ABSTRACT NUMBER 1367:

Novel Autoantibodies Might Circumvent the Need for Labial Biopsy in a Subset of Seronegative Sjögren's Disease Patients

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Background/Purpose: Sjögren's disease (SjD) is typically diagnosed by the presence of an anti-SSA antibody or focal lymphocytic sialadenitis in salivary gland tissue. Among SjD patients who are anti-SSA antibody negative (SSA-), a salivary gland biopsy is required for diagnosis. Our objective was to identify novel autoantibodies to non-invasively diagnose SSA- SjD.

Methods: IgG binding to a high density whole human peptidome array was quantified using sera from SSA- SjD (n=8) cases and age- and sex-matched healthy controls (n=8). The highest bound peptides from the array were confirmed by ELISA. Fifteen peptides were selected for external validation by ELISA using an independent cohort of subjects that were age-, sex-, and race-matched from the SICCA biorepository: SSA- SjD subjects (met 2016 ACR/EULAR criteria for SjD; n=76), sicca controls (sicca with negative ANA, rheumatoid factor, SSA, and focus score < 1; n=75), and autoimmune controls (positive ANA [≥1:320], rheumatoid factor, or SSA, but failed to meet 2016 ACR/EULAR criteria for SjD; n=41). Among all subjects, 85/192 (44.3%) had a positive focus score (FS ≥1). Peptide abundance was compared between groups using area under the ROC curve (*c*-index; AUC). Binary decision trees using R trees package were used to generate models predictive of SjD vs. controls. Adaptive Lasso was used for variable selection for binary logistic regression models.

Results: IgG against a peptide from DTD2 (D-aminoacyl-tRNA deacylase 2) and RESF1 (retroelement silencing factor 1) was bound more in SSA- SjD than in sicca controls (p=.004; p=0.045; Fig 1a) and more in SSA- SjD than in combined controls (sicca and autoimmune) (p=0.003, p=0.03; Fig 1b). The top performing classification tree model discriminating SjD vs. sicca control included peptides from proteins SCRB2, DTD2, LRCC1, and SLK (65% accurate on validation set; Fig 1c). The top performing tree (55% accuracy upon validation) to discriminate between SjD and sicca controls involved peptides from proteins DTD2, SCRB2, CYP7A1, LRCC1, and KNL1. The top performing (62% accuracy upon validation) to discriminate between SjD and combined controls included peptides from proteins DTD2, RESF1, SCRB2, LRBA, TEX15, CYP7A1, NPAT, and PDZD8 (Fig 1d). Next, we defined if we could predict a positive FS. IgG against peptides from proteins RESF1, DTD2, and SCRB2 were bound more in patients with FS positive vs. negative (p=.010; p=0.012; p=0.027; Figure 2a). We incorporated peptide binding into a regression model with clinical variables including platelet count, SSB, ANA≥1:320, rheumatoid factor, and unstimulated whole salivary flow. Our dependent variable was FS (positive vs. negative). The final model showed good discrimination between FS positive vs. negative (Fig 2b-

c). The AUC for this model is 71.6% (95% CI 63.9-78.2%) and allows flexibility to optimize for specificity, sensitivity, and positive and negative predictive value (Fig 2d-g).

Conclusion: We present novel autoantibodies inSSA- SjD compared to autoimmune- and sicca-controls that can be used to predict an abnormal FS on labial salivary gland biopsy with good predictive value.

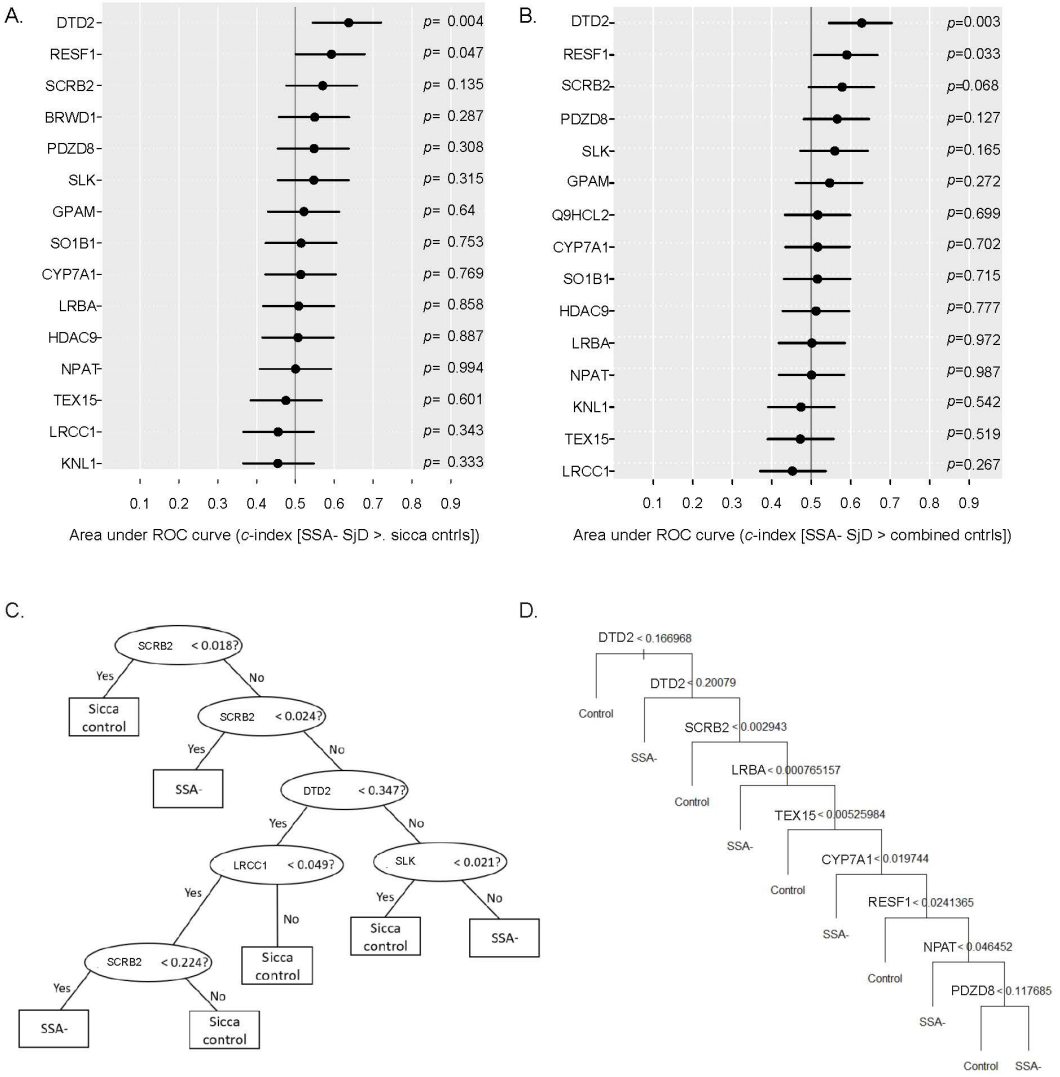
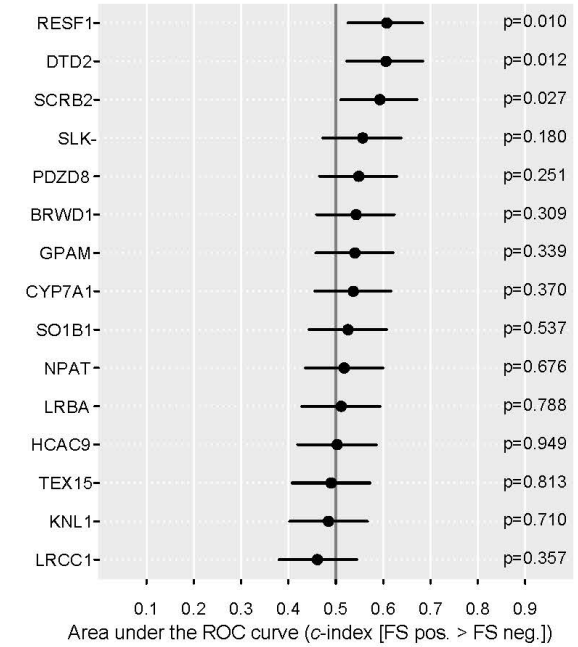


Figure 1. SSA- SjD subjects bind peptides from DTD2 and RESF1 more than controls. A) Area under the ROC curve (AUC) of the adjusted optical density of peptide groups between SSA- SjD (n=76) and sicca controls (n=75); B) AUC of the adjusted optical density of peptide groups between SSA- SjD (n=76) and a combined control comprising sicca and autoimmune controls (n=116); C) Best performing tree discriminating SSA- SjD vs. sicca controls; D) Best performing tree discriminating SSA- SjD vs. combined controls.

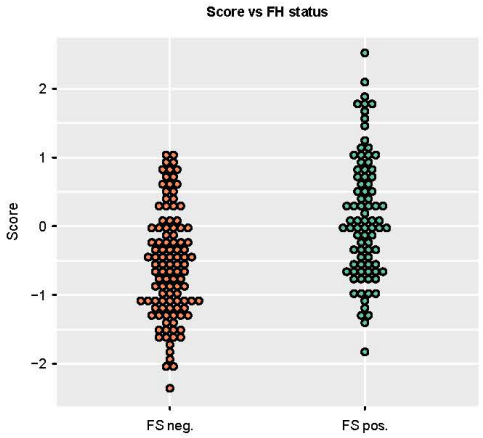
A.



B.

Term	Estimate	p-value	Single term deletion ΔAUC	Single term deletion ΔR^2_N
$\sqrt{\text{DTD2}}$	$2.42_{0.823}$	0.003	-3.91%	-4.17%
$\sqrt{\text{UWS}}$	$-1.04_{0.379}$	0.006	-3.36%	-3.63%
$\log_e$ (Platelet)	$-1.73_{0.626}$	0.006	-2.36%	-3.75%
High ANA (≥ 320)	$0.922_{0.433}$	0.033	-1.20%	-2.11%
Constant	$8.55_{3.46}$	—		

C.



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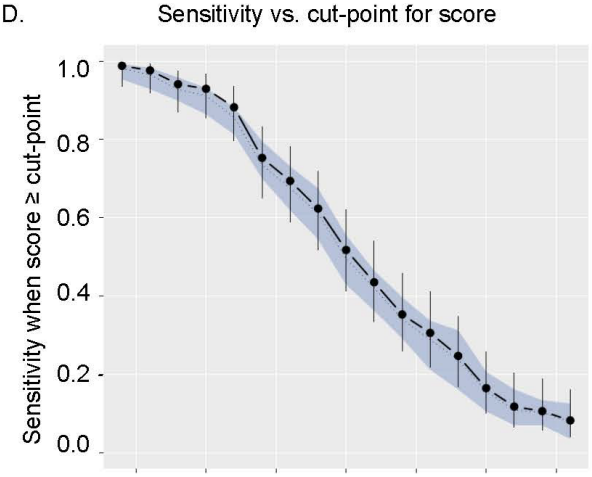

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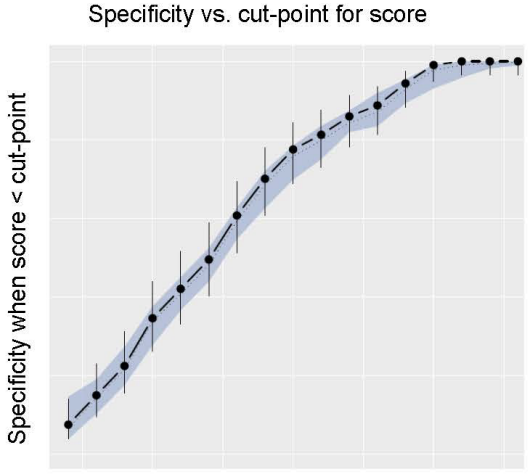

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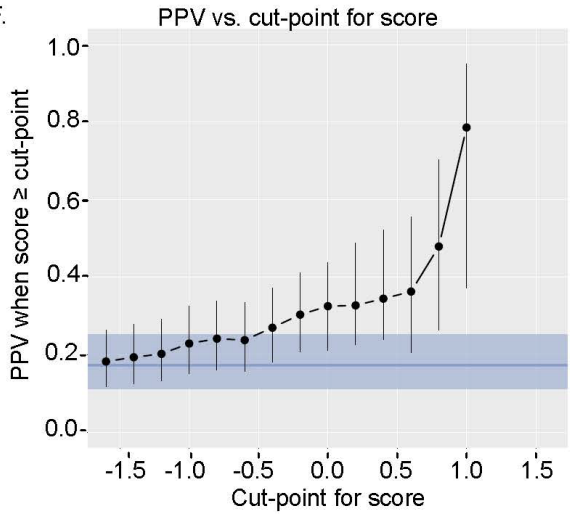

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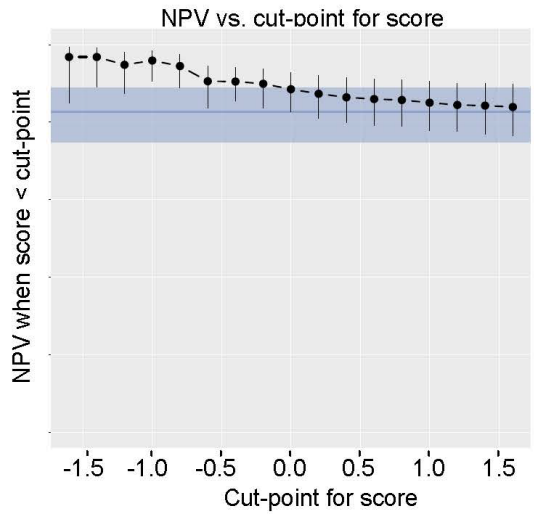

Figure 2. Models that incorporate binding to a peptide from DTD2 yield clinically relevant sensitivity, specificity, and positive and negative predictive value among SSA- patients. A) AUC comparing distributions of adjusted optical density of peptide groups comparing between focus score positive vs. negative (n=85 focus score positive and n=107 focus score negative). The forest plot shows the degree of IgG binding to the peptide of interest differed between focus score positive and focus score negative comparisons; B) Adaptive Lasso reduced variables from 33 to 5 (anti-SSB antibodies, RF, high ANA titer, IgG level, and platelet count) for separate binary logistic regression models. The model incorporated four predictors (binding a peptide form DTD2, unstimulated salivary flow, platelet count, and high ANA) with an AUC of 71.6% (95% CI 63.9-78.2%). The table shows estimated model coefficients and their standard errors in subscript; C) dot plot showing the separation between positive and negative focus score groups; D-E) Specificity and sensitivity graphed separately for cut-points of the score ranging from _1.6 to 1.6. Optimism-corrected values as dotted lines and differ from original values by at most 2.6 or 1.8% for sensitivity and specificity, respectively; F-G) Positive and negative predictive value graphed separately.

Disclosures: M. Parker: JangoBio, 3; **Z. Zheng**: None; **M. Lasarev**: None; **R. Alexandridis**: None; **M. Newton**: None; **M. Shelef**: None; **S. McCoy**: Bristol-Myers Squibb(BMS), 2, Horizon, 2, Kiniksa, 2, Novartis, 2, Otsuka/Visterra, 2, Target RWE, 2.