Letters to the Editor

Tear fluid measurement of anti-SS-A and anti-SS-B antibody in anti-SS-A and anti-SS-B seronegative Sjögren's syndrome patients

Sirs,

In the US/European Consensus Group (EUCG) classification criteria for Sjögren's syndrome (SS) objective items, such as focal sialadenitis and serum presence of anti-SS-A and anti-SS-B antibodies (anti-SS-A, anti-SS-B), are emphasized (1). In the sera of approximately 20% of SS patients no anti-SS-A or anti-SS-B can be detected at the time of diagnosis. However, in these 'seronegative' patients anti-SS-A or anti-SS-B may nevertheless be present within the affected exocrine glands or their excreta *e.g.* saliva or tear fluid (2-6). We hypothesised that if anti-SS-A or anti-SS-B synthesis also occurs within lacrimal glands tear fluid presence of these antibodies may occur, even in the absence of serum presence. To our knowledge, anti-SS-A or anti-SS-B presence in tear fluid of SS patients has indeed been reported once (7). The aim of this study was to confirm whether anti-SS-A and anti-SS-B could be detected in tear fluid samples of our seronegative SS patient population.

All patients gave their informed consent before blood or tear fluid withdrawal was carried out. Sera were analysed for anti-SS-A and anti-SS-B presence by counterimmuno-electrophoresis performed as described by Bunn and Kveder, using bovine spleen extract for detection of anti-SS-A, and rabbit thymus extract for detection of anti-SS-B (8). For confirmation, all sera were also tested using the ELISA that was used for tear fluid measurement. Tear fluid was collected from 13 anti-SS-A and anti-SS-B seronegative patients fulfilling the EUCG criteria for SS, and 3 anti-SS-A and anti-SS-B seronegative focal sialadenitis patients. Tear fluid samples of 3 anti-SS-A and anti-SS-B seropositive SS patients served as control. Tear fluid was obtained using Schirmer-test paper strips placed in the lower conjunctival sac for 10 minutes. The paper was then stored in 0.5ml phosphate buffered saline (PBS) and immediately stored at -70º Celsius. Presence of IgG anti-SS-A and IgG anti-SS-B in tear fluid samples was determined by ELISA using Quanta LiteTM SS-A 708570 and Quanta LiteTM SS-B 708575 assays (Inova Diagnostica, Inc. San Diego) respectively, both with anti-IgG conjugate antibodies. The ELISA was performed as recommended by the supplied manufacturers manual. Dilution of tear samples was not performed because the tear samples had already been diluted in PBS before storage.

Very low amounts of tear fluid (approximately 5 µl per sample) were obtained. Despite this, the ELISA showed anti-SS-A presence in tear fluid of 2 of 13 seronegative SS patients. From these two patients, tear fluid was afterwards collected and re-tested and this confirmed the presence of anti-SS-A. No anti-SS-B presence was observed. Both anti-SS-A and anti-SS-B were found in the tear fluid control samples of all three seropositive SS patients. Tear fluid samples of seronegative focal sialadenitis patients were negative for both anti-SS-A or anti-SS-B (Table I).

Table I. Tear fluid presence of anti-SS-A and anti-SS-B in patients with Sjögren's syndrome (SS) and Focal Sialadinitis (FS) (Table shows number of positive serum and tear fluid samples).

		serum		tear fluid	
	n	anti-SS-A	anti-SS-B	anti-SS-A	anti-SS-B
SS	3	3	3	3	3
SS	13	0	0	2	0
FS	3	0	0	0	0

Our results support the findings of Toker *et al*. (5) who, also using ELISA, demonstrated IgG anti-SS-A and anti-SS-B in tear fluid of SS patients. However, our study is limited by the small number of evaluated seronegative patients and the small volumes of tear fluid that were obtained. The latter did not allow evaluation using a confirmation technique for ELISA, such as CIE. Nevertheless, a remarkably low amount of tear fluid appeared to be enough to detect anti-SS-A by ELISA. Further studies should elucidate whether the sensitivity of these antibodies is higher in exocrine gland products than in sera.

Since apparently tear presence of anti-SS-A or anti-SS-B is not necessarily associated with serological presence, we conclude that combined and optimized measurement of both body fluids may facilitate clinical diagnosis of SS patients with a negative anti-SS-A and anti-SS-B serological profile.

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