LETTERS

New markers and an old phenomenon: prozone effect disturbing detection of filaggrin (keratin) autoantibodies

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Anti-filaggrin antibodies, also named anti-keratin antibodies (AKA), are serum IgG labelling the stratum corneum of the rat oesophagus epithelium, detectable by indirect immunofluorescence (IIF). They constitute a specific marker for the diagnosis of rheumatoid arthritis (RA).^{1–3}

A laminar staining pattern is seen with most AKA-positive sera (fig 1A), but occasionally the cornified layer is diffusely labelled, and the image also shows foamy staining (fig 1B). Although most authors consider sera as AKA positive only when a linear laminated pattern is observed,^{4,5} some consider the sera as AKA positive even when the staining is diffuse or foamy.⁶

On evaluating in our laboratory⁷ the performance of a second-generation enzyme immunoassay for detecting anti-cyclic citrullinated peptide (anti-CCP) antibodies, directed against the immunogenic target of the AKA,⁸ we noticed that some of the foamy AKA sera had very high titres of anti-CCP antibodies.

We then evaluated the anti-CCP antibodies of all the foamy AKA sera considered as AKA negative in our laboratory from November 2001 to March 2005. AKA were searched with rat oesophagus slides (Biomedical Diagnostics, Ann Arbor, Michigan, USA), sera diluted 1:20, and a rabbit anti-human fluorescein isothiocyanate-labelled IgG (Dako SA). Anti-CCP antibodies were assessed by ELISA (Euroimmun), according to

the manufacturer's instructions. Sera were considered positive if the anti-CCP antibodies were >5 arbitrary units per ml. Rheumatoid factors were also measured by nephelometry on a BNprospec (Dade Behring), using the commercial kit N Latex RF (Dade Behring). Sera were considered as positive for values >10 IU/ml. Clinical diagnoses are given when they are available. Table 1 lists the results. Only four patients were truly positive for AKA without anti-CCP antibodies (one with polymyalgia rheumatica, one with anti-phospholipid syndrome and two with uveitis—in one ankylosing spondylitis and in the other sarcoidosis).

Out of 20 sera with foamy staining, 12 were positive for anti-CCP antibodies and rheumatoid factors with high titres. Only 2 out of 12 patients were not diagnosed with RA, according to the physician. One of the two patients had an uncertain diagnosis: psoriatic arthritis or RA with cutaneous psoriasis (chronic inflammatory rheumatism; patient 8). The other one (patient 3) was an outpatient with no more clinical indication than Sjögren's syndrome associated with vasculitis.

These results reminded us of a very old phenomenon called the prozone effect,⁹ sometimes described in tests using the IIF

Abbreviations: AKA, antikeratin antibodies; anti-CCP, anti-cyclic citrullinated peptide; IIF, indirect immunofluorescence; RA, rheumatoid arthritis

Table 1 Rheumatological serology of patients with anti-keratin antibodies with foamy staining from November 2001 to March 2005

Patient	Birth date	Sex	Anti-CCP (AU/ml) positive >5	Rheumatoid factors (IU/ml) positive >10	Diagnosis
1	24/04/1943	F	>2000	435	RA
2	26/02/1917	F	356	326	RA
3	27/10/1955	M	265	874	Sjögren's syndrome
4	12/11/1939	M	173	591	RA
5	04/02/1919	F	171	17	RA
6	24/04/1964	M	>100	1470	RA
7	04/07/1922	F	>100	353	RA
8	30/09/1954	M	>100	60	Psoriatic arthritis or RA with cutaneous psoriasis
9	27/01/1937	F	99	117	RA
10	09/05/1949	F	93	551	RA
11	04/09/1964	F	92	132	RA
12	27/12/1922	M	68	46	RA
13	24/06/1934	M	47	<10	Polymyalgia rheumatica with Waldenström's disease
14	15/06/1975	F	<5	<10	Unclassified arthritis
15	24/08/1954	M	4.4	17	Crystal-induced synovitis
16	14/03/1926	F	3.2	17	Unavailable (outpatient)
17	26/08/2001	F	2.6	<10	Juvenile chronic arthritis
18	11/03/2002	M	1.9	<10	Inflammatory arthralgias
19	15/05/1953	F	1.4	<10	Spondyloarthropathy
20	16/03/1949	F	0.8	<10	Ocular vasculitis

CCP, cyclic citrullinated peptides; F, female; M, male; RA, rheumatoid arthritis.

Figure 1 Anti-keratin antibodies (AKA) detected by indirect immunofluorescence on rat oesophagus. (A) Typical linear laminated labelling pattern restricted to the upper cornified layer observed in the serum of patients with rheumatoid arthritis. (B) Foamy pattern due to prozone effect in rheumatoid serum, with high titres of AKA and anticyclic citrullinated peptide antibodies; when diluted, this serum gives the typical pattern as observed in (A).

technique. An excess of antibodies can result in diffuse or faint staining.¹⁰

To confirm this hypothesis, some of the anti-CCP positive sera, usually diluted 1:20 for AKA determination, were then diluted in phosphate-buffered saline up to 1:200. Patients 6, 7 and 10 then showed the characteristic linear laminated pattern. These patients who were first considered to be AKA negative were in fact AKA positive!

To conclude, testing for autoantibodies is highly relevant in the diagnosis or exclusion of many systemic autoimmune diseases, including RA. Our data show that IIF is an interesting diagnostic tool, provided the biologist has trained staff, quality controls, proficiency testing and keeps in mind the possible occurrence of prozone effects. As much as possible, AKA and anti-CCP antibodies should always be combined for optimum diagnostic performance. Solid-phase assays, which may be too expensive in some countries, can detect only anti-CCP antibodies, whereas IIF could give extra information such as the presence of antinuclear antibodies when examining for AKA.

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Successful treatment with bosentan for severe digital ischaemia in limited cutaneous systemic sclerosis

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We report the case of a patient with limited cutaneous systemic sclerosis, who developed progressive digital gangrene. This responded rapidly to bosentan.

A 38-year-old, previously well, male non-smoker presented with an ischaemic left index finger. On examination the distal left index finger was cyanosed; sclerodactyly and facial telangiectasia were also noted. Investigations revealed haemoglobin 10.7 g/dl (normocytic); erythrocyte sedimentation rate 94 mm in the first hour; normal C-reactive protein levels; positive antinuclear, anticentromere and anti-Ro antibodies;

normal complement components; no anticardiolipin antibodies, lupus anticoagulant activity or cryoglobulin; negative blood cultures; normal chest radiograph and ECG; arteriogram of the left arm revealed no arterial occlusion and was consistent with small vessel disease; and endoscopy revealed antral gastritis.

A diagnosis of limited cutaneous systemic sclerosis was made. The patient was treated with iloprost, nifedipine, clopidogrel, losartan, fluoxetine, lansoprazole and repeated blood transfusions. Despite this, he presented acutely with gangrene of seven fingers, constitutional symptoms and