Paraffin stimulation might not be necessary for the collection of saliva: effect on the rate and cellular distribution in primary Sjögren's syndrome

Sirs,

Saliva has favourable effects on oral health and comfort. There are many organic molecules, bacteria, immune system cells, and ions in this exocrine fluid. The structure and content of saliva can change in systemic diseases as well as in oral diseases. These changes were primarily demonstrated in infections localised to the tissues inside the mouth, such as periodontitis (1-3). Cellular components of the immune system were also detected in saliva at different rates in various disorders (1-4).

The flow cytometry method which detects the cells according to their surface markers determines the change in the rates of immune system cells in saliva. The first salivary flow cytometry study was performed by Aps *et al*. in 2001. In this study, the rates of epithelial cells, erythrocytes, leukocytes, and bacteria in the saliva were compared between patient groups with and without gingivitis by flow cytometry (1). Subsequently, the severity of periodontal inflammation and flow cytometry findings in the saliva were compared. In patients with an increased gingivitis score, the number of leukocytes in the saliva was higher (2). Furthermore, Vidovic *et al*. first reported the rates of leukocyte subtypes in saliva by flow cytometry in the healthy population. In this study, B and T lymphocytes and monocytes from leukocyte subtypes were analysed (3).

In these three studies, stimulated whole saliva was collected from the subjects by chewing paraffin to augment the amount of saliva.

Based on the previous publications of alterations in cellular components of saliva we followed the aforementioned methodology of stimulated saliva collection in our study that aims to address the presence and the rate of cells possibly relevant in the pathogenesis of the Sjögren's syndrome in the saliva. Stimulation of the salivary glands provides more material for flow cytometry, but this is more likely an increase in the serous components of saliva. In addition, chewing paraffin may increase the relative rate of epithelial cells in saliva due to minor intraoral traumas. However, it is essential to detect cellular components of the immune system of saliva by flow cytometry. One of the most common systemic diseases that can change the number and rates of immune system cells in the saliva is Sjögren's syndrome (SS). The centre of pathogenesis in SS is lymphocytic infiltration in the salivary glands (5). One may assume that this infiltrate may enter the salivary secretion, and may be detected by flow cytometry. Selifanova *et al*. detected lymphocyte subgroups in the parotid secretion of SS patients by flow cytometry (4).

We aim to share herein our observations of the effect of a stimulated and unstimulated whole saliva collection on the rates of immune cells detected by flow cytometry.

We analysed lymphocyte subgroups by flow cytometry in saliva from two primary SS (pSS) patients who were diagnosed with pSS according to the 2016 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) Classification Criteria and two healthy controls (6). Both unstimulated and stimulated saliva were collected from four subjects in these two groups. After collecting the unstimulated whole saliva, the salivary glands were stimulated by paraffin-chewing. Saliva was

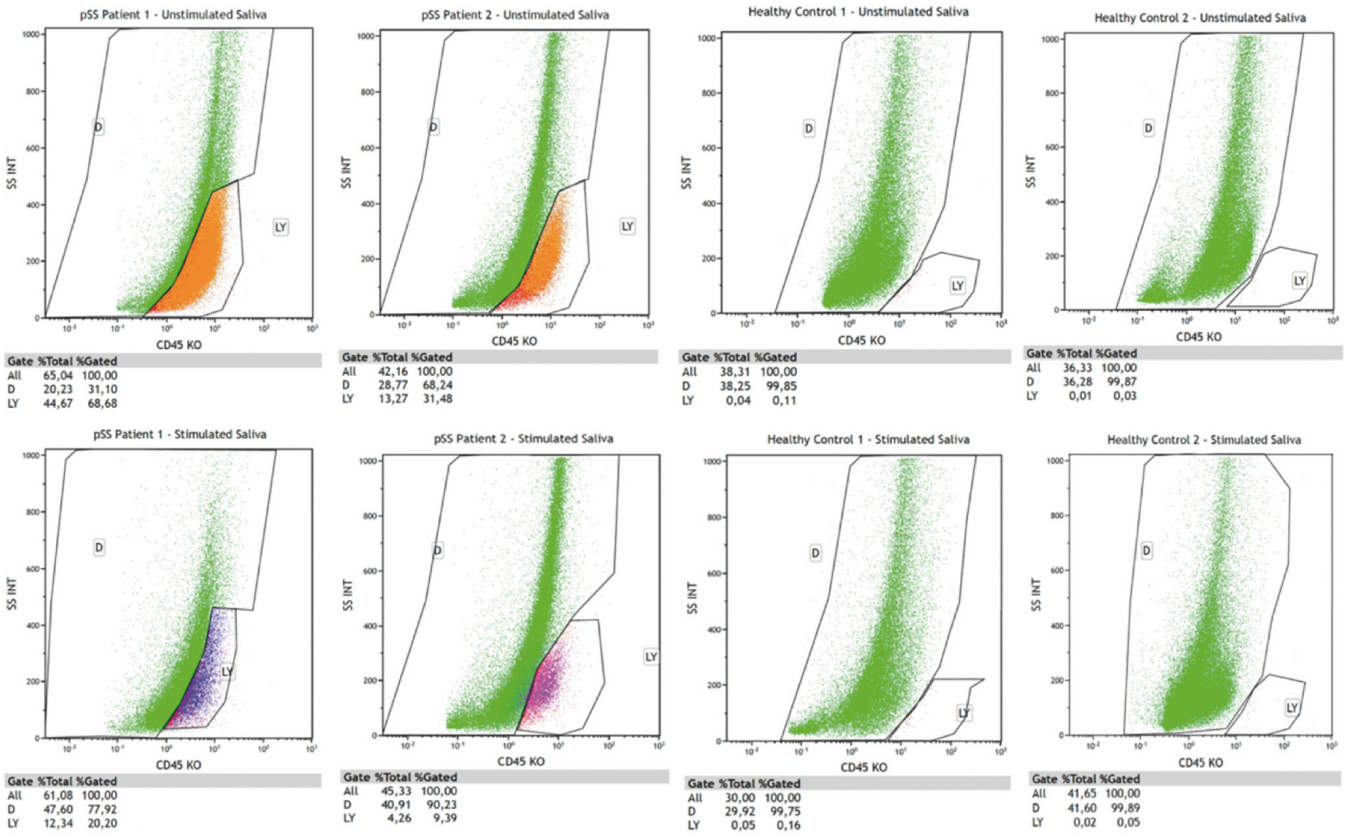

Fig. 1. Stimulated and unstimulated saliva flow cytometric results of two pSS patients and healthy controls.

collected between 9-11 a.m. and on an empty stomach. The minimum cell count for flow cytometric analysis of these four subjects' unstimulated and stimulated whole saliva was 100000.

In these four subjects' stimulated and unstimulated salivary flow cytometry analysis, the CD45+ leukocyte ratio in stimulated saliva was lower than that in unstimulated saliva (Fig. 1). Additionally, we observed in our first set of 16 pSS patients and six controls that the lymphocyte subgroup ratio in the saliva of pSS patients was considerably higher than that of healthy controls. And it was possible to collect whole saliva samples in appropriate amounts in all of the pSS cases with the aforementioned protocol without stimulation for flow cytometry analysis (manuscript in preparation).

In conclusion, stimulation for the whole saliva collection in pSS may be disadvantageous. The reason for this lowered ratio of lymphocytes may be the increase of epithelial cells in the whole saliva due to minor trauma. Unstimulated collection of the whole saliva was possible in pSS and was more accurate.

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