Letters

Serum immune complex containing thrombospondin-1: a novel biomarker for early rheumatoid arthritis

The diagnosis of rheumatoid arthritis (RA) is based on classification criteria set by the 2010 RA classification criteria including serological assessment of rheumatoid factor (RF) and anticitrulline-containing protein/peptide (anti-CCP) antibody.[1 2] Anti-CCP antibody is specific (94–99%) for RA; however, 25% of patients with established RA and 40% of patients with early RA are negative for this marker.[3 4] Novel biomarkers, especially for early RA and/or for RA lacking RF and anti-CCP antibody markers (ie, seronegative RA) are therefore urgently required. Circulating immune complexes (CICs) present in the human

body are likely to contain many different antigens that may reflect underlying disease, so antigens incorporated into CICs are promising candidates for diagnostic biomarkers. We developed a novel proteomic strategy (immune complexome analysis) to identify and profile antigens in CICs and used this method to analyse CICs in patients with established RA and controls (healthy donors and patients with osteoarthritis).[5] CIC-associated thrombospondin-1 (TSP-1) was found in 81% and CIC-associated platelet factor 4 (PF4) in 52% of patients with established RA, but neither protein was found in CICs from any of the controls.[5] Both proteins are known as endogenous inhibitors of angiogenesis[6–8]; the formation of CICs may promote angiogenesis. We evaluated the diagnostic potential of CIC-associated TSP-1 and CIC-associated PF4 in patients with early RA divided into seropositive and seronegative groups.

Serum samples were collected from 25 disease-modifying antirheumatic drug (DMARD)-naïve seropositive patients with early RA (mean±SD age 52.8±18.4 years; 21 women; disease duration 0.25–12 months; CRP 0.01–8.55 mg/dl) and 15 seronegative patients with early RA (mean±SD age 60.5±17.9 years; 8 women; disease duration 1–6 months; CRP 0.02–14.4 mg/dl) at Nagasaki University Hospital. All the seropositive patients were positive for RF and 20 were positive for anti-CCP antibody, while all the seronegative patients were negative for both RF and anti-CCP antibody. The diagnosis of RA was made by the 2010 RA classification criteria as well as administration of DMARDs within the first 12 months.[1][2] Serum samples from 16 patients with Sjögren's syndrome (SS) (mean±SD age 60.9±13.0 years) and 14 patients with systemic lupus erythematosus (SLE) (mean±SD age 42.6±12.4 years) who fulfilled the international criteria for the diagnosis of SS[9] and SLE[10] and 11 healthy donors (mean±SD age 49.5±10.3 years) were used as controls. CICs purified by magnetic beads with immobilised protein G were reduced and alkylated, followed by tryptic digestion. The peptide mixture (1 μl) was subjected to nano-liquid chromatography/electrospray ionization/tandem mass spectrometry. More details of the analytical method can be found in our earlier report.[5]

As shown in table 1, CIC-associated TSP-1 was found only in patients with early RA and was not found in disease controls (patients with SS or SLE) or healthy donors (100% specific). Twenty-two (55%) of the total of 40 patients with early RA (56% (14/25) of the seropositive patients and 53% (8/15) of the seronegative patients) had CIC-associated TSP-1. PF4-containing CICs were found in only three patients (8%) with early RA compared with 52% of the patients with established RA.[5] These PF4-containing CICs may therefore promote disease progression.

In conclusion, we have shown that CIC-associated TSP-1 has high potential as a novel biomarker for diagnosing early and/or seronegative RA. Further analyses using a large number of patients are warranted to determine the clinical benefit of using this novel biomarker.

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Table 1 Number of patients with early RA carrying CIC-associated TSP-1 or CIC-associated PF4

	Early RA patients (n=40)		SS patients (n=16)	SLE patients (n=14)	Healthy donors (n=11)
	Seropositive (n=25)	Seronegative (n=15)			
TSP-1	14	8	0	0	0
PF4	3	0	0	0	0

CIC, circulating immune complex; PF, platelet factor; RA, rheumatoid arthritis; SS, Sjögren's syndrome; SLE, systemic lupus erythematosus; TSP, thrombospondin.

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