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Original

Gross Findings in Lymphadenectomy Specimens: Diagnosis and Differentiation of Malignant Lymphoma

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Summary

In the diagnosis of malignant lymphoma (ML), lymphadenectomy specimens are separated as needed for histological diagnosis, flow cytometry, and various genetic analyses after determining the suitability of the specimens from gross findings. We investigated gross findings for cut surfaces of lymphadenectomy specimens. Of the 57 patients who underwent lymphadenectomies, 7 were diagnosed with reactive lymphoid hyperplasia (RLH), 19 with follicular lymphoma (FL), 20 with diffuse large B-cell lymphoma (DLBCL), and 11 with Hodgkin lymphoma (HL). The gross findings for fresh, unfixed lymph node specimens with the maximum cut surface were evaluated in terms of color homogeneity, unevenness of cut surface, aspect ratio, presence of internal nodules, and capsular disruption. After this evaluation, a histological diagnosis was performed. In a comparison of color homogeneity, significantly more cases in the ML group (30/50 [60.0%]) were homogeneous than in the RLH group (1/7 [14.2%]) (P = 0.039). Capsular disruption was not observed in the RLH group, whereas it was found in 42/50 (84.0%) cases in the ML group (P = 0.001). The internal nodules between the FL group (17/19 [89.5%]) and HL group (9/11 [81.8%]) occurred more frequently than those of the DLBCL group (11/20 [55.0%]) (P = 0.039). Moreover, the internal nodules tended to be smaller in FL and HL than in DLBCL (P = 0.007). The color homogeneity and capsular disruption findings were important in differentiating ML from RLH, and the presence and size of the internal nodules were important in differentiating the ML subtypes.

Key Words: Gross findings, lymphadenectomy specimen, malignant lymphoma, reactive lymphoid hyperplasia, malignant lymphoma subtypes

Introduction

Malignant lymphomas are diagnosed by taking account of all available information, including morphology, immunophenotype, genetic abnormalities, and clinical features[1)]. In the subtyping of malignant lymphomas, the molecular biological analysis of chromosomal and genetic abnormalities has been particularly important, and advances in molecular biological analysis have had a significant impact on treatment[1)]. Malig-

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Table 1 Clinicopathological characteristics of patients with lymphadenopathy (n = 57)

	Reactive lymphoid hyperplasia (%)	Follicular lymphoma (%)	Diffuse large B-cell lymphoma (%)	Hodgkin lymphoma (%)
Number of cases	7	19	20	11
Sex				
Male	4 (57.1)	11 (57.9)	14 (70)	7 (63.6)
Female	3 (42.9)	8 (42.1)	6 (30)	4 (36.4)
Age (year-old), median (range)	60 (25-78)	64 (44-83)	66 (31-83)	63 (42-74)
Symptoms				
Palpable lymphadenopathy	7 (100)	16 (84.2)	20 (100)	11 (100)
Fever	2 (28.6)	0 (0)	0 (0)	0 (0)
Tonsillar hypertrophy	0 (0)	0 (0)	2 (10)	0 (0)
Pleural effusion	0 (0)	0 (0)	1 (5)	0 (0)

nant lymphoma is diagnosed by examining excisional biopsy specimens of the swollen lymph node. The lymphadenectomy specimens are separated as needed for histological diagnosis, cytological diagnosis, flow cytometry, electron microscopy, and genetic analyses. The required analysis methods depend on the presumed malignant lymphoma subtype[2], and if the lymphadenectomy specimen is small, histological diagnosis is preferred. Therefore, it is necessary to determine the adequacy of the specimens from the gross findings before formalin fixation and separate the specimens into those needed for histological diagnosis and those needed for various ancillary diagnostic procedures. If diagnoses based on molecular biological abnormalities are further developed and subdivided in the future, the specimen submission methods may also be subdivided according to the presumed diseases.

Some lymph node lesions are known to have disease-specific gross findings[3,4]. In malignant lymphomas, gross findings may also be associated with histological diagnosis[5,6]. For example, follicular lymphoma is known to show gross nodular changes, often with a bulky appearance[6]. Gross observation is a very important examination for some diseases. However, few studies have clarified the gross findings of lymph nodes as to whether they are malignant lymphomas or not, and whether they vary by histological subtype.

The purpose of this study is to determine the differences in the gross findings of benign and malignant lymph node lesions and the differences in the gross findings of lymph node lesions by representative malignant lymphoma subtypes in order to prioritize the various types of testing.

Materials and Methods

Patients

The study involved 57 patients who had lymphadenopathy detected by computed tomography and had undergone a lymphadenectomy between April 2014 and March 2017 at Dokkyo Medical University Hospital. Of the 57 patients who underwent a lymphadenectomy, 7 were diagnosed with reactive lymphoid hyperplasia (RLH), 19 with follicular lymphoma (FL), 20 with diffuse large B-cell lymphoma (DLBCL), and 11 with Hodgkin lymphoma (HL). The histological subtype of all HL cases was nodular sclerosis classic HL. The clinicopathologic findings, including sex, median age, and clinical symptoms, are summarized in Table 1.

The study was approved by the Ethical Research Committee of Dokkyo Medical University (R-25-17J). All procedures conducted on human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Evaluation of gross findings

The gross findings were evaluated using fresh, unfixed lymph node specimens. The size of the lymphadenectomy specimen was measured and sections of the lymph node were cut with the maximum diameter to provide the best assessment of the nodal architecture. Each gross observation was performed by two investigators (Y. Nakazato and AT-O or YK), and a consensus was reached in all cases.

On the maximum cut surface of the lymph node, we

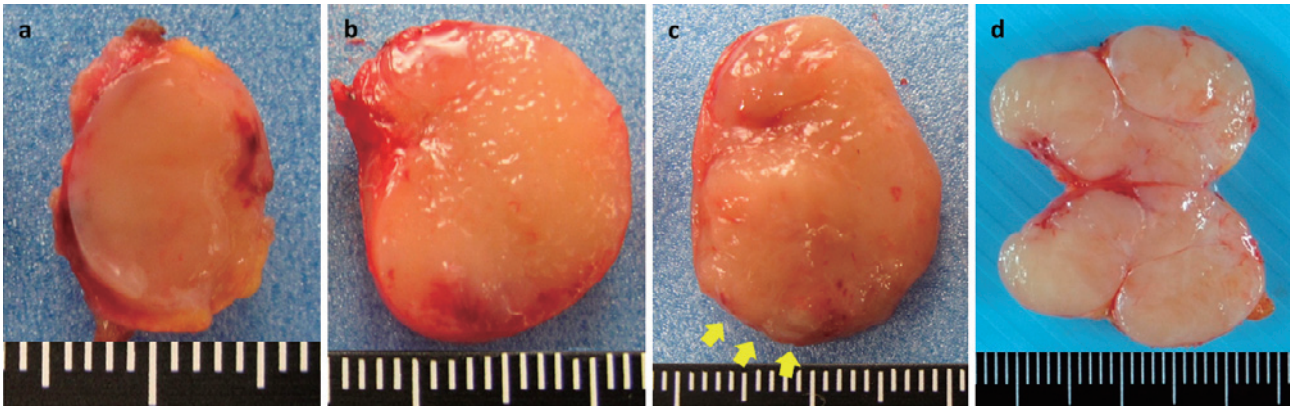

Figure 1 Representative gross findings of reactive lymphoid hyperplasia (RLH), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), and Hodgkin lymphoma (HL) on a cut surface. **(a)** RLH has heterogenous color and is flat, without capsular disruption. **(b)** FL has a bulging cut surface with homogeneous color and small internal nodules. **(c)** DLBCL also has a bulging cut surface with homogeneous color, vague nodules, and capsular disruption (arrows). **(d)** HL is a homogeneous whitish color with internal nodules measuring up to 15 mm. The aspect ratio exceeds 1.5.

evaluated the gross findings in terms of the following: color homogeneity, unevenness of cut surface, aspect ratio, presence of internal nodules, and capsular disruption. The color homogeneity of the lymph node cut surface was classified as homogeneous, which consists of one-color tone, or heterogenous, which consists of several color tones such as whitish, yellowish, and reddish. The unevenness of the lymph node cut surface was divided into three categories: flat, bulging, or collapsed. The aspect ratio of the cut surface was calculated by dividing the long diameter by the short diameter, with a boundary of 1.5 to indicate the irregularity of the lymph node shape. The presence of internal nodules in the cut surface was evaluated by confirming the presence or absence of a nodular structure. When internal nodules were evident, their largest diameter was measured. The capsular disruption was grossly classified according to whether the continuity of the lymph node capsule was preserved or destroyed.

Assessment of histologic diagnosis

After the evaluation of the gross findings, lymphadenectomy specimens for histological diagnosis were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Three-micrometer-thick sections were cut from formalin-fixed paraffin-embedded tissue and stained with hematoxylin-eosin (HE).

Immunohistochemistry was conducted using an autoimmunostainer (Leica BOND-III system: Leica Biosystems, Newcastle, UK). The histological diagnosis was performed by multiple pathologists based on the morphology, immunohistochemical findings, and results of light chain restriction by flow cytometry according to the criteria of the World Health Organization classification[6-8]. Fig. 1 and 2 show representative gross findings of RLH, FL, DLBCL, and HL, as well as histological findings, including immunohistochemical findings.

Statistical analysis

The analysis was performed with SPSS v 18.0.0.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was evaluated using the chi-square/Fisher's exact test, the Kruskal-Wallis test, and the Mann-Whitney *U* test. $P < 0.05$ was considered significant.

Results

Difference between gross findings in lymph node of RLH versus malignant lymphoma

Table 2 summarizes a comparison of the gross findings for patients with RLH and malignant lymphoma including FL, DLBCL and HL. The color homogeneity and the capsular disruption revealed significant differences between the RLH and malignant lymphoma groups, respectively ($P = 0.039$ and $P = 0.001$). In the comparison of color homogeneity, the RLH group had fewer homogeneous cases than heterogenous cases (1/7 [14.2%] and 6/7 [85.7%], respectively), while the malignant lymphoma group had more homogeneous cases than heterogenous cases (30/50 [60.0%] and 20/50

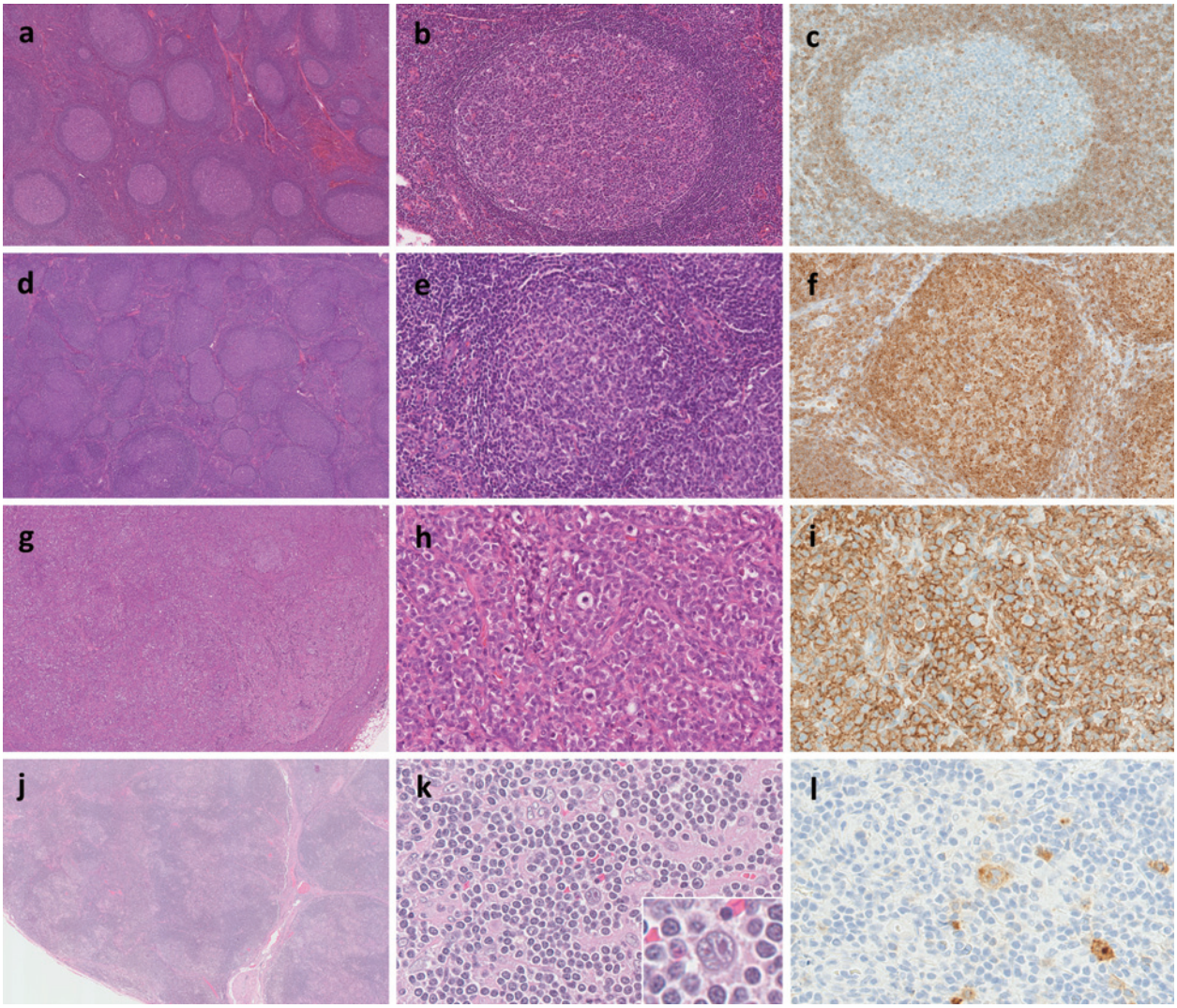

Figure 2 Representative histologic findings and immunoreactivity of reactive lymphoid hyperplasia (RLH), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), and Hodgkin lymphoma (HL). **(a-c)** RLH shows enlarged secondary follicles containing BCL2 negative germinal centers. **(d-f)** FL has predominant closely packed neoplastic follicles with BCL2 positive. **(g-i)** DLBCL exhibits total architectural effacement by medium- to large-sized neoplastic cells proliferation showing CD20 positive. **(j-l)** Classic HL involvement forms enlarged and firm lymph node. Lacunar cells **(k,** inset) and Reed-Sternberg cells with CD30 positive are scattered in the presence of small lymphocytes and other non-neoplastic inflammatory cells. Immunohistochemical findings indicate BCL2 **(c** and **f)**, CD20 **(i)**, and CD30 **(l)**. Original magnifications are ×20 **(a, d, g,** and **j)** and ×200 **(b, c, e, f, h, i, k,** and **l)**.

[40.0%], respectively). The capsular disruption was more common in the malignant lymphoma group (42/50 [84.0%]) than in the RLH group (0 [0%]). Indefinite cases of capsular disruption were found in both groups, although these were particularly common in the RLH group. The two groups exhibited no differences as regards the frequency of the unevenness of the cut surface, aspect ratio, and internal nodule.

Difference between gross findings in lymph nodes of FL, DLBCL, and HL

Gross findings by type of malignant lymphoma are shown in Table 3. In a comparison of the three malignant lymphoma groups, the internal nodules in the FL group (17/19 [89.5%]) and HL group (9/11 [81.8%]) occurred more frequently than those of the DLBCL group (11/20 [55.0%]) (P = 0.039). In particular, the internal nodules of the FL group occurred more frequently than those of the DLBCL group (P = 0.031). No

Table 2 Comparison of reactive lymphoid hyperplasia and malignant lymphoma in gross findings (n = 57)

		Reactive lymphoid hyperplasia, n = 7 (%)	Malignant lymphoma, n = 50 (%)	*P* value
Color homogeneity	Homogeneous	1 (14.2)	30 (60)	0.039
	Heterogenous	6 (85.7)	20 (40)	
Unevenness of cut surface	Flat	3 (42.9)	9 (18)	N.S.
	Bulge	4 (57.1)	40 (80)	
	Collapse	0 (0)	1 (2)	
Aspect ratio	1.5 ≥	2 (28.6)	34 (68)	N.S.
	1.5 <	5 (0)	16 (32)	
Internal nodule	Absent	1 (14.2)	13 (26)	N.S.
	Present	6 (85.7)	37 (74)	
Capsular disruption	Absent	4 (57.1)	5 (10)	0.001
	Present	0 (0)	42 (84)	
	Indefinite	3 (42.9)	3 (6)	

N.S., not significant

Table 3 Comparison of follicular lymphoma, diffuse large B-cell lymphoma, and Hodgkin lymphoma in gross findings (n = 50)

		Follicular lymphoma, n = 19 (%)	Diffuse large B-cell lymphoma, n = 20 (%)	Hodgkin lymphoma, n = 11 (%)	*P* value
Color homogeneity	Homogeneous	10 (52.6)	14 (70)	6 (54.5)	N.S.
	Heterogenous	9 (47.4)	6 (30)	5 (45.5)	
Unevenness of cut surface	Flat	2 (10.5)	5 (25)	2 (18.2)	N.S.
	Bulge	17 (89.5)	14 (70)	9 (81.8)	
	Collapse	0 (0)	1 (5)	0 (0)	
Aspect ratio	1.5 >	12 (63.2)	15 (75)	7 (63.6)	N.S.
	1.5 <	7 (36.8)	5 (25)	4 (36.4)	
Internal nodule	Absent	2 (10.5)	9 (45)	2 (18.2)	0.039
	Present	17 (89.5)	11 (55)	9 (81.8)	*0.031
Capsular disruption	Absent	2 (10.5)	1 (5)	2 (18.2)	N.S.
	Present	16 (84.2)	17 (85)	9 (81.8)	
	Indefinite	1 (5.3)	2 (10)	0 (0)	

*Significant difference between follicular lymphoma and diffuse large B-cell lymphoma.
N.S., not significant.

significant differences were found between the three malignant lymphoma groups as regards to color homogeneity, unevenness of cut surface, aspect ratio, and capsular disruption.

Comparison of internal nodular size in lymph nodes of FL, DLBCL, and HL

Next, we compared the largest sizes of internal nodule in the lymph nodes of FL, DLBCL, and HL, as shown in Fig. 3. The median sizes of the largest internal nodule of FL, DLBCL, and HL were 1.0 mm (0.5-16.0 mm), 6.0 mm (1.0-13.0 mm), and 3.0 (1.0-15.0 mm), respectively. The internal nodules tended to be smaller in FL and HL than in DLBCL ($P = 0.007$). In particular, the internal nodule size of FL was significantly smaller than that of DLBCL ($P = 0.022$).

Discussion

Lymphadenopathy is defined as an enlarged lymph node measuring 1 cm or more[9], and has various causes, including RLH and malignant lymphoma, as well as cancer metastases and infections[3,4,10]. Gross observation of a lymphadenectomy specimen is important not only for differentiating between benign and

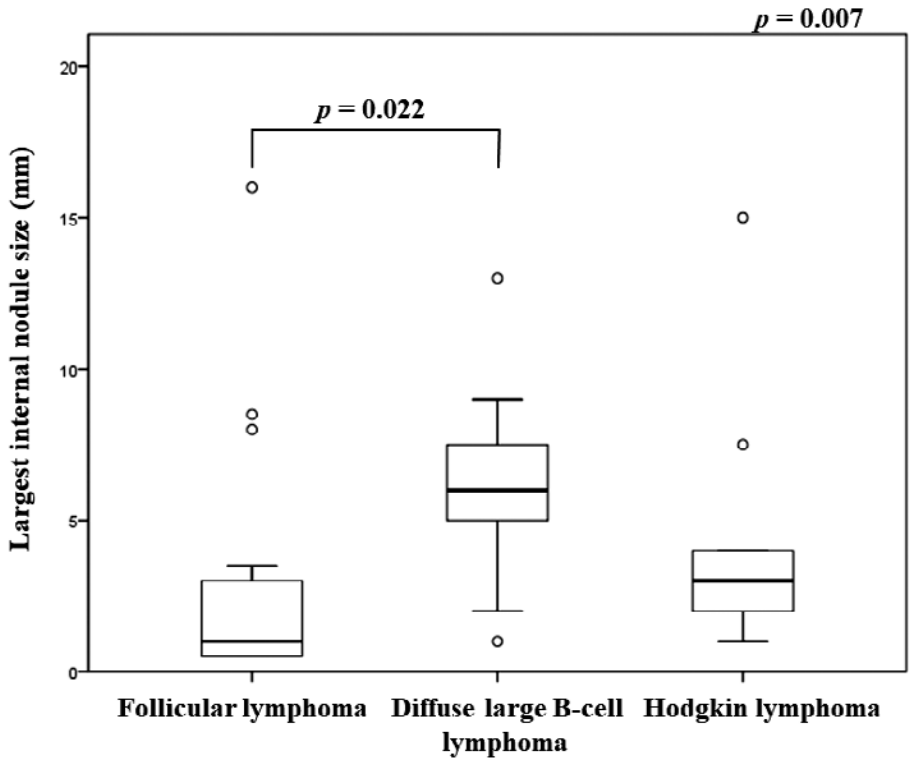

Figure 3 Comparison of the largest internal nodule size on a cut surface in lymph nodes among follicular lymphoma, diffuse large B-cell lymphoma, and Hodgkin lymphoma. This comparison is based on cases in which each internal nodule was seen (n = 37). Numbers indicate the largest internal nodule size.

malignant cases, but also for determining the required analysis and prioritization. In this study, malignant lymphoma was characterized by the capsular disruption compared with that of RLH. A capsular disruption is a characteristic gross finding in squamous cell carcinoma lymph node metastases[10]. The finding of a capsular disruption grossly suggests that the tumor has invaded beyond the capsule. This study also showed that RLH was more common in cases with heterogenous color. In cancer metastasis, the color homogeneity is often heterogeneous and diverse due to necrosis and fibrosis[11], which is different from that of malignant lymphoma. Therefore, if a capsular disruption is observed and the color is homogeneous, a malignant lymphoma must be assumed and the lymphadenectomy specimen must be submitted for the testing required for a malignant lymphoma as well as histological diagnosis.

The cut surface of the lymph node in HL is described as a banded, bulging nodule[8]. In this study, the cut surface of the lymph node was more bulging in cases of malignant lymphoma compared with those of RLH, especially in FL and HL. Malignant lymphomas are classified as low grade and high grade according to the degree of tumor growth[12]. Although the unevenness of the cut surface was expected to be due to the degree of cell proliferation, more cases of low-grade FL were found to be bulging than with high-grade DLBCL. The unevenness of the cut surface may be influenced by the histological subtype. HL is classified into four histological subtypes by the WHO classification[1], with lymphocyte-rich classic HL having better overall event-free survival than the others[13]. However, since all the HLs in this study were nodular sclerosis classic HLs, the differences in the findings of the cut surface according to the histological subtype of the HL is a subject for future study.

The frequency of cases with internal nodules varied according to the histological subtype of malignant lymphoma. FL is characterized by the histological findings of neoplastic follicular structures[1,6], which may be reflected in the gross findings of small internal nodules and the unevenness of the cut surface. The gross findings of HL were similar to those of FL. However, nodu-

lar sclerosis classic HL is characterized histologically by fibrous bands[14], and the small nodules may reflect different histological findings from those of FL. DLBCL had internal nodules in 55% of cases in this study, and the internal nodules were significantly larger than those of FL. The etiology of DLBCL is diverse, including de novo or transformation from a less aggressive lymphoma such as FL[15], which may be why the gross findings are not distinctive.

The limitations of this study are the small number of cases and the fact that only FL, DLBCL, and nodular sclerosis classic HL subtypes of malignant lymphoma were examined, in which selection bias is inherent. However, among the representative subtypes of malignant lymphoma, FL, DLBCL, and HL, which are more likely to exhibit lymph node involvement, were involved, and all FL, DLBCL, and HL of the study period were included and analyzed. The design of this study was such that the histological diagnosis was confirmed after gross observation. Studies involving larger cohorts are needed to make it possible to prioritize examinations based on gross findings.

In conclusion, we investigated gross findings for the cut surface of lymphadenectomy specimens. The presence or absence of capsular disruption and the color homogeneity were useful in differentiating malignant lymphoma from RLH. The malignant lymphoma subtyping was based on the presence or absence and size of internal nodules. Gross observation of the cut surface of a lymphadenectomy specimen may provide important information for appropriate allocation of the specimen for various types of testing.

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Author contributions

MO: development of acquisition of data, analysis and interpretation of data, and writing the manuscript. Y. Nakazato: concept and design, acquisition of data, development of acquisition of data, analysis and interpretation of data, and writing the manuscript. AT-O and YK: acquisition of data, development of acquisition of data, and development of methodology. MT, Y. Nozawa, SN: analysis and interpretation of data. HM and KM: development of methodology. KI: interpretation of data, development of methodology, study supervision, and writing the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

There are no conflicts of interest regarding the publication of this article.

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