Title of the paper

# Methotrexate-Associated Lymphoproliferative Disorder with Multiple Pulmonary Nodules and Bilateral Cervical Lymphadenopathy

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## Abstract

As has been well recognized, methotrexate (MTX) leads to a state of immunosuppression and can provide a basis for the development of lymphoproliferative disorders (LPDs). MTX-associated LPDs can affect nodal sites as well as extranodal sites, though the manifestation of an LPD in the form of multiple pulmonary nodules is rare. Here, we report two cases of MTX-associated LPD with multiple bilateral pulmonary nodules, which was a finding suggestive of lung cancer, and bilateral cervical lymphadenopathy. After withdrawal of MTX, the multiple bilateral pulmonary nodules and bilateral cervical lymphadenopathy disappeared without chemotherapy in both cases. From these results, patients with pulmonary nodules and cervical lymphadenopathy should be examined for head and neck malignant tumors. Also, physicians should carefully check the administration of MTX. In patients with an MTX-associated LPD, we

need to make an early diagnosis and consider discontinuing the administration of MTX as soon as possible.

Key words:

**Methotrexate; MTX-associated LPD; Pulmonary Nodules**

## Introduction

Methotrexate (MTX) is an effective immunosuppressive drug that is widely administered to patients with autoimmune diseases including rheumatoid arthritis (RA). In 1991, reported for the first time, a patient with RA who was taking low-dose weekly MTX developed lymphoma [1]. These days, there are a lot of reports that MTX leads to a state of immunosuppression and can provide a basis for the development of lymphoproliferative disorders (LPDs)[2-6]. MTX-associated LPDs can affect nodal sites as well as extranodal sites. Forty to fifty percent of the patients with MTX-associated LPDs have extranodal sites[7]. The skin, lungs, gastrointestinal tract, spleen, oral cavity, and kidneys may be involved as the extranodal LPD sites [3]. In the head and neck regions, the palatine tonsil, epipharynx, gingiva, tongue, salivary gland, thyroid gland, and nasal cavity have also been reported as the extranodal LPD sites [4, 5, 8].

The manifestation of an LPD in the form of multiple pulmonary nodules is rare. Here, we report two cases of MTX-associated LPD with bilateral cervical lymphadenopathy and multiple bilateral pulmonary nodules suggestive of lung cancer.

## Case report

Case 1

Before being referred to our hospital, a 67-year-old male had complained of bilateral parotid swelling for one week. He had a medical history of RA, diabetes, hypertension, stenosis of the left internal carotid artery, and right Bell's palsy. He had been receiving MTX (14 mg/week for 10 years), prednisolone (PSL), folic acid, and cilostazol. He did not have a history of smoking. He did not present with fever. A physical examination revealed superficial lymphadenopathy in the bilateral neck region, submandibular adenopathy, and parotid gland enlargement. He had no bilateral facial nerve palsy.

Laboratory data showed an LDH of 209 IU/L (within the normal range). The level of soluble interleukin-2 receptor was elevated at 1,180 U/mL (normal: 127–582 U/mL), and the C-reactive protein level was also elevated at 2.0 mg (normal: <0.3 mg). The serum anti-SSA and anti-SSB antibodies were negative.

Computed tomography (CT) demonstrated multiple bilateral

pulmonary nodules (0.6–2.7 cm diameter), which was a finding suggestive of lung cancer (Fig. 1A), mediastinal lymphadenopathy, and left hilar adenopathy. Contrast-enhanced neck magnetic resonance imaging (MRI) demonstrated multiple nodules of the bilateral submandibular gland and parotid gland, and bilateral cervical lymphadenopathy (Fig. 1B).

A fine needle aspiration was performed on nodules of the left submandibular gland and left parotid gland. The cytological specimen showed Class II. For a definite diagnosis, the right submandibular gland was removed under general anesthesia. MTX was discontinued before surgery. The bilateral superficial lymphadenopathy, submandibular adenopathy, and parotid gland enlargement improved rapidly after the cessation of the drug. A pathological examination showed the possibility of subsidence of necrotizing lesions after the withdrawal of the drug, and it also confirmed infiltrating foam cells and small lymphoid cells around the necrotizing lesions.

Five months after the surgery, CT demonstrated that the multiple bilateral pulmonary nodules, mediastinal lymphadenopathy, and left hilar adenopathy disappeared.

Case 2

Before being referred to our hospital, a 74-year-old female had complained of right pharyngeal discomfort including mild pain, which got worse during swallowing, for two months. She had a medical history of RA and osteoporosis. She had been receiving MTX (14 mg/week for 10 years), folic acid, penicillamine, sodium risedronate hydrate, and vitamin B complex. She did not have a history of smoking. She did not present with fever and fatigue. In nasal and pharyngo-laryngeal examinations with a flexible endoscope, there was an ulcerated elevated lesion with a white mass at the right posterior part of the tongue (Fig. 2A). A biopsy was performed in this region.

Laboratory data showed an LDH of 208 IU/L and a soluble interleukin-2 receptor level of 449 U/mL, both within the normal range. The C-reactive protein level was elevated at 1.7 mg (normal: <0.3 mg). Contrast-enhanced CT demonstrated multiple bilateral pulmonary nodules (0.4–2.6 cm diameter) and bilateral cervical lymphadenopathy (Fig. 2B, C).

The results of a pathological examination of the ulcerated elevated

lesion showed granulation tissue. For a definite diagnosis, the right lymph node was removed under local anesthesia. In a pathological examination, there was a collapsed basic structure of the lymph node with medium-sized atypical lymphoid cells (Fig. 3A). In situ hybridization of an Epstein-Barr virus-encoded small RNA (EBER) revealed that the atypical lymphoid cells were positive for EBER (Fig. 3B). A definitive diagnosis of MTX-associated LPD was established.

One month after withdrawal of the drug after the lymph node biopsy, CT demonstrated that the multiple bilateral pulmonary nodules and bilateral cervical lymphadenopathy had disappeared.

## Discussion

MTX-associated LPDs are categorized as either iatrogenic or immunodeficiency-associated diseases in the recent World Health Organization (WHO) classification of lymphoid neoplasms[7]. MTX-associated LPDs are defined as lymphoma or lymphomatoid lesions identified in an MTX-induced immunosuppressed state. Tokuhira, et al. found that there were three patterns of LPD development[6]. In the first

group, MTX-Regressive-LPDs, LPD regression occurred after withdrawal of MTX. In the second, MTX-Persistent-LPDs, LPD persisted after MTX withdrawal. In the last, Other-Mediated-LPDs, LPD developed only after MTX. Our two cases fit MTX-Regressive-LPDs.

Although the mechanism of onset is unknown, the combination of immunodeficiency as a result of RA and the immunosuppressive effect of MTX has been implicated in the pathogenesis of MTX-LPD. In RA, self-reactive T cells of a specific clone stimulate B cells that produce autoantibodies, such as rheumatoid factor. When MTX is administered, its immunosuppressive effect reactivates viral infections, including subclinical infections, and it is hypothesized that this triggers clonal proliferation of cells[9].

In particular, EBV is considered to be an important viral infection associated with LPD that develops in the context of rheumatological diseases. Activation of EBV is identified in the majority of MTX-LPD cases[5], including Case 2. Many reports have indicated that EBV contributes to the development of MTX-associated LPDs and that MTX-associated LPDs regress spontaneously after withdrawal of MTX[1, 2, 4, 7].

There was an EBV infection in the LPD in Case 2. EBV infections were observed in 37.7% of cases (40 of 106 cases)[7]. LPDs with spontaneous regression had a higher EBV positivity than LPDs without regression [10]. After the withdrawal of MTX, our both patients had a smooth remission fortunately, although there was no EBV infection in Case 1. If the withdrawal of MTX had not achieved remission of the LPD, chemotherapy like R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) would be needed [11].

The histologic patterns of the MTX-associated LPDs were heterogeneous and identical to several types of lymphomas. Mariette et al. reported 18 types of non-Hodgkin lymphoma (NHL) (16 B-cell NHLs and 2 T-cell NHLs) and 7 types of Hodgkin disease (HD) in 25 MTX-associated LPDs [5]. In Japan, Hoshida et al. reported 42 NHL types (38 B-cell, 3 T-cell, and 1 natural killer cell) and 6 HD types in 48 MTX-associated LPDs [4]. B-cell NHL and HD make up the majority of the histologic patterns of MTX-associated LPDs. Our cases are not compatible with a particular histological type of lymphoma. After the withdrawal of MTX, we did a biopsy in both cases, and the MTX-associated LPDs may have regressed

spontaneously. It is difficult to distinguish MTX-Regressive-LPDs from MTX-Persistent-LPDs in advance. For the choice of chemotherapy of MTX-Persistent-LPDs, we believe tissue examination is necessary. If LPD regression occurred after withdrawal of MTX, it is difficult to diagnose LPD, and that is why tissue examination should be done before cessation of MTX, if it is possible. The decision regarding operation was done 10 days before the day of the operation in Case 1. For preventing further deterioration, we decided to perform the operation after withdrawal of MTX.

The reported median age at diagnosis of an MTX-associated LPD is 60–70 years, and there is a female preponderance in MTX-associated LPDs [4, 5]. The mean duration of MTX treatment is over four years in the reported cases. However, there is no association between the onset of an MTX-associated LPD and the method of administration of MTX (dose and/or duration). A patient could develop an MTX-associated LPD a few months after starting to use MTX [4, 5].

Image findings of pulmonary lesions of MTX-LPD are nodular density, infiltrative shadow, ground-glass pattern, and so on about CT scan. These are non-specific findings, and that is why it is necessary to make a

final differential diagnosis by counting laboratory examinations such as tumor marker, pathological findings, and image findings of other body parts. In Case 2, it is unconventional way of progress for lung cancer to find no mediastinal lymphadenopathy and hilar adenopathy, but cervical lymphadenopathy.

To the best of our knowledge, only 14 cases of MTX-associated LPD with multiple pulmonary nodules have been reported in the English literature. Table 1 summarizes the clinicopathological features of these cases. The mean age ± standard deviation of the disease is 62.8 ± 11.9 years old (Table 1). The median MTX intake duration was 5 years (range: several weeks to 17 years). The patients exhibited involvement of the spleen (4/14), liver (2/14), ileum (1/14), skin (1/14), central nervous system (1/14), adrenal gland (1/14), retromolar triangle (1/14), kidney (1/14), and retroperitoneal lesions (1/14). The histologic patterns are diffuse large B-cell lymphoma (5/14), lymphomatoid granulomatosis (LYG) (4/14), extranodal NK/T-cell lymphoma (1/14), CD8-positive T-cell LPD (1/14), and non-classifiable patterns (3/14). LYG (B-cell derived lymphoproliferative disease) is rare; however, it often presents as pulmonary nodular lesions with a

histopathology of lymphatic invasion of the vascular wall [12]. Its development is thought to be associated with the immunosuppressive state [13]. LYG is a less-aggressive LPD, and regression of LYG following the discontinuation of MTX has been reported [12-14]. The only reported fatal case was an RA patient who was receiving MTX therapy and developed both LYG and interstitial pneumonia with the pathological features of diffuse alveolar damage [15]. Aside from this fatal case, the patients in the other 13 cases were alive after the cessation of MTX. Chemotherapy was needed in three cases, and an upper lobectomy was needed in two cases as additional therapy.

The multiple nodules demonstrated in the chest CT suggest multiple metastatic cancers or alveolar cell carcinoma in general. Another differential diagnosis issue that should be considered is the possibility of organizing pneumonia (OP), either primary or secondary to MTX or other disease-modifying antirheumatic drugs (DMARDs) [3]. Although all manifestations and pathological results point to MTX-associated LPDs, we should remember the possibility of OP in the subsequent follow-up on a patient after the regression of lung nodules. It is very important to carefully check

for the presence of an MTX-associated LPD in a patient who has been receiving MTX, has lesions like cervical lymphadenopathy in the head and neck regions, and also has lung tumors suspected to have metastatic lung cancer, before considering an aggressive treatment.

## Conclusion

We experienced two cases of MTX-associated LPD with multiple bilateral pulmonary nodules and bilateral cervical lymphadenopathy. Head and neck malignant tumors and/or lung cancer was suspected in the patients. From this experience, even though the manifestation of an MTX-associated LPD in the form of multiple pulmonary nodules is rare, physicians should carefully check for the presence of an MTX-associated LPD.

## Disclosure Statement

The authors declare that they have no conflict of interest.

## Figure legend

Fig. 1A: Chest CT at the initial visit (black arrows, multiple nodular lung lesions).

Fig. 1B: Contrast-enhanced neck MRI at the initial visit (black arrows, ring-enhancing lesion of the bilateral submandibular gland; white arrows, bilateral cervical lymphadenopathy).

Fig. 2A: Endoscopic finding in the pharynx at the initial visit (black arrow, ulcerated elevated lesion with a white mass at the right posterior part of the tongue).

Fig. 2B, C: Contrast-enhanced neck-chest CT at the initial visit (black arrows, bilateral cervical lymphadenopathy; white arrows, multiple nodular lung lesions).

Fig. 3A: Microscopic findings. Histopathology of a biopsied lymph node showing a collapsed basic structure of the lymph node with medium-sized atypical lymphoid cells (hematoxylin and eosin staining).

Fig. 3B: In situ hybridization findings. An Epstein-Barr virus-encoded small

RNA (EBER) revealed that atypical lymphoid cells were positive for EBER.

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