# Primary lung mucosa-associated lymphoid tissue lymphoma accompanied by multiple sclerosis: case report and molecular diagnosis

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*To the Editor:* Mucosa-associated lymphoid tissue (MALT) lymphoma arises at a wide range of different extranodal sides, including the stomach (70%), lung (14%), ocular adnexa (12%), thyroid (4%), and small intestine.[1,2] Most lung MALT lymphoma are marginal zone B-cell lymphoma with proliferation of small irregular monocytoid B cells around and between reactive lymphoid follicles, with extensive invasion of the lung parenchyma, forming lymphoepithelial lesions. Immunohistochemical analysis showed the positive staining for CD20 and Bcl-2 and negative staining for CD3, CD5, CD23, and CD10 in the neoplastic cells.

However, some cases were very difficult to discriminate between malignant and reactive lymphoproliferations. The diagnosis of lymphoid malignancies can be supported by clonality assessment based on the fact that all cells of malignancy had a common clonal origin. Polymerase chain reaction (PCR) genescan is necessary to confirm monoclonality, which helps for the final diagnosis in patients with lymphoproliferative disorders. The combined BIOMED-2 protocols are summarized in a guideline, which can now be implemented in routine lymphoma diagnosis. There are many factors that can affect the results of PCR genescan. The quality of samples, especially the content of tumor cells is the key to ensure the accuracy of the test.

Here we described a case of MALT lymphoma accompanied by multiple sclerosis, which was diagnosed via PCR-based clonality testing using micro-dissected tumor cells. We found that extensive fibrosis in the formalin-fixed paraffin-embedded (FFPE) specimens disturbed the real genetic analysis. These results underscored the importance of avoiding fibrosis when getting tumor cells for PCR-based clonality testing.

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A 56-year-old man was presented to Shanghai Chest Hospital after physical examination. Computed tomography demonstrated an oval homogeneous mass (4.8 cm × 2.7 cm) with mild enhancement after administration of a contrast media in the left anterior mediastinum near the arch of the aorta. The mass infiltrated the left upper lobe of the lung with an irregular border. Moreover, multiple pulmonary nodules scattered throughout both lungs. The patient suffered 5 kg weight loss recently. Next, the patient underwent thoracoscopic wedge resection of left upper lobe. Grossly the tumor size was about 4.5 cm × 4.0 cm × 2.5 cm, gray and hard, beneath the pleura. Histologically, small lymphoid cells were accumulated in multiple foci surrounded by broad fibrosis bands and formed lymphoepithelial lesion focally. Within and between follicular aggregates, the lymphocytes were pale staining and in a monocytoid and plasmacytoid interfollicular pattern. Immunohistochemical analysis showed that the tumor cells were positive for CD20 (L26, 1:800; Dako, Carpinteria, CA, USA) and CD79α (MB1, 1:500; Dako) but negative for CD3 (F7.2.38, 1:100; Dako) and Cyclin D1 (ZA-0101, 1:200; ZSGB, Beijing, China). And the positive rate of Ki67 (ZM0166, 1:300; ZSGB-BIO, Beijing, China) for tumor cells was 10% [Figure 1].

BIOMED-2 PCR assays for clonality analysis of immunoglobulin (*IG*) gene rearrangement were evaluated with FFPE specimens. Heteromorphic lymphocytes were microdissected from the paraffin specimens, and then DNA was extracted and purified using a QIAamp DNA mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. PCR amplifications were performed using commercial BIOMED-2 multiplex PCR master mixes and controls (Invivoscribe technologies, San Deigo, CA, USA) according to BIOMED-2 PCR protocols. Experiments were carried out in a 25-μL volume in duplicate. For purified DNA samples, 125 ng of DNA was used in PCR. The full set of reactions for *IG* gene

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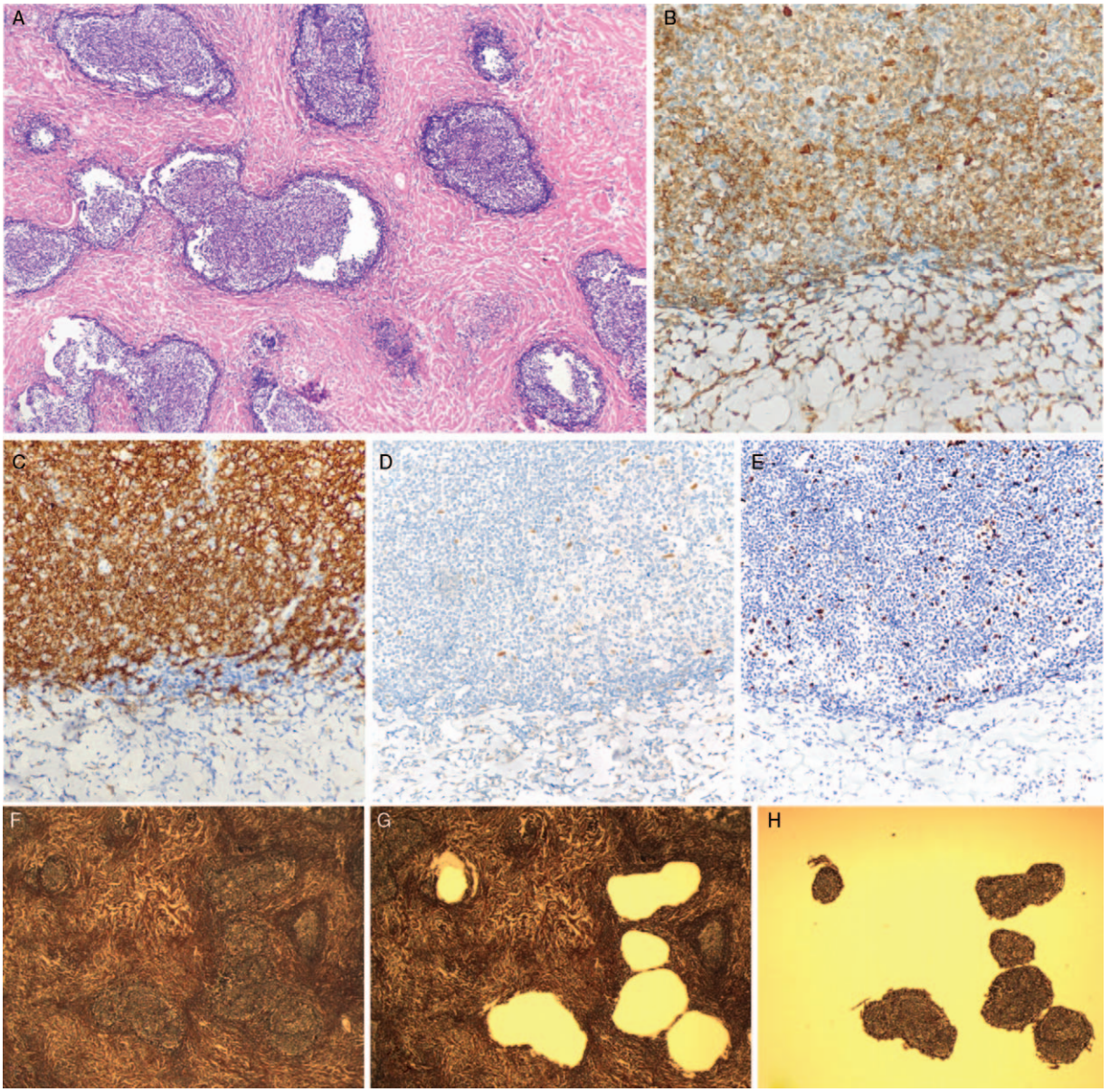


**Figure 1:** (A) Pathological findings indicating numerous clusters of small lymphocytes infiltrated in the peripheric pink sclerotic tissue by hematoxylin and eosin staining (original magnification ×100). Immunohistochemistry of the same sample showed positive staining of CD79α (B) and CD20 (C), but negative staining of CyclinD1 (D; original magnification ×200). And the positive rate of Ki67 (E) for tumor cells was 10% (original magnification ×200). Micro-dissection (original magnification ×200): Images showing the small lymphoid cells accumulated in multiple foci surrounded by broad fibrosis bands before laser micro-dissection (F); lymphoid cells were removed from samples (G); and the dissected tumor fractions (H).

arrangements included five reactions targeting immunoglobulin heavy chain (IGH: IGHA, IGHB, IGHC, IGHD, and IGHE), two reactions targeting immunoglobulin Kappa (IGK: IGKA and IGKB). The full set of reactions for T cell receptor (*TCR*) gene arrangements included three reactions targeting TCRB (TCRBA, TCRBB, TCRBC) and two reactions targeting TCRG (TCRGA, TCRGB) and one reaction targeting TCRD. Control PCR yielded a product with size of 400 bp. Polyclonal and monoclonal controls were included for each experiment. PCR products were denatured at 95°C for 5 min and rapidly transferred to 4°C for capillary electrophoresis by gene scanner.

PCR results showed IGH A and B with a defined monoclonal rearrangement peak. So the clonal *IGH* gene rearrangement was detected in this case [Figure 2]. Based on the histomorphologic features and PCR genescan results, a diagnosis of MALT was rendered.

MALT lymphoma of the lung is rare and typically shows single or multiple radiologically detected pulmonary nodules or consolidations. It occurs either in asymptomatic patients or in patients with non-specific respiratory complaints such as cough and dyspnoea.[3,4] Although central sclerosis may be a feature of MALT lymphoma, we found extensive fibrosis in this case which is rarely reported in the literature and may interfere with the normal pathological diagnosis.[5,6]

Pulmonary extranodal MALT originated lymphoma is composed of a morphologically heterogeneous infiltrate, which is made up of small B cells, monocytoid B cells,

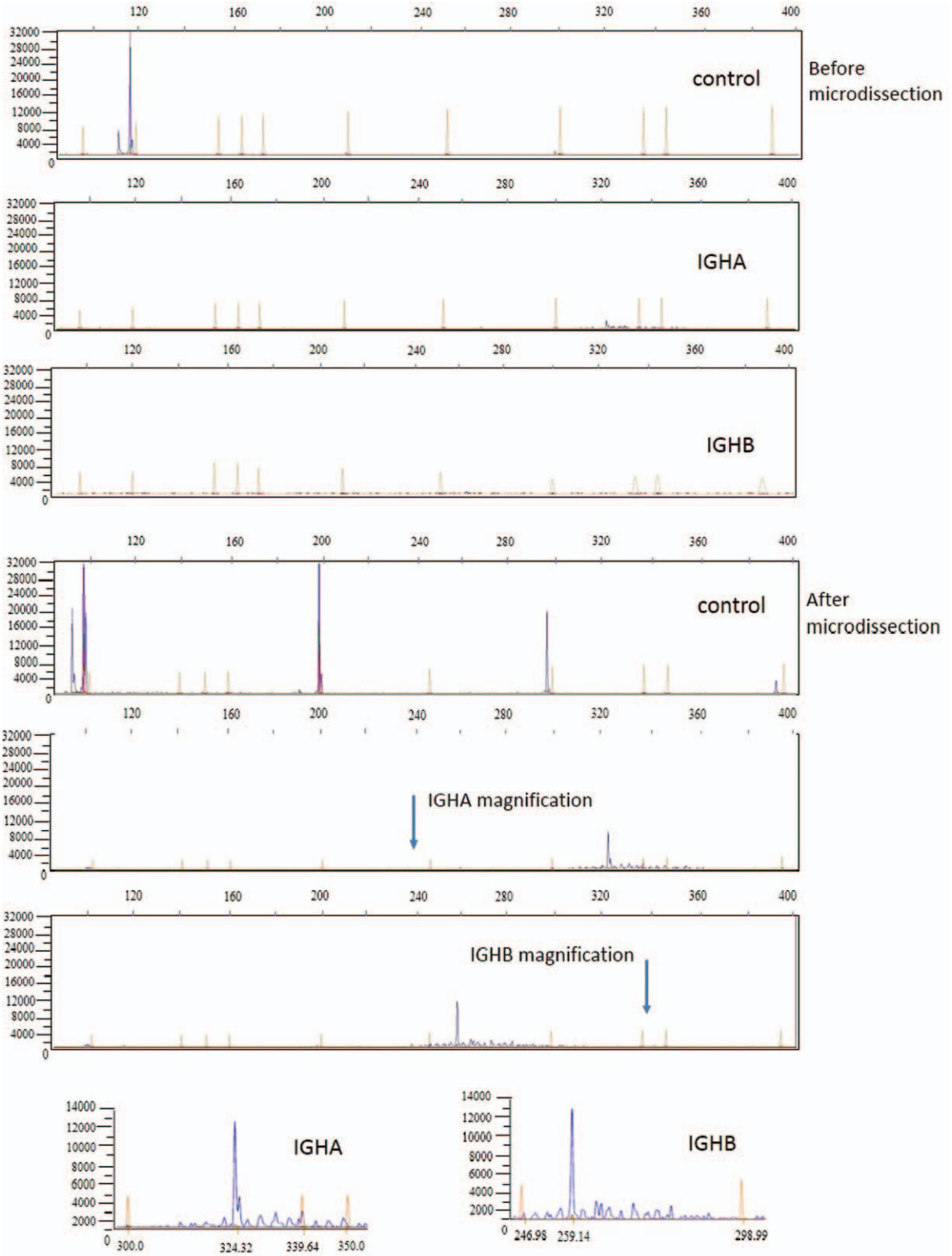


**Figure 2:** The results of multiplex PCR analyzed by gene scanning before and after micro-dissection. Before micro-dissection, the size of control DNA from FFPE specimen was only 100 bp, suggesting that the DNA has been fragmented. There was no clear signal in the IgH A and B. After micro-dissection, the size of control DNA from FFPE specimen was 400 bp, which was good for clonality testing. Clonal *IGHA* and *IGHB* gene rearrangement were detected with this sample. FFPE: Formalin-fixed paraffin-embedded; IGH: Immunoglobulin heavy chain; PCR: Polymerase chain reaction.

scattered immunoblasts, and centroblast-like cells. Under immunohistochemical stains, the neoplastic cells are positive for CD20 and CD79α. Further, CD21 staining showed a follicular dendritic cell network of different sizes. Bcl-2 positive cells in the network grew into the germinal center, in which follicular cells embedded. The lymphoma is associated with a crystal-storing histiocytosis, with a monomorphous population of plasmacytoid cells mixed with histiocytes filled with crystalloid structures.[7,8]

Molecular pathologic testing plays an important role for the diagnosis, prognostication, and decision of treatment in the diagnosis of lymphoproliferative diseases. We did PCR genescan test; however, the results were not good due to low quality of internal control. We thought that the accuracy of PCR results for gene rearrangement can be affected by the interference of fibrous tissue. So we used tumor cells microdissected from tissue for PCR genescan test. The results showed that IGH A and B were defined as a positive peak obviously. Our results also suggested that the quality of FFPE specimens, especially the tumor cells contained in the samples, was crucial for diagnosis of molecular pathology accurately. The molecular pathology of MALT lymphoma was complex; therefore, we should pay attention to the quality of samples in order to exclude interference factors and provide strategies at the same time for integrating this information into daily pathological practice. Only after we have done this can we make accurate molecular pathological diagnosis in clinical detection.

Fresh or frozen samples often had better quality than FFPE specimen, the amount of which may be too small to purify DNA. Furthermore, the quality of DNA extracted varies considerably due to the effect of tissue fixation and processing and many biopsies are small and inadequate for standard DNA purification procedures as suggested by the BIOMED-2 studies. PCR-based clonality testing between lymphoproliferations and lymphoma has now matured into a reliable method that can easily be used in the laboratory with routine molecular diagnostics.[9,10] Both knowledge and experiences are required for correct interpretation of PCR-based clonality diagnostics. It is important; however, that the test results are interpreted with full knowledge including immunobiology, *IG/TCR*-gene composition, and pathology of lymphomas, and thus close cooperation between the molecular biologist and the pathologist is crucial. Collectively, both accurate detection and professional interpretation will help to improve the general performance level of clinical management in patients with suspected lymphoproliferations.

## Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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## Conflicts of interest

None.

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