ABSTRACT NUMBER: 2830

Epigenetic Cell Counting: A Novel Tool to Quantify Immune Cells in Salivary Glands Detects Robust Correlations of T Follicular Helper Cells with Immunopathology

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Meeting: 2017 ACR/ARHP Annual Meeting

Date of first publication: September 18, 2017

Keywords: epigenetics and salivary gland, Sjogren's syndrome

SESSION INFORMATION

Date: Tuesday, November 7, 2017

Session Title: Genetics, Genomics and Proteomics

Session Type: ACR Concurrent Abstract Session

Session Time: 4:30PM-6:00PM

Background/Purpose: Histological analysis of salivary glands for decades has been a valuable tool in the characterization of patients with primary Sjögren's syndrome (pSS) and non-Sjögren's sicca (nSS) patients. Importantly, it has helped in understanding the immunopathology of sicca patients. Nonetheless, standardization of histological assessments, e.g. to quantify lymphocytic foci or germinal centers is lacking, contributing to improper classification of disease and assessment of risk of lymphoma for example. Also, detailed and reproducible quantification of the heterogeneity of inflammatory cells and their contribution to immunopathology is lacking. Recent progress in epigenetics has revealed that cell-specific DNA methylation profiles can be applied to reliably quantify numbers of cells in blood and tissues. The objective of this study was to investigate whether epigenetic cell counting can serve as a novel reliable tool to quantify immune cells in salivary glands of sicca patients.

Methods: DNA was isolated from frozen tissue sections of 13 nSS, 12 probable SS, 29 pSS and 7 overlap SS patients. Bisulfite conversion of demethylated DNA sites was followed by cell specific qPCR that was used to calculate the percentage of cell subsets related to the total number of cells quantified by housekeeping gene expression. Percentages of epigenetically counted cells were correlated to gene expression generated by RNA-seq analysis of matched salivary gland tissue and histological and clinical parameters (lymphocytic focus score (LFS), %IgA+ plasma cells, serum IgG, SSA positivity).

Results: Strongly increased percentages of epigenetically quantified percentages of CD3, CD4, CD8, B cells, T follicular helper (Tfh) cells and Treg cells in pSS vs nSS patients were observed (all $p<0.001$, CD8 $p<0.05$, B cells $p<0.01$). These inflammatory cell types all strongly correlated with LFS (all at least $p<0.001$, CD8 $p=0.015$), local B cell hyperactivity (% IgA+ cells, all $p<0.001$, except CD8 $p=0.060$ and B cells $p=0.127$) and systemic B cell hyperactivity (all at least $p<0.01$, except CD8 $p=0.052$). Th17 cells were not significantly different between nSS and pSS patients. Only CD8 T cells were significantly increased in probable SS patients as compared to nSS patients ($p<0.05$). Percentages of CD3 and B cells positively correlated with CD3 and CD19 RNA expression ($r=0.608$, $p<0.0001$; $r=0.598$, $p<0.0001$, resp.). Interestingly, percentages of Tfh cells correlated with CXCL13 ($r=0.789$, $p<0.0001$), IL7R, CXCR5 and ICOS RNA expression (all $p\leq0.0001$) and were strongly associated with autoimmunity (SSA positivity, $p<0.001$).

Conclusion: Epigenetic cell counting is a promising novel tool to reproducibly and easily quantify immune cells in the (inflamed) labial salivary gland of sicca patients with relatively low amount of tissue needed (<1 mm^3). Considering the potential of this technique to include a huge number of (cell-specific) biomarkers we believe this opens up new standardized ways for salivary gland analysis with high relevance for patient classification, understanding of immunopathology and clinical trials.

Disclosure: J. A. G. van Roon, None; **F. M. Moret**, None; **S. L. M. Blokland**, None; **A. A. Kruize**, None; **G. Bouma**, None; **A. van Maurik**, None; **S. Olek**, None; **U. Hoffmueller**, None; **T. R. D. J. Radstake**, None.

To cite this abstract in AMA style:

van Roon JAG, Moret FM, Blokland SLM, Kruize AA, Bouma G, van Maurik A, Olek S, Hoffmueller U, Radstake TRDJ. Epigenetic Cell Counting: A Novel Tool to Quantify Immune Cells in Salivary Glands Detects Robust Correlations of T Follicular Helper Cells with Immunopathology [abstract]. *Arthritis Rheumatol.* 2017; 69 (suppl 10). https://acrabstracts.org/abstract/epigenetic-cell-counting-a-novel-tool-to-quantify-immune-cells-in-salivary-glands-detects-robust-correlations-of-t-follicular-helper-cells-with-immunopathology/. Accessed May 15, 2019.
