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Original Article

Molecular pathology assists the diagnosis of lymphoepithelial sialadenitis, Sjögren's syndrome and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue

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KEYWORDS
Lymphoepithelial sialadenitis;
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FCRL4;
Clonal Ig gene rearrangement

Abstract *Background/purpose:* Lymphoepithelial sialadenitis (LESA), Sjögren's syndrome (SS), and salivary MALT lymphoma are diseases characterized by lymphoepithelial lesions, and the differential diagnosis between them in the salivary glands is challenging. This study aimed to explore clinicopathological and genetic characteristics of the three diseases.
Materials and methods: We retrospectively analyzed the clinical features, the histomorphology, immunohistochemistry, and genetic profiling by polymerase chain reaction (PCR) and next-generation sequencing (NGS).
Results: There included 68 LESAs, 25 SSs, and 62 MALT lymphomas. Ten cases relapsed in total, and 3 of MALT lymphomas died due to high-level transformation. Immunohistochemical double staining showed FCRL4 cells co-expressed Pax-5 and Ki-67, suggesting FCRL4 cells were

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proliferative B-cells. The expression level of the FCRL4 was significantly higher in MALT lymphoma than LESA and SS. The detection rates of clonal IGH, IGK, and IGL gene rearrangements in MALT lymphoma with a sensitivity of 83.33%. Monoclonal immunoglobulin gene rearrangements were confirmed in five suspected patients by NGS (100%).
Conclusion: FCRL4 B cells might play an important role in the formation of lymphoepithelial lesions and might be as a diagnostic positive marker of salivary MALT lymphoma. The application of multiple detection methods could significantly improve the diagnostic accuracy for MALT lymphomas from LESA and SS.

Introduction

Lymphoepithelial sialadenitis (LESA) is a benign autoimmune lesion, which is characterized by acinar atrophy, lymphocytic infiltration, ductal hyperplasia, and epimyoepithelial islands formation. LESA is closely related to Sjögren's syndrome (SS), and LESA is the cardinal components of SS, but not all LESA are consistent with the clinical manifestations of SS.[1] LESA can also occur as an isolated salivary gland lesion. Most LESA and SS cases have a benign clinical prognosis, but there is a risk of malignant transformation, which is about 40 times higher than that in the general population. The incidence rate of malignant lymphoma is about 5%—10%, among which extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is the most common lymphoma.[2,3]

The histological morphology of MALT lymphoma includes different degrees of the acinar atrophy and disappearance. The infiltrating tumor cells are mainly centrocyte-like cells, monocytoid B-cells and small lymphocytic cells, and a small number of plasma cells. It forms reactive lymphoid follicles, and tumor cells can infiltrate lymphoid follicles to form follicular colonization. The glandular epithelium is damaged, and lymphoepithelial lesions can be seen.[4] Salivary MALT lymphoma has an indolent behavior and remain localized to the primary site for a long time. And, the prognosis is usually good.[5] The aetiology of MALT lymphoma remains unclear, but infectious and autoimmune aetiologies have been proposed, including chronic gastritis (*Helicobacter pylori* [*Hp*] infection), Hashimoto's thyroiditis, and SS.[6] Salivary MALT lymphomas have the pathological characteristics of lymphoepithelial lesions, and belong to the inert lymphoma with no obvious heterogeneity and specificity of cells in the pathological features. Lacking of specific clinical manifestations, histopathological features, and immunophenotypes, salivary MALT lymphomas may be clinically confused with benign lymphoproliferative lesions (such as LESA and SS) and other B-cell lymphomas, which may lead to misdiagnosis, and delayed treatment, which in turn affect the prognosis.

Fc receptor—like 4 (FCRL4) also known as immunoglobulin superfamily receptor translocation-associated protein 1(IRTA1) is located on chromosome 1q21, and is one of the family of immunoglobulin-like proteins which mediate B-cell immune responses.[7,8] In normal lymphoid tissue, FCRL4 is mainly distributed near the epithelia of tonsil crypts. It can also be expressed in benign monocytoid B-cells, some marginal zone cells, and epithelium-associated B-cells.[9—11] There are many studies that have indicated that FCRL4 could express on SS and MALT lymphoma, which may play a role in the development of the diseases.[12—14] Haacke EA et al. indicated that FCRL4 cells exist in salivary gland tissue of patients with primary SS, but could not be observed in patients of sicca complaints. They also believed that the great majority of FCRL4 cells located in lymphoepithelial lesions of the salivary glands and a small part in the periductal infiltrate closely to the epithelium. So, the FCRL4 cells might play a role in the formation of lymphoepithelial lesions. Furthermore, FCRL4 has been discovered expressed in the MALT lymphomas and expressed with higher intensity in tumor cells that form lymphoepithelial lesions. FCRL4 B cells presented in primary SS and in almost all primary SS-related MALT lymphomas, which linked the FCRL4 cells to lymphogenesis. Ikeda JI et al. indicated that FCRL4 antibody could be used to discriminate MALT lymphoma from other low-grade B-cell lymphomas.[12—14]

The purpose of this study was to analyze the clinicopathological characteristics of various lymphoepithelial lesions of the salivary glands, and to explore the diagnostic importance of histopathological morphology, immunogenetic features, and molecular pathological features.

Materials and methods

The research was reviewed and approved by the Institutional Review Board of Peking University School and Hospital of Stomatology (No. PKUSSIRB-201949125). The requirement for informed consent was waived because this was a retrospective study and patients were anonymized.

Study population

This was a retrospective study and we collected the patients with the pathological characteristics of lymphoepithelial lesions in Peking University School and Hospital of Stomatology from February 1988 to August 2020. Then, we found out the medical records and HE sections of the corresponding patients and they were reviewed and diagnosed by at least two professional pathologists with LESA, SS, and salivary MALT lymphoma based on the 2002 American and European Consensus Group (AECG) and 2016 American College

of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria, and WHO (2017).[1,15,16] Notably, we also re-diagnosed patients before 2002 according to the above diagnostic criteria. The exclusion criteria were with an unclear medical history, only labial gland biopsy and no further treatment, and poor quality or unacceptable paraffin-embedded tissue specimens.

Clinical information was obtained from the patients' medical records, including age, sex, anatomical location of lesions, clinical symptoms, radiological features, and treatment methods. All patients were followed up by telephone for prognostic evaluation.

Hematoxylin-eosin staining

Paraffin-embedded tissue specimens were obtained from the Department of Oral Pathology of Peking University School and Hospital of Stomatology. The tissue specimens were cut into 3—4-μm slices and stained with Hematoxylin-eosin (HE) staining in accordance with the instructions of the manufacturer of the automated staining machine (Autostainer XL; Leica, Wetzlar, Germany). The HE slides were then reviewed by two specialized pathologists blinded to the original diagnosis to confirm the initial diagnosis using a consensus approach.

Immunohistochemistry (IHC)

The paraffin-embedded blocks were cut into 3—4-μm slices. Single-marker staining was performed using an automated immunohistochemical staining machine (BOND-III; Leica, Wetzlar, Germany). We also selected several immunohistochemical staining markers related to the disease. The CD20 (the common B cell marker), pan-cytokeratin (CKpan) (the common epithelial marker), kappa (κ light chain), lambda (λ light chain), and Ki-67 (the marker of cell proliferation) (ZSGB-BIO, Beijing, China) staining were carried out according to the manufacturer's instructions. Dual staining for FCRL4 (dilution = 1:250; Abcam, Cambridge, UK), combined with Pax-5 (the common B cell marker) (ZSGB-BIO), and Ki-67 was performed using the silver proteinate (SP) method according to the manufacturer's instructions. Phosphate-buffered saline (PBS) (ZSGB-BIO) was used instead of primary antibodies as the negative control.

For semiquantitative analysis, two specialist pathologists assessed each slide in five fields at a magnification of 400×, independently, and then their individual scores were averaged. Before the formal evaluation of immunohistochemistry scores, each professional pathologist should first evaluate 10 slices, and then conduct consistency test.

The immunohistochemical markers of CD20, CKpan, Pax-5 and FCRL4 were applied and evaluated according to the following scoring criteria. Scoring method of positive cell ratio score: 0 point for <5%; 1 point for 6%—25%; 2 points for 26%—75%; and 3 points for 76%—100%. Scoring method of staining intensity: negative = 0; weakly positive (light yellow) = 1 point; positive (yellow) = 2 points; and strongly positive (brown) = 3 points. Immunohistochemical scores (IHS) = positive cell ratio score × staining intensity score: 0 = negative; 1—3 = low expression; 4—9 = high expression. Ki67 score: 0 for positive cell ratio<5%, 1 for 6%—15%, 2 for 16%—25%, 26%—35% was 3 points, and>35% was 4 points; Negative staining intensity was 0, weakly positive was 1 point, positive was 2 points, strong positive was 3 points; IHS = positive cell ratio score × Staining intensity score. Kappa and lambda light chain expression was classified as 'restricted' if the kappa/lambda ratio was >10:1 or the lambda/kappa ratio was >2:1.

Polymerase chain reaction

DNA was extracted from the paraffin-embedded tissues in accordance with the manufacturer instructions (QIAamp DNA FFPE Tissue Kit; Qiagen, Hilden, Germany). Multiplex polymerase chain reaction (PCR) assay was designed and conducted according to the BIOMED-2 protocol.[17] An ABI3500 genetic analyser (Thermo Fisher, Waltham, MA, USA) was used for capillary electrophoresis, and the results were analyzed using GeneMapper software.

Next-generation sequencing

The DNA extracted from the paraffin-embedded tissue of the suspected patients was identified using agarose gel electrophoresis and sent to Beijing Biolancet Technology Ltd. (Beijing, China) for next-generation sequencing (NGS). The sequencing results were then analyzed.

Statistical analysis

All statistical analyses were performed using SPSS Statistics software (ver. 24.0; IBM Corp., Armonk, NY, USA). The Chi-square test, Fisher's exact test and Spearman correlation analysis were performed. A two-tailed P-value < 0.05 was considered statistically significant.

Results

Patients

In total, 555 patients presented with lymphoepithelial lesions between January 1988 and August 2020. We excluded 400 patients according to the exclusion criteria (the patients with an unclear medical history, only labial gland biopsy and no further treatment, and poor quality or unacceptable paraffin-embedded tissue specimens), and the remaining 155 patients underwent glandular resection or partial glandular resection. And they were re-diagnosed through histomorphology, immunohistochemical staining, PCR or NGS by two specialist pathologists. Then, the patients were enrolled in the study, including 68 LESA patients. The male to female ratio was 1:3.53, and the average age was 48.51 years. The parotid gland (75%) was the most common lesion site. Three patients (4.41%) relapsed. Twenty-five SS patients were included, with a male to female ratio of 1:4 and average age of 45.16 years. The parotid gland (92%) was the most common lesion site. One patient (4%) relapsed. Sixty-two patients were diagnosed with salivary MALT lymphoma, and the male to female ratio was 1:2.65, and the average age was 52.06 years. The parotid gland (66.13%) was also the most

common lesion. Twenty-nine patients (46.77%) had a history of SS. Seven patients (11.29%) relapsed, and four patients (6.45%) received continuous treatment (chemotherapy, radiotherapy, etc.). Three patients (4.83%) died due to transformation into diffuse large B-cell lymphoma (DLBCL) (Table 1).

Histomorphological characteristics and immunophenotypes

LESA and SS had similar histomorphological characteristics, and manifested mainly as glandular atrophy, lymphocytic infiltration, and lymphoepithelial lesions (Fig. 1A). The histopathological manifestations of SS were that the acini were destroyed and disappeared, and dense lymphocytes infiltrated, sometimes forming lymphoid follicles. But the outline of lobules remained. There was no repair of fibrous connective tissue in the glandular lobule. And the intralobular ductal epithelial hyperplasia, lymphoepithelial lesion formation (Fig. 1B). The salivary MALT lymphoma was diffusely infiltrated with proliferative tumor lymphocytes, including centrocyte-like cells, monocytoid B cells, and small lymphocytes. Destruction of the glandular epithelium led to lymphoepithelial lesions (Fig. 1C). Reactive lymphoid follicles and follicular implantations were found in some cases. LESA, SS and salivary MALT lymphoma could express CD20 and CKpan (Fig. 1D and E). No cases of LESA and SS showed kappa or lambda light chain restriction, but 10 of the 62 salivary MALT lymphoma patients showed. Among them, seven patients had a kappa/lambda ratio >10:1 (Fig. 1G and H), while three had a ratio <1:2. The sensitivity of IHC for the detection of kappa or lambda light chain restriction was about 16.67%. The proliferation activity was low, and the Ki-67 positivity rate was approximately 1%–5% in LESA and SS. As a low-grade malignant tumor, salivary MALT lymphoma generally had a low Ki-67 index (Fig. 1F). However, there were three patients with salivary MALT lymphoma who were subsequently malignant transformation into DLBCL, in whom the Ki-67 index reached approximately 30%.

FCRL4 was mostly expressed in lymphocyte cell membranes, and was mainly distributed in and around lymphoepithelial lesions, but FCRL4 expression was less found in other parts away from lymphoepithelial lesions (Fig. 2A and B). The FCRL4 positivity rate was higher in salivary MALT lymphoma patients (98.39%) compared to SS (76%) and LESA (70.59%), and were significantly higher than in LESA and SS ($P < 0.001$). However, there was no significant difference in FCRL4 expression between the LESA and SS patients ($P = 0.851$) (Supplementary Tables S1 and S2).

Among the three lesions, the immunohistochemical double staining showed that FCRL4+ cells co-expressed Pax-5 (Fig. 2C), the expression levels of which were significantly correlated ($r = 0.505$, $P < 0.001$). FCRL4+ cells also co-expressed Ki-67 (Fig. 2D), The Ki-67 index was

Table 1 Basic clinical data of the patients. LESA, Lymphoepithelial sialadenitis; SS, Sjögren's syndrome; MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.

Characteristics		LESA	SS	Salivary MALT lymphoma
		(%)	(%)	(%)
Gender				
	Female	53 (77.94%)	20 (80%)	45 (72.58%)
	Male	15 (22.06%)	5 (20%)	17 (27.42%)
Age (years)				
	<20	3 (4.41%)	2 (8%)	2 (3.23%)
	20–40	22 (32.35%)	8 (32%)	10 (16.13%)
	40–60	19 (27.94%)	10 (40%)	29 (46.77%)
	>60	24 (35.3%)	5 (20%)	21 (33.87%)
Sites				
	Parotid gland	51 (75%)	23 (92%)	41 (66.13%)
	Submandibular gland	12 (17.65%)	0	2 (3.23%)
	Sublingual gland	0	1 (4%)	1 (1.61%)
	Palatine gland	3 (4.41%)	0	5 (8.06%)
	Two or more salivary glands	0	1 (4%)	5 (8.06%)
	Other glands	2 (2.94%)	0	8 (12.91%)
History of SS				
	Yes	*	*	29 (46.77%)
	No	*	*	33 (53.23%)
Prognosis				
	No recurrence	25 (36.77%)	12 (48%)	38 (61.29%)
	Recurrence	3 (4.41%)	1 (4%)	7 (11.29%)
	Continuous treatment	0	0	4 (6.45%)
	Death	0	0	3 (4.83%)
	Death from other diseases	1 (1.47%)	0	2 (3.23%)
	Loss to follow-up	39 (57.35%)	12 (48%)	8 (12.91%)
Total	155	68	25	62

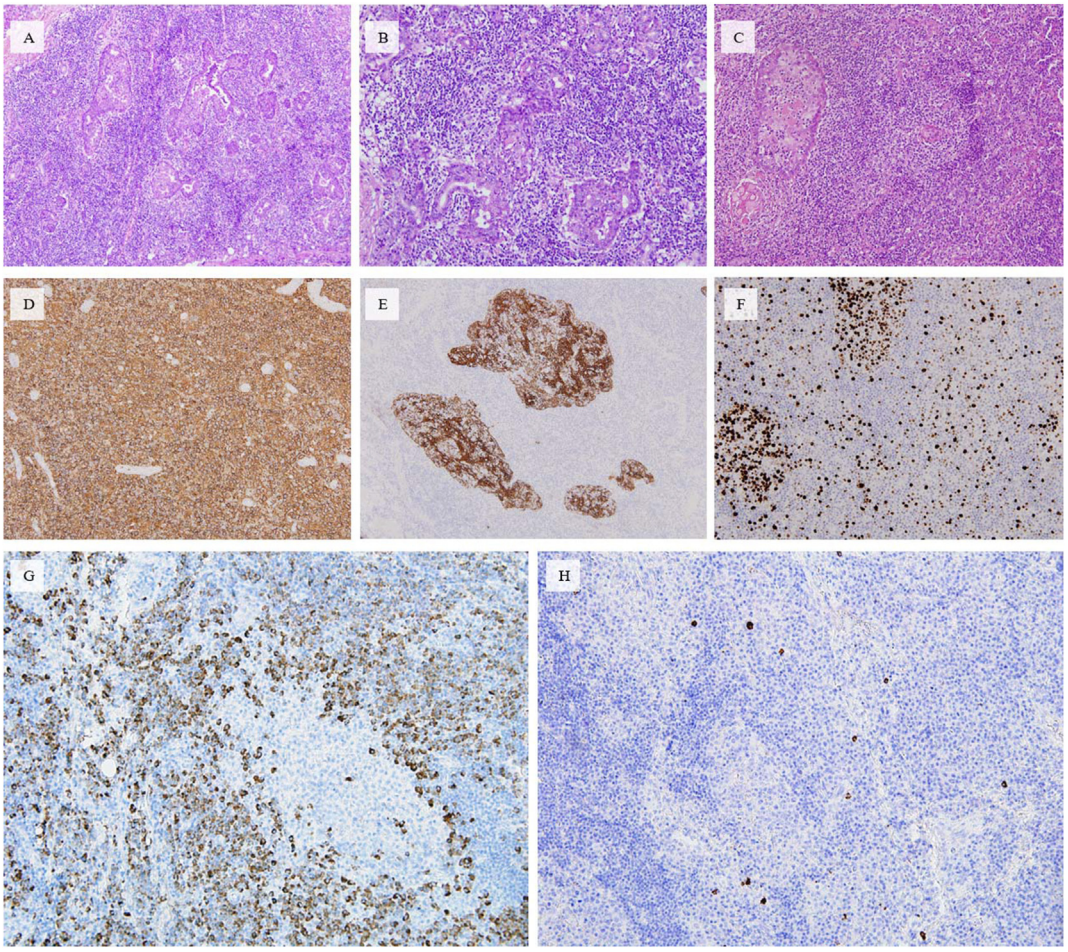

Figure 1 The histomorphological characteristics of LESA, SS and salivary MALT lymphoma, and the immunophenotype of salivary MALT lymphoma. A. LESA (HE × 100); B. SS(HE × 200); C. Salivary MALT lymphoma (HE × 200); D. CD20 (IHC × 200) of salivary MALT lymphoma; E. CKpan (IHC × 200) of salivary MALT lymphoma; F. Ki-67 (IHC × 200) (about 10%) of salivary MALT lymphoma; G. Kappa (IHC × 200) of salivary MALT lymphoma; H. Lambda (IHC × 200) of salivary MALT lymphoma. LESA, Lymphoepithelial sialadenitis; SS, Sjögren's syndrome; MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; HE, Hematoxylin-eosin; IHC, Immunohistochemistry.

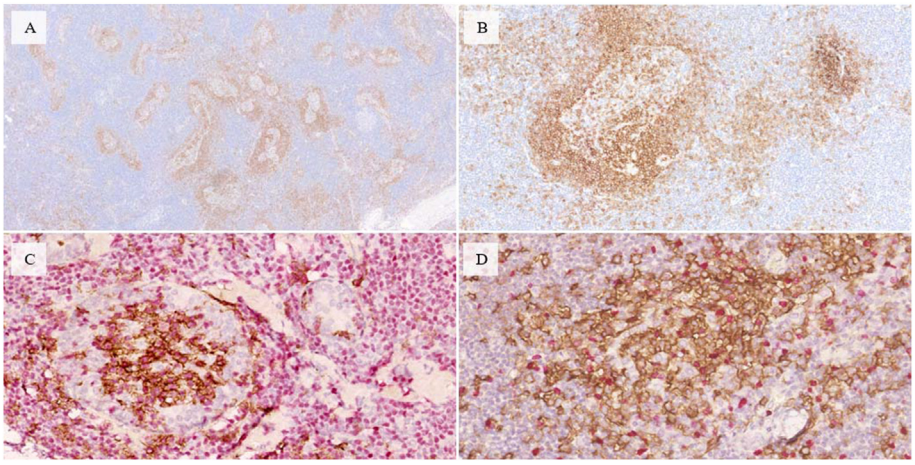

Figure 2 The expression of FCRL4 and Pax-5, Ki-67 in salivary MALT lymphoma. A. FCRL4 (IHC × 40); B. FCRL4 (IHC × 200); C. FCRL4-Pax-5 (IHC × 400); D. FCRL4-Ki-67 (IHC × 400). FCRL4, Fc receptor—like 4; MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; IHC: Immunohistochemistry.

significantly correlated with FCRL4 expression ($r = 0.564$, $P < 0.001$).

Evaluation of FCRL4 expression as a diagnostic marker for salivary MALT lymphomas by receiver operating characteristic curve analysis

Receiver operating characteristic (ROC) curve analysis was used to evaluate FCRL4 expression as a potential diagnostic marker for salivary MALT lymphomas. The area under the curve (AUC) value was 0.889 ($P < 0.001$), which indicated that FCRL4 had high predictive value (95% CI: 0.836–0.941) and could be used clinically. The optimal IHS cut-off value was 3.5 (between the low [1–3 points] and high [4–9 points] FCRL4 expression categories) (Fig. 3).

Clonal immunoglobulin gene rearrangement

Among the 62 salivary MALT lymphoma patients, PCR was performed with the BIOMED-2 primer set to detect clonal immunoglobulin (Ig) gene rearrangements, and 50 of the 60 salivary MALT lymphoma patients had monoclonal Ig gene rearrangements (sensitivity was about 83.33%). And the other 5 patients were diagnosed by kappa or lambda light chain restriction with the poor quality in paraffin-embedded tissue samples by PCR, and there were 2 patients only performed and diagnosed by kappa or lambda light chain restriction without performing PCR. NGS was performed for verification in five patients suspected of monoclonal Ig gene rearrangements. Monoclonal immunoglobulin heavy chain (IGH) gene rearrangement was detected in 46 patients (76.67%). The detection rates of IGH FR1-JH, IGH FR2-JH, IGH FR3-JH, IGH DH1-6-JH, and IGH DH7-JH were 25%, 36.67%, 43.33%, 25%, and 0%, respectively. IGH FR3-JH gene had the highest detection rate. The IGH DH7-JH gene was not detected. We detected monoclonal IGK gene rearrangement in 22 of the 60 patients (36.67%). The detection rate of IGK Vκ-Jκ (28.33%) was higher than that of IGK Vκ-Kde + intron Kde gene (18.33%). However, monoclonal rearrangement of the IGL Vλ-Jλ gene was detected in only seven patients; the detection rate was 11.67%, which was far lower than those for the IGH and IGK genes. In addition, the detection rate with combined application of the IGH and IGK probes was 81.67%. With combined application of the IGH, IGK, and IGL probes, the sensitivity was 83.33% (Supplementary Table S3).

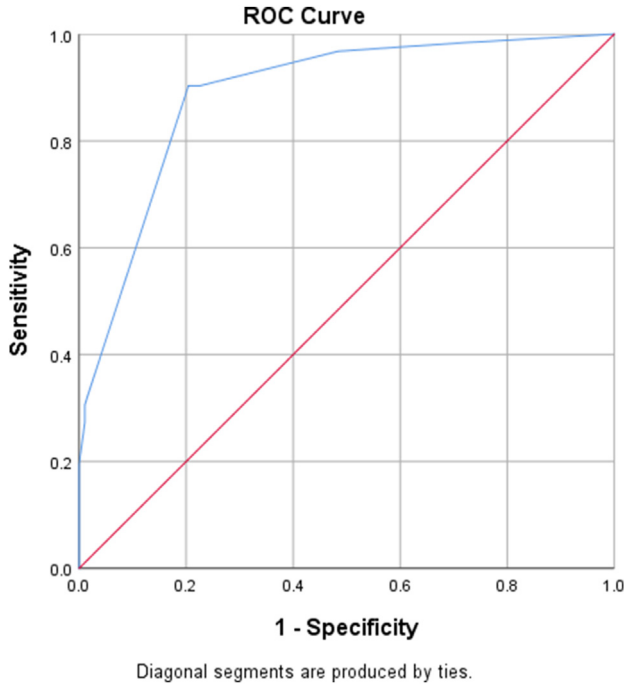

Figure 3 The ROC curve of FCRL4 in diagnosis of salivary MALT lymphoma. ROC curve, Receiver operating characteristic curve; FCRL4, Fc receptor–like 4; MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue.

Next-generation sequencing

NGS was performed in five patients with suspected monoclonal Ig gene rearrangements using the BIOMED-2 primer set. Two patients had both monoclonal IGH and monoclonal IGK gene rearrangements. The remaining three patients had monoclonal rearrangements for the IGH, IGK, and IGL genes. Thus, all five patients showed NGS-positive results with the sensitivity of 100% (Supplementary Fig. S1 and Supplementary Table S4).

Discussion

In this study, the recurrence rates of LESA and SS were 4.41% and 4%, respectively. Meanwhile, the incidence of salivary MALT lymphoma in lymphoepithelial lesions was about 11.17% (62/555), similar to a previous report.[3] Twenty-nine patients diagnosed with salivary MALT lymphomas had a history of SS. Although most salivary MALT lymphomas have indolent clinical manifestations and biological behaviors, a small proportion of patients may transform into highly malignant DLBCL. In this study, three patients died due to subsequently transformation into DLBCL, with the high Ki-67 indices approximately 30%. And kappa or lambda light chain restriction was detected in two of those three patients. Clinical practice guidelines for the diagnosis, treatment, and follow-up of marginal zone lymphomas were issued by ESMO in 2020.[18] Although there is no currently standard definition, the guidelines state that marginal zone B-cell lymphoma (MZL) with large B-cell transformation should be diagnosed when "isolated sheets" of large cells account for more than 20% of the tumor cells.[18] Therefore, patients with LESA, SS and salivary MALT lymphoma should be checked regularly over long-term follow-up for recurrences and high-grade malignant transformations. So, it would get early detection and treatment, thus improving survival rates.

Although, the mechanism underlying the role of FCRL4 in malignant transformation is not clear, many studies have demonstrated that the development of MALT lymphoma is related to activation of the nuclear factor kappa B (NF-κB) signaling pathway.[19] Some genes related to the NF-κB signaling pathway have been detected in FCRL4+ B-cells, which suggests that FCRL4+ B cells might play a role in the

development of lymphomas.[19,20] In primary SS, TNFAIP3/A20 is an important negative feedback regulatory gene encoding the NF-κB signaling pathway, and the diverse range of mutations of this gene is related to the development of lymphomas, especially in the early stages.[21] In addition, chemokines may activate both FCRL4+ B-cells and epithelial cells, which might provide an environment conducive to lymphoepithelial pathologies and the development of lymphoma. In this study, FCRL4 expressed in LESA, SS and salivary MALT lymphoma patients, and FCRL4+ cells mainly clustered in and around lymphoepithelial lesions and showed an obvious epithelial tendency. This suggested that FCRL4 might play a role in the formation of lymphoepithelial lesions, which was consistent with the results of previous studies.[12,13,21] In this study, the expression of FCRL4 in salivary MALT lymphoma patients was significantly higher than that in LESA and SS. There was no significant difference between the LESA and SS patients. This study showed that FCRL4 might play a role in the development of salivary MALT lymphomas, and the ROC curve analysis was performed to determine whether FCRL4 could be used as an auxiliary diagnostic marker for salivary MALT lymphoma. The AUC was 0.889 ($P < 0.001$), indicating that FCRL4 had high predictive value. So, the FCRL4 could be used as a positive marker to differentiate salivary MALT lymphoma cases from LESA and SS. And patients with lymphoepithelial lesions who have high expression of FCRL4 should receive more attention from pathologists, and a high degree of suspicion for salivary MALT lymphomas should be maintained.

Among the three lesions, the immunohistochemical double staining technique ("cocktail double staining") was used to explore the relationships among the expression levels of Pax-5, Ki67, and with FCRL4. The FCRL4+ cells could co-express Pax-5 and Ki-67, the expression levels of which were both correlated. Therefore, this study suggested that FCRL4+ cells were the actively proliferative B cells, which were mainly distributed in and around lymphoepithelial lesions. We also found that FCRL4 expression increased by the Ki-67 index, indicating FCRL4 might be used as a diagnostic marker for salivary MALT lymphomas with LESA and SS.

Owing to a lack of distinctive histomorphological and immunophenotypical features, MALT lymphomas, benign lymphoproliferative lesions, and other small B-cell lymphomas are mainly differentiated based on exclusionary diagnosis. To improve diagnostic accuracy, MALT lymphomas should be diagnosed based on histomorphological and IHC findings, combined with molecular pathology. Monoclonal gene rearrangements can be detected by IHC, PCR, NGS, and fluorescence in situ hybridization (FISH). The levels of clonal B-cell proliferation in MALT lymphomas can be assessed based on restricted Ig light chain (kappa/lambda) expression using IHC.[22] In this study, only 10 of the 62 salivary MALT lymphoma patients showed restricted kappa or lambda expression (16.67%). The low detection rate at the protein level might be due to poor differentiation of some B-cells, fixation or preservation of paraffin tissue, or the limitations of the antibodies, resulting in the failure of clonal Ig gene rearrangement. Currently, the BIOMED-2 primer set is the gold standard for detecting clonal Ig gene rearrangements for the diagnosis of B-cell lymphomas.[17,23] Clonal Ig gene rearrangements are "level II" markers for the diagnosis of extranodal marginal zone lymphomas (non-primary gastric lymphomas) according to the 2021 Chinese Society of Clinical Oncology (CSCO) diagnostic and treatment guidelines for malignant lymphomas.[24] In this study, the BIOMED-2 primer set was used to detect clonal Ig gene rearrangements, and salivary MALT lymphoma with the sensitivity of 83.33% when the probes were applied together. At present, IGH FR3-JH is the most commonly used primer, given its high detection rate for Ig gene rearrangements (in turn due to relatively short gene segments that can be easily amplified).[25,26] A combination of IGH, IGK, and IGL probes could improve the diagnostic accuracy for salivary MALT lymphomas.

In this study, five patients were suspected of monoclonal Ig gene rearrangements, so we used NGS for further verification. We performed NGS with paraffin-embedded tissue samples and confirmed that all five patients had salivary MALT lymphomas (100%). Therefore, NGS verified the BIOMED-2 primer results and improved the diagnostic accuracy for salivary MALT lymphomas. The EuroClonality-NGS working group proposed the use of NGS to detect clonal Ig gene rearrangements. Amplification of small gene fragments can increase sensitivity and diagnostic accuracy by avoiding the false-negative results caused by small DNA fragments and poor DNA quality when using the BIOMED-2 primer set.[27] In the era of precision medicine, NGS has been widely applied for diagnosis and classification of lymphomas due to its high throughput and sensitivity, and potential to detect unknown mutations. It can also provide data informing targeted treatments and prognosis.[28] However, NGS remains cumbersome and expensive, so its clinical use is still limited. We believe that further developments may lead to wider recognition of the utility of NGS for clinical diagnosis, treatment, and research on lymphomas and other diseases.

The application of multiple detection methods could significantly increase the diagnosis rate of salivary gland MALT lymphomas, and reduce the rates of missed diagnoses and misdiagnoses. FCRL4+ B-cells play an important role in the formation of lymphoepithelial lesions. FCRL4 could be used as a marker aiding differential diagnosis of salivary MALT lymphomas from LESA and SS. Combining PCR with analysis of "restricted" kappa/lambda light chain expression to detect clonal Ig gene rearrangements could improve the diagnosis of salivary MALT lymphomas. NGS could be used to detect false-negative results for clonal Ig gene rearrangements caused by poor DNA quality when using the BIOMED-2 primer set; in turn, this could improve the diagnostic accuracy.

In conclusion, this study introduced a diagnostic method for salivary lymphoepithelial lesions. For differential diagnosis of diseases characterized by lymphoepithelial lesions, including LESA, SS, and salivary MALT lymphoma, a series of diagnostic methods may be needed (Figs. 4A and 4B). We also summarized the process for diagnosing MALT lymphomas. Generally, a preliminary diagnosis should be made on the basis of the histological morphology, followed by IHC, PCR, and NGS for definitive diagnosis. In this manner, an appropriate treatment plan could be made, and the quality of life and survival rates of patients could thus be improved (Figs. 4A and 4B).

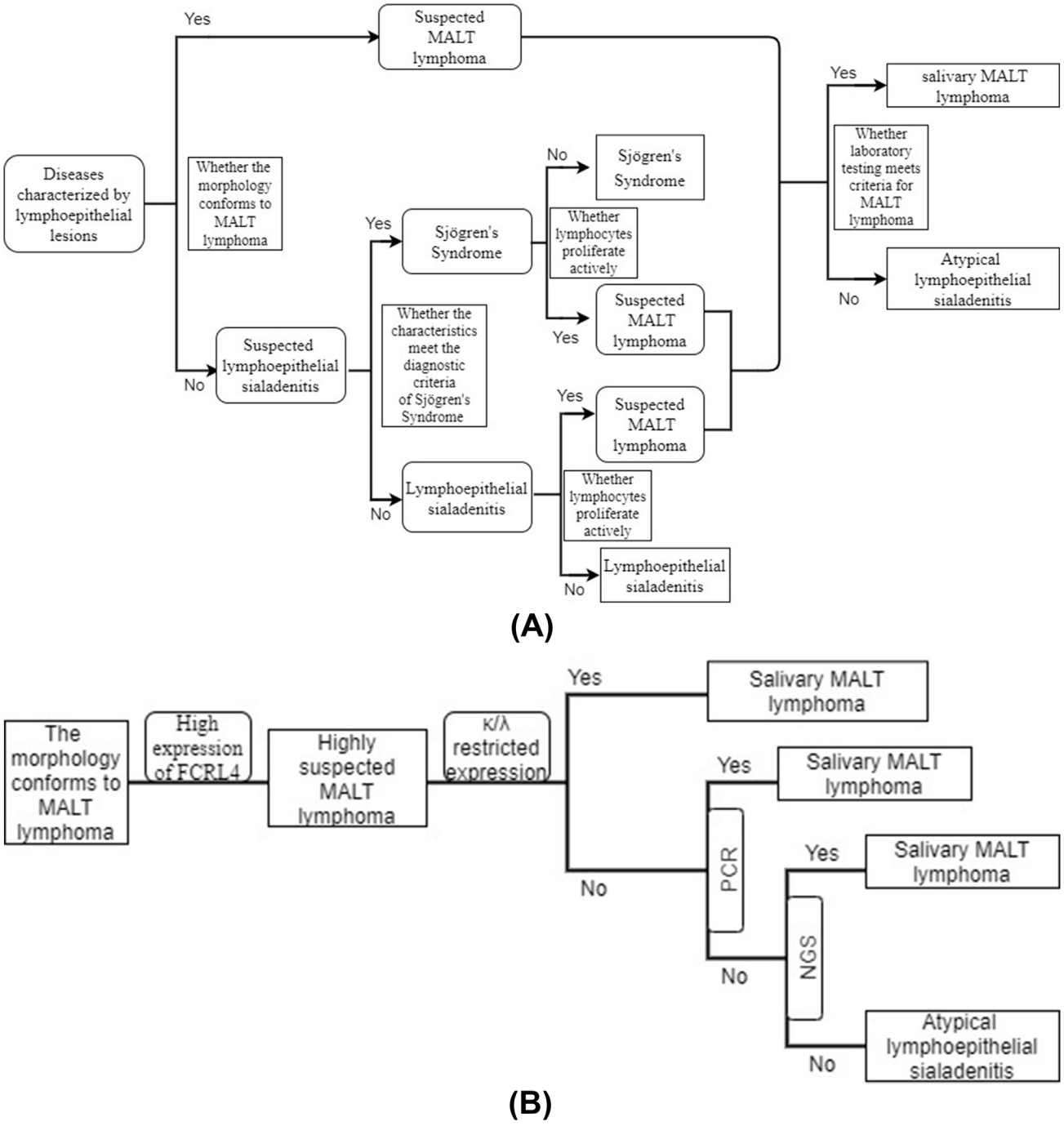

Figure 4 A. The diagnostic flow chart of diseases with lymphoepithelial lesions. MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue. B. The diagnostic flow chart of MALT lymphoma. MALT lymphoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue; FCRL4, Fc receptor—like 4; κ, kappa; λ, lambda; PCR, Polymerase chain reaction; NGS, Next-generation sequencing.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2023.05.018.

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