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Effectiveness of Profiling Serum IL-18 and Neopterin in Diagnosis of Adult-Onset Still's Disease Complicated by Pulmonary Tuberculosis: A Case Report

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Adult-onset Still's Disease (AOSD) is a systemic inflammatory disorder characterized by high fever, skin rashes, and joint pains, and is extremely rare in patients over 80 years of age. An 88-year-old woman was admitted with high fever lasting for > 2 weeks and arthritis of the right knee and bilateral wrists. Further examination revealed that the patient fulfilled the Yamaguchi criteria, the most sensitive and extensively used classification criteria for AOSD. After ruling out other causes and considering a greatly raised serum interleukin-18 (IL-18) level, the patient was diagnosed with AOSD. Before prednisolone therapy, active tuberculosis was excluded using chest computed tomography (CT) and an interferon-gamma release assay (IGRA). After starting the treatment, serum levels of IL-18 and acute-phase reactants were decreased gradually. However, during prednisolone tapering, fever relapsed along with increasing serum acute phase reactant levels. Her serum IL-18 level was decreased but remained at a high level, and the neopterin level was further increased. These findings suggested the onset of another disease, but not AOSD recurrence. A chest CT scan revealed new lung infiltrates. Despite the initial negative IGRA result, cultures and polymerase chain reaction tests of bronchoalveolar lavage and sputum were positive for *Mycobacterium tuberculosis*. She was placed on a 9-month course of anti-tuberculosis therapy and continued prednisolone tapering. She showed steady improvement and her cytokine profile showed a decrease in the IL-18 and neopterin levels. In conclusion, cytokine profiling is useful in making the diagnosis of AOSD and subsequent pulmonary tuberculosis developed during steroid therapy.

Keywords: adult-onset Still's disease; cytokine profiling; interleukin-18; neopterin; tuberculosis
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Introduction

Adult-onset Still's disease (AOSD) is the adult presentation of systemic juvenile idiopathic arthritis (s-JIA). Patients reported repeated fever, a salmon-colored rash, and certain phenotypic characteristics including polyarthritis every day. Due to the lack of tools for definitive diagnosis, AOSD is diagnosed by excluding a wide variety of diseases with similar clinical manifestations. Among the several sets of diagnostic criteria that have been developed to facilitate the diagnosis of AOSD, the Yamaguchi criteria (Yamaguchi et al. 1992) are regarded as the most sensitive set and are therefore used extensively (Masson et al. 1996). The Yamaguchi criteria require the exclusion of other diseases including infectious diseases such as rickettsia and those caused by viruses, collagen diseases such as systemic vasculitis, and malignant tumors such as malignant lymphoma. In Japan, the mean age of AOSD onset is 46 years (Asanuma et al. 2015), and the onset of AOSD has been reported rarely in patients older than 80 years of age (Asanuma et al. 2015). Therefore, the diagnosis of AOSD in elderly patients requires the exclusion of other diseases such as malignancies and auto-inflammatory diseases, and failure to do so renders the definite diagnosis of AOSD very difficult.

Although used widely, the Yamaguchi criteria are lim-

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ited to the clinical manifestations of AOSD and do not reflect the underlying disease mechanism. The main pathogenesis of AOSD involves the activation of macrophages and T cells and the overproduction of cytokines; among the cytokines, serum concentrations of interleukin (IL)-18 are highly elevated (Yamaguchi et al. 1992; Shimizu et al. 2010). IL-18 is produced by activated macrophages and induces other inflammatory cytokines such as interferon-γ and tumor necrosis factor (TNF)-α (Efthimiou et al. 2006). As the pathogenesis of AOSD involves an auto-inflammatory process, measurement and profiling of serum cytokine concentrations are proposed to be useful in AOSD diagnosis (Shimizu et al. 2010).

Neopterin is produced by macrophages when they are stimulated by interferon-γ from activated T cells. Neopterin levels are known to be high in both pulmonary and extrapulmonary tuberculosis patients and decline after the start of treatment. A previous study reported the serum neopterin levels of 69.54 ± 29.42 nmol/L in active pulmonary tuberculosis cases before treatment (Turgut et al. 2006).

In this report, we present a rare case of AOSD with onset at an advanced age; the diagnosis of our patient was based on cytokine profiling. Importantly, during steroid treatment, the patient developed pulmonary tuberculosis, which was diagnosed based on the persistently elevated serum concentrations of neopterin.

Case Presentation

An 88-year-old woman with hypertension and spondylolisthesis presented with high fever lasting for over two weeks and arthritis of the right knee and bilateral wrists. During her physical examination on admission, the following vital signs were noted: temperature, 38.3°C; blood pressure, 101/57 mmHg; pulse rate, 81 beats/min; respiration rate, 14 breaths/min; and peripheral capillary oxygen saturation, 98% in room air. Arthritis of the right knee and bilateral wrists as well as pharyngitis was observed. While there was no lymphadenopathy, hepatosplenomegaly and rash were present. Her initial blood workup results were as follows: white blood cell count, 27,800/μL with 95.3% granulocytes; C-reactive protein, 28.42 mg/L; aspartate aminotransferase, 100 U/L; alanine aminotransferase, 94 U/L; lactate dehydrogenase, 328 U/L; and ferritin, 9,619 ng/ml (Table 1). The results of all serological tests to distinguish AOSD from other diseases mimicking AOSD were

Table 1. Laboratory data at the time of first admission.

Parameter	Recorded value
White blood cell count	27,800/μL
Neutrophils	95.3%
Hemoglobin	11.1 g/dL
Platelet count	32.9×10^3/μL
C-reactive protein	28.42 mg/L
Total protein	5.5 g/dL
Albumin	1.6 g/dL
Total bilirubin	1.0 mg/dL
Aspartate aminotransferase	100 U/L
Alanine aminotransferase	94 U/L
Lactate dehydrogenase	328 U/L
Blood urea nitrogen	21.0 mg/dL
Creatinine	0.76 mg/dL
Ferritin	9,619 ng/mL

Table 2. Laboratory test results for hepatitis and other potential infections causative agents.

Parameter	Recorded value	Standard value
T-SPOT. Tb	Negative	Negative
Antinuclear antibody	< 40	< 40
HHV-6 IgM	< 10	< 10
HHV-6 IgG	× 30.5	< 10
CMV-IgM	0.7	0-0.7
CMV-IgG	114.0	0-1.0
EBVCA-IgM	0.9	0-0.4
EBVCA-IgG	2.3	0-0.4
EBNA-IgG	3.7	0-0.4
RF	4 U/mL	0-15 U/mL
Anti-CCP antibody	Negative	Negative
MPO-ANCA	Negative	Negative
PR3-ANCA	Negative	Negative
IL-2 receptor	1,600 U/mL	135-483 U/mL

ANCA, antineutrophil cytoplasmic antibody; CCP, cyclic citrullinated peptide; CMV, cytomegalovirus; EBNA, Epstein-Barr nuclear antigen; EBVCA, Epstein-Barr virus capsid antigen; HHV-6, Human herpesvirus 6; Ig, immunoglobulin; IL, interleukin; MPO, myeloperoxidase; PR3, proteinase 3; RF, rheumatoid factor.

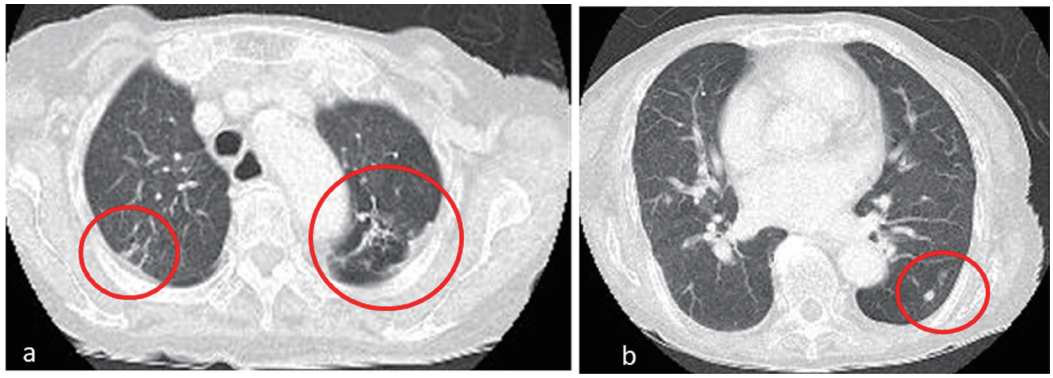

Fig. 1. Chest computed tomography images on admission.
Red circles show old inflammatory shadows of the lungs.

Table 3. Criteria for the diagnosis of adult-onset Still's disease.

Major criteria	1) Fever ≥ 39°C lasting ≥ 1 week, 2) Arthralgia or arthritis lasting ≥ 2 weeks, 3) Typical nonpruritic salmon-colored rash, 4) Leukocytosis ≥ 10,000/mm, with granulocytes ≥ 80%
Minor criteria	1) Sore throat, 2) Lymphadenopathy, 3) Splenomegaly, 4) Abnormal liver function tests, 5) Abnormal liver function tests, 6) Negative for antinuclear antibody and rheumatoid factor
Exclusion criteria	Infection, Malignancy, Other rheumatic disease (vasculitis)

Adult-onset Still's disease is diagnosed if five or more criteria are present, including two or more major criteria. No exclusion criteria are present.

negative (Table 2).

Chest computed tomography (CT) revealed old inflammatory shadows (Fig. 1). All cultures of blood, urine, and sputum were negative for bacterial growth. No abnormal lesions were found by gastric and colon fiberscopy. Skin biopsy revealed no signs of intravascular lymphoma. Bone marrow biopsy showed no evidence of hemophagocytic lymphohistiocytosis. The cultures for *Mycobacterium* and polymerase chain reaction (PCR) of sputum and bone marrow aspirate were both negative. There was no thickening of the vessel walls or angiostenosis of bilateral temporal arteries by ultrasonography. These results excluded infectious diseases, malignancies, and auto-inflammatory diseases. The patient met three major and two minor Yamaguchi criteria (Table 3), including fever over 39°C lasting for more than one week, arthritis lasting two weeks or longer, and leukocytosis (≥ 10,000 leukocytes/μL) with at least 80% granulocytes as the major criteria and sore throat and abnormal liver functions as the minor criteria. The serum concentrations of inflammatory cytokines were as follows (Table 4): IL-6, 25 pg/mL; IL-18, 43,500 pg/mL; neopterin, 21.2 nmol/L; soluble TNF receptor I (sTNF-RI), 8,200 pg/mL; and sTNF-RII, 14,500 pg/mL. Based on these results, the definitive diagnosis was AOSD.

Following the initiation of prednisolone at 45 mg/day (1 mg/kg/day), her serum levels of IL-18, ferritin, and acute phase reactants started to decline. Approximately 80 days after the initiation of prednisolone treatment, at which time the prednisolone dose was at 27.5 mg/day, the patient suffered a relapse of high fever with the elevated serum levels of acute phase reactants. The inflammatory cytokine profiling revealed an elevated serum neopterin level despite the slightly decreased serum IL-18 level (Table 4), suggesting the development of another disease. Chest CT revealed new, active infiltrations of the lungs (Fig. 2). Despite the negative result of interferon-gamma release assay (IGRA), PCR results of both bronchoalveolar lavage fluid and sputum were positive for *Mycobacterium tuberculosis*. Thus, the patient was diagnosed with pulmonary tuberculosis. Of note, the sputum culture for *M. tuberculosis* was later determined to be positive as well. While the prednisolone dose was tapered, the patient was initiated on of antituberculosis drugs: iscotin 200 mg/day, rifampicin 400 mg/day, and ethambutol 625 mg/day for two months, followed by iscotin 200 mg/day and rifampicin 400 mg/day for seven months. After nine months of treatment for pulmonary tuberculosis,

Table 4. Cytokine profile at admission, at the outbreak of *Mycobacterium tuberculosis*, and at 2 months after treatment for *M. tuberculosis*.

Parameter (Standard value)	Recorded value at hospitalization	Recorded value at the development of *M. tuberculosis*	Recorded value 2 months treatment for *M. tuberculosis*
Neopterin (≤ 5 nmol/L)	**21.2**	**77.7**	**38.0**
IL-6(≤ 5 pg/mL)	25	≤ 3	5
IL-18(≤ 500 pg/mL)	**43,500**	**14,100**	780
sTNF-RI(484-1,407 pg/mL)	8,200	4,000	2,620
sTNF-RII(829-2,262 pg/mL)	14,500	10,300	6,600
sTNF-RII/RI(< 5)	1.77	2.58	2.52

IL, interleukin; sTNF-R, soluble tumor necrosis factor receptor.

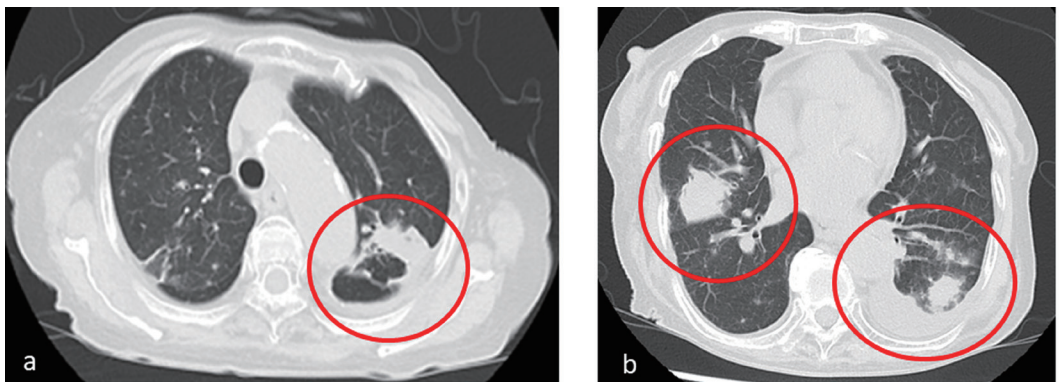

Fig. 2. Chest computed tomography images at the development of tuberculosis. Red circles show new, active infiltrations of the lungs.

the serum levels of neopterin, other cytokines, and acute phase reactants were decreased (Table 4), and no recurrence of tuberculosis was observed after the completion of treatment. Furthermore, two years after the initiation of prednisolone treatment, the management of AOSD in the current patient has been satisfactory with the current dose of 5 mg/day prednisolone.

Ethics approval and consent for this case report were waived. On the other hand, written informed consent was obtained from the patient for publication of this case report and accompanying images.

Discussion

We herein present a rare case of AOSD that developed in a patient with advanced age who exhibited the typical AOSD cytokine profile of highly elevated IL-18 levels that was complicated by pulmonary tuberculosis, which was considered to be induced by prednisolone treatment for AOSD. Two issues complicated her diagnosis and treatment. First, AOSD usually affects young and middle-aged individuals, and the onset of AOSD in individuals above 80 years of age is extremely rare (Asanuma et al. 2015). Second, following the tapering of prednisolone treatment for AOSD, the patient's fever relapsed along with an increase in serum acute phase reactant levels. However, owing to inflammatory cytokine profiling, at hospitalization, we could detect that her serum IL-18 level was extremely high, which mimicked the typical AOSD cytokine profile. Moreover, when fever relapsed, we also detected that the neopterin levels were not decreased along with the IL-18 level, indicating the possibility of another inflammatory disease. This assisted our diagnosis of AOSD and the identification of the development of pulmonary tuberculosis at an early stage. A recent large-scale study found that the immune response was dysregulated in s-JIA, the childhood counterpart of AOSD (Inoue et al. 2016). Indiscriminate activation of the antigen-presenting cells including macrophages and increased cytotoxic T cell response are proposed as the pathogenic mechanisms that underlie both s-JIA and AOSD; therefore, both disorders are hypothesized to define the same disease entity. The activation of these cellular immune responses is associated with increased production of several inflammatory cytokines. Specifically, the serum levels of IL-1, IL-6, and IL-18 are elevated in both AOSD and s-JIA (Shimizu et al. 2010). Therefore, the measurement of these cytokines may aid in the diagnosis. Cytokine profiling of serum levels of IL-6, IL-18, neopterin, sTNF-RI, and sTNF-RIIshows specific radar chart patterns which are useful in differentiating between s-JIA/AOSD, Epstein-Barr virus-induced hemophagocytic lymphoistiocytosis, Kawasaki disease and s-JIA with macrophage activation syndrome (Shimizu et al. 2010; Goda et al. 2020). sTNF-RI and sTNF-RII play an important role as modulators of the biological function of TNF-α (Shimizu et al. 2018). Further, it has been reported that a ratio of sTNF-RII/sTNF-RI of ≥ 5 is a characteristic of

AOSD or s-JIA with macrophage activation syndrome (Shimizu et al. 2018). Serum IL-18 levels are also increased in other diseases, including systemic lupus erythematosus; however, maximum serum IL-18 levels in this disease could be 10^3 pg/mL at highest (Wu et al. 2016). In contrast, s-JIA/AOSD is characterized by very high serum IL-18 levels of more than 10^4 pg/mL (Wu et al. 2016).

The advanced age of disease onset in the current patient hindered the definitive diagnosis of AOSD, and other diseases including malignancies such as malignant lymphoma and gastric cancer and giant cell arteritis had to be ruled out to avoid misdiagnosis. The negative test results for other diseases and the typical cytokine profiling with highly elevated serum IL-18 levels supported the AOSD diagnosis. Neopterin levels have been reported to elevate in AOSD and to decrease in parallel with IL-18 levels after treatment initiation in patients with AOSD (Shimizu et al. 2010). In the current case, after the start of AOSD treatment, serum IL-18 and acute phase reactants levels were decreased gradually, which indicated that the treatment was effective. Furthermore, the sTNF-RII/sTNF-RI ratio was found to be < 5, indicating that the increased neopterin levels were not due to macrophage activation syndrome (Shimizu et al. 2018). Only neopterin levels, which were previously reported to decrease along with IL-18 after the initiation of AOSD therapy (Goda et al. 2020), were persistently high. This situation suggested the presence of another inflammatory disease, which led to reassessments for potential comorbidities. Neopterin levels are known to be high in both pulmonary and extrapulmonary tuberculosis patients and decline after the start of treatment. A previous study reported serum neopterin levels of 69.54 ± 29.42 nmol/L in active pulmonary tuberculosis cases before treatment (Turgut et al. 2006). Another study reported serum levels of 18.6 ± 14.2 nmol/L (Ozdemir et al. 2006). In our case, on development of pulmonary tuberculosis, the serum neopterin level was increased from 21.2 nmol/L at hospitalization to 77.7 nmol/L. Owing to these facts and the new infiltration in the bilateral lungs, we eventually diagnosed the patient with pulmonary tuberculosis (induced by prednisolone administration for AOSD) because of the previous pulmonary tuberculosis revealed by CT. However, after the diagnosis of AOSD, *M. tuberculosis* was excluded by negative cultures and PCR of sputum and bone marrow aspirate as well as IGRA, which was negative even after the development of tuberculosis. The sensitivity and specificity of IGRA for the diagnosis of active tuberculosis are approximately 90% and > 95%, respectively (de Visser et al. 2015). Approximately 8%-19% of patients presenting with active tuberculosis have negative IGRA results (de Visser et al. 2015). Several risk factors are associated with negative IGRA results including immunodeficiency, young or advanced age, negative tuberculin skin test, extrapulmonary tuberculosis, disseminated tuberculosis, concomitant tuberculosis treatment, and smoking (de Visser et al. 2015). Tuberculosis has been reported to mimic AOSD (Sood et al. 2015). We confirmed that the patient was tuberculosis-free based on a negative IGRA result, the absence of active lung infiltrations, and negative cultures of sputum and bone marrow aspirate before the diagnosis of AOSD. However, the patient developed tuberculosis after the initiation of prednisolone. Therefore, a careful follow-up examination is necessary in patients with immunodeficiency and those with advanced age as some patients might have active tuberculosis despite a negative IGRA result. The current patient was responding well to the AOSD treatment, as evidenced by a reduction in the serum IL-18 levels; however, new active lung infiltrations emerged. Transient pulmonary infiltrates are observed in 30%-40% of patients with AOSD (Cheema and Quismorio 1999). In the absence of worsening AOSD despite the emergence of active infiltrations, other comorbidities such as tuberculosis should be considered. As illustrated in the current case, inflammatory cytokine profiling to determine elevated levels of serum neopterin together with a decline in the serum IL-18 levels is particularly beneficial for the diagnosis of pulmonary tuberculosis at an early disease stage.

In conclusion, we herein present a rare case of AOSD that developed in a patient at an advanced age complicated by pulmonary tuberculosis, which was considered to be induced by prednisolone treatment for AOSD. The typical cytokine profile showing extremely high serum IL-18 levels is beneficial as a new etiology-oriented diagnostic criterion for AOSD, and a negative IGRA result accompanied by high serum neopterin levels is useful in recognizing the pulmonary tuberculosis development during steroid therapy of AOSD.

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Author Contributions

M.K. managed the case and redaction and correction of the manuscript. T.K. assisted with redaction, correction, and reconstruction of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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