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Reviewer A

This is a small retrospective case series reporting 4 cases with MALT lymphoma diagnosed by TBLC samples.
As bronchopulmonary MALT lymphoma cases are rare, reports on these cases are of some interest. However, there are some concerns about the scientific conclusions and the methodic approach.

Comment 1:
The number of reported cases is low. In the methods section it is described that patients have been included where MALT lymphoma had been suspected. This suggests that it has been suspected before the endoscopy. As there are no specific radiological signs of MALT-Lymphoma, this is a differential diagnosis in every case of persisting pulmonary infiltrate. I guess, that there were much more cases of persisting pulmonary infiltrate seen in the institution than 4 cases. By this, I guess that the authors only included cases with proven MALT lymphoma which is a different inclusion criteria.
We already know from previous publications, that MALT lymphoma may be diagnosed by TBLC.
Reply 1:
Thank you for your suggestion. The selection of cases was as described in the Methods section, and cases in which the disease was suspected based on CT findings were included. Because of the lack of detail in the description, which could be misleading, we have added "duration of the study" and more detail on the "imaging findings that led us to suspect the disease".
Changes in the text:
We retrospectively analyzed the CT findings for four patients who underwent cryobiopsy using a GS for suspected pulmonary MALT lymphoma at our institution between February 2022 and January 2023. Notably, all patients had CT findings characteristic of pulmonary MALT lymphoma, such as lung nodules, ground-glass opacity, lung mass, and/or airspace consolidation. The lesions had peribronchovascular distributions with air bronchograms [9].
(see Page 9, lines 8–13)
We also noted that this is the first report of TBLC using a 1.1 mm cryoprobe in

combination with GS, although there have been previous reports of TBLC using conventional cryoprobes. Therefore, we have added the following sentences.

Changes in the text:

A 1.1 mm cryoprobe has recently become available, and its usefulness has been increasingly reported. Use of a conventional cryoprobe for TBLC in diagnosing pulmonary MALT lymphoma has been previously reported; however, there are no reports on the use of a 1.1 mm ultrathin cryoprobe and guide sheath (GS).

(see Page 3, lines 7–11 in Abstract)

Furthermore, there have been previous reports on the usefulness of conventional TBLC in the diagnosis of pulmonary MALT lymphoma; however, this is the first report describing the use of a 1.1 mm cryoprobe with a GS.

(see Page 9, lines 2–5)

Due to the addition of the text (see Page 3, lines 7–11 in Abstract) to the abstract, the following text was revised due to the word limit.

Changes in the text:

In all four cases, 8-16 biopsy specimens were obtained using a cryoprobe. Histopathological analysis of two cases revealed the infiltration of small lymphocytes with numerous lymphoepithelial lesions, confirming MALT lymphoma.

（Page 4, lines 5–8 in Abstract）

Comment 2:

Detailed information about the clinical background and the endoscopic approach is lacking and is only provided for Pt 1. In these cases results from TBB are available which is not reported for the other three cases.

Reply 2:

Thank you for your advice.

This is a Brief report, and although the clinical background of Cases 2-4 and the endoscopic approach cannot be described in detail due to word limit, we have added text as much as possible.

Changes in the text:

Case 2 was a 69-year-old male former smoker with hypertension. He was asymptomatic and noticed abnormal shadows during the routine physical examination. Case 3 was a 78-year-old female never-smoker with a history of osteoporosis. She had symptoms of shortness of breath. Case 4 was an 86-year-old female never smoker. She had Sjogren's syndrome with symptoms of dry eyes and dry mouth, hypertension and previous chronic subdural hematoma. All cases had the CT findings described in the Methods section, and

were performed using a 1.1 mm cryoprobe in combination with with a GS described in the Methods section.
(see Pages 12–13, lines 12–2)
The reason for performing the TBB in case1 is described in Methods and Results for better understanding by the reader.
Changes in the text:
TBB using single-use 1.8 mm diameter flexible biopsy forceps (Radial Jaw™4 Pulmonary Biopsy Forceps; Boston Scientific, Tokyo, Japan) was added only in Case 1 to compare the size and quality of the specimens obtained using TBLC and TBB.
(see Pages 11, lines 3–6)
TBB was also performed in Case 1 to compare the specimen size and quality.
(see Page 14, lines 1–2)

Comment 3:
To estimate whether the TBLC approach is a "valuable" tool, suspicious cases should undergo a TBLC procedure and in case of inconclusive results should be forwarded for VATS to establish a diagnosis.
Reply 3:
Thank you for your comment. In cases 2 and 4, it was difficult to diagnose pulmonary MALT lymphoma histopathologically because most of the specimens were dominated by organization and fibrosis. However, the results of molecular biological testing performed at the same time were obtained and considered to have contributed to the diagnosis. As you pointed out, this study was conducted on MALT lymphoma, a lesion that is generally difficult to diagnose by bronchoscopy, and we also believe that if sufficient evidence cannot be obtained for the diagnosis, it is necessary to consider the necessity of performing VATS.

Comment (summary):
In summary, the reported results do not contribute to the scientific field as the feasibility is already reported in previous publications. However sensitivity and specificity data are lacking. I would like to encourage the authors to intensify their studies in this area (TBLC in persisting pulmonary infiltrates).
Reply to comment (summary):
Thank you for your comment. This study suggests that the combination of 1.1 mm cryoprobe and GS is useful for the diagnosis of pulmonary MALT lymphoma, which is difficult to diagnose and requires a sufficient volume of specimen, in that a large number

of biopsies can be performed more accurately, more easily, and more safely.
Although this is a retrospective study of a small number of cases, we believe that this may be one of the endoscopic approaches.

Reviewer B

In this study, the authors conclude that cryobiopsy with a 1.1-mm diameter cryoprobe and a 2.7-mm diameter guide sheath is a useful and safe method for diagnosing pulmonary MALT lymphomas.
This biopsy technique, which can safely obtain a good quality tissue sample, may be useful in the diagnosis of MALT lymphoma as well as lung cancer.

However, there are problems that need to be resolved.

Comment 1:
First, in the cases in this paper, specimens are collected through the guide sheath by setting a short freezing time. While this technique allows safe specimen collection, the specimen size is limited to the area that can pass through the guide sheath. On the other hand, a larger specimen can be collected by freezing the 1.1-mm cryoprobe for nearly 10 seconds and extracting the specimen and bronchoscope together from the airway. Doing so might have made the diagnosis clearer in Cases 2 and 4. It has also been reported that the risk of bleeding is not high with 1.1-mm cryoprobes, even with longer freezing times. I suggest clarifying the reason for setting the cryoprobe freezing time short.
Reply 1:
Thank you for your precious suggestion. No reports we searched, or from the developer, Amco Incorporated, have reported that the 1.1 mm cryoprobe does not bleed easily even with longer freezing times. In previous reports using the 1.1 mm cryoprobe, the longer the freezing time, the larger the tissue obtained, and there are reports of bleeding. Although the size of specimens with shorter freezing times is smaller per specimen than those which obtain a small number of larger specimens with longer freezing times, GS allows a large number of biopsies to be performed more easily and accurately. We reported this study because we believe that this advantage may be useful in the diagnosis of pulmonary MALT lymphoma.

We also noted that this is the first report of TBLC using a 1.1 mm cryoprobe in combination with GS, although there have been previous reports of TBLC using

conventional cryoprobes. Therefore, we have added the following sentences.
Changes in the text:
A 1.1 mm cryoprobe has recently become available, and its usefulness has been increasingly reported. Use of a conventional cryoprobe for TBLC in diagnosing pulmonary MALT lymphoma has been previously reported; however, there are no reports on the use of a 1.1 mm ultrathin cryoprobe and guide sheath (GS).
(see Page 3, lines 7–11 in Abstract)
Furthermore, there have been previous reports on the usefulness of conventional TBLC in the diagnosis of pulmonary MALT lymphoma; however, this is the first report describing the use of a 1.1 mm cryoprobe with a GS.
(see Page 9, lines 2–5)
Due to the addition of the text (see Page 3, lines 7–11 in Abstract) to the abstract, the following text was revised due to the word limit.
Changes in the text:
In all four cases, 8-16 biopsy specimens were obtained using a cryoprobe. Histopathological analysis of two cases revealed the infiltration of small lymphocytes with numerous lymphoepithelial lesions, confirming MALT lymphoma.
（Page 4, lines 5–8 in Abstract）

Comment 2:
Second, when using a 2.7 mm guide sheath, it should be possible to collect large specimens using a 1.7 mm or 2.4 mm cryoprobe. In the case of malignant lymphoma, a large specimen should be necessary for diagnosis. I suggest adding a reason for choosing the 1.1mm probe size instead of 1.7mm or 2.4mm.
Reply 2:
Thank you for your advice. We have added the following sentences as advised.
Changes in the text:
If the freezing time is too long with a 1.1 mm cryoprobe, the sample is too large to pass through the 2.7 mm GS and gets stuck. However, a 1.1 mm cryoprobe was used because the specimens would not pass through the GS unless the freezing time was shortened, although it is theoretically possible to combine a 1.7 mm or 2.4 mm cryoprobe with a GS.
(see Pages 10–11, lines 16–3)

Comment 3:
Third, does freezing of specimens affect the results of immunostaining, IGR, flow cytometry, etc.? I suggest that the author provide an additional explanation on this point

in the text.

Reply 3:

Thank you for your suggestion. Bianchi et al. reported that immunohistochemical staining and molecular testing are available, even in TBLC specimens [6].There have been no previous reports of flow cytometry using TBLC specimens. Although flow cytometry also contributed to the diagnosis in Case 1, more cases need to be accumulated to determine whether TBLC specimens can be used for flow cytometry. It may be better to perform flow cytometry with non-frozen TBB specimens. Therefore, we have added the following text.

Changes in the text:

Bianchi et al. reported that immunohistochemical staining and molecular testing are available, even in TBLC specimens [6]. We performed immunohistochemical staining in all cases and evaluated the proliferation of B-cells and light-chain restriction by immunohistochemical staining. Clonal IGR was also demonstrated using molecular testing in cases 1, 2, and 4. There have been no previous reports of flow cytometry using TBLC specimens. Although flow cytometry also contributed to the diagnosis in Case 1, more cases need to be accumulated to determine whether TBLC specimens can be used for flow cytometry. It may be better to perform flow cytometry with non-frozen TBB specimens.

(see Page 16, lines 4–13)

Comment 4:

Finally, the use of a thick bronchoscope in combination with a guide sheath means that accessibility to the lesion is reduced compared to a thin bronchoscope. All the cases in this study had large lesions, but how should the size of the bronchoscope be selected for smaller lesions?

Reply 4:

Thank you for your comment. for small lesions, the method in this study seems to be limited in that the lesion cannot be reached. In that case, I would try other methods, such as trying a 1.1mm cryoprobe (Oki M, et al. Respirology 2023:28:143-151) with ultrathin bronchoscope.

Comment 5:

Also, as a minor comment, the meaning of the following sentence on page 11, line 17 to page 12, line 1 is unclear. Please correct it to a clear sentence.

“In each case, 1 or 2 biopsies were performed on peripheral pulmonary lesions (PPLs)

ranging from 28 to 67 mm in size."
Reply 5:
Thank you for your suggestion. We have added the following sentences as advised.
Changes in the text:
In each case, biopsies were performed on one or two peripheral pulmonary lesions (PPLs) ranging from 28 to 67 mm in size.
(see Page 13, lines 3–5)

Reviewer C

Comment 1:
(Page 7, 13~14) Although diagnosis using noninvasive examinations is preferable, the diagnostic yield of TBB with bronchoscopy is low.
Please add the diagnostic yield of TBB and references.
Reply 1:
Thank you for your suggestion. We have added the following sentences as advised.
Changes in the text:
A Diagnosis using noninvasive examinations is preferable; however, the diagnostic yield of TBB for pulmonary MALT lymphoma is low, with Borie et al. and Farrell et al. reporting a diagnostic yield of approximately 30% (19/61 cases) and 30% (2/8 cases), respectively [3,5].
(see Page 7, lines 12–16)

Comment 2:
(Page 8, 7~9) However, TBLC is associated with the risk of bleeding and pneumothorax owing to the removal of the entire bronchoscope when obtaining specimens.
En-bloc revomal of scope and cryoprobe together after freezing can increase severe bleeding, but does it also increase pneumothorax? Check if there is any relevant content in the presented references, and if not, you can just add increased bleeding.
Reply 2: Thank you for your comment. As you pointed out, there is no clear statement in the references regarding an association between the removal of the entire bronchoscope and an increase in pneumothorax. For the sake of accuracy, we have removed the sentence to an increase in pneumothorax.
Changes in the text:
However, TBLC is associated with the risk of bleeding owing to the removal of the entire bronchoscope when obtaining specimens [6,7].

(see Page 8, lines 9–11)

Comment 3:
(Page 9~10, Procedures)
Please add the types and sizes of conventional forceps used for TBB. Since the internal diameter of GS is large, there is a possibility that a fairly large tissue can be obtained by repeatedly obtaining tissue using 1.8-1.9 mm forceps. Is there a reason why TBB was performed only 3 times (Case 1)?
Reply 3:
Thank you for your advice. Types and sizes of TBB forceps are listed. TBB was performed only three times to compare the quality and size of each sample with those obtained by TBLC. So the reason for performing the TBB in Case1 is described in Methods and Result for better understanding by the reader.
Although TBB specimens can be used for molecular testing by increasing the number of times, the quality of the specimens is poor and morphological diagnosis is difficult.
The advantage of this study is that the TBLC specimens are slightly larger in size than TBB specimens and the quality is overwhelmingly better.
Changes in the text:
TBB using single-use 1.8 mm diameter flexible biopsy forceps (Radial Jaw™4 Pulmonary Biopsy Forceps; Boston Scientific, Tokyo, Japan) was added only in Case 1 to compare the size and quality of the specimens obtained using TBLC and TBB.
(see Pages 11, lines 3–6)
TBB was also performed in Case 1 to compare the specimen size and quality.
(see Page 14, lines 1–2)

Comment 4:
(In results section)
The diameter of the cryobiopsy specimen in Figure 1 (D) is larger than the inner diameter of the GS, and in general, even if the thick GS is used, it is thought that a smaller tissue can be obtained. Show the average or median values for the sizes of the cryobiopsy specimens. (If possible)
Reply 5:
Thank you for your suggestion. The specimens are not spheres, but are wrapped around the cryoprobe, so the maximum diameter is larger than the inner diameter of the GS. The size of cryobiopsy specimens correlates with freezing time, and the specimens used in the figure are larger with longer freezing times. Because the sizes were not measured during

the examination and some specimens were submitted to fresh specimens for molecular testing, some specimens were not formalin-fixed, and an average value cannot be obtained.