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Preoperative diagnostic algorithm of primary thyroid lymphoma using ultrasound, aspiration cytology, and flow cytometry

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Abstract. The aims of this report were to clarify the diagnostic significance of ultrasound (US), fine needle aspiration cytology (FNAC), and flow cytometry for primary thyroid lymphoma, and to establish a preoperative diagnostic algorithm of primary thyroid lymphoma. We retrospectively examined US, FNAC, and flow cytometry in 43 patients with benign lymphoproliferative lesions and 32 patients with primary thyroid lymphoma, who underwent US, FNAC, and flow cytometry at Kuma Hospital between May 2012 and December 2015. Primary thyroid lymphomas included 27 mucosa-associated lymphoid tissue lymphomas, 4 diffuse large B-cell lymphomas, and 1 follicular lymphoma. Flow cytometry had the highest specificity (88.4%) and sensitivity (75.0%). The specificity of US was the lowest (32.6%). Both the positive predictive value (90.5%) and negative predictive value (94.7%) were the highest for FNAC. A scoring system was defined as follows: US, low suspicion 0, intermediate suspicion 1, and high suspicion 2; FNAC, benign 0, undetermined 1, malignant 2; and flow cytometry, 0.33< κ/λ ratio <3 0, κ/λ ratio ≤0.33 2, and κ/λ ratio ≥3 2. We propose that a score ≥4 indicates the need for thyroid resection for diagnosing primary thyroid lymphoma. In such a situation, the case of diffuse large B-cell lymphoma, which was aggressive, was not excluded. Approximately one-fifth of mucosa-associated lymphoid tissue lymphomas may be overlooked, but the patients could be followed up with because of an indolent course.

Key words: Thyroid lymphoma, MALT, Flow cytometry, κ/λ light chain ratio, Aspiration cytology

PRIMARY THYROID LYMPHOMA is a rare condition that accounts for 5% of thyroid malignancies [1-3]. Women are more commonly affected than men are. Patients typically present in the sixth or seventh decade of life. Primary thyroid lymphoma typically arises in the setting of Hashimoto thyroiditis. Most thyroid lymphomas are of B-cell origin [1-3]. The most common subtype of primary thyroid lymphoma is diffuse large B-cell lymphoma (DLBCL), followed by mucosa-associated lymphoid tissue (MALT) lymphoma [1-3].

An ultrasound (US) is the initial diagnostic modality used to detect primary thyroid lymphoma, and the next step is fine needle aspiration cytology (FNAC). Approximately half of patients suspected of having thyroid lymphoma based on US were pathologically confirmed as having lymphoma [4]. FNAC has an established procedure for diagnosing thyroid tumor, but the diagnostic accuracy has varied among reports (40 to 100%) [1, 5]. To improve the limited diagnostic tools, ancillary techniques have been recently used. Flow cytometry (FC) can detect small-sized primary thyroid lymphoma and may contribute the diagnosis of lymphoid lesions that are difficult to interpret with FNAC alone [6]. The aims of this report were to clarify the diagnostic significance of US, FNAC, and FC for primary thyroid lymphoma, and to establish a preoperative diagnostic algorithm for primary thyroid lymphoma by combining the use of these three diagnostic tools.

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Materials and Methods

We reviewed 227 patients who underwent US, FNAC, and FC at Kuma Hospital between May 2012 and December 2015. Among them, we selected 84 patients in whom both FNAC and a sampling for FC were simultaneously performed by one pathologist (M.H.), and resection of the lesions for histological examination or follow-up examination for more than 1 year was performed. Of 84 patients, 45 underwent total thyroidectomy, lobectomy, or surgical open biopsy to confirm the diagnosis. The diagnoses included Hashimoto thyroiditis (4 patients), other benign conditions (2), primary thyroid lymphoma (32), and other malignant tumors (7 cases), including 3 patients with PTC, 2 patients with mucoepidermoid carcinoma, 1 patient with metastatic renal cell carcinoma, and 1 patient with CASTLE (carcinoma with thymus-like differentiation). The diagnosis of primary thyroid lymphoma was determined by histological examination and immunohistochemical examination using antibodies for L26, UCHL-1, Bcl-2, CD10, CD23, κ light chain, λ light chain, and cytokeratin AE1/AE3, as well as results of FC based on CD45 and side scatter-based gating, G-banding chromosomal examination, and immunoglobulin heavy chain JH DNA rearrangement analysis. Primary thyroid lymphomas included 27 MALT lymphomas, 4 DLBCLs, and 1 follicular lymphoma. The remaining 37 patients who had been followed up without progression for more than 1 year were regarded as having benign lymphoproliferative lesions in this study. Thus, we retrospectively examined US, FNAC, and FC findings in 75 patients, including 32 with primary thyroid lymphoma and 43 with benign lymphoproliferative lesions.

Clinical data were obtained from patients' medical records at Kuma Hospital. US was performed using the APLIO SSA-770A (Toshiba Medical Systems Co., Ltd., Otawara, Japan) or APLIO 500 TUS-A500 (Toshiba) with the PLT-805AT (Toshiba) or PLT-1005BT probe (Toshiba). Ultrasonography interpretation was performed according to ATA nodule sonographic patterns [7], including low suspicion, intermediate suspicion, and high suspicion, which are shown in Fig. 1.

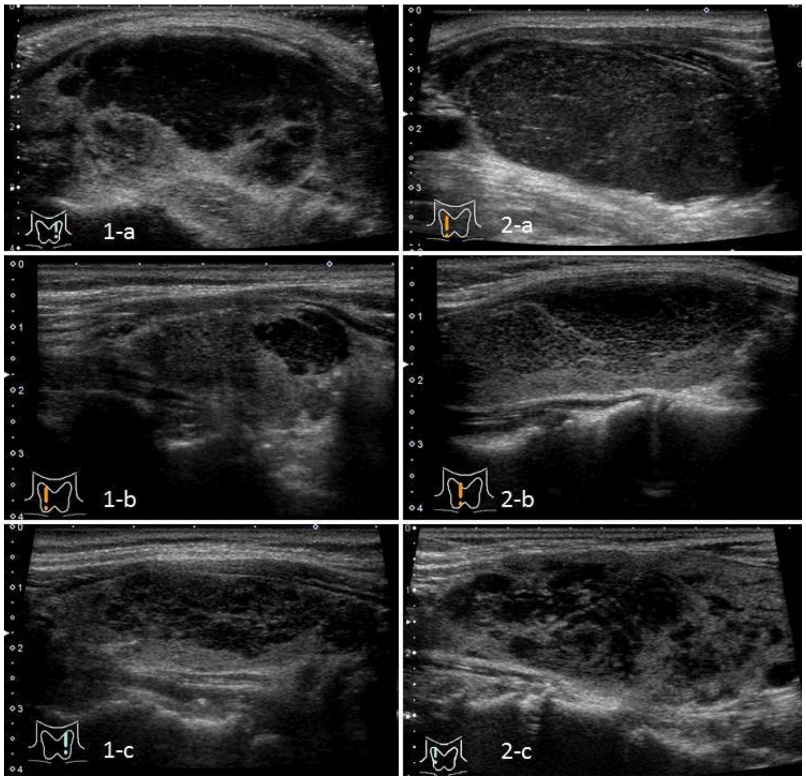

Fig. 1 Ultrasonogram findings typical of high suspicion **(a)**, intermediate suspicion **(b)**, and low suspicion **(c)** lesions of primary thyroid lymphoma (1; nodular type, 2; diffuse type) (B-mode).

FNAC was performed by using a 22-gauge needle under ultrasound guidance. Cytological slides were prepared by expressing the aspirated materials from the needle onto slide glasses and compressing them with a second slide, and they were immediately fixed with Cytorop (Alfresa Pharma Co., Osaka, Japan), a cytological fixative. They were stained using the Papanicolaou method. FNAC reports were categorized into benign, atypia of undetermined significance, suspicious for malignancy, and malignant, based on the criteria of The Bethesda System for Reporting Thyroid Cytopathology [8]. The last two categories were considered as malignant in this study. Fig. 2 shows cytological findings typical of MALT lymphomas and DLBCL.

The specimens for FC were obtained from second aspirates using the same method for the first one. Immediately after the aspirates were obtained, the materials were placed into cell preservative liquid (5 mL, H00, Nissui Pharmaceutical, Tokyo, Japan) and transported to the FC laboratory (SRL, Tokyo, Japan). The materials were analyzed using the FACSCanto™ II (BD Biosciences, San Jose, CA, USA), with antibodies of CD45 (CD45-per-cp, BD Biosciences), κ light chain/CD19(K-L(Rb-F(ab')2)/FITC+CD19(HD37)/RPE, Yamasa, Tokyo, Japan), and λ light chain/CD19 (L-L(Rb-F(ab')2)/FITC+CD19(HD37)/RPE, Yamasa, Tokyo, Japan). To identify monoclonal proliferation, we defined light chain restriction when the κ/λ light chain ratios of the lymphoid cells counted by gating were greater than 3:1 or less than 1:3 on CD45 gating (Fig. 3).

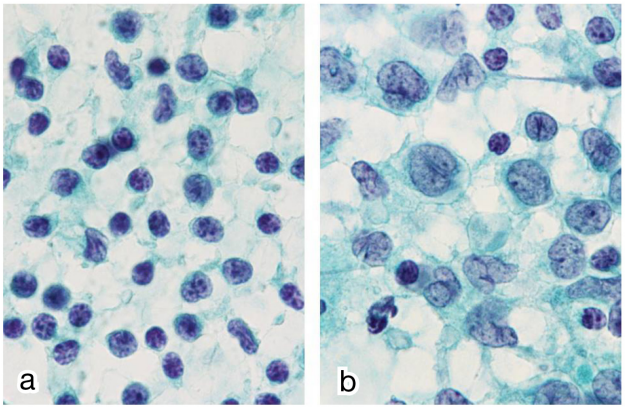

Fig. 2 Cytological findings of mucosa-associated lymphoid tissue lymphoma **(a)** and diffuse large B-cell lymphoma **(b)** (Papanicolaou stain, × 1,000).

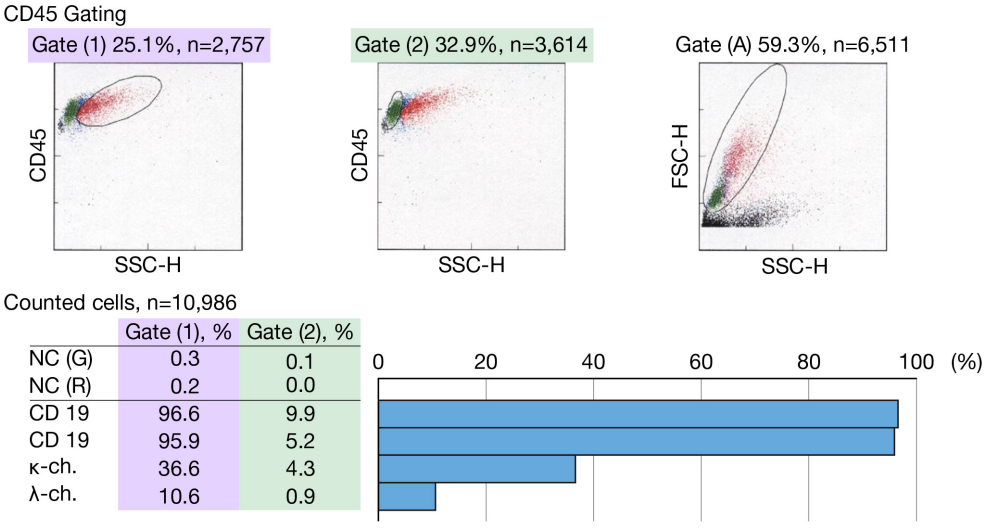

Fig. 3 The κ/λ light chain ratios of the lymphoid cells counted by gating are greater than 3:1 by CD45 gating. FSC-H, forward scatter height; SSC-H, side scatter height.

Results

US (Table 1)

Of 43 cases of benign lymphoproliferative lesions assessed by US, 14 (32.6%), 22 (51.2%), and 7 (16.3%) were classified as low suspicion, intermediate suspicion, and high suspicion, respectively. Of 32 cases of primary thyroid lymphomas, 1 (3.1%), 11 (34.4%), and 20 (62.5%) were classified as low suspicion, intermediate suspicion, and high suspicion, respectively. One case of primary thyroid lymphoma that was classified as low suspicion was MALT lymphoma.

FNAC (Table 2)

Over half of the cases of benign lymphoproliferative lesions were classified as undetermined. Eighteen (41.9%) and two (4.7%) cases of benign lymphoproliferative lesions were benign and malignant, respectively. Nineteen (59.4%) of 32 cases of primary thyroid lymphoma were malignant. One (3.1%) patient with primary thyroid lymphoma that was MALT lymphoma was diagnosed as having a benign lesion. All 4 patients with DLBCL were diagnosed as having a malignant lesion.

FC (Table 3)

The number of counted lymphoid cells varied from 64 to 6,001 (median 686). Median numbers of the counted cells in cases of benign lymphoproliferative lesions, DLBCL, and MALT lymphoma were 719, 626.5, and 353, respectively. Light chain restriction was demonstrated in 75.0% (24) of cases of primary thyroid lymphoma. There were five times more cases of primary thyroid lymphoma with κ-predominant light chain restriction than those with λ-predominant light chain restriction. In the remaining 8 cases of primary

Table 1 Results of ultrasound in 75 lymphoma-suspected cases

	BLL	Lymphoma			
			(MALT-L)	(DLBL)	(FL)
Low suspicion	14 (32.6%)	1 (3.1%)	1	0	0
Intermediate suspicion	22 (51.2%)	11 (34.4%)	10	1	0
High suspicion	7 (16.3%)	20 (62.5%)	16	3	1
Total	43	32	27	4	1

BLL, benign lymphoproliferative lesion; MALT-L, mucosa-associated lymphoid tissue lymphoma; DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

Table 2 Results of fine needle aspiration cytology in 75 lymphoma-suspected cases

	BLL	Lymphoma			
			(MALT-L)	(DLBL)	(FL)
Benign	18 (41.9%)	1 (3.1%)	1	0	0
Undetermined	23 (53.5%)	12 (37.5%)	11	0	1
Malignant	2 (4.7%)	19 (59.4%)	15	4	0
Total	43	32	27	4	1

BLL, benign lymphoproliferative lesion; MALT-L, mucosa-associated lymphoid tissue lymphoma; DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

Table 3 Results of flow cytometry in 75 lymphoma-suspected cases

	BLL	Lymphoma			
	(43)	(32)	(MALT-L)	(DLBL)	(FL)
0.33< κ/λ ratio <3	38 (88.4%)	8 (25.0%)	7	1	0
κ/λ ratio ≤0.33 or ≥3	5 (11.6%)	24 (75.0%)	20	3	1
κ/λ ratio ≤0.33	1	4	2	1	1
κ/λ ratio ≥3	4	20	18	2	0
	43	32	27	4	1

BLL, benign lymphoproliferative lesion; MALT-L, mucosa-associated lymphoid tissue lymphoma; DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

thyroid lymphoma (25.0%), light chain restriction was not demonstrated, although a sufficient number of lymphoid cells were counted. Cases of primary thyroid lymphoma without light chain restriction showed that the lesions of primary thyroid lymphoma and Hashimoto thyroiditis were histologically mixed. Five (11.6%) cases of benign lymphoproliferative lesions had light chain restriction. In all of these cases, the lesions were not enlarged more than 1 year after the FC examination.

Diagnostic accuracy (Table 4)

Among the three diagnostic tools, FC had the highest specificity (88.4%) and sensitivity (75.0%). Specificity of US was the lowest (32.6%). Both the positive predictive value (PPV) (90.5%) and negative predictive value (NPV) (94.7%) were highest for FNAC.

Diagnostic algorithm (Table 5)

We defined a score to establish the diagnostic algorithm of primary thyroid lymphoma as follows: US, low suspicion 0, intermediate suspicion 1, and high suspicion 2; FNAC, benign 0, undetermined 1, and malignant 2; and FC, 0.33< κ/λ ratio <3 0, κ/λ ratio ≤0.33 2, and κ/λ ratio ≥3 2. When scores ≥2 were an indication of diagnostic resection, all cases of primary thyroid lymphoma were included, but 48.8% (21/43) of cases of BLL were also included (Table 6). Concerning scores ≥3, 1 (3.1%) case of primary thyroid lymphoma was excluded, and 8 (18.6%) cases of benign lymphoproliferative lesions were included. Scores ≥4 included 4 (9.3%) cases of benign lymphoproliferative lesions and 26 (81.3%) cases of primary thyroid lymphoma. In either setting, the case of DLBCL, which was aggressive, was not excluded. To avoid overlooking the case of aggressive DLBCL and excessive resection for Hashimoto thyroiditis, it was preferable to use scores ≥4.

Discussion

Primary thyroid lymphoma is divided into two separate clinicopathological entities: DLBCL and MALT lymphoma [1, 3, 5]. DLBCL exhibits an aggressive clinical course, and multimodal treatment should be considered as treatment. In contrast, MALT lymphoma exhibits an indolent course, and its management is more conservative [1-3]. DLBCL is the most common, and it accounts for approximately two-thirds of cases

Table 4 Diagnostic accuracy of the ultrasound, aspiration cytology, and flow cytometry in 75 lymphoma-suspected cases

	Specificity	Sensitivity	PPV	NPV
US	32.6% (14/43)	62.5% (20/32)	74.1% (20/27)	93.3% (14/15)
Aspiration cytology	41.9% (18/43)	59.4% (19/32)	90.5% (19/21)	94.7% (18/19)
Flow cytometry	88.4% (38/43)	75.0% (24/32)	82.8% (24/29)	82.6% (38/46)

US, ultrasound; PPV, positive predictive value; NPV, negative predictive value.

Table 5 Diagnostic scores for the diagnosis of primary thyroid lymphoma

	Score
Ultrasound	
Low suspicion	0
Intermediate suspicion	1
High suspicion	2
Fine needle aspiration cytology	
Benign	0
Undetermined	1
Malignant	2
Flow cytometry	
0.33< κ/λ ratio <3	0
κ/λ ratio ≤0.33 or ≥3	2

Table 6 Results using the diagnostic scores of thyroid malignant lymphoma

Score	BLL (43)	PTL (32)			
			MALT-L	DLBL	FL
0	4	0			
1	18	0			
2	13	1	1		
3	4	5	5		
4	3	6	5	1	
5	1	12	10	1	1
6	0	8	6	2	
Total	43	32			

BLL, benign lymphoproliferative lesion; MALT-L, mucosa-associated lymphoid tissue lymphoma; DLBL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

of primary thyroid lymphoma [3]. Mizokami *et al*. reported that all cases of primary thyroid lymphoma that developed during the ultrasonographic follow-up of Hashimoto thyroiditis were MALT lymphoma [9]. These findings indicate that there is a strong association between Hashimoto thyroiditis and MALT lymphoma. As a component of MALT lymphoma can be seen in one-third of cases of DLBCL, some cases of DLBCL may be transformed from a preexisting MALT lymphoma [3]. In our study, of 32 cases of primary thyroid lymphoma, 27 (84.4%) were MALT lymphoma, and 4 (12.5%) were DLBCL. We think that earlier detection of the lesion and an accurate diagnosis of primary thyroid lymphoma increased the proportion of MALT lymphomas in cases of primary thyroid lymphoma in our series.

US is the initial diagnostic modality for primary thyroid lymphoma [7]. The findings show three patterns: well-defined nodular lesions with hypoechoic and homogeneous internal echoes (nodular pattern), bilateral diffuse hypoechoic lesions with indistinct borders (diffuse pattern), and multiple patchy hypoechoic lesions (mixed pattern) [1, 4]. The presence of enhanced posterior echoes helps distinguish primary thyroid lymphoma from other thyroid lesions [4]. Wang *et al*. described that a central blood flow pattern highly suggests the diagnosis of primary thyroid lymphoma [10]. There are few reports on the diagnostic accuracy of primary thyroid lymphoma in large series. PPVs of the nodular, mixed, and diffuse type were 64.9%, 63.2%, and 33.7%, respectively [4]. In a prospective study, 47.9% of patients suspected of having primary thyroid lymphoma were pathologically confirmed as having lymphoma [4], and the PPV was 74.1%, which was higher compared to that of a previous report [4]. However, specificity of US was the lowest (32.6%) among the three diagnostic tools.

The diagnostic accuracy of FNAC for primary thyroid lymphoma is not high enough to rely solely on it. Several series have reported that in 50% to 90% of patients with primary thyroid lymphoma, the diagnosis has been made by FNAC [1, 11, 12]. This is mainly because of the morphological similarities between Hashimoto thyroiditis and primary thyroid lymphoma, especially MALT lymphoma. MALT lymphoma is usually composed of heterogeneous cells and associated with Hashimoto thyroiditis. The diagnosis of DLBCL is easier to make because of the presence of large monotonous atypical cells [11]. The presence of lymphoglandular bodies is also helpful [12]. Therefore, the diagnostic accuracy of FNAC for primary thyroid lymphoma significantly depends on the proportion of MALT lymphoma and DLBCL in the series. In our series, the PPV (90.5%) and NPV (94.7%) of FNAC were satisfactory; however, the specificity (41.9 %) and sensitivity (59.4 %) were low, because 37.5% of cases of primary thyroid lymphoma were classified as undetermined. Considering the fact that cases of MALT lymphoma outnumber that of DLBCL, our results are understandable, and an additional diagnostic tool should be used for undetermined cases.

It has been demonstrated that ancillary techniques improve the cytological diagnosis of primary thyroid lymphoma. Assessment of the κ/λ light chain ratio with FC is one of the most important procedures, enabling differentiation between benign lymphoproliferative lesions and lymphoma. When the κ/λ light chain ratio is greater than 3 to 4:1 or less than 1:2 to 3, clonal proliferation is present [6, 13-15]. However, some lymphomas do not satisfy this criterion [16, 17]. Conversely, light chain restriction has been reported in a minority of patients with Hashimoto thyroiditis [18-20]. In addition, inadequate sampling for FNAC remains a limiting factor for performing FC [14]. In fact, 8 (25.0%) of 32 cases of primary thyroid lymphoma did not have light chain restriction in our series. In 5 (11.6 %) of 43 cases of Hashimoto thyroiditis, κ/λ light chain ratios were greater than 3:1 or less than 1:3. As some of them did not undergo a histological examination, it was unclear whether they had primary thyroid lymphoma. However, this is not significant, because they were followed up with and had no progression for more than 1 year. Therefore, we think that κ/λ light chain restriction is not an absolute indication of thyroid resection for a diagnosis of primary thyroid lymphoma.

When primary thyroid lymphoma is suspected, core-needle biopsy may be considered as a first-line histologic diagnostic tool [1]. However, we prefer lobectomy or open biopsy rather than core-needle biopsy since the materials obtained by core-needle biopsy are insufficient for ancillary studies, including flow cytometry, G-banding chromosomal examination, and immunoglobulin heavy chain JH DNA rearrangement analysis. The specimen is also too small to detect an early lesion of MALT lymphoma. In addition, the procedure is risky for the small lesions. To establish the indication of thyroid resection for a diagnosis of primary thyroid lymphoma, we used

a scoring system for US, FNAC, and FC. To avoid overlooking cases of aggressive DLBCL and excessive resection for Hashimoto thyroiditis, we propose that a score ≥4 indicates thyroid resection for a diagnosis of primary thyroid lymphoma. In such a situation, approximately one-fifth of MALT lymphomas may be overlooked, but the patients could be followed up with because of an indolent course. We think that the diagnostic algorithm we proposed is not theoretically affected by the proportion of MALT lymphoma and DLBCL at each institution.

Disclosure

The authors have no conflicts of interest to declare regarding grant support or financial relationships.

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