A new autoantibody to valyl transfer RNA synthetase associated with anti-synthetase syndrome

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Dear, Editor,

The aminoacyl tRNA synthetase (ARS) antibody is the most frequently detected myositis-specific autoantibody (MSA) in idiopathic inflammatory myopathy [1]. ARS antibody-positive patients frequently have myositis, as well as interstitial lung disease (ILD), polyarthritis, Raynaud's phenomenon, fever, and mechanic's hands. Together, these symptoms define "antisynthetase syndrome (ASSD)" [2]. ARS binds amino acids to tRNA in the presence of ATP and catalyzes the synthesis of aminoacyl-tRNA. In humans, there are 20 different types of ARSs, one for each amino acid. Eight of these tRNA synthetases have been reported as myositis-specific autoantigens [3], Jo-1 (histidyl), PL-7 (threonyl), PL-12 (alanyl), EJ (glycyl), OJ (isoleucyl), KS (asparaginyl), Zo (phenylalanyl), and Ha (tyrosyl), and are associated with the ASSD phenotype, with the latter two being the least commonly involved [4, 5]. In this study, we identified a new anti-ARS directed against valyl tRNA synthetase in a patient with typical features of ASSD.

A 43-year-old man was admitted to our hospital with occasional fever, myalgia, and arthralgia lasting 2 months. He also reported Raynaud's phenomenon. Physical examination revealed swollen fingers and weakness of the deltoid and quadriceps muscles. Laboratory studies revealed the following: lactate dehydrogenase (LDH), 732 U/L; creatine phosphokinase (CK), 2472 U/L; aldolase, 46.4 IU/L; C-reactive protein (CRP), 1.5 mg/dL; and Krebs von den Lungen (KL-6), 396 U/mL. Fluorescent antinuclear antibody (FANA) was also positive (titer 1:640, with a nucleolar and cytoplasmic pattern) but disease-specific autoantibodies were negative. Chest CT revealed ILD, with a pattern classified as a non-specific interstitial pneumonia. Fat-saturated T2-weighted MRI revealed diffuse high-intensity signals in the deltoid and iliopsoas muscles, and needle electromyography of these muscles revealed myogenic changes. A muscle biopsy of the left quadriceps femoris showed necrotic muscle fibers and endomysial infiltration of mononuclear cells, consistent with myositis. Based on these findings, the patient was clinically diagnosed with ASSD. CT also revealed a swollen mediastinal lymph node that was pathologically diagnosed as an undifferentiated carcinoma. Immunohistochemical analysis of the lymph node did not identify it as a lymphoma, sarcoma, or specific type of carcinoma. 18F-fluorodeoxyglucose PET did not reveal any lesions

other than the swollen mediastinal lymph node. Thus, the patient was diagnosed with ASSD coexisting with a cancer of an unknown origin.

The patient was treated for cancer of unknown origin with chemotherapy (carboplatin and paclitaxel) and concurrent radiotherapy. The CK value peaked at the beginning of chemotherapy and gradually decreased with cancer treatment. He died of cancer 8 months after first admission.

This case was consistent with ASSD, but the patients' serum did not show any known disease-specific autoantibodies. However, we found that the serum precipitated some autoantigens by RNA-immunoprecipitation (IP) and protein-IP. Analysis of RNA-IP from HeLa cell extracts with urea-10% polyacrylamide gel electrophoresis and silver staining showed a band corresponding to tRNA, which differed from those precipitated by previously reported ARS antibodies (figure 1 a). Analysis of protein-IP using proteins extracted from ^{35}S-labeled HeLa cells with sodium dodecyl sulfate-8 % polyacrylamide gel electrophoresis revealed a 140-kDa, different from those reported previously such as a melanoma differentiation-associated gene 5 antibody (figure 1 b). Among the 20 ARS enzymes, VRS was listed as a candidate antigen based on its molecular weight; therefore, we hypothesized that the novel band represented a VRS antibody. Western blot analysis showed that the protein precipitated by the patients' serum reacted with an antibody to valyl tRNA synthetase (VRS) (Santa Cruz Biotechnology, Texas, USA) (Supplementary Figure 1). Moreover, the serum also reacted with recombinant VRS protein synthesized by an *in vitro* transcription and translation system using a pET-21a (+) vector carrying VRS cDNA (figure 1 c). The patient's serum was therefore demonstrated to contain an anti-VRS antibody.

This case of ASSD coexisted with a cancer of an unknown origin. However, the association between ASSD and malignancy remains controversial. The presence of Jo-1 antibodies has been reported to be a protective factor against malignancy [6]. In contrast, a meta-analysis showed no association between ARS antibodies and malignancy [7]. Hamaguchi et al. reported that 12% of Japanese anti-ARS-positive patients had malignancies [8]. In that report, the types of cancer found in order of frequency were: colon, gastric, breast, lung, and others; however, there were no cancers with unknown origin. The association between VRS antibodies and malignancy remains unclear. However, considering that the CK levels decreased after cancer

treatment began, there is probably an association between myositis and malignancy.

Herein, we report the first case of ASSD with VRS antibodies. The frequency of such antibodies in ASSD and the association between their presence and malignancy remain unclear. It is important to identify more cases of ASSD presenting with VRS antibodies to elucidate clinical characteristics.

Key message:

Autoantibodies to valyl tRNA synthetase are novel antibodies found in ASSD.

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Figure legend

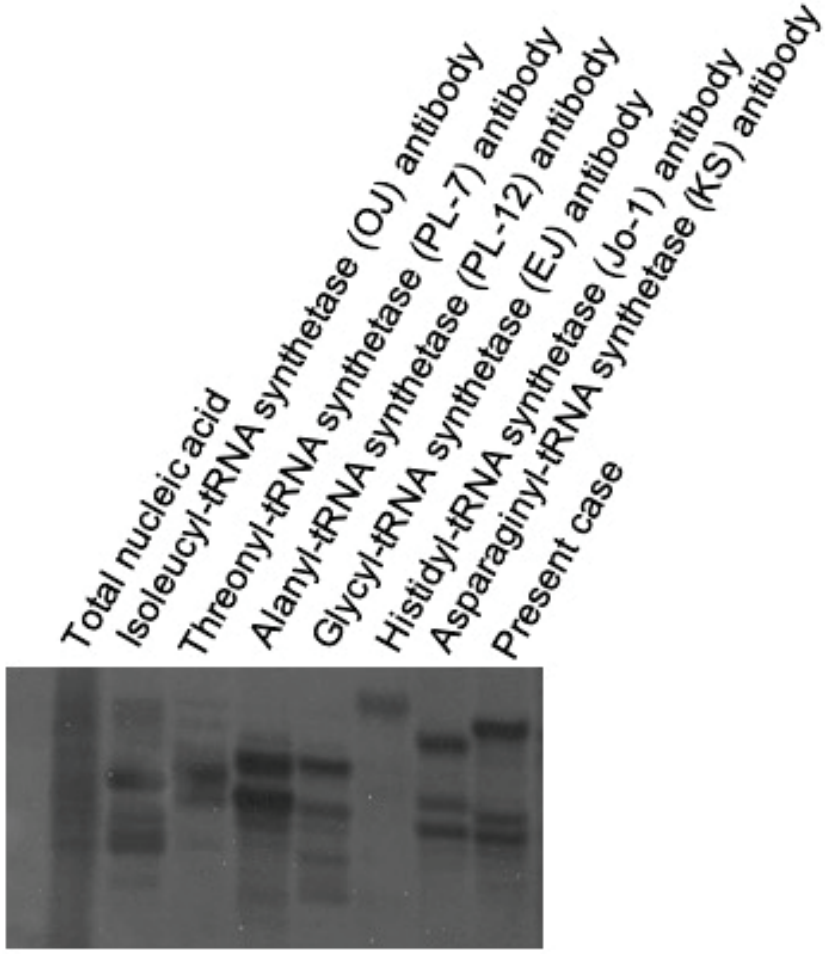
Figure 1. Detection of VRS antibodies
(a) Serum from the present case produced a band corresponding to tRNA different from those previously reported with tRNA synthetase antibodies, in RNA-immunoprecipitation.

(b) The present case's serum displayed a band with slightly higher molecular weight compared to 140 kDa (an anti-MDA5-positive patient) in protein immunoprecipitation.

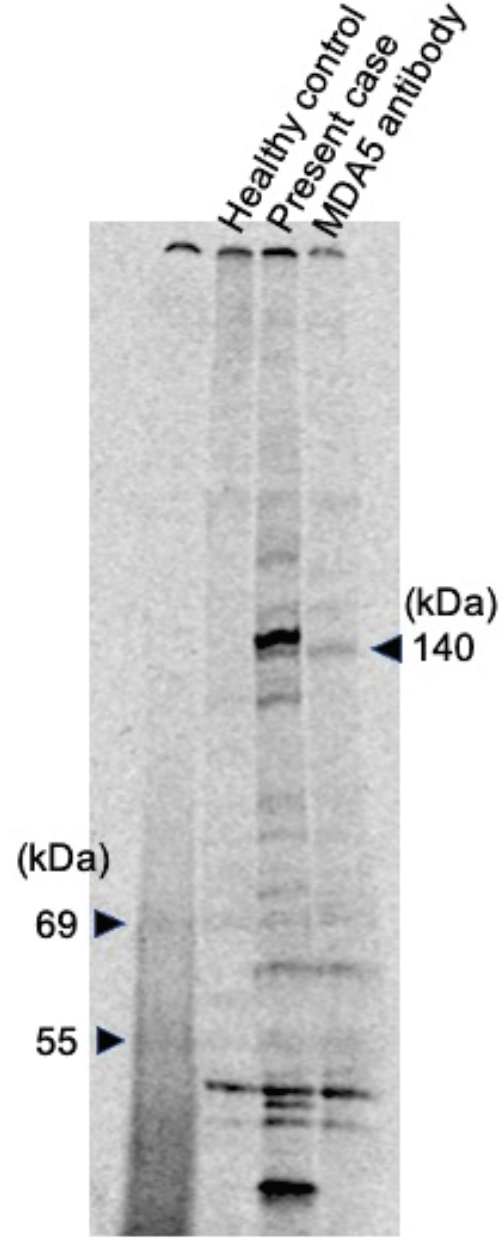
(c) Serum from the present case (lane 1), a healthy human (lane 2), and synthetic VRS protein without serum (lane 3) are shown. The lane 1 sample reacted to the VRS antigen.

MDA5, melanoma differentiation-associated gene 5; VRS, valyl-tRNA synthetase.

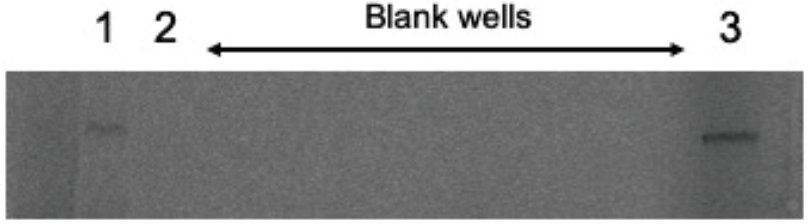
(a)


Total nucleic acid
Isoleucyl-tRNA synthetase (OJ) antibody
Threonyl-tRNA synthetase (PL-7) antibody
Alanyl-tRNA synthetase (PL-12) antibody
Glycyl-tRNA synthetase (EJ) antibody
Histidyl-tRNA synthetase (Jo-1) antibody
Asparaginyl-tRNA synthetase (KS) antibody
Present case

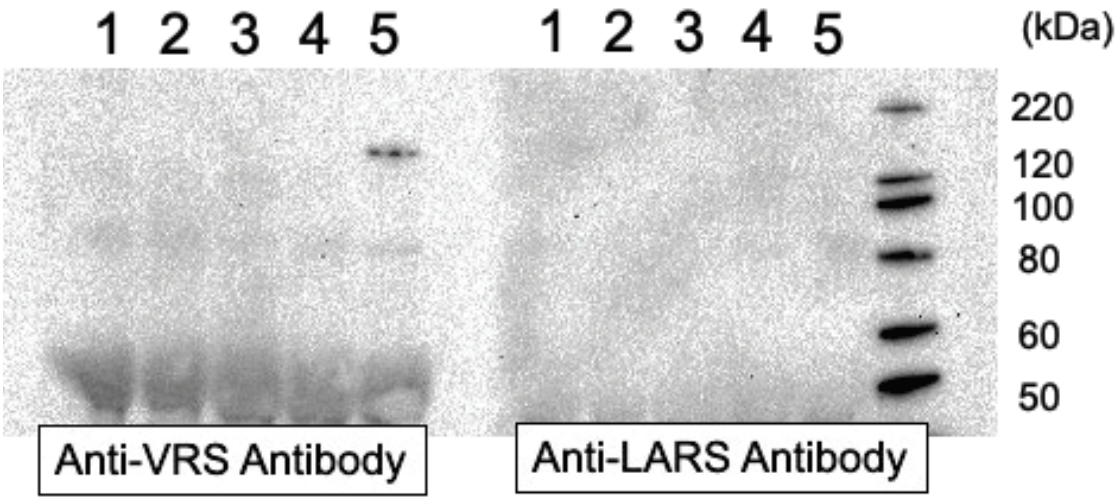
(b)


Healthy control
Present case
MDA5 antibody
(kDa)
140
(kDa)
69
55

(c)


1
2
Blank wells
3

Supplementary Figure 1. Detection of VRS antibodies in Western blot.



Western blot with HeLa cell extract immunoprecipitated with human sera probed with VRS and LARS antibodies (both purchased). Sera from healthy humans (lanes 1-4) and the present case (lane 5, reacting with anti-VRS antibody) are shown.
VRS, valyl-tRNA synthetase; LARS, leucyl-tRNA synthetase.