The Korean Journal of Pathology 2011; 45: 406-411
http://dx.doi.org/10.4132/KoreanJPathol.2011.45.4.406

Fine-Needle Aspiration Cytology of the Nodal Marginal Zone Lymphoma

Seung Kyu Choi · Ji Eun Kwon[1]
Young-Hyeh Ko[2]

Department of Pathology, Eulji Hospital, Eulji University School of Medicine, Seoul; [1]Department of Pathology, Dankook University Hospital, Dankook University College of Medicine, Cheonan; [2]Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Received: March 3, 2011
Accepted: June 21, 2011

Corresponding Author
Young-Hyeh Ko, M.D.
Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, Korea
Tel: +82-2-3410-2762
Fax: +82-2-3410-0025
E-mail: yhko310@skku.edu

Background: Nodal marginal zone lymphoma (NMZL) is a rare B-cell neoplasm consisting of heterogeneous cellular components and residual B-cell follicles. Because of such histological features, it is difficult to diagnose NMZL by fine needle aspiration (FNA) cytology. We reviewed FNA cytology of NMZL to identify a cytological clue to avoid misdiagnosing NMZL. **Methods:** Histological, cytological, and clinical findings of seven cases of NMZL were reviewed. **Results:** Most cases showed nodular aggregates of lymphohistiocytes derived from the germinal center irrespective of histological pattern. The cellular components were heterogeneous and composed of mature small lymphocytes, intermediate and large lymphocytes, immunoblasts, tingible body macrophages, and follicular dendritic cells. Intermediate-sized neoplastic cells with a pale nucleus were observed but difficult to identify because of admixed non-neoplastic cells, which outnumbered neoplastic cells. Except for one case with a high proportion of intermediate-sized cells, the other six cases were initially diagnosed as reactive hyperplasia. A flow cytometric analysis was performed in two cases and failed to demonstrate a monoclonal B-cell population. **Conclusions:** The FNA showing a reactive hyperplasia-like smear pattern should be carefully observed by experienced cytopathologists to identify intermediate-sized neoplastic cells. Clinical information including the size of the lymph nodes is important to avoid a misdiagnosis.

Key Words: Lymphoma, B-cell, marginal zone; Lymph nodes; Biopsy, fine-needle

Fine needle aspiration (FNA) cytology is a simple and useful tool to screen malignant lymphoma involving the lymph node; however, it may give rise to false negative or false positive results particularly in low grade lymphoma.[1,2] Additional studies including flow cytometric immunophenotypic analysis and gene rearrangement studies may be useful to improve the diagnostic accuracy of FNA cytology.[3-7] However, such adjunct studies result in increased medical costs, so they are usually not performed for a routine FNA diagnosis of the lymph nodes. Currently, immunophenotypic and genetic studies have become more important for diagnosing malignant lymphoma; therefore, an FNA diagnosis depending only on the cytological findings is limited in diagnostic accuracy for low grade lymphomas such as nodal marginal zone lymphoma (NMZL). Histological findings and cellular components of NMZL are heterogeneous. The pattern of neoplastic infiltrates is diffuse, nodular, perifollicular, or interfollicular, and commonly shows residual hyperplastic or colonized follicles. In addition to the heterogeneous histological architecture, the tumors consist of mixtures of small to medium-sized cells with variable numbers of large lymphocytes and plasma cells.[8] In this study, we analyzed the cytological findings of NMZL and correlated them with histological architecture to identify a diagnostic clue to avoid a misdiagnosis.

MATERIALS AND METHODS

Inclusion criteria for the cases were NMZLs with a primary presentation in lymph nodes without extranodal or splenic involvement. Eighteen cases were diagnosed at our institution between 2001 and 2010. FNA cytology was performed in nine patients prior to biopsy, among which seven cases with fair quality cytology samples were enrolled in the study. The FNA cytology diagnosis of six cases was reactive hyperplasia and one case was suspicious of malignant lymphoma. A flow cytometric study was performed for two cases at the time of aspiration. Immunohistochemical studies for CD20 (Novocastra, Newcastle, UK), CD3 (Dako, Glostrup, Denmark), CD10 (Novocastra), BCL6 (Novocastra), and Ki-67 (Dako) were performed for all seven biopsy samples, and an *IgH* gene rearrangement study was conducted for seven biopsy samples. Retrieved histological and cytological materials were reviewed. The histological biopsy patterns were divided into a diffuse pattern, a follicular/nodular pattern, a perifollicular pattern, and a interfollicular pat-

tern according to Salama *et al.*[9] FNA cytology was analyzed for smear pattern (follicular/nodular pattern or diffuse pattern), follicular components (tingible body macrophages and follicular dendritic cells) and cellular components (% of small cells, intermediate-sized cells, large cells, and plasma cells). Cellular components were counted in up to 500 cells under a 400× magnification field. Clinical information including age, gender, biopsy site, symptoms, size of lymph node, stage, treatment, and relapse were obtained from the medical records.

RESULTS

Patient clinical information is summarized in Table 1. Ages ranged from 16 to 47 years with a median of 34 years. The patients presented with lymphadenopathy without systemic symptoms and one patient had multiple lymphadenopathy in the neck and abdomen. After FNA, a computed tomography scan of the head and neck region was performed in all cases. The size of the biopsied lymph nodes ranged from 1 to 2.3 cm. Four patients underwent chemotherapy or radiotherapy. Only one patient (case 1) relapsed from the disease at 1 and 6 years after initial treatment.

Histological pattern

As described previously, the diffuse pattern was characterized by sheets of small to medium-sized neoplastic cells with no residual follicular structure (Fig. 1A). The nodular/follicular pattern was noted in three cases and characterized by well-formed nodules that were replaced by small neoplastic cells without a visible normal germinal center (Fig. 1B). The interfollicular pattern was characterized by a widened interfollicular zone containing heterogeneous intermediate and large lymphocytes and plasma cells and retained secondary follicles (Fig. 1C). Tumor cells were limited to the interfollicular area. A perifollicular pattern was noted in three cases and characterized by annular distribution of the neoplastic cells around normal secondary follicles (Fig. 1D). In four cases, a mixed pattern, consisting of more than one pattern, was observed in the same lymph node.

Cytological findings

Except case 1, the FNA of all cases (cases 2-7) revealed nodular lymphohistiocytic aggregates in a background of a diffuse lymphocytic smear (Fig. 2A). Many tingible body macrophages and follicular dendritic cells derived from residual germinal centers were associated with the nodular aggregates. Admixed

Table 1. Clinical data of seven nodal marginal zone lymphoma cases

Case	Age (yr)/Sex	Symptom and sign	LN size (cm)	Flow cytometry	IgH	Stage	Treatment	Relapse
1	21/F	Multiple neck mass	1.3	ND	MC	IIIA	CT	Yes
2	34/M	Submental mass	1.8	ND	MC	IA	RT	No
3	47/M	Neck mass	1.6	ND	MC	IA	ND	No
4	16/M	Submental mass	1.8	ND	MC	IA	ND	No
5	39/M	Infraauricular mass	1.0	ND	MC	IA	RT	No
6	45/M	Neck mass	1.6	NC	MC	IA	CT, RT	No
7	34/M	Submental mass	2.3	NC	MC	IA	ND	No

LN, lymph node; IgH, immunoglobulin heavy chain gene rearrangement; F, female; ND, not done; MC, monoclonal; CT, chemotherapy; M, male; RT, radiotherapy; NC, not conclusive for monoclonality by flow cytometry.

Table 2. Cytological characteristics and histological patterns of seven nodal marginal zone lymphoma cases

Case	Biopsy	FNA cytology				
	Biopsy pattern	FNA pattern	Small/Intermediate/Large cell/ Plasma cell	TBM/FDC	Initial Dx	Review Dx
1	D+IF	D	30/10/50/10	-/-	Lymphoma	Lymphoma
2	PF	F	40/10/50/0	+++/+++	RH	Suspicious
3	IF	F	30/50/20/0	+++/+++	RH	Suspicious
4	IF	D/F	85/10/5/0	+/++	RH	RH
5	F+D	F	50/20/30/0	++/++	RH	RH
6	F+PF	F	40/40/20/0	++/++	RH	RH
7	F+PF	F	40/0/60/0	+++/+++	RH	Suspicious

FNA, fine needle aspiration; TBM, tingible body macrophage; FDC, follicular dendritic cell; Dx, diagnosis; D, diffuse; IF, interfollicular; PF, perifollicular; F, follicular/nodular; RH, reactive hyperplasia.

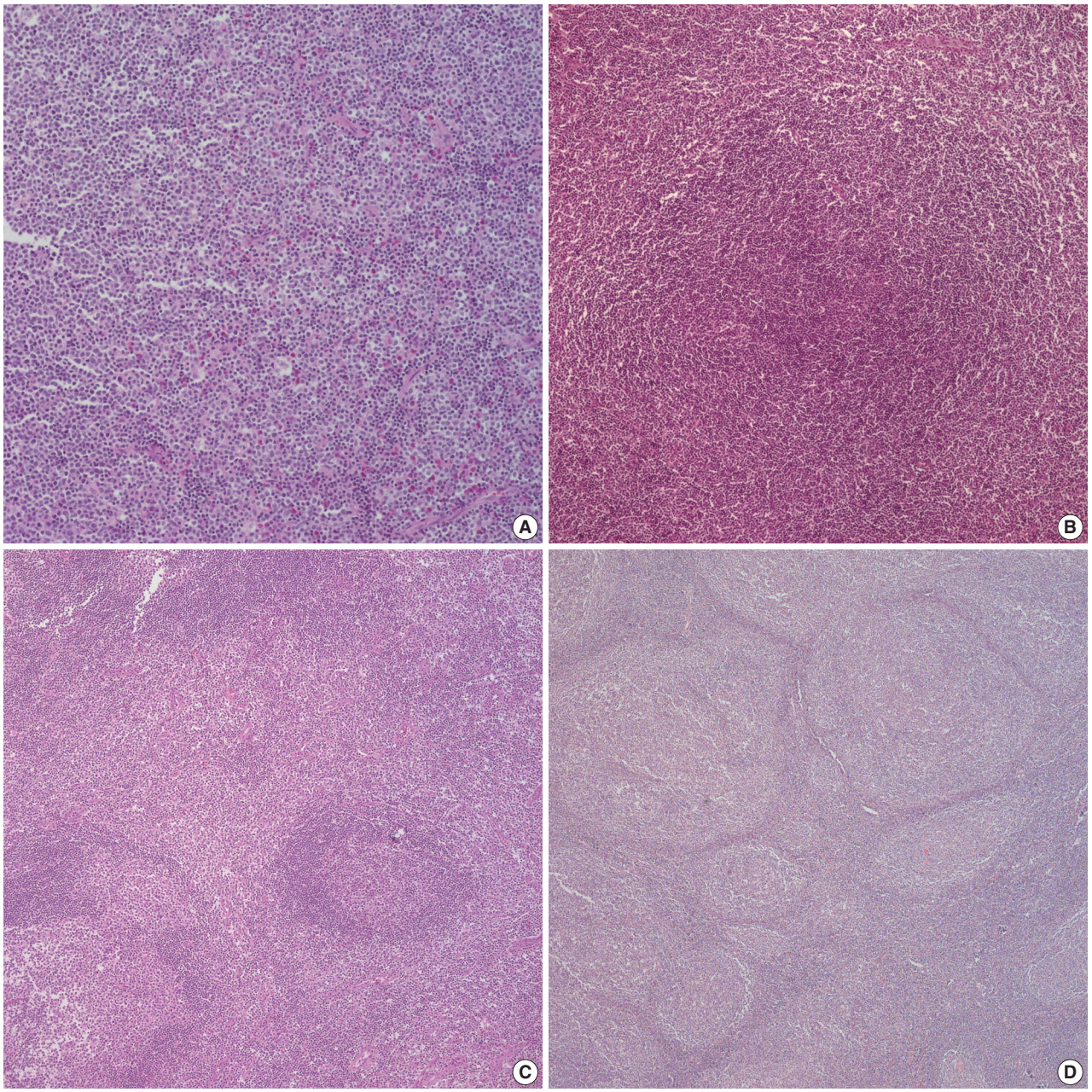

Fig. 1. Histological patterns of nodal marginal zone B-cell lymphoma show the diffuse pattern (A, case 1), the nodular pattern (B, case 7), the interfollicular pattern (C, case 4), and the perifollicular pattern (D, case 2).

with these cells, heterogeneous lymphoid cells of small lymphocytes, intermediate-sized lymphocytes and large lymphocytes were smeared in various proportions (Fig. 2B). Intermediate cells were two or three times larger than small lymphocytes with round nuclei, open chromatin, and moderate cytoplasm. Large cells had a round nucleus, open chromatin, and abundant cytoplasm. Because the cellular infiltrates were heterogeneous and admixed with normal germinal center cells, the FNA smear appeared to be reactive hyperplasia. In particular, when the small cells outnumbered the intermediate and large cells, it was impossible to make a diagnosis of malignant lymphoma. However, in cases 2, 3, and 7 intermediate and large cells were more frequent, accounting for more than 50% of the lymphoid elements, and the smears looked more monotonous (Fig. 2C). These cases were previously diagnosed as reactive hyperplasia, but on retrospective review of the FNA, an abnormal increase in the number of intermediate and large lymphoid cells was recognized and classified as "suspicious." The case showing a diffuse histo-

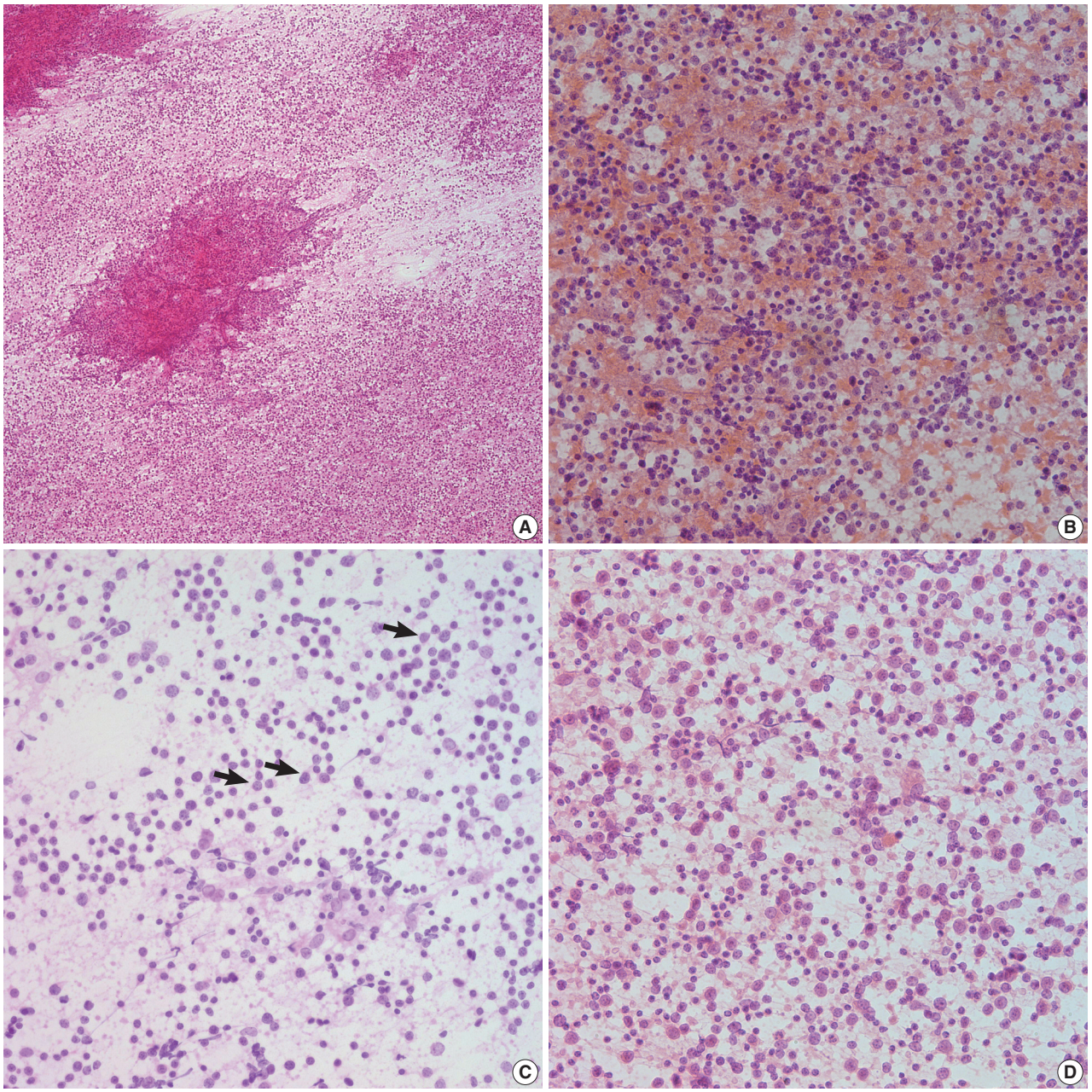

Fig. 2. (A) The majority of cases show nodular lymphohistiocytic aggregates in a background of a diffuse lymphocytic smear. (B) Heterogeneous lymphoid cells of small lymphocytes, intermediate-sized lymphocytes, and large lymphocytes smeared in various proportions. Tingible body macrophages and follicular dendritic cells derived from residual germinal centers are seen. The overall pattern simulates reactive hyperplasia. (C) Careful observation reveals many intermediate-sized lymphocytes with open chromatin and a round nuclear contour (arrows). (D) Large lymphocytes are predominant in case 1. This case shows a diffuse histological pattern on biopsy. Based on the biopsy, an FNA smear shows no nodular aggregates or tingible body macrophages.

logical pattern in the biopsy (case 1) had no nodular aggregates on the FNA smear and exhibited no tingible body macrophages or follicular dendritic cells (Fig. 2D). In case 1, intermediate and large cells outnumbered small cells, which led to a correct diagnosis at the initial examination (Table 2).

Flow cytometric analysis

Two cases (cases 6 and 7) were subjected to flow cytometry. Monoclonal antibodies for CD19, CD3, CD4, CD8, and the kappa and lambda light chain were applied. The CD45-positive population was gated, and the ratio between kappa-light chain

positive cells and lambda-light chain positive cells was calculated. Among the CD20-positive lymphocytes, the kappa to lambda ratios were 0.96 and 1.04, respectively, and no monoclonal population was identified.

DISCUSSION

NMZL is an uncommon type of malignant lymphoma and accounts for 1.5-1.8% of all malignant lymphomas.[10] Clinically, the majority of patients present with a low clinical stage at diagnosis (59% patients with clinical stage I-II).[11] In our series, all patients except one presented with lymphadenopathy localized to the neck area.

NMZL reveals similar histological changes to those of an extranodal marginal zone lymphoma (EMZL) or splenic marginal zone lymphoma.[10] Tumors consist of medium-sized monocytoid cells, small centrocyte-like cells, plasma cells, transformed large B-cells, and small lymphocytes that are admixed in various proportions. Tumor cells encircle reactive follicles and gradually expand to the interfollicular area, perisinusoidal area, and intrafollicular zone, eventually diffusely effacing the entire lymph node architecture.[12] Therefore, cases, except those with diffusely infiltrated lymph nodes, retain the residual germinal center. Even cases showing completely colonized follicles in neoplastic cells retain the follicular dendritic cell meshwork that is identified by CD21 immunohistochemistry.[9] Such a follicular dendritic cell meshwork can disappear in cases showing a diffuse pattern. In consideration of such architecture, it is understandable that nodular lymphohistiocytic aggregates consisting of follicular dendritic cells, tingible body macrophages, centrocytes, and centroblasts are frequently present in a FNA smear, and that the smear pattern may be similar to that of follicular hyperplasia, follicular lymphoma, or other lymphomatous lesion involving lymphoid follicles. Reactive follicular hyperplasia is the most important differential diagnosis. Six of seven cases in our study were misdiagnosed as reactive hyperplasia at the initial assessment. Reports on cytological analyses of NMZL are rare, and EMZL and NMZL are usually analyzed together.[4,13] Matsushima *et al.*[13] reported FNA results of five EMZL cases and four NMZL cases. In all four nodal cases, the initial cytological diagnosis was reactive lymphadenitis. The cytological specimens showed polymorphous proliferation comprising a predominant population of intermediate-sized lymphoid cells with centrocyte-like or monocytoid features, transformed cells, and variable numbers of plasma cells. These findings, while highly suggestive of mucosa-associated lymphoid tissue lymphoma, may be more difficult to distinguish from reactive conditions in lymph nodes. In one study from Korea, the diagnostic accuracy for EMZL and NMZL was 50%, which was lower than other types of malignant lymphoma.[2] These results support the limitation of FNA for a diagnosis of NMZL.

Flow cytometric analyses are a useful adjunct to improve the diagnostic accuracy of FNA. However, even with flow cytometry, only 7/15 (47%) marginal zone lymphomas in the series of Dong *et al.*[3] were initially recognized as neoplastic, with a distinct monotypic population. Six additional cases had a small monotypic population or a slight light-chain excess, which was not diagnostic of malignancy, and they lacked sufficient cytological atypia to be recognized as malignant on morphological grounds alone.[3] In other studies, flow cytometry successfully demonstrated the monoclonal B-cell population in 50% of EMZL cases.[4,14,15] Such lower accuracy of flow cytometry seems to be due to the heterogeneous cellular components of NMZL including relatively low numbers of neoplastic cells in the samples analyzed and a lack of immunohistochemical markers specific for NMZL. In our series, lymph node size ranged from 1 to 2.3 cm. Considering that the diameter of a normal lymph node is usually less than 1 cm, lymph nodes larger than 1 cm should be carefully examined even though the smear mimics reactive lymphadenitis. Clinical information is important to improve the diagnostic accuracy of FNA. A multiple lymphadenopathy and B symptoms would be valuable information; however, the majority of patients with NMZL present as stage I without systemic symptoms.

Taken together, a diagnosis of NMZL using FNA cytology remains a difficult issue. Identifying intermediate-sized neoplastic cells associated with lymphohistiocytic nodules in an enlarged (> 1 cm) lymph node may be the key leading to an accurate diagnosis and require an experienced cytopathologist. Despite such difficulties, FNA is useful as a simple test for initial screening, follow-up after chemotherapy, or a staging work-up; ancillary diagnostic tests such as gene rearrangement studies can reduce false negative results. Clinicians should be aware of the limitations of FNA in a diagnosis of NMZL and provide precise information to the cytopathologist.

REFERENCES

1. Wakely PE Jr. Fine-needle aspiration cytopathology in diagnosis and classification of malignant lymphoma: accurate and reliable?

Diagn Cytopathol 2000; 22: 120-5.
2. Lee SS. Fine needle aspiration cytology of non-Hodgkin lymphomas predominating small lymphoid cells : differential diagnosis of small lymphoid cell neoplasm. Korean J Cytopathol 2006; 17: 87-98.
3. Dong HY, Harris NL, Preffer FI, Pitman MB. Fine-needle aspiration biopsy in the diagnosis and classification of primary and recurrent lymphoma: a retrospective analysis of the utility of cytomorphology and flow cytometry. Mod Pathol 2001; 14: 472-81.
4. Chhieng DC, Cohen JM, Cangiarella JF. Cytology and immunophenotyping of low- and intermediate-grade B-cell non-Hodgkin's lymphomas with a predominant small-cell component: a study of 56 cases. Diagn Cytopathol 2001; 24: 90-7.
5. Mourad WA, Tulbah A, Shoukri M, *et al.* Primary diagnosis and REAL/WHO classification of non-Hodgkin's lymphoma by fine-needle aspiration: cytomorphologic and immunophenotypic approach. Diagn Cytopathol 2003; 28: 191-5.
6. Safley AM, Buckley PJ, Creager AJ, *et al.* The value of fluorescence in situ hybridization and polymerase chain reaction in the diagnosis of B-cell non-Hodgkin lymphoma by fine-needle aspiration. Arch Pathol Lab Med 2004; 128: 1395-403.
7. Venkatraman L, Catherwood MA, Patterson A, Lioe TF, McCluggage WG, Anderson NH. Role of polymerase chain reaction and immunocytochemistry in the cytological assessment of lymphoid proliferations. J Clin Pathol 2006; 59: 1160-5.
8. Jaffe ES, Harris NL, Vardiman JW, Campo E, Arber DA. Hematopathology. Philadelphia: Saunders-Elsevier, 2010; 319-32.
9. Salama ME, Lossos IS, Warnke RA, Natkunam Y. Immunoarchitectural patterns in nodal marginal zone B-cell lymphoma: a study of 51 cases. Am J Clin Pathol 2009; 132: 39-49.
10. Swerdlow SH, Campo E, Harris NL, *et al.* WHO classification of tumors of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press, 2008; 218-9.
11. Camacho FI, Algara P, Mollejo M, *et al.* Nodal marginal zone lymphoma: a heterogeneous tumor: a comprehensive analysis of a series of 27 cases. Am J Surg Pathol 2003; 27: 762-71.
12. Campo E, Miquel R, Krenacs L, Sorbara L, Raffeld M, Jaffe ES. Primary nodal marginal zone lymphomas of splenic and MALT type. Am J Surg Pathol 1999; 23: 59-68.
13. Matsushima AY, Hamele-Bena D, Osborne BM. Fine-needle aspiration biopsy findings in marginal zone B cell lymphoma. Diagn Cytopathol 1999; 20: 190-8.
14. Murphy BA, Meda BA, Buss DH, Geisinger KR. Marginal zone and mantle cell lymphomas: assessment of cytomorphology in subtyping small B-cell lymphomas. Diagn Cytopathol 2003; 28: 126-30.
15. Crapanzano JP, Lin O. Cytologic findings of marginal zone lymphoma. Cancer 2003; 99: 301-9.