Future prospects for salivary proteomics in rheumatology: the example of eosinophil granulomatosis with polyangiitis

Sirs,

Since the onset and clinical picture of rheumatic diseases may be unclear, salivary proteomics may offer a non-invasive approach to search for informative protein biomarkers for the diagnosis and prognosis of rheumatic diseases (1-10). So far, a large amount of data have supported the application of salivary proteomics to the study of Sjögren’s syndrome and other diseases prevalent oral involvement (11-14). More recently, the observation that salivary proteins may derive not only from salivary glands but also from plasma has led to the analysis of the human salivary proteome as a source for novel easy-to-obtain biomarkers for systemic pathological conditions (15). This proof of concept study was aimed at verifying whether salivary proteomics could represent a useful technique to identify disease biomarkers for eosinophil granulomatosis with polyangiitis/Churg-Strauss syndrome (EGPA). This rare and systemic small-vascular necrotising vasculitis is characterised by sustained and elevated blood eosinophilia, with evidence of eosinophil-induced organ damage and asthma and might be particularly challenging to early diagnose from refractory asthma and hypersensitivity disorders (16-18). Fourteen EGPA consecutive patients (8 M: 6 F, mean age 61±13 yrs, mean disease duration 6.6±5.0 yrs) and 10 age and sex matched healthy volunteers were prospectively enrolled in the study. Unstimulated whole saliva samples were collected and analysed under standard conditions. Two-dimensional electrophoresis (2DE) was performed using the Immobiline-Dryloc polyacrylamide system with pH 3-10 L, 17 cm IPG strips (Protean IEF Cell Biorad). The second dimension (SDS-PAGE) was performed using 15% polyacrylamide gels. The analytical gels were stained with colloidal Coomassie and scanned using the GS800 Densitomer. Subsequently, the gels were analysed with PDQuest advanced software. MALDI-TOF mass spectrometry was used to identify the spots of interest. All the patients underwent detailed evaluation, including a complete history, physical examination and laboratory analysis. EGPA disease activity was assessed according to the Birmingham Vasculitis Activity Score (BVAS). Asthma severity was evaluated according to GINA guidelines, by means of spirometry test, and asthma control test (ACT). Particularly, forced expiratory volume in 1s (FEV1) and the ratio FEV1/slow vital capacity (SVC) were assessed. None of the patients had current or previous renal involvement. At study entry, all the patients were in clinical remission (BVAS=2.5±2.9, VDI=2±1.1), treatment consisted of low dose steroids (<7.5 mg) and weekly methotrexate (<15 mg/w). Asthma was poorly or partially controlled in 12/14 patients. We observed that 6 salivary proteins were differentially expressed in whole saliva of EGPA patients with respect to healthy volunteers, supporting our work hypothesis that saliva could be a useful biological fluid for the identification of biomarkers in systemic rheumatologic disorder, irrespectively of a direct involvement of salivary glands. More specifically, we found that the expression of three proteins, i.e. Immunoglobulin J chain, kallikrein-1 and lipocalin-1, was significantly increased, while the expression of cystatins S, SA and C was apparently reduced (see Table 1). We focused the attention on Kallikrein-1 since this serine protease has been identified as the major kininogenase in the airways influencing a number of biological processes such as vasodilation, vascular permeability, and bronchoconstriction—all of which contribute to asthma pathophysiology (19). Since Kallikrein-1 is secreted in a variety of glandular tissue (19), including both salivary glands and serous cells of tracheobronchial submucosal glands this finding represents an intriguing intersection between salivary biomarkers and the airway inflammation observed in EGPA. To better validate these hypothesis, we performed a Western Blot analysis on a polyclonal antibody to kallikrein 1 (Abcam). In the study also 14 patients with poorly or partially controlled asthma were included as pathological controls. Our findings showed that salivary kallikrein-1 was equally increased in EGPA (salivary kallikrein-1=0.21±0.16 mean levels±SD) and asthma (salivary kallikrein-1=0.25±0.22 mean levels±SD) with respect to healthy controls (salivary kallikrein-1=0.05±0.03 mean levels±SD) (p<0.05), probably reflecting kinin-mediated airway inflammation (Fig. 1).

In conclusion, this preliminary study, for the first time, describes a different salivary proteomic profile in patients with CSS with respect to healthy controls. Salivary kallikrein-1 seems to correlate with partially/poorly controlled asthma in EGPA thus mirroring airway hyper-responsiveness. Additional studies are required in order to clarify whether these preliminary data have any implication for EGPA management.

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References

Table 1. Identification by MALDI-TOF-MS of salivary proteins differently expressed in patients with EGPA in comparison with healthy controls.

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Accession no</th>
<th>Gene name</th>
<th>Theor Mr</th>
<th>Theor pl</th>
<th>Coverage (%)</th>
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<tbody>
<tr>
<td>Kallikrein-1</td>
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<td>Ig J chain</td>
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<td>IgJ</td>
<td>18087</td>
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<tr>
<td>Lipocalin-1</td>
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<td>LCN1</td>
<td>19239</td>
<td>5.39</td>
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<tr>
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<td>CST3</td>
<td>15799</td>
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<td>49%</td>
</tr>
<tr>
<td>Cyst SA</td>
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<td>CST2</td>
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<td>4.95</td>
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</tr>
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Fig. 1. Western blot validation of the expression of salivary kallikrein-1 levels in patients with EGPA, asthma and in healthy controls. Salivary kallikrein-1 levels were significantly higher in patients with either EGPA and asthma in comparison with healthy controls (p<0.05).
Letters to the Editors


