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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 infection using transcriptional profiles or single cytokine assays.

## Objectives

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

## Aim

We aimed to simultaneous measure 63 cytokines from clinical specimens from men who were infected with syphilis

## METHODS

The study was conducted at two sexual health clinics, Epicentro and Barton HealthCenters in Lima, Peru.

## Participants

Sera were collected from 5 HIV-negative individuals infected with syphilis (rapid plasma reagin titer  $\geq 1:32$ , *Treponema pallidum* particle agglutination assay positive, mean age  $32 \pm 8.8$  years), and 5 HIV-negative, syphilis-negative (negative serologic tests, mean age  $35.6 \pm 7.9$  years).

## Experimental procedure

Using a multiplex bead-based enzyme-linked immunosorbent assay, we conducted a pilot study comparing the median fluorescence intensity (MFI) of 63 cytokines in the sera specimens.

MFI values between groups were compared using a Wilcoxon rank-sum test.

P-value of  $<.05$  was considered statistically significant.

## Ethics statement

Institutional review boards at Universidad Peruana Cayetano Heredia and Barton Health Center approved the study protocol.

All participants gave written informed consent for participation.

## TABLE

Cytokine	Notes	Median MFI of Syphilis Infected	Median MFI of Syphilis Uninfected	p-value
VEGF	Vascular endothelial growth factor	353	227	0
IL7*	Interleukin 7	986	592	0.01
VEGFD**	Vascular endothelial growth factor D	218	71	0.01
MIP1B**	Macrophage inflammatory protein 1B or chemokine (C-C motif) ligand 4 (CCL4)	216	133	0.01
IL12P70**	Interleukin 12 active heterodimer	46	38	0.02
IL15	Interleukin 15	52	45	0.02
IP10*	Interferon gamma-induced protein 10 or (C-X-C motif) chemokine 10 (CXCL10)	561	388	0.02
LEPTIN*	Leptin	1795	869	0.02
MCP3*	Monocyte-specific chemokine 3 or chemokine (C-C motif) ligand 7 (CCL7)	80	67	0.02
NGF*	Nerve growth factor	26	23	0.03
EOTAXIN*	Eotaxin	337	398	0.04
GMCSF*	Granulocyte macrophage colony-stimulating factor	2463	1495	0.04
IL10	Interleukin 10	43	23	0.04
IL1B	Interleukin 1 beta	28	23	0.04
PDGFBB*	Platelet-derived growth factor (BB chain of a dimeric protein)	2087	687	0.04
TNFA	Tumor necrosis factor alpha	452	341	0.04
HGF	Hepatocyte growth factor	411	262	0.06
IL1RA	Interleukin 1 receptor antagonist	134	56	0.06
MIG	Monokine induced by gamma interferon or chemokine (C-X-C motif) ligand 9 (CXCL9)	227	122	0.06
TRAIL	TNF-related apoptosis-inducing ligand	156	123	0.06
ENA78	(C-X-C motif) chemokine 5 (CXCL5)	974	1112	0.09
ICAM1	Intercellular adhesion molecule 1	10997	4733	0.09
IFNG	Interferon gamma	77	60	0.09
IL13^	Interleukin 13	55	44	0.09
IL17A	Interleukin 17A	57	56	0.09
IL21	Interleukin 21	55	46	0.09
IL4^^	Interleukin 4	289	116	0.09
IL5^	Interleukin 5	52	36	0.09
IL9	Interleukin 9	306	255	0.09
LIF	Leukemia inhibitory factor	50	46	0.09
IL2^^	Interleukin 2	29	27	0.11
FGFB	Basic fibroblast growth factor	83	76	0.13
IL12P40	Interleukin 12 subunit beta	2022	923	0.13
IL18	Interleukin 18	544	459	0.13
IL22	Interleukin 22	80	61	0.13
IL31	Interleukin 31	133	119	0.13
IL8	Interleukin 8	329	253	0.13
MIP1A^^	Macrophage inflammatory protein 1 alpha or chemokine (C-C motif) ligand 3 (CCL3)	172	127	0.13
SDF1A	Stromal cell-derived factor 1	423	358	0.13
SCF	Stem cell factor	39	37	0.15
RANTES	Regulated on activation, normal T cell expressed and secreted or chemokine (C-C motif) ligand 5 (CCL5)	5366	6467	0.17
IL1A	Interleukin 1 alpha	221	198	0.17
TNFB	Tumor necrosis factor beta	369	335	0.17
CD40L	or CD154	289	262	0.23
FASL	Fas ligand	166	162	0.23
IL27	Interleukin 27	40	36	0.23
IL6^^	Interleukin 6	52	45	0.23
MCSF	Macrophage colony-stimulating factor	102	95	0.23
PIGF1	Placental growth factor	421	430	0.23
TGFB	Transforming growth factor beta	161	130	0.23
IFNB	Interferon beta	90	94	0.27
BDNF	Brain-derived neurotrophic factor	8554	7647	0.3
RESISTIN	Resistin or adipose tissue-specific secretory factor (ADSF)	6229	5633	0.3
PAI1	Plasminogen activator inhibitor-1	12027	12734	0.3
IL17F	Interleukin 17F	72	69	0.38
IL23^^	Interleukin 23	52	53	0.38
IFNA	Interferon alpha	211	213	0.38
EGF	Epidermal growth factor	551	582	0.46
GCSF	Granulocyte-colony stimulating factor	105	113	0.46
GROA	also GRO1 or chemokine (C-X-C motif) ligand 1 (CXCL1)	118	126	0.46
MCP1^^	Monocyte chemoattractant protein 1 or chemokine (C-C motif) ligand 2 (CCL2)	1348	1231	0.46
TGFA	Transforming growth factor alpha	70	85	0.46
VCAM1	Vascular cell adhesion protein 1	18506	18815	0.46

\*Previously unidentified cytokines

\*\*Previously unidentified cytokines subunits

^Previously described as having no association with syphilis

^^Previously described in literature as being syphilis-associated

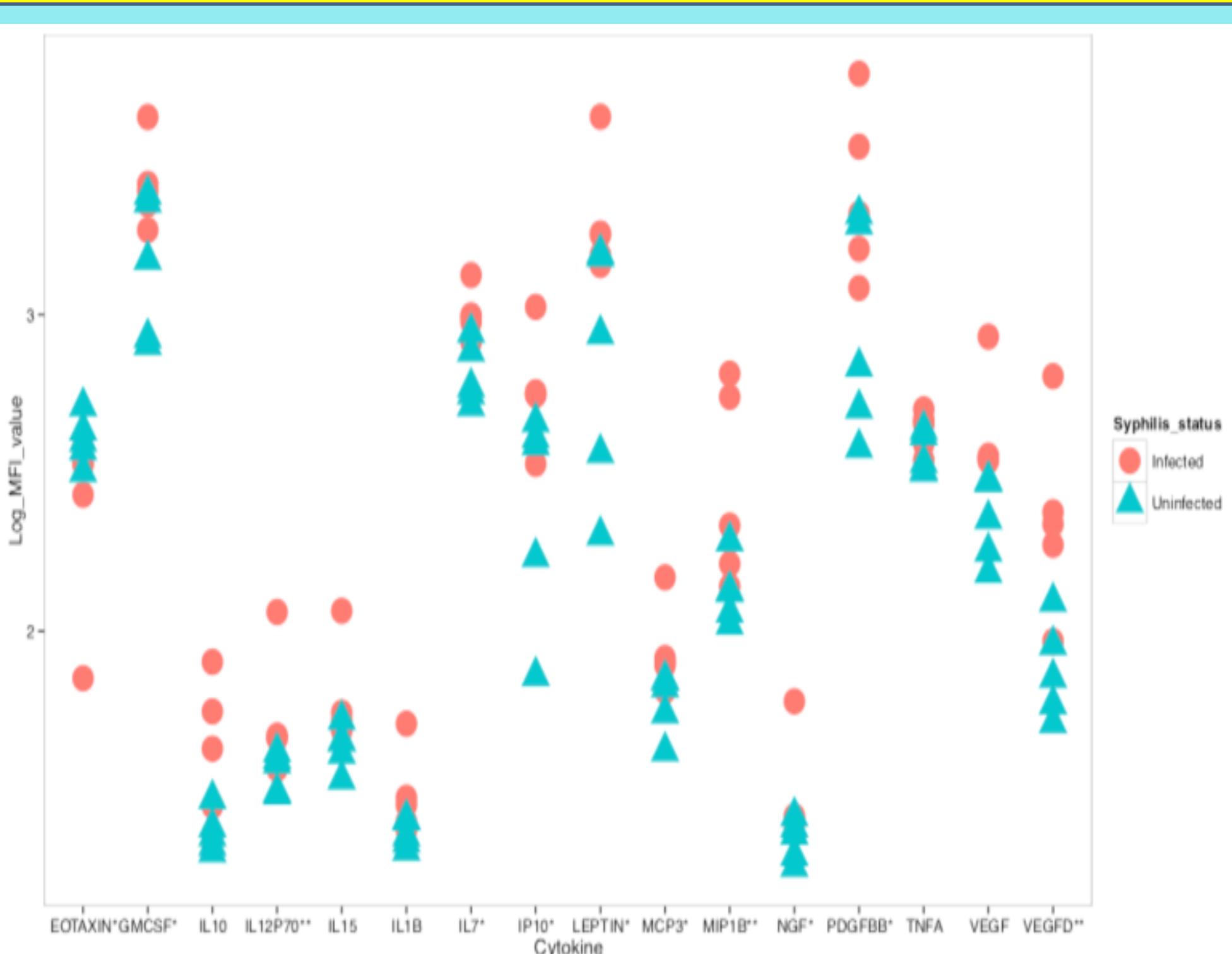
## RESULTS

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-specific chemokine 3, nerve growth factor, eotaxin, granulocyte macrophage colony-stimulating factor, and platelet-derived growth factor.

Cytokines associated with syphilis could be used to better understand the pathogenesis of the disease and may play a role in future diagnostic testing

## FIGURE



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

\*cytokine not previously described in the literature to be associated with syphilis

\*\*cytokine protein subunit not previously described in the literature to be associated with syphilis

## DISCUSSION

Our pilot study found at least eight previously unidentified candidate 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.

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