A Pilot Study of 63 Sera Cytokines in Patients with and without Syphilis

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Background
• Syphilis, while curable, may present either with symptoms or without symptoms.
• Distinguishing between active versus treated infection can be difficult in the asymptomatic stage.
• New tests formulated on the basis of a host cellular response may allow for the differentiation of active or treated infection.
• Prior studies have linked certain cytokines with active or treated infection.

Objectives
• To better understand the pathogenesis of syphilis by investigating sera cytokine levels in men infected with syphilis.
• Explore cytokine markers of syphilis.

Aims
• We aimed to simultaneous measure multiple cytokines, i.e. 63 or more, from clinical specimens from men who were infected with syphilis.

Methods
From 2013 to 2014, MSM seeking testing or care, aged 18 years or older, were recruited for the PICASSO study in one of two STI clinics in Lima, Peru—Alberto Barton Clinic and Epicentro Salud. The study was conducted using sera collected from five HIV-negative individuals infected with syphilis (average age 32 ± 8.8 years), and five HIV-negative, syphilis-negative participants (average age 35.6 ± 7.9 years).

Whole blood samples via venipuncture were collected from five male patients with syphilis (RPR titer 1:32, TPPA positive) and from five syphilis-negative participants (Determine TP negative, TPPA negative). Whole blood was held at room temperature for clot formation and sera was collected following centrifugation, then frozen, stored at -80 °C and then shipped on Dry Ice to the Human Immune Monitoring Center at Stanford University.

Using a multiplex bead-based enzyme-linked immunosorbent assay, we conducted a pilot study comparing the median fluorescence intensity (MFI) between groups. Measurement of cytokines and chemokines in sera was performed using a Luminex multiplex assay (ebiosciences/affymetrix, San Diego, California, USA). A Wilcoxon rank-sum test was used to compare cytokine MFI values between groups (specimens from study participants with and without syphilis). P-values < .05 were considered statistically significant.

Table. Cytokine log-transformed MFI values with significant differences between specimens from active syphilis cases compared to syphilis negative controls.

Results
We identified significant differences in 16 cytokines between groups. Of those cytokines, 8 were not previously described in the literature: interleukin 7, interferon gamma-induced protein 10, leptin, monocyte chemotactic protein-3, nerve growth factor, eotaxin, granulocyte macrophage colony-stimulating factor, and platelet-derived growth factor-BB.

Conclusions
• Our pilot study found at least eight previously unidentified cytokines associated with active-syphilis infection.
• Longitudinal studies and studies with larger sample sizes are needed to confirm our findings and conduct analyses using groups of cytokines.
• Cytokines associated with syphilis could be used to better understand the pathogenesis of the disease and may play a role in future diagnostic testing.