted for 24% cases (36 patients).

Summary of CNB Procedures

In total, among 72 patients who underwent CNB in 2017, an 18-gauge core needle was utilized most frequently at 67% (48 patients), followed by a 20-gauge and a 13-gauge core needle, which were used at 4% (3 patients) each. Information about the gauge of core needle was unavailable from medical records for the remaining 18 cases. The most frequent number of passes was 3 (range, 1 to 10 passes) in 49% (35 patients), followed by 4 in 19%, 2 in 16% (12 patients), and 1 in 11% (8 patients).

Table 1. Distribution of histologic subtypes

| Histology                        | N   | %   |
|----------------------------------|-----|-----|
| Total                            | 151 |     |
| B-cell neoplasms                 |     |     |
| CLL/SLL                          | 2   | 1.3 |
| DLBCL                            | 61  | 40.3|
| FL                               | 40  | 26.5|
| High-grade B-cell lymphoma       | 2   | 1.3 |
| Low-grade B-cell lymphoma        | 4   | 2.6 |
| LPL                              | 1   | 0.7 |
| MALT                             | 11  | 7.3 |
| MCL                              | 4   | 2.6 |
| Plasmablastic lymphoma           | 2   | 1.3 |
| Nodal MZL                        | 1   | 0.7 |
| NK/T-cell neoplasms              |     |     |
| AITL                             | 3   | 2.0 |
| ALCL                             | 2   | 1.3 |
| ATL                              | 3   | 1.3 |
| CTCL                             | 1   | 0.7 |
| ENKLG                            | 5   | 3.3 |
| Hodgkin                          | 9   | 6.0 |

AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ATL, adult T-cell leukemia/lymphoma; CTCL, cutaneous T-cell lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; ENKL, extranodal NK/T-cell lymphoma, nasal type; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; FISH, fluorescence in situ hybridization.

Fig. 1. Transition of the frequency of CNB and EB over the 15 years from 2002 to 2017. EB, excisional biopsy; CNB, core-needle biopsy.

1 Despite full immunohistochemical studies including CD5, CD10, BCL2, and Cyclin D1 and FISH studies, these 4 cases could not be subclassified.
Table 2. Patient characteristics, use of ancillary tests, and days from first visit to biopsy of those having CNB or EB from anterior mediastinal lesion or superficial and intra-abdominal lymphadenopathy

|                         | CNB \(n = 47\) (%) | EB \(n = 13\) (%) | \(p\) value |
|-------------------------|---------------------|------------------|------------|
| Age, age, median (range)| 67.00 (26.00, 89.00) | 61.00 (18.00, 80.00) | 0.66       |
| Sex                     |                     |                  |            |
| Male                    | 27 (57.4)           | 7 (53.8)         | 1          |
| Female                  | 20 (42.6)           | 6 (46.2)         |            |
| Subtype                 |                     |                  |            |
| Aggressive\(\textsuperscript{1}\) | 27 (57.4) | 8 (61.5) | 1          |
| Indolent\(\textsuperscript{2}\) | 20 (42.6) | 5 (38.5) |            |
| Histology               |                     |                  |            |
| DLBCL                   | 17 (36.2)           | 3 (23.0)         |            |
| Cell of origin (only DLBCL) |           |                  |            |
| GCB                     | 9                   | 0                |            |
| Non-GCB                 | 6                   | 2                |            |
| Unclassified            | 2                   | 1                |            |
| FL                      | 19 (40.4)           | 4 (30.8)         |            |
| Other B-cell lymphomas  | 2 (4.3)             | 1 (7.7)          |            |
| T/NK-cell lymphoma      | 4 (8.5)             | 2 (15.4)         |            |
| Hodgkin lymphoma        | 5 (10.6)            | 3 (23.1)         |            |
| Distribution\(\textsuperscript{3}\) |           |                  |            |
| Superficial             | 22 (46.8)           | 12 (92.3)        | 0.004      |
| Deep                    | 25 (53.2)           | 1 (7.7)          |            |
| Complication            |                     |                  |            |
| Minor bleeding          | 2 (4.3)             | 1 (7.7)          | 0.526      |
| None                    | 45 (95.7)           | 12 (92.3)        |            |
| Nodal size, cm median (range) | 4.20 (1.00, 15.00) | 2.80 (0.70, 4.80) | 0.002  |
| Number of immunohistochemistry | 6 (4, 14) | 7 (6, 14) | 0.12      |
| Ancillary tests         |                     |                  |            |
| Flow cytometry          |                     |                  |            |
| Yes                     | 4 (8.5)             | 10 (76.9)        | <0.001     |
| No                      | 43 (91.5)           | 3 (23.1)         |            |
| G-banding               |                     |                  |            |
| Yes                     | 4 (8.5)             | 7 (53.8)         | <0.001     |
| No                      | 43 (91.5)           | 6 (46.2)         |            |
| FISH                    |                     |                  |            |
| Yes                     | 4 (8.5)             | 0                | 0.57       |
| No                      | 43 (91.5)           | 13 (100)         |            |
| Days from the first visit to biopsy |           |                  |            |
| Median (range)          | 5.5 (0.0, 185.0)    | 15.0 (0.0, 67.0) | 0.01       |
| Days from the first visit to diagnosis |           |                  |            |
| Median (range)          | 15.0 (6.0, 192.0)   | 22.0 (12.0, 80.0) | 0.004     |

DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell; FL, follicular lymphoma; EB, excisional biopsy; CNB, core-needle biopsy; FISH, fluorescence in situ hybridization.

\(\textsuperscript{1}\) Aggressive lymphoma included DLBCL, T/NK-cell lymphoma, Hodgkin lymphoma, and plasmablastic lymphoma.

\(\textsuperscript{2}\) Indolent lymphoma included FL and low-grade B-cell lymphoma.

\(\textsuperscript{3}\) Superficial lymphadenopathy includes cervical, axillary, or inguinal lesions. Deep lymphadenopathy includes anterior mediastinal and abdominal lymphadenopathy (mesenteric, para-aortic, or pelvic).
18%, and 1 and 5 in 6% each. Two patients experienced 7 and 10 passes each. In terms of diagnostic yield, only one out of 72 patients underwent biopsy with CNB and subsequent EB due to insufficiency. Despite unsuccessful classification in 4 low-grade lymphoma cases, they were treated based only on CNB results. Therefore, they were not counted as cases that lacked adequate tissue for diagnosis.

Biopsy Procedure-Related Complications

In 2017, overall, 2 (2.7%) out of 72 patients who underwent CNB experienced minor bleeding, and 1 (7.1%) out of 14 patients who underwent EB had minor bleeding. All these complications were graded as grade IIIa according to the Clavien-Dindo classification.

Comparison between CNB and EB in Biopsy for Lymphadenopathy

We also studied patients who underwent biopsy for truncal lymphadenopathy to compare the characteristics of CNB with those of EB. For this analysis, we focused on truncal lymphadenopathy including superficial lymphadenopathy (cervical, axillary, or inguinal) and abdominal lymphadenopathy (mesenteric, para-aortic, or pelvic), and anterior mediastinal lymphadenopathy to exclude potential bias related to biopsy sites. The results are summarized in Table 2.

Patient age was found to be comparable between the CNB group and the EB group (median 67 years old, range 26–89 and median 61 years old, range 18–80 years, \( p = 0.66 \)). Irrespective of the subtypes of malignant lymphomas such as aggressive or indolent, the frequency of CNB and EB was consistent. However, CNB was chosen significantly more frequently for biopsy for deep lesions, which include anterior mediastinal mass and abdominal lymph node, than for biopsy for superficial lymph nodes (53 vs. 8%, \( p = 0.004 \)). The size of the target lesion resected by CNB measured by imaging was larger than that resected by EB (median 4.2 cm, range 1.0–15.0 cm and median 2.8 cm, range 0.7–4.8 cm, respectively, \( p = 0.002 \)).

In terms of diagnostic yield, all the biopsies with either EB or CNB resulted in actionable diagnosis (Table 2). The number of immunohistochemical slides required for an actionable diagnosis was almost the same in both the CNB group and the EB group (median 6, range 4–14 and median 7, range 6–14 respectively, \( p = 0.12 \)). Regarding DLBCL, the cell of origin was determined successfully in 17 out of 20 patients (17 in the CNB group and 3 in the EB group) in this cohort. On the contrary, ancillary examination including G-banding, FISH, and flow cytometry was performed less frequently in patients diagnosed with CNB. More precisely, ancillary tests were performed in only 4 out of 47 patients who underwent biopsy with CNB. One patient with follicular lymphoma and 3 with diffuse large B-cell lymphoma underwent FISH on formalin-fixed paraffin-embedded slides. However, flow cytometry and G-banding were performed in 10 and 7 out of 13 patients who underwent biopsy with EB, respectively. None underwent FISH examination in the EB group. The median time from the first outpatient visit to biopsy was significantly shorter with CNB than with EB (median 5.5 days, range 0–185 and median 15 days, range 0–67 days, \( p = 0.01 \)). The time required for the diagnosis from the first visit was also significantly shorter with CNB than with EB (median 15 days, range 6–192 and median 22 days, range 12–87 days, \( p = 0.004 \)). During the 15 years, the time necessitated for the diagnosis from the first visit was consistently shorter in the CNB group than in the EB group, as shown in Table 3.

Table 3. Variation in the proportion of biopsies performed by either CNB or EB among patients with lymphadenopathy between 2002 and 2017

| Total | 2002 (n = 33) | 2007 (n = 28) | 2012 (n = 44) | 2017 (n = 60) |
|-------|--------------|--------------|--------------|--------------|
| CNB, n (%) | 4 (12) | 7 (25) | 18 (41) | 47 (77) |
| EB, n (%) | 29 (88) | 21 (75) | 26 (59) | 13 (23) |
| Days from the first visit to diagnosis, median (range) | | | | |
| CNB | 14.0 (12.0, 17.5) | 13.0 (7.0, 21.0) | 13.5 (7.0, 1853.0) | 15.0 (6.0, 192.0) |
| EB | 21.0 (18.0, 32.0) | 25.0 (10.0, 63.0) | 21.5 (7.0, 271.0) | 22.0 (12.0, 80.0) |
| \( p \) value | 0.0438 | 0.001 | 0.189 | 0.004 |

The comparison of the time spent on diagnosing between CNB and EB during the same period. EB, excisional biopsy; CNB, core-needle biopsy.
Discussion

In the present study, we have shown that the use of CNB has been increasing as the initial diagnostic approach during the past 15 years. As far as we know, this is the first report to describe the trend in increasing use of CNB for lymphoma diagnosis, availability of pathological slides for immunostaining, and the use of ancillary diagnostic tests using samples obtained through CNB in a clinical practice setting.

Although the frequency of performing ancillary tests was lower in the CNB group than in the EB group, the number of slides used for immunohistochemical staining was comparable between the groups. Moreover, CNB aided in obtaining samples from representative and/or deep-seated lesions. Furthermore, CNB was able to shorten the time required to make an actionable diagnosis from the first visit by approximately 10 days. The advantages and disadvantages of CNB and EB as a biopsy method for lymphoma diagnosis are summarized in Table 4.

Representative guidelines recommend EB or tissue resection to diagnose malignant lymphomas; however, several studies have revealed that CNB has been increasingly used as the primary diagnostic modality for malignant lymphomas. At our institute, the frequency of CNB has increased dramatically >4-fold over the past 15 years. Regarding safety, no serious complications related to CNB were observed in our study. Two out of 72 patients (2.7%) experienced minor bleeding, which is consistent with the previously reported incidence of 0.14–7.5% [15–17]. Therefore, this study showed that CNB is a biopsy method that has minimal complication and less physical burden on patients.

To assess the feasibility of CNB to obtain sufficient specimens for an actionable diagnosis compared with that of EB, we showed the number of immunohistochemical slides in CNB was comparable with that in EB. Previous studies showed that the volume of tissue samples could correlate with the needle gauge and the number of passes obtained [18, 19]. While there was no significant difference, CNB with larger core needles (19 gauge or larger) tend to obtain greater tissue volume, which increases the diagnostic accuracy [18]. At our institution, among patients who had biopsy for lymphadenopathy or anterior mediastinal mass, >80% of CNB was performed with 18-gauge core needles and with at least >3 passes. Our findings support the concept that larger samples obtained using a larger core needle may be helpful to diagnose malignant lymphomas. However, in the ECOG/ACRIN 1412, in which nearly half of all patients were diagnosed by CNB, over 40% of all the rejected specimens were obtained by CNB because of insufficient material for diagnosis and/or necessary molecular/genetic examinations [20]. In the present study, there was only 1 patient with insufficient tissue obtained with CNB. It is certain that ancillary tests that require fresh samples, such as flow cytometry and conventional karyotyping, were performed less frequently for CNB than for EB. With additional passes we could have done more ancillary tests. However, it was policy at our institution to limit the number of passes for CNB during the study period. Therefore, we consider that sample volume obtained by CNB, at least with the procedure that we have performed, may be sufficient for an actionable diagnosis. But it is difficult to conclude tissue volume obtained by CNB is enough for ancillary tests.

In terms of clinical benefits, in this study, the time needed for an actionable diagnosis from the first visit in patients with CNB was approximately 10 days shorter than that in patients with EB. This is largely because CNB can be performed in the outpatient clinic or IR room. However, it is generally required to schedule the operat-

| Method | CNB | EB |
|--------|-----|----|
| Advantages | Less invasive procedure | Larger tissue volume (preferred for evaluation of histologic architecture, additional studies including genetic study, and banking of tissue for future research) |
| | The time required for the diagnosis from the first visit is shorter | The time required for the diagnosis from the first visit is longer |
| Disadvantages | Smaller tissue volume (limitation in evaluation of histologic architecture, additional studies including genetic study, and banking of tissue for future research) | More invasive |
| | Skilled radiologist is required | General anesthesia is required for patients with deep-seated lesion |
| | Often difficulty in coordinating surgery appointment such as surgery slot | Often difficulty in coordinating surgery appointment such as surgery slot |

CNB, core-needle biopsy; EB, excisional biopsy.
Moreover, CNB was significantly preferred for sampling comparable between the CNB group and the EB group. The number of slides for immunohistochemical staining was our institution. Regarding pathological evaluation, the primary diagnostic modality for lymphoma diagnosis at our institution during the study period. For example, we did not routinely perform flow cytometry in CNB samples. Therefore, EB is still a standardized biopsy method for lymphoma diagnosis, but CNB can be an alternative option in clinical scenarios where EB is difficult to perform.

**Statement of Ethics**

The present study was approved by the Ethics Committee of the National Cancer Center (Approval number: 2017-161). All procedures followed were in accordance with the ethical standards of the responsible committee and with the Helsinki Declaration. The need for written informed consent was waived by the Ethics Committee because of the retrospective nature of the study.

**Conflict of Interest Statement**

The authors declare the following competing interests: S.M. reports personal fees from Bristol-Myers Squibb, Celgene, Chugai, Daiichi-Sankyo, Eisai, Novartis, SymBio, and Takeda. S.F. reports personal fees from Chugai, Otsuka, Takeda, and Zenyaku kogyo. T.S. reports personal fees from Chugai. D.M. reports grants and personal fees from Bristol-Myers Squibb, Celgene, Chugai, Janssen, Ono, and Takeda; and reports grants from Amgen Astellas BioPharma, Astellas Pharma, Merck, Novartis Pharma, Otsuka, and Sanofi; and reports personal fees from Eisai, Kyowa Kirin, NIPPON SHINYAKU, Symosia Biopharma, and Zenyaku kogyo. K.I. reports receiving research support from MSD, AstraZeneca, Abbvie, Incyte, Celgene, Novartis, Janssen, Yakult, Daiichi-Sankyo, Chugai, and Beigene; and reports personal fees from Chugai, Celgene, Takeda, Novartis, Abbvie, Janssen, Kyowa Kirin, Eisai, MSD, AstraZeneca, FUJIFILM Toyama Chemical, and Ono.

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**Author Contributions**

K.I. and Y.I. performed the research, analyzed the data, and wrote the initial manuscript. A.M.M. and H.T. contributed to pathologic assessment. M.S. performed CNB and supervised the biopsy procedures. The remaining authors reviewed the manuscript and provided patient care.
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