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Original Article

Immunohistochemistry for IRTA1 and MNDA helps differentiate gastric MALT lymphoma from chronic gastritis/reactive lymphocyte hyperplasia

Yoshiyuki Ayada,[1)] Takuro Igawa,[1)] Yusuke Naoi,[1)] Kyosuke Horikawa,[1)] Tetsuya Tabata,[2)] Takehiro Tanaka,[1)] Tadashi Yoshino[1)]

It is difficult to histologically differentiate extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) from chronic gastritis (CG)/ reactive lymphoid hyperplasia (RLH). To determine whether immunohistochemistry for IRTA1 and MNDA can differentiate gastric MALT lymphoma from CG/RLH, we investigated 81 stomach biopsy specimens [Wotherspoon grade (WG) 1, 11 cases; WG 2, 9 cases; WG 3, 20 cases; WG 4, 31 cases; and WG 5, 10 cases]. According to a previously reported algorithm involving PCR for immunoglobulin heavy (IgH) chain locus rearrangement, all 81 cases were divided into three groups: CG/RLH (55 cases), MALT lymphoma (19 cases) groups, and IgH undetectable group (7 cases). We analyzed the CG/RLH and MALT lymphoma groups. The median percentage of IRTA1-positive cells was 0% (range 0%–90.6%) in the CG/RLH group and 43.5% (range 0%–97.6%) in the MALT lymphoma group ($p < 0.0001$). The median percentage of MNDA-positive cells was 32.4% (range 0%–97.6%) in the CG/RLH group and 55.1% (range 0%–97.6%) in the MALT lymphoma group ($p = 0.0044$). These results indicate that immunohistochemistry for IRTA1 and MNDA can help differentiate gastric MALT lymphoma from CG/RLH.

Keywords: MALT lymphoma; chronic gastritis; reactive lymphoid hyperplasia; IRTA1; MNDA

INTRODUCTION

Extranodal marginal zone lymphoma (MZL) of mucosa-associated lymphoid tissue (MALT lymphoma) is classified as a subtype of MZL in the World Health Organization (WHO) classification of lymphoid tumors.[1] MALT lymphoma is a common low-grade lymphoma occurring in various extranodal organs, such as salivary glands, lungs, and gastrointestinal tract.[1] MALT lymphoma most commonly develops in the stomach: accounting for 20%–40% of all the extranodal lymphomas and 40%–50% of primary gastric malignant lymphomas.[2–4] In recent years, immunohistochemistry for immunoglobulin superfamily receptor translocation-associated 1 (IRTA1) and myeloid nuclear differentiation antigen (MNDA) has been utilized to histopathologically diagnose MZL.[5–9] IRTA1, also known as Fc receptor-like 4 (FCRL4), is expressed on monocyte-like B cells, marginal zone cells, and intraepithelial B cells in normal lymphoid tissues.[10–14] Conversely, MNDA is expressed in myelomonocytic cells and found in normal splenic marginal zone B cells.[7,15–17]

Differentiating gastric MALT lymphoma from non-neoplastic lesions, such as chronic gastritis (CG) or reactive lymphoid hyperplasia (RLH), is often challenging in routine practice. The tissue-grading score proposed by Wotherspoon *et al.*[18] has been widely used for the biopsy tissue diagnosis of gastric MALT lymphoma. The Wotherspoon grade (WG) ranges from grade 1 to grade 5, including normal condition (WG0), CG (WG1-2), and obvious neoplastic lesion (WG5). WG3 and WG4 represent ambiguous categories to determine neoplasia; WG3 rather suggests a reactive lesion, whereas WG4 rather suggests a neoplastic lesion. In WG3 and WG4, additional testing such as immunohistochemistry for CD20, CD3, and cytokeratin is often required to prove or rule out malignancy.[19] The usefulness of immunohistochemistry for IRTA1 and MNDA for non-neoplastic lesions such as CG and

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[1)]Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan, [2)]Department of Pathology, National Hospital Organization Okayama Medical Center, Okayama, Japan

Corresponding author: Dr. Takuro Igawa, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan. E-mail: igawa-t@okayama-u.ac.jp

RLH has not been adequately explored, even though it could help differentiate MALT lymphoma from other types of gastric lymphomas, including the diffuse large B-cell lymphoma.[6,8,9,16,20]

Herein, we aimed to clarify the usefulness of IRTA1 and MNDA in the histopathological differential diagnosis between gastric MALT lymphoma and CG/RLH with immunohistochemistry.

MATERIALS AND METHODS

Case selection

We used 81 gastric biopsy specimens from the surgical pathology consultation files of the Department of Pathology of Okayama University, Japan. Seventeen cases were endoscopically suspected of MALT lymphoma before a histological examination, whereas another 11 were histologically suspected of MALT lymphoma without any clinical suspicion. Fifty-three follow-up cases of pre-existing MALT lymphoma were also included. The details of 53 cases were as follows: watch & wait: five cases, *Helicobacter pylori* eradication only (including unsuccessful eradication): 41 cases (unsuccessful eradication: one case), radiation therapy after *H. pylori* eradication: six cases, and chemotherapy only: one case. These 81 cases comprised 20 CG/RLH (WG 1: 11 cases, WG 2: 9 cases), WG 3: 20 cases, WG 4: 31 cases, and ten histologically apparent MALT lymphoma (WG5). According to a previous study,[19] cases of WG 3-4 without immunoglobulin heavy chain (IgH) rearrangement were designated as CG/RLH, whereas cases of WG 3-4 with IgH locus rearrangements were designated as MALT lymphoma. Demographic information for the examined patients is shown in Table 1. We also examined three normal stomach tissue samples as normal controls.

Immunohistochemistry

Specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial, 3-μm thick sections were cut from the paraffin-embedded tissue blocks for staining. Sections were either stained with hematoxylin and eosin (H&E) or immunohistochemically stained with antibodies specific for IRTA1 (FcRL4; EPR21961, rabbit monoclonal, ab239076, 1:50; Abcam plc, Cambridge, UK) and MNDA (253A, mouse monoclonal, ab188566, 1:100; Abcam plc). Staining was performed using an automated Bond-III Stainer (Leica Biosystems GmbH, Wetzlar, Germany), according to the manufacturer's instructions. Immunohistochemistry with each antibody was performed for all 81 specimens. The positivity rate of IRTA1 was determined by computing the percentage of localized or sheet-like IRTA1-positive cells to the total infiltrating lymphocytes. Rate zero was assigned if no staining was observed or only scattered positive cells were detected. The positivity rate of MNDA was determined at one high-power field in the hotspot. Strongly positive cells that appeared to be granulocytes or histiocytes were excluded from scoring.

Polymerase chain reaction (PCR) assays of the IgH locus rearrangements

Tissue sections were scraped from gastric mucosal tissue biopsy specimens, and DNA was extracted using the QIAamp® DNA Micro Kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's instructions. DNA was quantified using the NanoDrop ND1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). All gene rearrangement analyses were performed using a previously described method.[21] All primers were procured from Sigma–Aldrich (St. Louis, MO, USA). The PCR products were analyzed using the ABI PRISM® 310 Genetic Analyzer with GeneScan® Analysis and GeneMapper® Software (Thermo Fisher Scientific Inc.).[21] For IgH rearrangements, DNA was amplified using Framework Region II (FR2) and III (FR3) primers. The JH consensus primer was fluorescently labeled (6-carboxyfluorescein).[21] IgH rearrangements were analyzed and evaluated using the BIOMED-2 protocol.[21] The fragment analysis results were interpreted as monoclonal, oligoclonal, or polyclonal. The exponential PCR amplification of single peak, two, and multiple high peaks indicate monoclonal, oligoclonal, and polyclonal cell samples, respectively. If the peaks were not visible, the samples were considered to have undetectable IgH expression levels.

Table 1. Demographic characteristics of gastric biopsy cases

Wotherspoon Grade	1 (n=9)	2 (n=11)	3 (n=20)	4 (n=31)	5 (n=10)
Age					
median(min-max)	72(60-83)	60(34-85)	67(36-90)	73(40-87)	73(52-83)
mean±SD	71.7±8.65	60.8±12.5	62.5±13.6	68.9±10.8	68.9±11.5
Sex					
male	5 (56)	7 (64)	8 (40)	17 (55)	4 (40)
female	4 (54)	4 (36)	12 (60)	14 (45)	6 (60)
history of MALT lymphoma					
No history	0	3 (30)	9 (45)	12 (38)	4 (40)
Previous history	9 (100)	8 (80)	11 (55)	19 (62)	6 (60)

Note: Data are presented as n (%) for categorical measures. *SD* standard deviation

Statistical analysis

Statistical analyses, including the Mann–Whitney U test and receiver operating characteristic (ROC) curves, were performed using STATA/SE ver17.0 (StataCorp LLC, College Station, TX, USA). A p-value < 0.05 was considered statistically significant, and data is presented as the mean ± standard deviation.

RESULTS

Control

The expression of IRTA1 and MNDA in normal gastric mucosal tissue specimens is shown in Figure 1. No IRTA1-positive cells were observed in any of the three specimens. In contrast, MNDA expression was observed only in granulocytes and macrophages of the three specimens.

IRTA1 and MNDA expression

The results of the immunohistochemical analysis of IRTA1 and MNDA expression for each WG are summarized in Table 2. Lymphocyte infiltration was limited, and the expression of IRTA1 was sporadic in many WG 1–2 specimens (Figure 2A and C). In contrast, neoplastic lymphoid cells were densely infiltrated, and IRTA1-positive cells were widely distributed in WG 5 specimens (Figure 2B and D). The mean percentage of IRTA1 positive cells in each WG was 0 (2.33 ± 7.00) in WG 1, 0 (1.52 ± 5.04) in WG 2, 0 (22.5 ± 33.3) in WG 3, 0 (17.7 ± 26.1) in WG 4, and 40.35 (45.6 ± 26.9) in WG 5. MNDA expression was higher in WG 5 specimens than in WG 1–2 specimens (Figure 2E, F). The mean percentage of MNDA-positive cells in each WG was 15.2 (23.7±17.4) in WG 1, 25.8 (29.3±18.3) in WG 2, 23.1 (27.3±19.3) in WG 3, 39.8 (42.0±21.3) in WG 4, and 68.0 (65.0±19.4) in WG 5.

IgH locus rearrangements and categorization as CG/RLH or MALT lymphoma

All 51 specimens with WG 3–4 were assessed for IgH locus rearrangements by performing PCR. Out of 20 specimens with WG 3, monoclonality was detected in six specimens (30%). Out of six specimens where IgH (FR2) PCR analysis was successful, monoclonality was detected in two

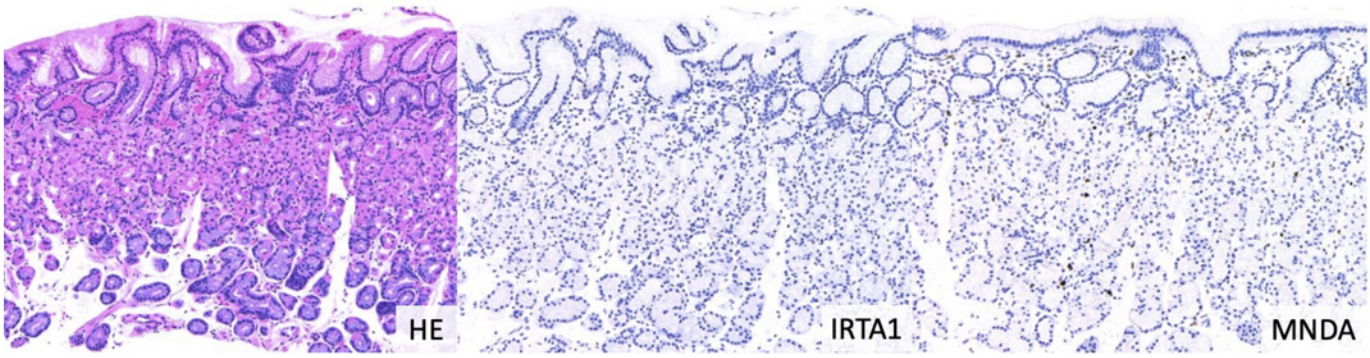

Fig. 1. Expression of immunoglobulin superfamily receptor translocation-associated 1 (IRTA1) and myeloid nuclear differentiation antigen (MNDA) in normal gastric mucosa. No IRTA1-positive cells are found; MNDA is only expressed among granulocytes and macrophages.

Table 2. Results of mmunohistochemistry and IGH locus rearrangement in gastric biopsy cases

Wotherspoon Grade	1 (n=9)	2 (n=11)	3 (n=20)	4 (n=31)	5 (n=10)
IRTA1(%)					
median(min-max)	0 (0-21)	0 (0-16.7)	0 (0-96.5)	0 (0-90.6)	40.35 (19.1-97.6)
mean±SD	2.33±7.00	1.52±5.04	22.5±33.3	17.7±26.1	45.6±26.9
MNDA(%)					
median(min-max)	15.2 (5.1-56.1)	25.8 (0-57.2)	23.1 (0-72)	39.8 (2.6-87.4)	68 (29.4-85.8)
mean±SD	23.7±17.4	29.3±18.3	27.3±19.3	42.0±21.3	65.0±19.4
IGH clonality					
Oligo/poly	ND	ND	9	26	ND
Mono	ND	ND	6	3	ND
UD	ND	ND	5	2	ND
Categorization*					
CG/RLH	9	11	9	26	0
MALT lymphoma	0	0	6	3	10

SD standard deviation, *Mono* monoclonal, *Oligo* oligoclonal, *Poly* polyclonal, *UD* undetected, *ND* not done
*Categorizeation according to the algorithm of previous study[19]

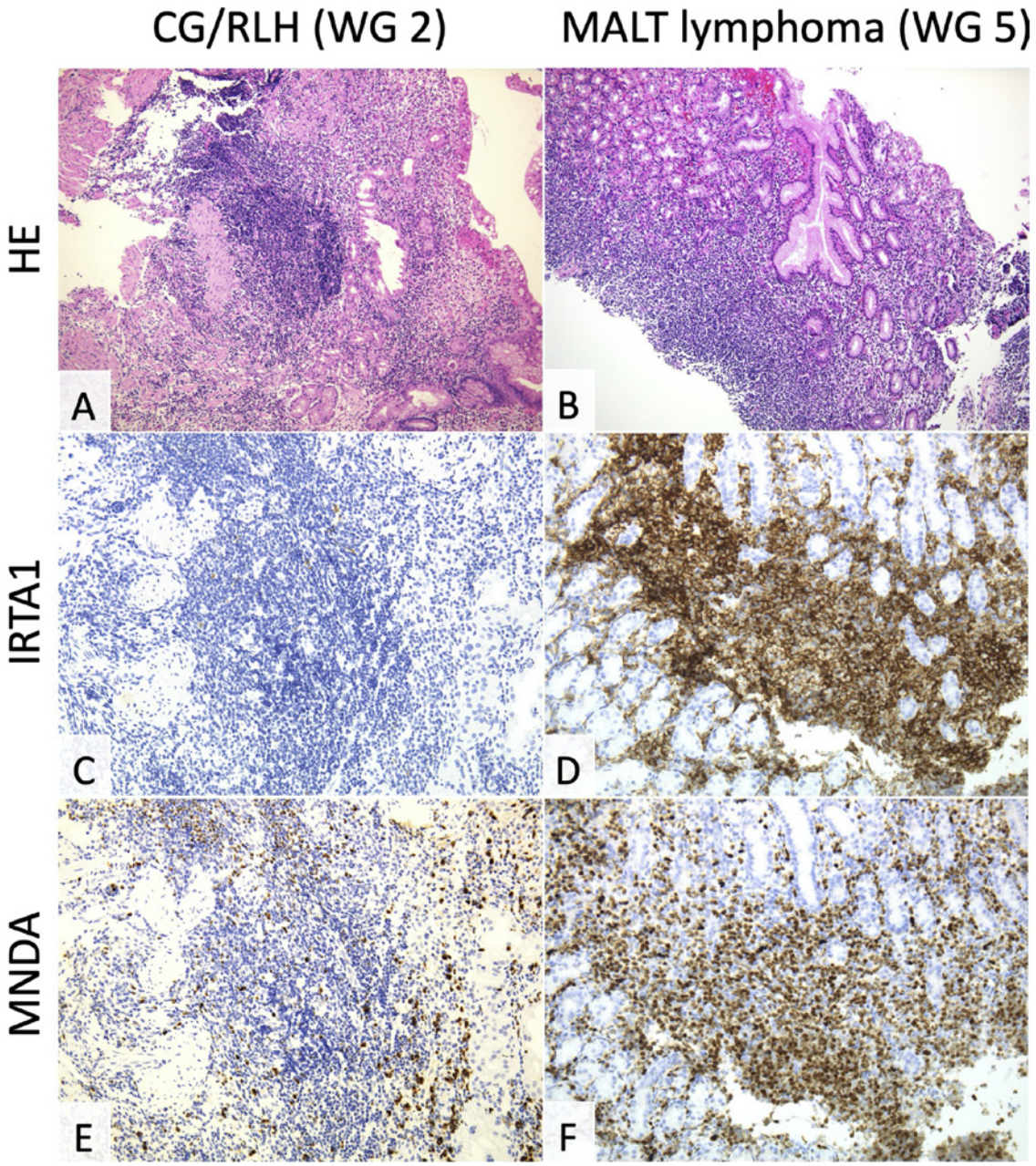

Fig. 2. Representative examples of immune receptor translocation-associated protein 1 (IRTA1) and myeloid nuclear differentiation antigen (MNDA) staining in cases of chronic gastritis (CG)/reactive lymphoid hyperplasia (RLH) (WG 1-2) (***A***, ***C***, ***E***) and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (WG 5) (***B***, ***D***, ***F***). Expression of IRTA1 is only scattered in many specimens of WG 1–2, determined as GC/RLH (***C***). In contrast, positive cells are widely distributed in WG 5 specimens, determined as MALT lymphoma (***D***). MNDA expression shows a higher density of positive cells in MALT lymphoma specimens than in CG/RLH specimens (percentage of MNDA expression in hotspot; ***E***: 36.6%, ***F***: 58.7%).

specimens (33%); out of 15 specimens where IgH (FR3) PCR analysis was successful, monoclonality was detected in six specimens (40%). Among the 31 specimens with WG 4, IgH rearrangement was detected in three specimens (9%). Out of 14 specimens where IgH (FR2) PCR was successful, monoclonality was detected in two specimens (14%); out of 28 specimens where IgH (FR3) PCR was successful, monoclonality was detected in two specimens (7%).

Subsequently, 74 cases were classified as either CG/RLH or MALT lymphomas (Table 2); 20 cases with WG 1–2 and 10 cases with WG 5 were classified as CG/RLH and MALT lymphoma, respectively. Consulting a previous study,[19] 35 cases with WG 3–4 but without IgH rearrangement were determined to be CG/RLH; nine cases with WG 3–4 and IgH locus rearrangements were determined to be MALT lymphoma. The other seven cases with WG 3–4 in which either IgH (FR2) or (FR3) was not detected by PCR were excluded from the following analysis.

Analysis between CG/RLH and gastric MALT lymphoma

Differences in the expression of IRTA1 and MNDA were analyzed in 55 specimens in the CG/RLH group and 19 specimens in the MALT lymphoma group (Table 3, Figure 3). There were significant differences in IRTA1 and MNDA expression between the two groups. The median IRTA1 expression in the CG/RLH group was 0% (range 0%–90.6%), while the median IRTA1 expression in the gastric MALT lymphoma group was 43.5% (range 0%–97.6%; $p < 0.0001$). The median MNDA expression was 32.4% (range 0%–72.0%) in the CG/RLH group, while that in the gastric MALT lymphoma group was 55.1% (range 7.8%–87.4%; $p = 0.0044$).

ROC curves were generated based on the expression levels of IRTA1 and MNDA for the differential diagnosis of gastric MALT lymphoma from CG/RLH (Figure 4). The ROC curve was based on Youden index (sensitivity + specificity – 1).[22] IRTA1 had 17.8% cut-off, 84.2% sensitivity, and 78.2% specificity; MNDA had 58.7% cut-off, 47.4% sensitivity, and 87.2% specificity. The area under the curve (AUC) was 0.8081 for IRTA1 and 0.7201 for MNDA. Based on these data, 20% and 60% appear to be practical cutoffs for IRTA1 and MNDA, respectively. The sensitivity and specificity of immunohistochemistry for IRTA1 using a 20% cut-off were 73.7% and 78.2%, respectively, and those for MNDA using a 60% cutoff were 36.8% and 90.9%, respectively.

Table 3. The expression of IRTA1 and MNDA in CG/RLH and MALT lymphoma

	CG/ RLH (n=55)	MALT lymphoma (n=19)
IRTA1 (%)		
median(min-max)	0(0-90.6)	43.5(0-97.6)
mean±SD*1	11.9±21.8	44.2±32.3
		P<0.0001*2
MNDA (%)		
median(min-max)	32.4(0-72.0)	55.1(7.8-87.4)
mean±SD*1	33.8±19.2	53.7±27.6
		P=0.0044*2

*1 SD: standard deviation *2 *P*<0.05

DISCUSSION

Immunohistochemistry for IRTA1 and MNDA has been a valuable marker in the histological diagnosis of MALT lymphoma; however, its potential application in differentiating MALT lymphoma from non-neoplastic lesions has not yet been explored. This study clearly showed that the immunohistochemical expression of IRTA1 and MNDA was significantly higher in gastric MALT lymphoma than that in CG/RLH cases, indicating that the procedure can successfully differentiate MALT lymphoma from CG/RLH.

To the best of our knowledge, this is the first study to determine the usefulness of immunohistochemistry for IRTA1 and MNDA in assessing whether lymphocytes infiltrating into the gastric mucosa are neoplastic or non-neoplastic. In previous studies, the expression of IRTA1 and MNDA was assessed based on the ratio of positive cells to tumor cells.[6–8,20] However, this evaluation method cannot be applied to the differential diagnosis of gastric MALT lymphoma from CG/RLH because inflammatory cells, including small lymphocytes, infiltrate the gastric mucosa in non-neo-

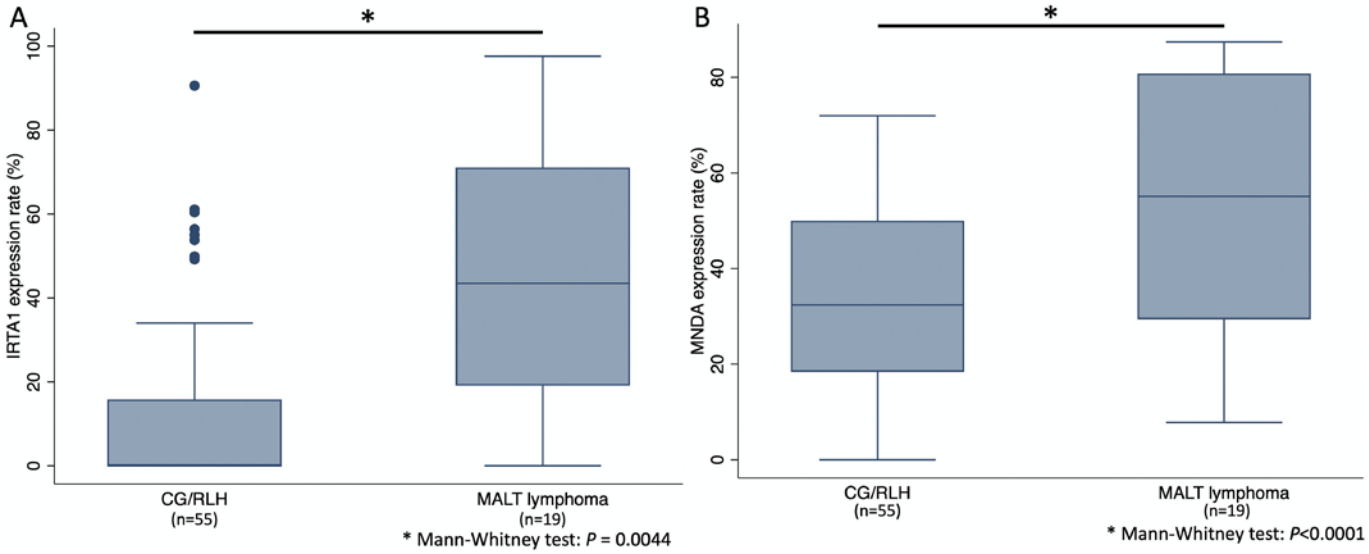

Fig. 3. Comparison of immunoglobulin superfamily receptor translocation-associated 1 (IRTA1) and myeloid nuclear differentiation antigen (MNDA) in chronic gastritis (CG)/reactive lymphoid hyperplasia (RLH) and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. ***A***: Differences and distribution of IRTA1 in CG/RLH and gastric MALT lymphoma. ***B***: Differences and distribution of MNDA in CG/RLH and gastric MALT lymphoma.
Box plot explanation: upper horizontal line of box: 75th percentile; lower horizontal line of box: 25th percentile; horizontal bar within box: median; upper horizontal bar outside box: 95th percentile; and lower horizontal bar outside box: 5th percentile.

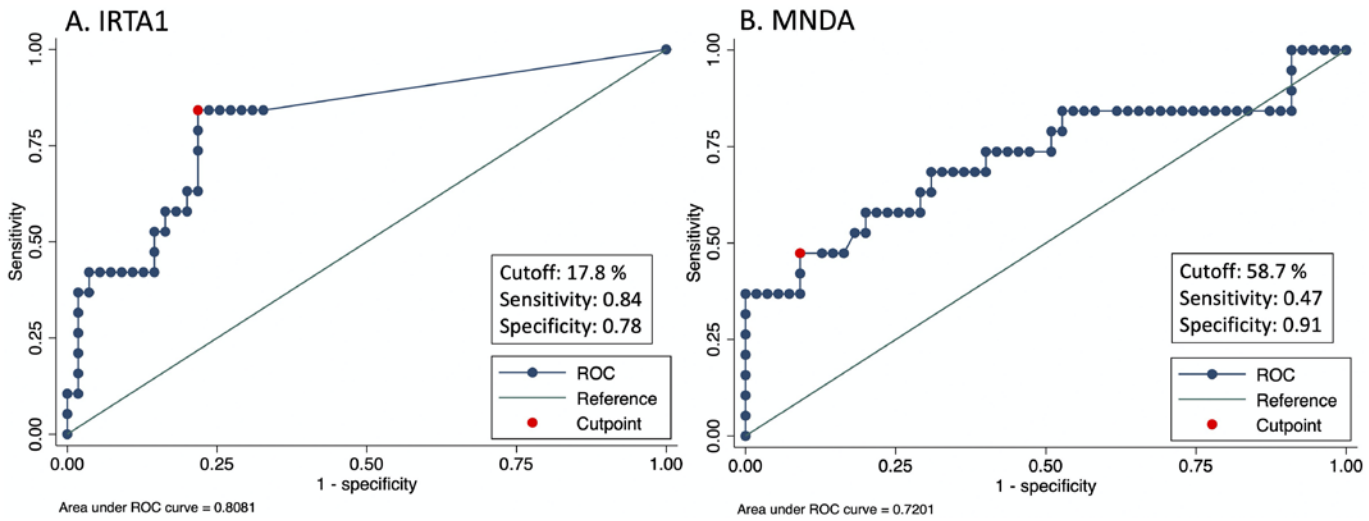

Fig. 4. Receiver operating characteristic curve for differential diagnosis of gastric mucosa-associated lymphoid tissue lymphoma from chronic gastritis/reactive lymphoid hyperplasia based on the expression of immunoglobulin superfamily receptor translocation-associated 1 and myeloid nuclear differentiation antigen.

plastic cases. Thus, all small lymphocytes were evaluated in this study regardless of their neoplastic potential. The positivity rates of IRTA1 and MNDA increased with the WG score. Significantly more IRTA1- and MNDA-positive lymphocytes were observed in the MALT lymphoma group than in the CG/RLH group.

In previous studies, the cut-off values for IRTA1 and MNDA to diagnose MALT lymphoma were often set at 20%–30% for IRTA1 and 10%–15% for MNDA.[6–9,16,20] IRTA1- or MNDA-positive cells can be observed in non-neoplastic lymphoid tissues. For example, IRTA1 can be seen in 30%–50% of non-neoplastic monocyte-like B cells, while MNDA can be seen in 10%–50% of non-neoplastic mantle/marginal zone cells.[8] Thus, the previously reported cut-off values are considered unsuitable for differentiating neoplastic from non-neoplastic lesions. We now propose a new cut-off value of 20% for IRTA1 and 60% for MNDA when diagnosing MALT lymphoma in a gastric biopsy, based on the ROC curves calculated from the positivity rates of immunohistochemistry for IRTA1 and MNDA. Our proposed cut-off value of 60% for MNDA significantly differs from the previously reported cut-off values of 10–15%; it can be a novel suggestion for histopathological diagnosis. Furthermore, the ROC curve offers another notable suggestion: the large AUC of IRTA1 indicates that IRTA1 is more useful than MNDA in diagnosing MALT lymphoma. This is partly because MALT lymphoma is characterized by an interaction between the epithelium and neoplastic lymphocytes—lymphoepithelial lesions.[1] Interestingly, it has been reported that tumor cells adjacent to the epithelium tend to express IRTA1 more strongly than those away from the epithelium.[6] In low-grade B-cell lymphoma, IRTA1 has been reported to have higher specificity than MNDA for MZL,[6,8,9] as MNDA can also be expressed in other types of lymphoma, such as chronic lymphocytic leukemia/small lymphocytic lymphoma, and mantle cell lymphoma.[7,8] Therefore, when using MNDA in histopathological analysis, MNDA and other markers such as CD5 and cyclin D1 should be used.[1]

These findings indicate that immunohistochemistry for IRTA1 and MNDA can help determine whether infiltrating lymphocytes are neoplastic or non-neoplastic, especially in WG 3 or 4 cases. Moreover, IRTA1 may be more beneficial than MNDA for diagnosing gastric MALT lymphoma. This insight will be helpful for pathologists, as distinguishing the two conditions using H&E staining is often difficult in routine practice. Furthermore, we believe that our study has clinical importance because a precise and prompt diagnosis of MALT lymphoma is required for appropriate treatments, such as chemotherapy or radiation therapy, to be provided to the patients.

CONCLUSION

IRTA1 and MNDA were significantly expressed in gastric MALT lymphoma cases compared with CG/RLH cases. We propose new cut-off values for immunohistochemistry of IRTA1 and MNDA (IRTA1: 20%, MNDA: 60%). These findings will be useful for the diagnosis of gastric MALT lymphoma in routine clinical practice.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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