High levels of TCF-1 are associated with primary Sjögren's disease

Sir,

T cell factor 1 (TCF-1; encoded by *TCF7*) is a transcription factor essential for T-lymphocyte development within the thymus (1). We conducted an exploratory study to evaluate TCF-1 expression in CD8⁺ T cells within minor salivary glands (MSG) and serum TCF-1 levels in Sjögren's disease (SjD).

Patients were derived from a parent study in which individuals with connective tissue diseases (CTDs) were systematically and randomly assessed for SjD (2). Study groups included primary SjD (pSjD) (n=24), systemic sclerosis-associated SjD (SSc/SjD) (n=5), systemic lupus erythematosus-associated SjD (SLE/SjD) (n=6), and rheumatoid arthritis-associated SjD (RA/SjD) (n=8). Control groups comprised patients with systemic sclerosis (n=6), systemic lupus erythematosus (n=6) and rheumatoid arthritis (n=4) without SjD, along with two non-SjD sicca controls.

Paraffin-embedded MSG sections (4 μm) were deparaffinised, rehydrated and subjected to heat-mediated antigen retrieval. Endogenous enzyme activity and nonspecific binding were blocked prior to overnight incubation at 4°C with a monoclonal anti-TCF-1 antibody, followed by incubation with an anti-CD8 antibody.

Antigen detection was performed using HRP/DAB for TCF-1 and Warp Red chromogen for CD8, with haematoxylin counterstaining. Double-positive TCF-1 (brown)/CD8 (pink) cells were quantified by blinded morphometric analysis in at least three high-power fields (×320) per sample using Image-Pro Plus software. Interobserver variability was assessed independently by two blind observers (ICC >0.85). Results were expressed as the mean ± SEM of the percentage of immunoreactive cells.

Serum TCF-1 levels were quantified using a commercial ELISA kit (Human Transcription Factor 7 ELISA Kit, MyBioSource, San Diego, CA, USA).

Descriptive statistics were used, and group comparisons were performed by one-way ANOVA with Dunn's or Holm-Sidak *post hoc* tests, as appropriate. A two-tailed $p<0.05$ was considered significant. Analyses were conducted using SPSS v22.0 and GraphPad Prism v6. The study was approved by the local Institutional Review Boards (IRBs).

Immunohistochemical analysis (Fig. 1 A and C) revealed that the frequency of TCF-1⁺/CD8⁺ double-positive cells were higher in pSjD (14.6±0.5%) than in non-SS sicca controls (1.8±0.3%; $p<0.001$), RA/SjD (7.3±0.4%); SLE/SjD (6.8±0.4%); SSc/SjD (4.9±0.2%), and CTD without SjD (RA: 6.7±0.4%; SLE: 6.3±0.5%; SSc: 5.7±0.4%). Serum TCF-1 levels (Fig. 1B) were increased in pSjD patients (3.1±0.8 ng/ml) compared to non-SjD sicca controls (1.1±0.3 ng/ml; $p=0.02$), RA/SjD (1.1±0.04 ng/ml; $p=0.03$) and SSc/SjD cases (1.0±0.4 ng/ml; $p=0.03$).

TCF-1 is essential for the generation of CD8⁺ T-cell memory responses. Moreover, within CD8⁺ T-cell lineages, TCF-1 is required for stem-like self-renewal and for the differentiation of follicular helper T cells and follicular regulatory T cells (3-4), which play a critical role in SjD pathogenesis by promoting ectopic germinal centre formation and supporting B-cell activation (5).

We observed increased TCF-1 expression in CD8⁺ T cells within MSGs, along with elevated circulating TCF-1 levels in patients with SjD, particularly in primary SjD. These findings suggest altered transcriptional programming of infiltrating T cells, potentially contributing to glandular immune activation and tissue damage.

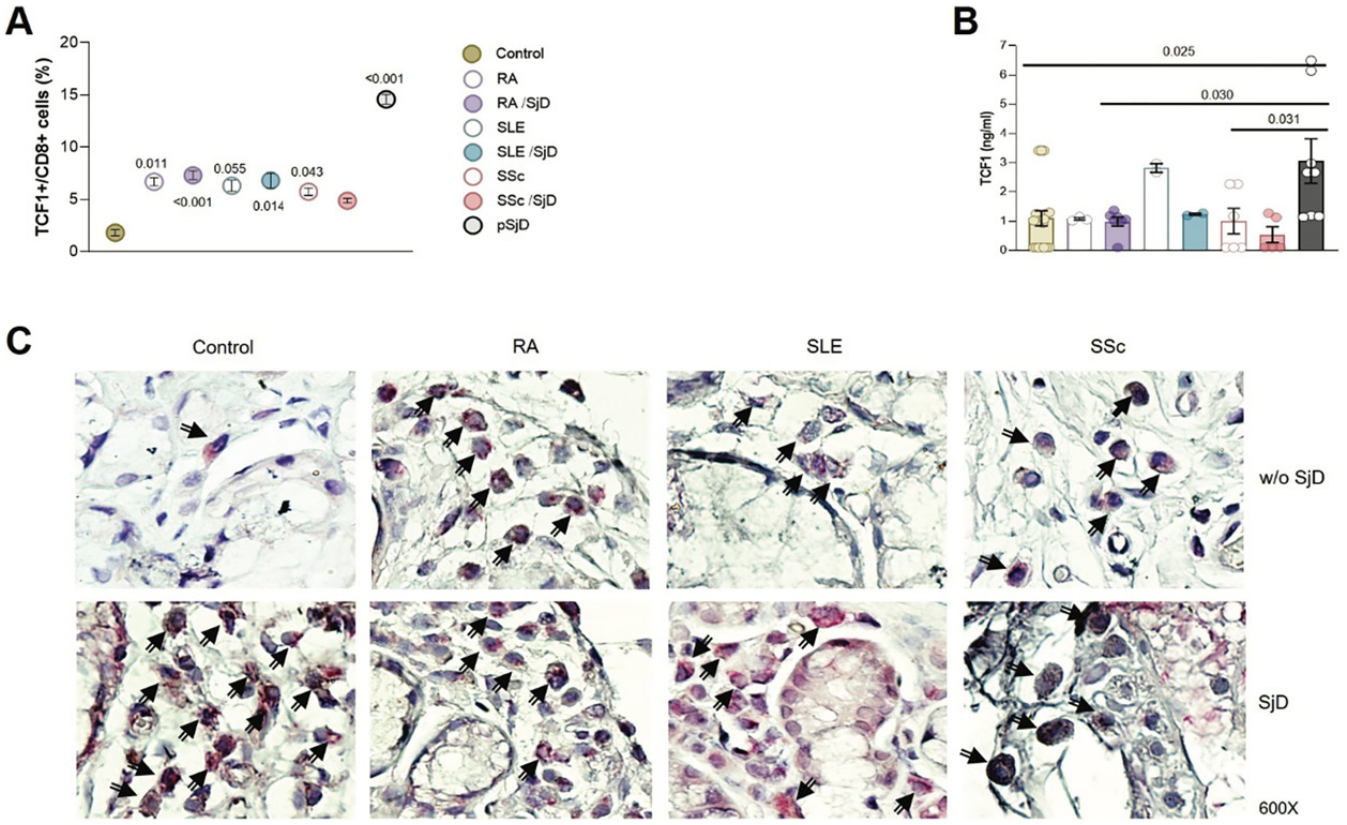

Fig. 1. A. Percentage TCF-1-expressing cells in MSG from patients with pSjD, associated SjD, SLE, RA, SSc, and non-SjD sicca controls. **B**. Serum TCF-1 levels. Bars represent mean values ± SEM. **C**. Representative immunoperoxidase analysis of TCF-1. Arrows depict burgundy cells (TCF-1 in brown, and CD8+ in pink).
Upper panel: Sicca non-SjD, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (SSc) without SjD. Lower panel: primary SjD, RA/SjD, SLE/SjD, and SSc/SjD. The original magnification was X600.

Several limitations should be acknowledged. First, the small sample size and lack of functional or mechanistic analyses limit our findings to a hypothesis-generating level and warrant validation in larger cohorts. Second, TCF-1 expression may have been influenced by treatment, although this variability is inherent to the diseases studied; notably, in patients with SLE, serum TCF-1$^+$ regulatory T-cell levels have been reported to be similar before and after treatment (4). Despite these limitations, this is, to our knowledge, the first report exploring TCF-1 expression in SjD. Elucidating the role of TCF-1 and related transcription factors in T-cell dysfunction may provide insight into SjD pathogenesis.

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Funding. This work was supported by funds from the Department of Immunology, Instituto Nacional de Cardiología Ignacio Chávez, and the Department of Experimental Surgery, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México.

Competing interests: none declared.

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