The potential usefulness of sputum cytology in the conclusive diagnosis of methotrexate-associated lymphoproliferative disorders: A case report

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Abstract:	Methotrexate (MTX) has been used as an anchor drug for the treatment of rheumatoid arthritis (RA) and is considered to be a cause of MTX-associated lymphoproliferative disorder (MTX-LPD). Spontaneous regression can occur after withdrawal of MTX and may be associated with Epstein-Barr virus (EBV) positivity and non-diffuse large B cell lymphoma (non-DLBCL) histological type. MTX-LPDs are often diagnosed pathologically by lung biopsy. To the best of our knowledge, there have been no studies on the cytological diagnosis of MTX-LPD using sputum smears. A 70-year-old man, who was diagnosed with RA 13 years previously and who had been treated with MTX, presented shortness of breath and productive cough. MTX-LPD was suspected as the sputum cytology showed many atypical lymphoid cells with hyperchromatic enlarged nuclei, foamy cytoplasm and distinct nucleoli. Chest computed tomography revealed multiple nodular shadows with interstitial pneumonia in the bilateral lower lung field. A lung biopsy specimen contained atypical lymphoid cells that were immunohistochemically-positive for CD20 and MUM-1, and weakly positive for bcl-6, but negative for CD3 and CD10. ISH-EBER showed no EBV-infectious lymphoid cells. He was finally diagnosed with MTX-LPD (Non-germinal center B-cell-like DLBCL histological type). Most of the nodules disappeared spontaneously following the withdrawal of MTX. A cytologically conclusive diagnosis of MTX-LPD may be reached using sputum smears and clinical information.

Case report

The potential usefulness of sputum cytology in the conclusive diagnosis of methotrexate-associated lymphoproliferative disorders: A case report

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Abstract

Background: Methotrexate (MTX) has been used as an anchor drug for the treatment of rheumatoid arthritis (RA) and is considered to be a cause of MTX-associated lymphoproliferative disorder (MTX-LPD). Spontaneous regression can occur after withdrawal of MTX and may be associated with Epstein-Barr virus (EBV) positivity and non-diffuse large B cell lymphoma (non-DLBCL) histological type. MTX-LPDs are often diagnosed pathologically by lung biopsy. To the best of our knowledge, there have been no studies on the cytological diagnosis of MTX-LPD using sputum smears.

Case: A 70-year-old man, who was diagnosed with RA 13 years previously and who had been treated with MTX, presented shortness of breath and productive cough. MTX-LPD was suspected as the sputum cytology showed many atypical lymphoid cells with hyperchromatic enlarged nuclei, foamy cytoplasm and distinct nucleoli. Chest computed tomography revealed multiple nodular shadows with interstitial pneumonia in the bilateral lower lung field. A lung biopsy specimen contained atypical lymphoid cells that were immunohistochemically-positive for CD20 and MUM-1, and weakly positive for bcl-6, but negative for CD3 and CD10. ISH-EBER showed no EBV-infectious lymphoid cells. He was finally diagnosed with

MTX-LPD (Non-germinal center B-cell-like DLBCL histological type). Most of the nodules disappeared spontaneously following the withdrawal of MTX.

Discussion and Conclusion: A cytologically conclusive diagnosis of MTX-LPD may be reached using sputum smears and clinical information.

Key words: MTX-associated lymphoproliferative disorders (MTX-LPD), sputum

Background:

MTX has been used as an anchor drug for the treatment of RA and is considered to be a cause of MTX-LPD. The spontaneous regression of MTX-LPD can occur after the withdrawal of MTX and may be associated with EBV positivity and the non-DLBCL histological type [1]. Although the exact incidence is unknown, it is reported that 0.8% of RA patients develop LPD (RA-LPD) and approximately 80% of RA-LPD patients show a close association with MTX [2]. MTX-LPDs are often diagnosed pathologically using lung biopsy specimens. To the best of our knowledge, no studied have been reported on the cytological diagnosis of MTX-LPD using sputum smears.

Case:

A 70-year-old man, who had been diagnosed with RA 13 years previously and who have been treated with 6mg MTX per week and 1.5mg tacrolimus per day, presented shortness of breath and productive cough. Before the presentation, RA was controlled well without any symptoms such as arthritis, which could mean that activity of the disease was low. There were no other clinical symptoms such as fever up. A blood test showed high CRP

(1.32 mg/dl), LD (269 U/l) and sIL-2R (1860 U/ml) levels. EBV antibody tests confirmed prior infection. Other results of blood test were as follows: RBC (3910000 /μl), Hb (13.5 g/dl), WBC (6300 /μl), AST (14 U/l), ALT (12 U/l), BUN (15 mg/dl), Cr (0.63 mg/dl). Chest computed tomography revealed multiple nodular shadows with interstitial pneumonia in the bilateral lower lung fields (Figure 1a, left). Since infectious pneumonia was suspected, sputum cytology was performed. Macroscopically the sputum was colorless and showed no atypical findings. Necrotic debris was observed in the background of the sputum cytology (Figure 1b). The sputum cytology showed many atypical lymphoid cells with low cell connectivity, a high nuclear to cytoplasmic (N/C) ratio, foamy cytoplasm, coarsely granular chromatin, and distinct nucleoli (Figure 1 b,c). Cytomorphologically, reactive lymphoid hyperplasia, lymphoproliferative disorder, adenocarcinoma and neuroendocrine tumor were considered as the differential diagnoses. Bronchoscopy showed protuberating mucosa with vascular proliferation and bleeding tendency. Lung biopsy revealed atypical mid- to large-sized lymphoid cells with a high N/C ratio, cleaved nuclei and distinct nucleoli (Figure 1d). Immunohistochemical staining of the lung biopsy specimen showed that the atypical lymphoid cells were positive for CD20 (Figure 2a) and MUM-1 (Figure 2b), and weakly positive for bcl-6, but negative for CD3 (Figure 2c) and CD10. ISH-EBER showed no EBV-

infectious lymphoid cells (Figure 2d). The patient was finally diagnosed with MTX-LPD (Non-germinal center B-cell-like DLBCL histological type). Most of the nodules disappeared spontaneously about 3 weeks after the withdrawal of MTX (Figure 1 a, right); thereafter, only tacrolimus was used. Total follow-up period after MTX withdrawal was 15 months and there is no progression of the disease.

Discussion:

In the World Health Organization (WHO) classification of lymphoid neoplasms, MTX-LPDs are categorized as other iatrogenic immunodeficiency-associated lymphoproliferative disorder (OIIA-LPD). Forty percent of MTX-LPD patients are EBV-positive. Ichikawa et al. reported that approximately 40% of MTX-LPDs occurred at extranodal sites and approximately 19% occurred in the lung. In approximately 83% of RA-LPD patients, the condition was associated with MTX [3]. Approximately 55% of MTX-LPD patients were diagnosed with the DLBCL histological type. Spontaneous regression of the lesions occurred in approximately 60% of MTX-LPD patients after the withdrawal of MTX, while progression after MTX withdrawal was observed in approximately 40% of MTX-LPD

patients. Spontaneous regression following MTX withdrawal has been associated with EBV positivity and a non-DLBCL histological type of LPD, whereas age >70 years and the DLBCL histological type are associated with shorter survival. In this case, it is interesting that spontaneous regression occurred after the withdrawal of MTX, considering that the patient was 70 years of age, was diagnosed with MTX-LPD (DLBCL histological type), and was EBV-negative. No recurrence was reported.

Sensitivity of sputum cytology is highest for detecting squamous cell carcinoma and malignant lymphoma of lung is usually reported on bronchoscopic specimen such as bronchial brushings and washings [4]. Moreover, to the best of our knowledge, there are few reports of malignant lymphoma diagnosed by sputum cytology. Although the exact rate is not clear, frequency of malignant lymphoma diagnosed by sputum cytology is probably low.

The cytological, histological and immunohistochemical findings of MTX-LPD are similar to those of malignant lymphoma in non-immunosuppressed hosts; thus, the diagnosis of the entity is difficult with cytology alone, and clinical information is necessary. In this case, with low cell cohesiveness, the atypical cells in the sputum smear were considered to be lymphocytes. Although reactive lymphoid hyperplasia was a differential diagnosis, neoplastic

LPD was suspected based on the monotonous proliferation and high nuclear atypia of the cells, and the necrotic background. Cell size and morphology are important criteria in cytological diagnosis of malignant lymphoma. Considering size of atypical cells in sputum specimen in this case, marginal zone lymphoma (MALT lymphoma), mantle cell lymphoma and follicular lymphoma are included in differential diagnosis of histological type of MTX-LPD. On the other hand, the most frequent type of MTX-LPD is DLBCL and that of MALT lymphoma, mantle cell lymphoma and follicular lymphoma is very low, therefore, DLBCL is also differential diagnosis. In our case, sputum cytology reported possibility of MTX-LPD and suggested necessity of lung biopsy which led to diagnosis as DLBCL type. In addition to our case, there have been some previous case reports of patients diagnosed with malignant lymphoma. These patients were diagnosed with Hodgkin lymphoma [5] or Adult T-cell leukemia/ lymphoma [6] based on sputum test and immunocytochemistry. Therefore, our case indicates that the diagnosis of MTX-LPD based on sputum cytology is possible.

Conclusion:

We reported that cytological diagnosis of MTX-LPD was achieved using a sputum smear with clinical information. The early detection of MTX-LPD is considered to be a prognostic factor. Thus, simple and rapid sputum cytology is a useful tool for diagnosing MTX-LPD occurring in the lung. This report shows the potential application of sputum cytology in making a conclusive diagnosis of MTX-LPD without invasive lung biopsy.

List of Abbreviations used

MTX, methotrexate; RA, rheumatoid arthritis; LPD, lymphoproliferative disorders EBV, Epstein-Barr virus; DLBCL, diffuse large B cell lymphoma; N/C, nuclear to cytoplasmic; WHO, World Health Organization; OIIA-LPD, other iatrogenic immunodeficiency-associated lymphoproliferative disorder.

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Ethics approval and consent to participate

Not applicable.

Consent for Publication

Written informed consent for the publication of this case report and any accompanying images was obtained from the patient and his family on admission.

Availability of Data and Materials

The dataset supporting the findings and conclusions of this case report is included within the article.

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Conflicts of Interests

The authors declare that there is no conflict of interest in association with the present study.

Author contributions

SM, KM and SY participated in the conception of the idea and writing of the manuscript. SM, KM, MY, HM and SY performed the clinical investigation and pathological/immunohistochemical examination. All authors have read and approved the final manuscript.

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Figure 1.

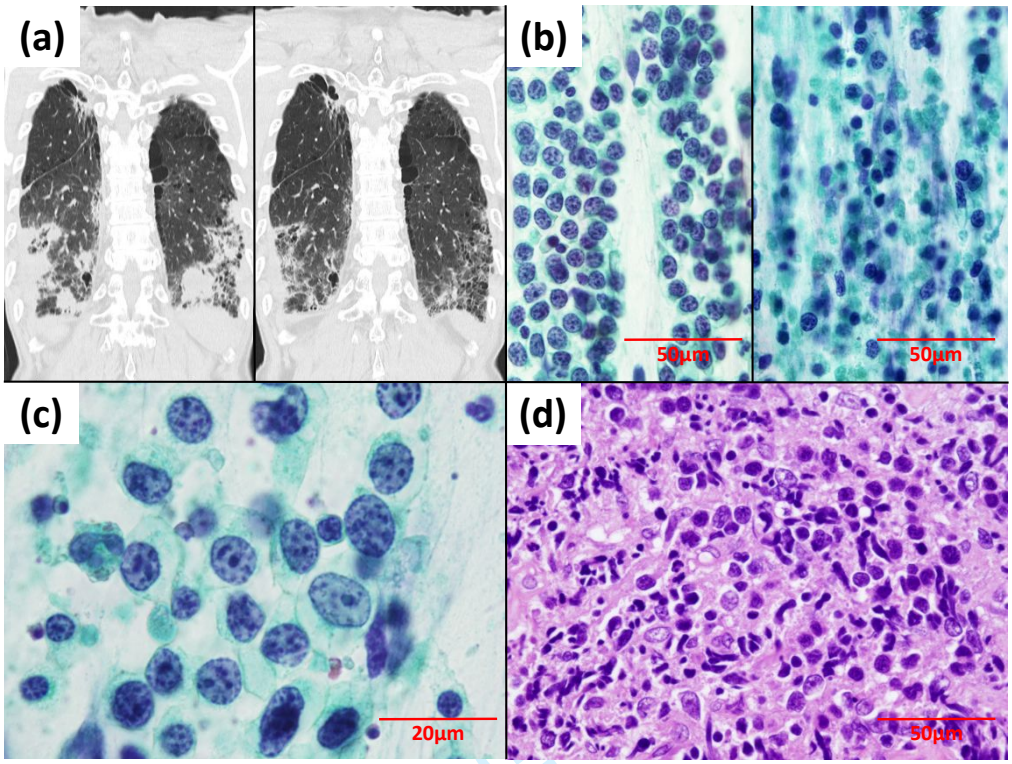

Figure 2.

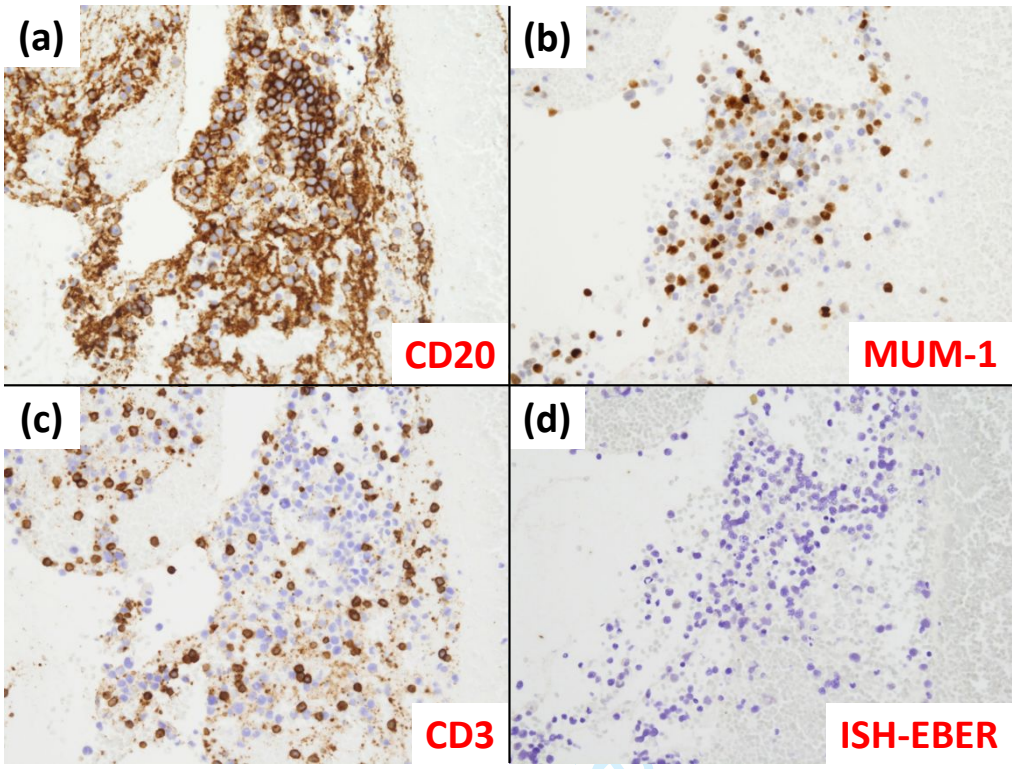

Figure Legends

Figure 1. Computed tomography, the cytological findings of the sputum, and the histological findings of the lung biopsy specimen.

(a) Computed tomography on admission showed multiple nodular shadows in the bilateral lower lung field (left). Most of the nodular shadows disappeared spontaneously after the withdrawal of MTX (right).

(b) A sputum smear on admission showed many atypical small cells with low cell cohesion (left). Necrotic debris was observed in the background of the sputum cytology (right) (Papanicolau staining; original magnification, ×400).

(c) A high-power view of the sputum revealed that the atypical cells had a high N/C ratio, foamy cytoplasm, increased chromatin and distinct nucleoli. (Papanicolau staining; original magnification, ×1,000).

(d) Lung biopsy after sputum cytology revealed atypical lymphoid cells with high N/C ratio, cleaved nuclei and distinct nucleoli (HE staining) (original magnification: ×400).

Figure 2. Immunohistochemical staining of a lung biopsy specimen showed that the atypical lymphoid cells were positive for CD20 (a) and MUM-1 (b), but negative for CD3 (c). ISH-EBER (d) was negative (original magnification: ×200).

(1) Ethics approval

Our institution does not require ethical approval for reporting individual cases or case series.

(2) Informed consent

Written informed consent was obtained from the patient for their anonymized information to be published in this article.