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LETTER TO THE EDITOR

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Novel cytokine and chemokine markers of hidradenitis suppurativa reflect chronic inflammation and itch

To the Editor,

Hidradenitis suppurativa (HS) is an auto-inflammatory skin disease characterized by recurrent, chronic painful and pruritic inflammatory nodules, abscesses and sinus tracts in predominantly the axillary, inguinal, and gluteal areas. A key element of the HS pathophysiology is occlusion of the follicular infundibulum and subsequent cyst formation, followed by rupture of the cyst inducing an intense inflammatory response.[1] Accordingly, identification of inflammatory markers is important for the clinical stratification of HS and may help refining treatment choices. To date, no studies have investigated inflammatory protein levels in the serum/plasma and skin *in parallel* in a cohort of HS patients. Therefore, the primary aim of this study was to simultaneously detect important cytokines and chemokines in, respectively, the plasma and lesional skin of patients with HS at a single time point.

Blood and skin samples from 20 patients with a dermatologist-verified diagnosis of HS and 10 healthy controls (Data S1) were prospectively collected in the Department of Dermatology of the Erasmus University Medical Center and Sint Franciscus Hospital in Rotterdam, the Netherlands. Skin samples of HS patients suffering of Hurley I to III disease severity were taken from actively inflamed, non-fluctuating, indurated, erythematous lesions, or plaques recurring on fixed locations. The research protocol was approved by the local Institutional Review Board (reference MEC-2013-337/NL45264.-078.13). All participants provided written informed consent.

Punch biopsies of 4 mm in diameter were obtained and immediately snap-frozen in liquid nitrogen. Venous blood was collected in vacuum EDTA tubes under sterile conditions, and after separation of the plasma samples were aliquoted and stored at −80°C until analysis. Samples were analyzed using the Meso Scale Discovery (MSD) V-PLEX™ Human Cytokine 30-plex kit (K15054D; Meso Scale Discovery, Gaithersburg, MD, USA) according to the manufacturers' instructions (Data S1). Moreover, three chemokines, which have not previously been reported to be overexpressed in HS patients, were additionally analyzed by immunohistochemistry (Data S1).

Plasma protein concentrations were expressed as picogram (pg) per milliliter (mL), whereas skin protein levels were normalized for milligram (mg) tissue dry weight (pg/mg). In case, a protein level was below the detection limit, the lowest limit of quantification (LLOQ) was used for further calculations. If more than 50% of the samples per analyzed protein in either the HS or the healthy control group had values below the LLOQ, values were substituted by two categories: detectable vs non-detectable, that is above or below the LLOQ, respectively. For the primary objective, either the Mann-Whitney *U* test or Fisher's exact test was used to assess the null-hypothesis that there was no difference in the levels of individual markers between control and HS samples. Secondly, correlations between protein levels of plasma and lesional HS skin were calculated (Data S1). Statistical analyses were conducted using SPSS Statistics 24.0 (IBM Corporation, Armonk, NY, USA). A two-sided *P* value below 0.05 was considered significant. This level was corrected by a false discovery rate using the Benjamini-Hochberg test for multiple comparisons.

In plasma, 20 of 30 (66.7%) analytes were detected. In the skin, 25 of 26 (96.2%) proteins were detected, while four proteins (IL-4, IL-7, VEGF, GM-CSF) were not analyzed because they have not been validated for skin-derived samples. In plasma, CCL-26 was detected significantly more often in HS patients (16 of 20) compared with healthy controls (2 of 10), $P = 0.004$ (Table 1). Accordingly, the median CCL-26 level in HS patients was 24.9 pg/mL, interquartile range 19.1−37.0 (Figure S1). In contrast, plasma CXCL-10 levels were significantly lower in HS patients, $P = 0.003$. In lesional skin, IL-16 ($P < 0.001$), IL-17A ($P < 0.001$), CXCL-8 ($P = 0.001$), plus IL-8 HA ($P = 0.011$), representing very high CXCL-8 concentrations, IL-12/23p40 ($P = 0.007$), CCL-4 ($P = 0.011$), CXCL-10 ($P = 0.011$) showed higher levels in HS patients compared with healthy controls (Table 2, Figure S2). The elevated CCL-4 and CXCL-10 protein levels in HS lesions were confirmed by immunohistochemistry (Figure S3). A strong staining of CCL-26 was observed in lesional skin, despite the fact that CCL-26 protein was not detected in lesional HS skin by the MSD assay (Table 2, Figure S3). Only weak correlations were observed between protein levels in HS plasma and lesional skin (Data S1, Table S1).

Chemokine CCL-26 (also known as eotaxin-3) is a newly identified inflammatory marker in HS patients. Significant elevation of this chemokine in the serum has previously been reported in atopic dermatitis and cutaneous T-cell lymphoma, which are characterized by the infiltration of eosinophils, basophils, and specific subpopulations of T cells,[2,3] and all, like HS,[4] diseases characterized by high pruritus scores. Interestingly, CCL-26 was found in

Abbreviations: CCL, C-C motif ligand; CRP, C-reactive protein; CXCL, C-X-C motif ligand; EDTA, ethylenediaminetetraacetic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; HS, hidradenitis suppurativa; IL, interleukin; LLOQ, lowest limit of quantification; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

TABLE 1 Inflammatory protein expression in the plasma of healthy control subjects and HS patients

	Protein pg/mL	NN (n = 10) Median (IQR) or x/total	HS (n = 20) Median (IQR) or x/total	LLOQ pg/mL	Unadjusted *P* value
1	CXCL-10 (IP-10)	402.7 (328.7-550.5)	277.4 (236.0-328.8)	2.40	0.003*
2	CCL-26 (Eotaxin-3)	2/10	16/20	18	0.0041*
3	IL-12/23p40	132.7 (97.7-182.5)	104.0 (74.6-127.2)	1.30	0.055
4	IL-1α	1.8 (1.7-3.6)	4.2 (2.4-10.2)	0.62	0.055
5	CCL-4 (MIP-1β)	119.4 (66.6-176.0)	78.5 (59.9-102.5)	2.10	0.091
6	TNF-β	2/10	0/20	0.28	0.103
7	IL-1β	3/10	13/20	0.24	0.122
8	CCL-22 (MDC)	926.2 (716.5-1212.4)	1312.7 (1000.8-1538.6)	38	0.155
9	INF-γ	8.8 (5.3-14.0)	6.9 (4.8-8.9)	2.20	0.155
10	IL-15	2.0 (1.7-2.3)	1.7 (1.5-2.1)	0.32	0.198
11	IL-7	18.6 (14.8-24.2)	22.3 (17.0-30.3)	0.32	0.214
12	IL-10	0.3 (0.2-0.4)	0.2 (0.2-0.3)	0.16	0.231
13	CCL-3 (MIP-1α)	2/10	1/20	15.60	0.251
14	CXCL-8 (IL-8)	8.7 (7.2-9.7)	7.1 (6.0-9.1)	3.80	0.286
15	IL-16	208.0 (191.9-287.3)	257.9 (186.1-317.9)	4.20	0.475
16	CCL-11 (Eotaxin-1)	135.9 (95.6-181.4)	151.6 (118.5-210.1)	5.60	0.502
17	IL-6	1.3 (0.9-2.6)	1.1 (0.7-2.6)	0.36	0.530
18	IL-13	1/10	1/20	0.98	0.532
19	IL-17A	4/10	6/20	2.10	0.690
20	CCL-17 (TARC)	385.8 (222.6-511.6)	325.9 (259.9-653.7)	2.80	0.713
21	TNF-α	2.6 (2.3-3.2)	2.5 (2.2-3.0)	0.64	0.779
22	CCL-13 (MCP-4)	188.0 (160.3-234.8)	210.6 (120.9-238.3)	4.80	0.880
23	CCL-2 (MCP-1)	85.0 (75.3-99.1)	83.0 (62.9-114.9)	0.22	0.983
24	VEGF	140.1 (116.0-200.0)	155.0 (103.6-250.7)	7	0.983
25	IL-2	ND	ND	0.68	-
26	IL-4	ND	ND	0.38	-
27	IL-5	ND	ND	0.40	-
28	IL-12p70	ND	ND	0.74	-
29	GM-CSF	ND	ND	1.80	-
30	IL-8 HA	ND	ND	344	-

IL-8 HA (human antibody) has been validated for the MSD V-PLEX™ kit and is recommended when high CXCL/IL-8 levels are anticipated.
HS, hidradenitis suppurativa patients; IQR, interquartile range; LLOQ, lowest level of quantification; ND, not detected; NN, healthy controls; x, number of samples with a detectable value.
*Significant after correction with the Benjamini-Hochberg test ($P < 0.0042$).

abundance in the HS infiltrate by immunohistochemistry, but was not detected in skin homogenates, possibly because CCL-26 is too strongly bound to its receptor on the many eosinophils present in the HS infiltrate.[4]

The cutaneous upregulation of IL-16 and chemokines CCL-4 and CXCL-10 is not surprising because they are produced by many immune cells and play a crucial role in the induction and modulation of immune responses during infection and inflammation.[5,6] In addition, our results obtained in the skin confirm previous findings demonstrating overexpression of IL-17 pathway-associated cytokines and chemokines such as IL-17A, IL-23p40, and CXCL-8 in HS.[1] The importance of neutrophils in the HS pathogenesis is underlined by the increased levels of CXCL-8 that can be cleaved by neutrophil elastase to activate Th17 cells to produce bioactive IL-17.[7] Some previously published results, that showed significant upregulation of TNF-α, IL-1ß, and IL-10 in (peri)lesional HS skin, could not be confirmed statistically.[8,9] This can be explained by the different approaches as in our study biopsies were homogenized for in situ assessment, while van der Zee et al and Kelly et al cultured the skin biopsies for, respectively, 24 and 3 hours. This step of ex vivo culturing of skin samples allows for a prolonged production of cytokines that may lead to higher cytokine levels in the culture media.

This study has several strengths including the *in parallel* assessment of inflammatory markers in skin and plasma using a

TABLE 2 Inflammatory protein expression in the skin of healthy control subjects and HS patients

	Protein pg/mg skin tissue	NN (n = 10) Median (IQR) or x/total	HS (n = 20) Median (IQR) or x/total	Unadjusted *P* value
1	IL-16	10.90 (7.67-13.09)	57.54 (38.50-120.81)	<0.001*
2	IL-17A	0/10	15/20	<0.001*
3	CXCL-8 (IL-8)	0.30 (0.21-1.30)	5.90 (1.25-19.48)	0.001*
4	IL-12/23p40	0.10 (0.08-0.17)	0.25 (0.14-0.47)	0.007*
5	CCL-4 (MIP-1β)	0.13 (0.08-0.15)	0.62 (0.19-1.83)	0.011*
6	CXCL-10 (IP-10)	0.66 (0.18-1.10)	1.80 (1.07-3.32)	0.011*
7	IL-8 HA	0/10	10/20	0.011*
8	TNF-β	1/10	9/20	0.101
9	CCL-3 (MIP-1α)	2/10	11/20	0.119
10	INF-γ	3/10	13/20	0.122
11	TNF-α	0/10	5/20	0.140
12	IL-1β	0.13 (0.07-0.18)	0.21 (0.08-0.73)	0.155
13	CCL-13 (MCP-4)	0.66 (0.53-0.72)	0.36 (0.25-0.66)	0.172
14	IL-10	0.009 (0.005-0.011)	0.006 (0.004-0.008)	0.183
15	CCL-17 (TARC)	2/10	9/20	0.246
16	IL-5	0.024 (0.019-0.039)	0.017 (0.013-0.029)	0.322
17	IL-1α	1.28 (0.92-2.10)	1.54 (0.86-4.40)	0.350
18	IL-2	0.035 (0.016-0.081)	0.031 (0.023-0.039)	0.530
19	IL-6	0.26 (0.02-0.41)	0.08 (0.03-0.54)	0.530
20	CCL-2 (MCP-1)	3.13 (0.30-4.82)	1.43 (0.42-3.35)	0.588
21	IL-15	0.029 (0.026-0.039)	0.035 (0.026-0.045)	0.588
22	CCL-11 (Eotaxin-1)	4/10	11/20	0.700
23	CCL-22 (MDC)	1.80 (1.44-3.44)	1.82 (1.23-3.25)	0.983
24	IL-13	0/10	1/20	1.000
25	IL-12p70	3/10	6/20	1.000
26	CCL-26 (Eotaxin-3)	ND	ND	-
27	IL-7	NA	NA	-
28	VEGF	NA	NA	-
29	IL-4	NA	NA	-
30	GM-CSF	NA	NA	-

IL-8 HA (human antibody) has been validated for the MSD V-PLEX™ kit and is recommended when high CXCL/IL-8 levels are anticipated.
HS, hidradenitis suppurativa patients; IQR, interquartile range; LLOQ, lowest level of quantification; NA, not analyzed, not validated for skin samples; ND, not detected; NN, healthy controls; x, number of samples with a detectable value.
*Significant after correction with the Benjamini-Hochberg test ($P < 0.014$).

sensitive and accurate detection technique. Limitations of this study are the limited sample size, which did not allow for a subgroup analysis by Hurley disease severity, and the use of a predefined panel of 30 cytokines and chemokines, which did not measure all previously reported HS biomarkers including antimicrobial peptides.

In conclusion, CCL-26 is a newly identified inflammatory marker that is upregulated in the circulation of HS patients. Besides previously demonstrated overexpression of IL-17A, IL-23p40, CXCL-8 in HS lesions, this study found IL-16, CCL-4, CXCL-10, and CCL-26 as novel and potentially important players in the pathogenesis of HS. The local and systemic upregulation of CCL-26 in HS patients can be linked to the high pruritus score in HS. Furthermore, our results demonstrate that plasma gives a limited reflection of the activated local cutaneous inflammatory milieu.

CONFLICTS OF INTEREST

ARJVV, HHvdZ, LCT, XX, JEG, and EPP have no conflicts of interest to declare. MD is a shareholder of AstraZeneca and Corvidia Therapeutics.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.