Brief communication

Salivary β2-microglobulin positively correlates with ESSPRI in patients with primary Sjögren's syndrome

A β2-microglobulina salivar se correlaciona positivamente com o ESSPRI em pacientes com síndrome de Sjögren primária

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Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder largely distinguished by lymphocytic exocrinopathy.[1] Salivary and lachrymal glands are mainly impaired in pSS. Saliva is therefore considered an optimal biological fluid that closely reflects the underlying autoimmune exocrinopathy.[1,2] For instance, in those with pSS, β2-microglobulin (B2M) is increased in whole saliva.[2] This protein is significantly correlated with lymphocytic infiltration in labial salivary glands.[3] The EULAR Sjögren's syndrome Patient Reported Index (ESSPRI) is a patient-centered measure of symptoms. This index has been recently shown to be valid, reliable and sensitive to change in a large cohort of patients with pSS.[1] The ESSPRI correlated significantly with serum B2M in patients with pSS.[4] However, serum and salivary levels of B2M were not correlated in a previous study.[5] It is currently unknown whether the ESSPRI is also correlated with salivary B2M in those with pSS. This is of particular importance since, if these two parameters are indeed associated, a treatment which could decrease inflammatory activity would improve the patients' symptoms, and also because the treatment of chronic diseases finally aims to improve quality of life and patient-perceived health status.[6] Due to the above issues, this study was conducted. Salivary B2M was further subanalysed according to the patient-acceptable symptom state (PASS). A cut-off value for salivary B2M to distinguish those who were above the threshold of unsatisfactory symptom state (ESSPRI ≥ 5) was also determined.

In this observational study, adult patients (≥18-year-old) with pSS were enrolled. Those with history of HIV, hepatitis B and C, head and neck radiation therapy, sarcoidosis, amyloidosis, chronic kidney disease, lymphoma, or multiple myeloma were excluded. pSS was diagnosed according to

the American College of Rheumatology (ACR)/Sjögren's International Collaborative Clinical Alliance (SICCA) criteria.[7] All patients completed the ESSPRI. This index is a mean score of 0–10 numerical scales for pain, fatigue and dryness features (including oral, ocular and global dryness).[1] PASS was defined as the value beyond which patients consider themselves well (i.e., ESSPRI < 5).[6] The degree of histopathological change was assessed using the grading standard for labial salivary gland biopsy.[8] Written informed consent was obtained from all patients, and the study was approved by the local ethics committee.

Our study protocol was similar to that described previously by Castro et al.[5] Saliva samples were collected over 15 min by passive spitting into containers. Unstimulated whole saliva samples were centrifuged at $14{,}000 \times g$ for 20 min at $+4\,^{\circ}\mathrm{C}$ prior to assaying. B2M was determined by an enzyme-linked immunosorbent assay (Abcam, Cambridge, UK) and results were expressed as nanograms per milliliter (ng/mL). This assay was performed according to the manufacturer's instruction manual.

Normality of data distribution was assessed using the Shapiro–Wilk test. The measurement of the strength of the association between the variables was explored using the Kendall's correlation coefficient. Continuous variables were compared between groups with the Mann–Whitney U test. A receiver operating characteristic (ROC) curve was generated to determine the cutoff value in the B2M with the highest level of accuracy in identifying patients with unsatisfactory symptom state (ESSPRI $\geq$ 5). Sensitivity and specificity were thus calculated. Unless indicated otherwise, all results are expressed as mean ± standard deviation or median (interquartile range). Statistical analyses were conducted using SigmaStat (v. 3.5, Erkrath, Germany) or MedCalc (v. 14.12.0, Ostend, Belgium), and a p-value below 0.05 ($p < 0.05$) was considered significant.

We included 71 consecutive female patients with pSS aged 53.05 ± 12.19 years. They had sicca syndrome for 48 (24–81) months. In our patients, the median ESSPRI was 6.3 (3.3–8.6), while the median B2M was 0.671 (0.284–1.072) ng/mL. A significant positive correlation between ESSPRI and salivary B2M was found (0.759, 95% CI 0.656–0.837, $p < 0.0001$) (Fig. 1). The degree of histopathological change was not significantly correlated with salivary B2M (0.0485, 95% CI −0.123 to 0.237, $p = 0.55$). Data were then subanalysed into PASS ($n = 28$) and non-PASS groups ($n = 43$). Salivary B2M was comparatively higher in those who were above the threshold of unsatisfactory symptom state (0.878 [0.682–1.263] vs 0.219 [0.10–0.324] ng/mL, $p < 0.0001$). The ROC curve analysis for salivary B2M showed an area under the curve of 0.965 (95% CI 0.891–0.994, $p < 0.0001$) with an optimal cutoff value of 0.472 ng/mL (Fig. 2). Sensitivity and specificity were 97.67% and 96.30%, respectively.

B2M is a nonglycosylated low-molecular-weight protein, which is part of the major histocompatibility complex I and is particularly expressed in lymphocytes. This protein is regulated by interferon and, in turn, this pathway activation is thought to be related to the disease progression of pSS.[9,10] The increased expression of B2M in whole saliva might thus represent both systemic B cells activation and increased intraglandular immunoglobulin synthesis, which are peculiar facets of pSS.[10,11] As noted before, the ESSPRI has been validated recently in an international multicenter
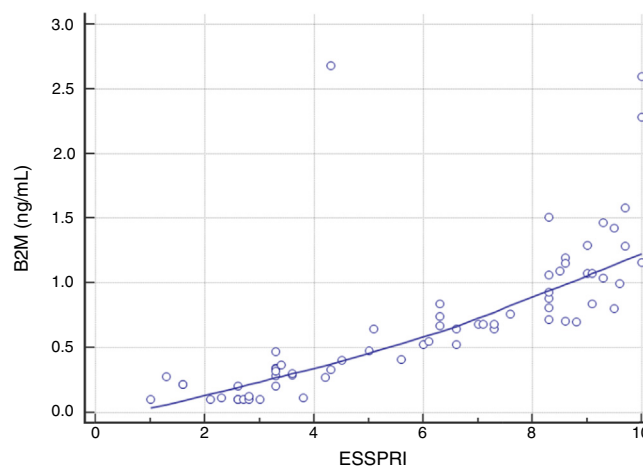

Fig. 1 – A significant positive correlation between the ESSPRI and salivary B2M was found (Kendall's tau 0.759, 95% CI 0.656–0.837, $p < 0.0001$).

cohort of patients with pSS. Two Spanish-speaking countries were included in the latter study (including one from Latin America).[1] The ESSPRI has been also validated into Brazilian Portuguese.[12] The median ESSPRI in our study is similar to that already described by Seror et al. in their international multicenter study.[1] The PASS is similar to the concept of low-disease activity for the EULAR SS disease activity index (ESSDAI; i.e., ESSDAI < 5). Nevertheless, these two indexes did not necessarily overlap, particularly in pSS where disease activity (ESSDAI) and patients' symptoms (ESSPRI) did not correlate.[6,13] Hence, these two indexes complement each other in the evaluation of patients with pSS.[1]
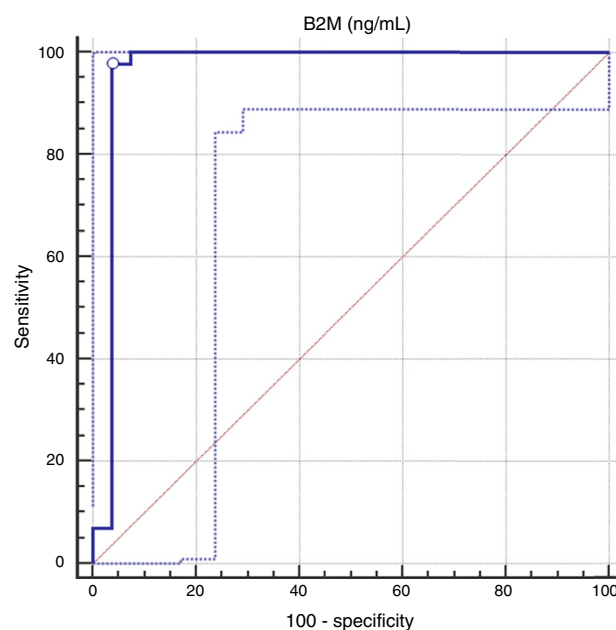

Fig. 2 – ROC curve analysis for salivary B2M. It showed an area under the curve of 0.965 (95% CI 0.891–0.994, $p < 0.0001$) with an optimal cutoff value of 0.472 ng/mL. Sensitivity and specificity were 97.67% and 96.30%, respectively.

Our results support the notion that salivary B2M might act as a biomarker for an unsatisfactory symptom state (Fig. 2). Patients with systemic features have higher scores on ESSPRI, which means that they are more symptomatic.[1] Additionally, a significant association between salivary B2M (measured by enzyme-linked immunosorbent assay) and serum anti-Ro/SSA antibodies has been previously reported.[11] This is relevant because, in patients with pSS, these antibodies are likely to be strongly involved in the clinical severity of keratoconjunctivitis sicca,[14] and also because higher levels of these antibodies have been reported in patients with systemic features (e.g., purpura).[15] Therefore, considering these two findings together, higher concentrations of salivary B2M may also distinguish those with systemic features, who could benefit most from treatment.[6]

In summary, there is a significant positive correlation between the ESSPRI and salivary B2M. In addition, salivary B2M is comparatively higher in those who were above the threshold of unsatisfactory symptom state. Our results suggest that, in pSS, the intensity of the symptoms is indeed associated with sB2M. sB2M might thus represent a simple and objective method to aid in the identification of those with an unsatisfactory symptom state. The latter is especially important in chronic diseases, such as pSS, since their treatment aims to improve patients' quality of life.

Conflicts of interest

The authors declare no conflicts of interest.

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