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Increased levels of platelet-associated immunoglobulin G in a patient with mixed connective tissue disease

混合結締體素病患者的血小板關聯免疫球蛋白G水平增加

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We report the case of a 71-year-old Japanese woman with mixed connective tissue disease and increased levels of platelet-associated immunoglobulin G. After administration of oral prednisolone, platelet-associated immunoglobulin G levels decreased with a simultaneous increase in the number of platelets, suggesting that the thrombocytopenia observed in this patient was mediated by immune mechanisms. This is the first reported case of increased platelet-associated immunoglobulin G levels in a patient with mixed connective tissue disease.

我們報告一名71歲日本婦女患上混合結締體素病，及血小板關聯免疫球蛋白G水平增加的病例。於口服脫氫皮質醇後，在血小板數目增加的同時，血小板關聯免疫球蛋白G的水平降低，顯示在該患者中觀察到的血小板減少是受到免疫機制的調節。這是在混合結締體素病患者中血小板關聯免疫球蛋白G的水平增加的首宗報告病例。

Introduction

Mixed connective tissue disease (MCTD) was first described by Sharp et al¹ in 1972. It is characterised by high titres of antibodies specific for ribonucleoproteins (anti-RNP Ab) and a combination of several defined connective tissue diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and polymyositis.

Several platelet membrane glycoproteins (GP), such as GPIIb/IIIa, GP Ib, and GP V, are known to act as target antigen sites for attachment of platelet-associated immunoglobulin G (PAIgG). Platelet-associated immunoglobulin G is elevated in idiopathic thrombocytopenic purpura (ITP), as well as in other disorders including autoimmune diseases (SLE, Hashimoto's disease), liver diseases (cirrhosis, chronic hepatitis), immunological bowel diseases (Crohn's disease, ulcerative colitis), and aplastic anaemia. Drug-induced thrombocytopenic purpura with increased PAIgG levels has been reported in association with quinidine and tranilast use.² In this case we report an increased level of PAIgG in a patient with MCTD.

Case report

A 71-year-old Japanese woman was admitted complaining of Raynaud's phenomenon, exertional dyspnoea, and thirst. Her family history was unremarkable. Physical examination revealed 'sausage-like', swollen fingers with multiple ulcers and sclerodactyly. There was no evident proximal scleroderma or muscle weakness, and no cardiac or respiratory abnormalities were detected on auscultation.

Serological examinations revealed elevated serum immunoglobulin G (19.0 g/L; normal range, 8.0-18.0 g/L), anti-nuclear antibody (1280 [speckled]), and autoantibody to the U1-70 Kd polypeptide (A and C) of ribonucleoprotein (RNP) [155 U/mL] levels. Immunoglobulin M levels (0.59 g/L; normal range,

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Scleroderma, systemic;
Sjogren's syndrome

關鍵詞：

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0.5-2.2 g/L), and the complement components C3 (0.3 g/L; normal range, 1.2-1.5 g/L), C4 (0.06 g/L; normal range, 0.35-0.60 g/L), and CH50 (<10 U/mL; normal range, 29-48 U/mL) were markedly reduced. Antibody testing for anti-DNA, anti-neutrophil cytoplasmic antibodies, perinuclear anti-neutrophil cytoplasmic antibodies, anti-Scl-70, anti-Sm, anti-mitochondrial, anti-SS-A, and anti-SS-B antibodies were all negative. Pancytopenia was observed—white blood cell count, $2.9 \times 10^9/L$ (normal range, $4.5-11.0 \times 10^9/L$); lymphocyte count, $1.5 \times 10^9/L$ (normal range, $1.0-4.8 \times 10^9/L$); red blood cell count, $3.6 \times 10^{12}/L$ (normal range, $3.9-5.5 \times 10^{12}/L$); haemoglobin level, 113 g/L (normal range, 120-150 g/L); haematocrit count, 0.33 (normal range, 0.35-0.45); platelet count, $49 \times 10^9/L$ (normal range, $150-450 \times 10^9/L$)—but bone marrow aspirates showed a normal number of megakaryocytes.

Chest X-ray and computed tomography indicated that pleural thickening and fibrosis were present. Pulmonary function (FVC [% predicted], 56.3; FEV1/FVC, 98.2%) revealed a restrictive lung disease pattern and mild hypoxia (PaO₂, 74.7 mm Hg; PaCO₂, 42.9 mm Hg). Proteinuria (100 mg/dL; normal level, <10 mg/dL) and microhaematuria (50 mg/dL; normal level, <15 mg/dL) were observed. Serum urea nitrogen (4.6 mmol/L) was normal and creatinine (44 mmol/L) was slightly reduced. Gam test (6 mL/15 min) and Schirmer test (5 mm/15 min) showed decreased secretion of the salivary glands. Left parotid glandography showed obstruction of several branches of ducts, and histology of a minor salivary gland (Fig 1) showed fibrotic changes in the interstitium, periductal infiltration of lymphocytes and plasma cells, and atrophy, consistent with Sjogren's syndrome. The patient was therefore diagnosed with MCTD.

Platelet-associated immunoglobulin G was measured by an enzyme-linked immunosorbent assay (ELISA). Briefly, platelet samples or control buffer was added into a 96-well plate coated with human IgG. Peroxidase-conjugated goat anti-human IgG F(ab')₂ was then added, and the plate was incubated for 2 hours at 37°C. Excess antibody was washed

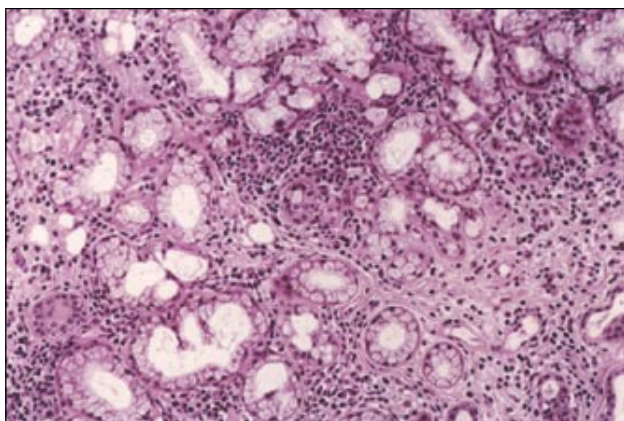


Fig 1. Histological findings in a small salivary gland showing fibrotic change in the interstitium, periductal infiltration by lymphocytes and plasma cells, and cellular atrophy (H&E, x200)

off, and the plate was incubated for an additional hour with orthophenylenediamine. Colour was developed with H₂SO₄, and chemiluminescence was quantified by an auto-reader (Sanko Chemicals, Tokyo, Japan). Platelet-associated immunoglobulin G was elevated to 203.5 ng/10⁷ platelets (normal range, 5.0-25.0 ng/10⁷ platelets) in this patient.

After the diagnosis, oral prednisolone (PSL) 40 mg/day was commenced and then subsequently decreased as the number of platelets increased; 45 days after the induction of PSL, the levels of PAIgG and anti-RNP Abs had recovered to within the normal range. The increase in the number of platelets suggests that the decrease in platelet count was due to, at least in part, increased PAIgG. Complement components C3, C4, and CH50 also returned to normal levels. This response to treatment is illustrated in Fig 2.

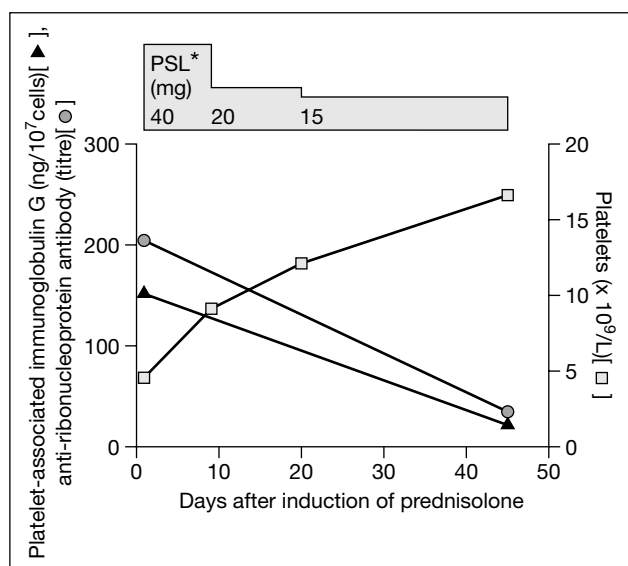
Discussion

This patient fulfilled the criteria for MCTD,³ with Raynaud's phenomenon, mixed findings of connective tissue disease (SLE-like findings and SSc-like findings), and an elevated level of anti-RNP Ab. However, whether MCTD is a defined disease entity or a transitional condition that ends in SLE or SSc is the subject of debate.

One reason for this controversy is that antibody to heterogeneous RNP has been reported in association with Raynaud's phenomenon and oesophageal dysmotility in SLE patients.⁴ It has also been reported that scleromatous manifestations are often unresponsive to corticosteroid treatment, suggesting evolution from a mixed clinical picture to that of predominantly SSc. Recently, Burdt et al⁵ reported findings of a long-term study indicating that the typical clinical, immunogenetic, and serological findings of MCTD did not evolve into SLE or SSc. Frandsen et al⁶ completed a longitudinal follow-up study of 84 patients with 'undifferentiated' connective tissue disease with high-titre antibody against U1RNP. They reported that 58 patients developed MCTD, four developed progressive systemic sclerosis, and two developed SLE.

Comparison of serum IgG, C-reactive protein, and cytokines (IFN- γ , IL-10, and TNF- α) in MCTD and SLE patients has demonstrated that MCTD shares some distinct immunological properties with SLE.⁷ However, patients with MCTD were reported to be more likely to have anti-A and anti-C U1snRNP than patients with SLE,⁸ as noted in the patient described here. It seems that decreased levels of complement, one of the diagnostic criteria for SLE,⁹ may be involved in the pathogenesis of this patient's condition, since C3 and C4 levels recovered after administration of PSL.

There have been no previous reports of PAIgG elevation in MCTD. Among autoimmune diseases, SLE is most commonly associated with elevated PAIgG, and the pathogenesis



*PSL prednisolone

Fig 2. Response to steroid therapy

The number of platelets increased following the administration of prednisolone, in parallel with a decrease in platelet-associated immunoglobulin G and anti-ribonucleoprotein antibody

of thrombocytopenia in patients with SLE is thought to be similar to that of ITP.¹⁰ Although the pathogenesis of the elevated PAIgG in SLE remains controversial, the target for PAIgG in both ITP and SLE is GPIIb/ IIIa.¹¹ In patients with quinidine-induced thrombocytopenic purpura, the GPIIb/IX complex is the predominant target for drug-dependent platelet antibodies.¹² Doria et al¹³ reported that antiphospholipid antibody was associated with thrombocytopenia in patients with MCTD. In this case, antiphospholipid antibody levels were not measured. Although both antiphospholipid antibody and PAIgG can be measured by ELISA, serum is used for the quantification of antiphospholipid antibody, whereas platelets are used for the quantification of PAIgG. Therefore, PAIgG may reflect platelet-bound IgG more specifically; how much antiphospholipid antibody was bound to platelets could not be determined. However, it should be realised that the finding of an elevated PAIgG in thrombocytopenia, and its reduction during remission do not prove causation. Treatment with high-dose steroids may have broad effects on antibody production, and other antibodies that were not measured, such as antiphospholipid antibodies could also have been responsible. It should also be noted that the presence of an antibody against a certain cellular antigen does not

necessarily lead to disease. Many relatives of patients with Grave's disease, for example, have antibodies against thyroid antigens but remain euthyroid, and the same is true for diabetes and anti-glutamic acid decarboxylase antibodies. Further study is required to determine the target of PAIgG in patients with MCTD who have a high titre of PAIgG.

References

1. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR. Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 1972;52:148-59.
2. Nagae S, Hori Y. Immune thrombocytopenia due to Tranilast (Rizaben): detection of drug-dependent platelet-associated IgG. *J Dermatol* 1998; 25:706-9.
3. Kasukawa R, Tojo T, Miyawaki S. Preliminary diagnostic criteria for classification of mixed connective tissue disease. In: Kasukawa R, Sharp G, editors. *Mixed connective tissue disease and anti-nuclear antibodies*. Amsterdam: Elsevier; 1987:41-7.
4. Montecucco C, Caporali R, Cobiainchi F, Negri C, Astaldi-Ricotti GC. Antibodies to hn-RNP protein A1 in systemic lupus erythematosus: clinical association with Raynaud's phenomenon and esophageal dysmotility. *Clin Exp Rheumatol* 1992;10:223-7.
5. Burdt MA, Hoffman RW, Deutscher SL, Wang GS, Johnson JC, Sharp GC. Long-term outcome in mixed connective tissue disease: longitudinal clinical and serologic findings. *Arthritis Rheum* 1999; 42:899-909.
6. Frandsen PB, Kriegbaum NJ, Ullman S, Hoier-Madsen M, Wiik A, Halberg P. Follow-up of 151 patients with high-titer U1RNP antibodies. *Clin Rheumatol* 1996;15:254-60.
7. Bakri Hassan A, Ronnelid J, Gunnarsson I, Karlsson G, Berg L, Lundberg I. Increased serum levels of immunoglobulins, C-reactive protein, type 1 and type 2 cytokines in patients with mixed connective tissue disease. *J Autoimmun* 1998;11:503-8.
8. Margaux J, Hayem G, Palazzo E, et al. Clinical usefulness of antibodies to U1snRNP proteins in mixed connective tissue disease and systemic lupus erythematosus. *Rev Rhum Engl Ed* 1998;65:378-86.
9. Smith EL, Shmerling RH. The American College of Rheumatology criteria for the classification of systemic lupus erythematosus: strengths, weaknesses, and opportunities for improvement. *Lupus* 1999;8: 586-95.
10. Kurata Y, Hayashi S, Kosugi S, et al. Elevated platelet-associated IgG in SLE patients due to anti-platelet autoantibody: differentiation between autoantibodies and immune complexes by ether elution. *Br J Haematol* 1993;85:723-8.
11. Warner MN, Moore JC, Warkentin TE, Santos AV, Kelton JG. A prospective study of protein-specific assays used to investigate idiopathic thrombocytopenic purpura. *Br J Haematol* 1999;104:442-7.
12. Nieminen U, Kekomaki R. Quinidine-induced thrombocytopenic purpura: clinical presentation in relation to drug-dependent and drug-independent platelet antibodies. *Br J Haematol* 1992;80:77-82.
13. Doria A, Ruffatti A, Calligaro A, et al. Antiphospholipid antibodies in mixed connective tissue disease. *Clin Rheumatol* 1992;11:48-50.