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

A.R.J.V. Vossen
H.H. van der Zee
L.C. Tsoi
X. Xing
M. Devalaraja
J.E. Gudjonsson
E.P. Prens

Allergy – accepted for publication in modified form

ABSTRACT

Background

A key element of hidradenitis suppurativa (HS) is an aberrant immune response. Identification of inflammatory markers is important for the clinical stratification of HS and may help refining treatment choices.

Objective

To simultaneously detect important cytokines and chemokines in respectively the plasma and lesional skin of patients with HS.

Methods

A multiplex electrochemiluminescent immunoassay platform (Meso Scale Discovery) was used to quantify the *in vivo* protein levels of 30 cytokines and chemokines in twenty HS patients and ten healthy controls. Immunohistochemistry was performed for newly identified markers. Additionally, a correlation between individual plasma and lesional skin protein levels was calculated within the HS patients.

Results

In the circulation of HS patients, CCL-26 (eotaxin-3) was significantly elevated and CXCL-10 significantly lower compared with healthy controls. In the skin, protein levels of IL-16, IL-17A, CXCL-8, IL-12/23p40, CCL-4, and CXCL-10 were significantly higher in HS patients than in controls. Immunohistochemistry demonstrated overexpression of CCL-4, CXCL-10, and CCL-26 in the HS infiltrate. There was no significant correlation between protein levels in patient plasma and lesional skin with correlation coefficients varying between –0.53 and +0.42.

Conclusion

The cytokine and chemokine profile of HS patients, including newly identified IL-16, CCL-4, CXCL-10 and CCL-26, reflects the ongoing skin inflammation in 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.

INTRODUCTION

Hidradenitis suppurativa (HS) is an auto-inflammatory skin disease characterised by recurrent or chronic painful and pruritic inflammatory nodules, abscesses and sinus tracts in predominantly the axillary, inguinal and gluteal areas.[1] Diagnosis at present is based largely on the clinical appearance and location of lesions, and their chronicity. Well-known symptoms are acute and chronic pain, discomfort, and a purulent, foul-smelling discharge, which, overall, contribute to a decreased quality of life.[2,3] More recently, itch, also known as pruritus, was found to be a frequent and bothersome symptom in HS patients.[4,5]

The pathogenesis of HS is complex with multiple factors contributing to the onset and progression of the disease including genetics, smoking, obesity, and mechanical friction.[6] 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 acute inflammatory response. Hereby a broad range of immune cells such as T cells, natural killer (NK) cells, neutrophils, eosinophils, monocytes, and dendritic cells are recruited.[4,7] Accordingly, elevated levels of multiple pro-inflammatory cytokines such as TNF-α, IL-1ß, IL-6, IL-8 (CXCL-8), and IL-17 have been detected in the skin and/or serum of HS patients.[8-11] Unravelling the role of cytokines and chemokines in disease initiation and progression is important for the clinical and therapeutic stratification of HS. Identification of biomarkers in HS could help in personalising treatments, and may aid in monitoring the efficacy of treatments.

To date, no studies have investigated the inflammatory protein levels in the serum/plasma and skin *in parallel* in a cohort of HS patients at a single time point. 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.

MATERIALS AND METHODS

Ethical statement

The research protocol was approved by the local Institutional Review Board (reference MEC-2013-337/NL45264.078.13). The collection of plasma and skin was conducted in the Department of Dermatology of the Erasmus University Medical Center and Sint Franciscus Hospital in Rotterdam, The Netherlands. All participants provided written informed consent in accordance with the Declaration of Helsinki principles for using their plasma and skin tissue in this study.

Hidradenitis suppurativa patient and healthy control samples

Blood and skin samples were prospectively collected from 20 patients with a dermatologist-verified diagnosis of HS and 10 healthy controls. HS patients, of which 10 were female, had a mean (± SD) age of 41.5 ± 10.6 years, a mean body mass index of 32.1 ± 7.6kg/m^2, and a mean HS duration of 16.4 ± 10.9 years. Seventeen patients were current or past smokers. Disease severity was assessed according to Hurley staging: two patients suffered Hurley I, 14 patients suffered Hurley II, and four patients suffered Hurley III. The healthy control subjects consisted of otherwise healthy patients undergoing dermatologic and plastic surgery who had no family history of HS. Participants receiving any systemic immunomodulators or immunosuppressants, and non-HS patients with any significant inflammatory disease were excluded. Punch biopsies of 4 mm in diameter were obtained and immediately snap-frozen in liquid nitrogen. Skin samples of HS patients were taken from actively inflamed, non-fluctuating, indurated, erythemous lesions or plaques recurring on fixed locations. 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.

In vivo protein quantification in skin homogenates

Plasma and skin samples were analysed using the Meso Scale Discovery (MSD) V-PLEX™ Human Cytokine 30-plex kit (K15054D; Meso Scale Discovery, Gaithersburg, MD) according to the instructions of the manufacturer. MSD assays use electrochemiluminescent labels that allows for analysis of multiple analytes in a variety of sample types.[12]

The plasma and skin samples were thawed at room temperature. Skin biopsies were weighed, thereafter homogenised in 250 µL phosphate-buffered saline (PBS) and grinded using micro pestles. The homogenates were subsequently transferred to 1.5 mL Eppendorf tubes and centrifuged at 13,000 g for 5 minutes at room temperature. Just before analysis, the plasma and supernatant samples were diluted two times for inflammatory cytokines and four times for chemokine analysis in sample diluents. The diluted samples were incubated on the MSD plates for two hours at room temperature with gentle shaking. The plates were rinsed and incubated for an additional two hours with detection antibodies. For CXCL/IL-8 two antibodies were used, both recognizing the same domain of its target. The difference between these antibodies relies on the dynamic range being measured. In addition to the regular antibody, the 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.

After rinsing twice, read buffer T was added to each well and the plate was analysed using the Sector Imager 6000. Calibrator concentrations and skin and plasma samples were analysed in duplicates. The calibrator concentrations were plotted as log signal

unit on the vertical (Y) axis versus log concentration on the horizontal (X) axis using the MSD Workbench software. A weighted four parameter logistic fit (4PL) equation was used for curve fitting and back calculation of sample concentrations.

Immunohistochemistry

Three chemokines, which have not previously been reported to be overexpressed in HS patients, were additionally analysed by immunohistochemistry (IHC). First, paraffin embedded tissue sections were heated at 60°C for 30 minutes, de-paraffinised, and rehydrated. Slides were subsequently placed in pH6 antigen retrieval buffer and heated at 95°C for 20 minutes in a hot water bath. After cooling, slides were treated with 3% H_2O_2 (5 minutes) and blocked using 10% goat serum (30 minutes). Overnight incubation (4°C) was then performed using the primary antibody at a concentration of 10µg/mL. Lastly, slides were washed, treated with secondary antibody, peroxidase (30 minutes) and diaminobenzidine substrate. The following antibodies were used: anti-CXCL10 (Thermofisher Scientific, cat#701225), anti-CCL4 (ThermoFisher Scientific, cat#PA5-23681), anti-CCL26 (Thermofisher Scientific, cat# PA5-75690), and Rabbit IgG control (Lifespan Biosciences, cat#LS-C149375).

Statisical analyses

Plasma protein concentrations were expressed as picogram (pg) per mL. Protein levels of the skin samples were normalised for 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 analysed protein in either the HS or healthy control group had values below the LLOQ, values were substituted by two categories: detectable versus non-detectable, i.e. above versus 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 healthy control and HS samples. For the secondary objective, to assess the correlation between individual plasma and skin protein levels within the HS patients, we used the Spearman's rho for the non-parametrically continuous variables and categorical variables (detectable versus non-detectable).[13] Alternatively, in case of a mismatch in the type of variable (continuous, categorical) between protein levels in skin and plasma, logistic regression models were used to estimate a Nagelkerke's R-squared.

Statistical analyses were conducted using SPSS Statistics 24.0 (IBM Corporation, Armonk, NY). A two-sided *p*-value below 0.05 was considered significant. The level of significance was corrected by a false discovery rate using the Benjamini Hochberg test for multiple comparisons in the plasma and skin samples separately.

RESULTS

Detection of cytokines and chemokines

In total, 30 plasma samples and 30 skin samples from 20 HS patients and 10 healthy controls were analysed by MSD multiplex assay. The protein levels for 30 inflammatory markers in plasma and skin are summarised in Table 1 and Table 2, respectively.

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	-

* Significant after correction with the Benjamini Hochberg test ($p < 0.0042$). 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.

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 analysed because they have not been validated for skin-derived samples.

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	-

* Significant after correction with the Benjamini Hochberg test ($p < 0.014$). 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 analysed, not validated for skin samples. ND: not detected. NN: healthy controls. x: number of samples with a detectable value.

Seven inflammatory markers were significantly elevated in HS patients

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 (IQR) 19.1-37.0 (Figure 1). In contrast, CXCL-10 levels were significantly lower in HS patients, $p = 0.003$. In addition, there was a trend for higher protein levels of IL-1α and lowered levels of IL-12/23p40 in HS patients. 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 2). The median IL-17A protein concentration in lesional HS skin was 0.18 pg/mg tissue (IQR 0.10-0.39). Besides CCL-4, CCL-3 (MIP-1α) was also detected in the majority (11/20) of HS lesional skin samples versus two of ten in the healthy control samples ($p = 0.119$). The elevated CCL-4 and CXCL-10 protein levels in HS lesions were confirmed by IHC (Figure 3). 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 multiplex assay (Table 2, Figure 3).

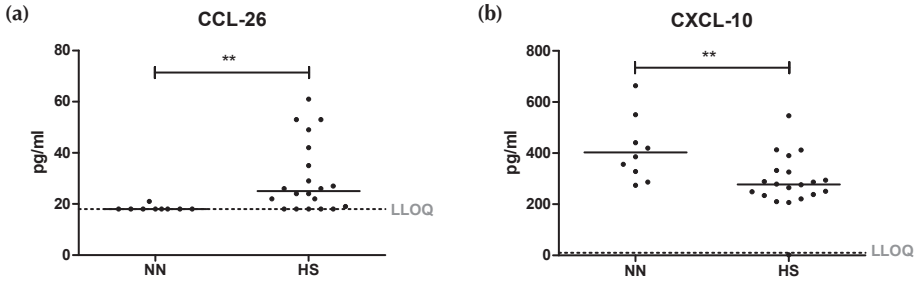

Figure 1. Levels of six elevated inflammatory proteins in lesional skin of HS patients in comparison with healthy control patients. **(a)** CCL-26 ($p = 0.004$). **(b)** CXCL-10 ($p = 0.003$), for NN one data point is out of the y-axis range. Horizontal bars display the median. ** $p < 0.01$. HS: hidradenitis suppurativa patients. LLOQ: lowest limit of quantification. NN: healthy controls.

Weak correlations between protein levels in the plasma and lesional skin of HS patients

Of the 30 proteins, nine were not detected/analysed in either plasma or skin. In 16 analytes, a correlation coefficient (r) for protein concentrations in HS plasma and skin samples was calculated. In general, weak correlations between the protein levels in plasma and skin samples were observed: 13 analytes displayed a negative r (range –0.053 to –0.532), and three analytes had a positive r (range 0.198 to 0.423) (Table 3). The top-3 upregulated

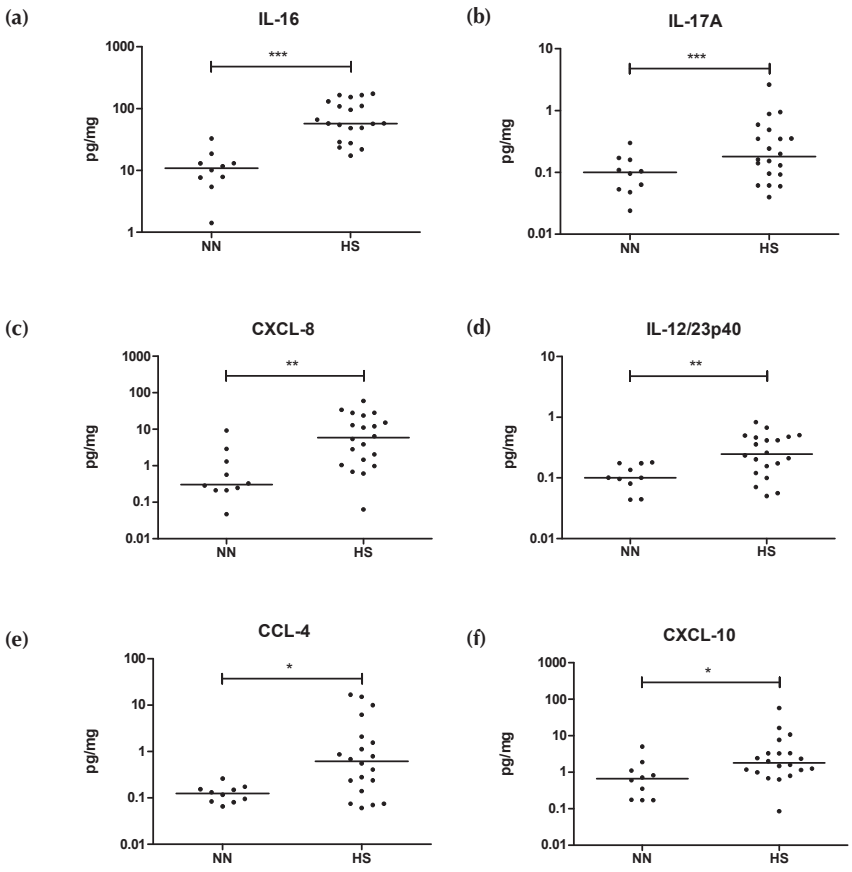

Figure 2. Levels of six elevated inflammatory proteins in lesional skin of HS patients in comparison with healthy control patients. **(a)** IL-16 ($p < 0.001$). **(b)** IL-17A ($p < 0.001$). The LLOQ was used to calculate the concentration of IL-17A in the healthy controls (0 of 10 detected). **(c)** CXCL-8 ($p = 0.001$). **(d)** IL-12/23p40 ($p = 0.007$). **(e)** CCL-4 ($p = 0.011$). **(f)** CXCL-10 ($p = 0.011$). The Y-axis displays a logarithmic scale with the concentrations expressed as pg per mg skin tissue. Horizontal bars display the median. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. HS: hidradenitis suppurativa patients. NN: healthy controls.

cytokines/chemokines in the lesional HS skin in which a correlation was calculated, showed a plasma-skin correlation coefficient of respectively $r = 0.409$ (CCL-4), $r = -0.358$ (CXCL-8), $r = -0.340$ (CXCL-10). None of the correlations was statistically significant ($p > 0.05$). Of note, CCL-2 ($p = 0.016$, unadjusted) and CCL-13 ($p = 0.028$, unadjusted) had no statistically significant plasma-skin correlations after correction for multiple testing. For IL-1ß, TNF-α, IFN-γ, CCL-11, CCL-17 there was a mismatch in the type of variable (continuous, categorical) in plasma and skin. The R-squared values for these five inflammatory markers ranged from 0.004 to 0.096, all statistically nonsignificant.

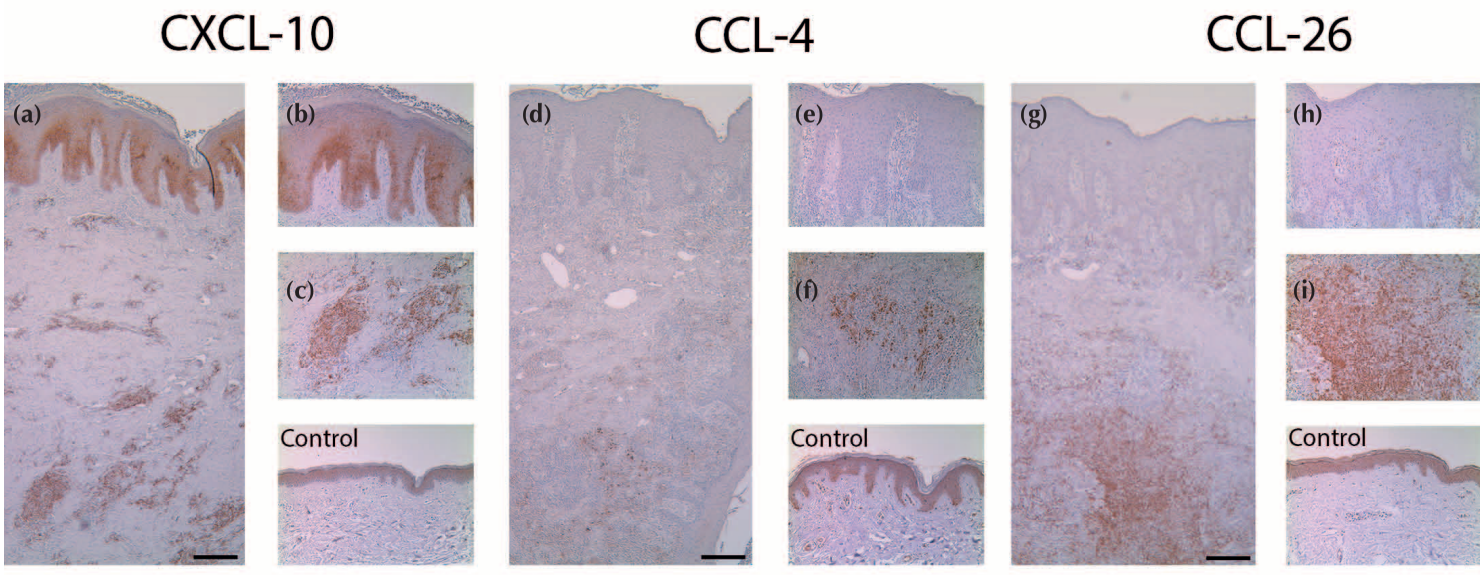

Figure 3. Immunohistochemical detection of CCL-4, CXCL-10 and CCL-26 in HS lesional skin and healthy control skin. CCL-4, CXCL-10 and CCL-26 were not expressed in healthy skin (control panels). CCL-4 **(d, e ,f)** was localised to the inflammatory HS infiltrate, whereas CXCL-10 **(a, b, c)** and CCL-26 **(g, h ,i)** were expressed at a higher intensity. DAB with haematoxylin counterstain. Bar inserted corresponds with 100μM.

Table 3. Correlation between protein expression in plasma and skin of HS patients. None of the inflammatory markers were statistically significant after Benjamini Hochberg correction.

	Protein	Coefficient (r) plasma vs skin	Unadjusted *p*-value
1	**CCL-2 (MCP-1)**	–0.532	0.016
2	**CCL-13 (MCP-4)**	–0.490	0.028
3	**CCL-22 (MDC)**	0.423	0.063
4	**CCL-4 (MIP-1β)**	0.409	0.073
5	**CXCL-8 (IL-8)**	–0.358	0.121
6	**CXCL-10 (IP-10)**	–0.340	0.143
7	**IL-1α**	–0.275	0.240
8	**CCL-3 (MIP-1α)**	–0.254	0.281
9	**IL-12/23p40**	0.198	0.402
10	**TNF-ß**	–0.189	0.317
11	**IL-17A**	–0.126	0.597
12	**IL-6**	–0.101	0.672
13	**IL-16**	–0.098	0.682
14	**IL-10**	–0.090	0.705
15	**IL-15**	–0.078	0.743
16	**IL-13**	–0.053	0.826

r: correlation coefficient (range -1, +1) calculated using the Spearman rho test. Nine markers were not tested for a correlation as these markers were not detected in either skin or plasma: IL-2, IL-4, IL-5, IL-7, IL-12p70, IL-8-HA, CCL-26, VEGF, GM-CSF.

DISCUSSION

In this study we simultaneously measured the protein levels of 30 important inflammatory markers, including Th1 and Th17 cytokines and chemokines, in the plasma and lesional skin of a well-defined cohort of HS patients. In the circulation, CCL-26 was detected significantly more often in HS patients. In lesional skin, the levels of IL-16, IL-17A, IL-23p40, CCL-4, CXCL-8, and CXCL-10 were significantly elevated compared with healthy controls. Remarkably, only weak correlations were found between the protein levels in the plasma and skin of HS patients.

In plasma, the chemokine CCL-26 (also known as eotaxin-3 or MIP-4α) was a newly identified inflammatory marker in HS patients. Significant elevation of this chemokine in the serum has previously been reported in atopic dermatitis, cutaneous T-cell lymphoma and HIV-associated eosinophilic folliculitis, which are characterised by the infiltration of eosinophils, basophils, and specific subpopulations of T cells,[14-16] and all, like HS, diseases characterised by high pruritus scores.[4]

No cytokines were found upregulated in the circulation despite the inclusion of severe cases of HS with 18 of 20 patients with Hurley stage II and III disease severity. Previously, increased serum levels of general inflammatory markers including (high sensitive) CRP, erythrocyte sedimentation rate, neutrophils, monocytes, and IgE have been demonstrated in HS patients.[8,10,17,18] In addition, six studies have reported on specific inflammatory serum markers in HS, of which IL-6, IL-17A, TNF-α, TNF-α receptor I, soluble IL-2 receptor, S100A8, S100A9 and MMP-8 have been found to be upregulated compared with healthy controls.[8,10,19-22]

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.[11,23,24] However, 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.[24,25] This can be explained by the different approaches as in the current study biopsies were homogenised for in situ assessment, while van der Zee *et al.* and Kelly *et al.* cultured the skin biopsies for 24 and three hours respectively. 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. Interestingly, CCL-26 was found in abundance in the HS infiltrate by IHC, 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]

Upregulation of CXCL-10 in the skin is a remarkable finding, because only limited levels of IFN-γ, despite the significant T cell infiltration, have been detected in lesional HS skin. The interleukin IL-16 and chemokines CCL-4 and CXCL-10 are produced by many cell types including macrophages, dendritic cells, B cells, mast cells, eosinophils and T lymphocytes, and play a crucial role in the induction and modulation of immune responses during infection and inflammation.[26,27] In the context of a strong upregulation of CXCL-8, IL-17A and IL-23, we hypothesise that IL-16, CCL-4 and CXCL-10 may participate in the recruitment of leucocyte subsets, especially neutrophils, eosinophils, monocytes and dendritic cells, into the inflamed lesional HS skin.[28,29] The importance of neutrophils in the pathophysiology of HS 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.[30] Furthermore, it has been demonstrated that activated neutrophils induce chemotaxis of Th17 cells by a reciprocal cross-talk.[31]

The poor correlation between cutaneous cytokine and chemokine protein levels was reflected by all coefficients except for CCL-2 being below (+/–)0.50, i.e. a low to negligible correlation.[13] Moreover, most of the analytes demonstrated a negative (inverse) correlation between expression in plasma and skin. The overexpression of CXCL-10 in HS lesions might explain the lower plasma levels as CXCL-10 could have been attracted from the circulation into lesional skin and bound by target cells to fuel

local inflammation. A similar observation was reported in a recent study, in which plasma concentrations of complement factor C5a and C5b9 were higher among patients with mild disease than those with moderate or severe HS.[32] Collectively, our findings indicate that enhanced levels of cytokines and chemokines in lesions of HS patients is generally not reflected in the plasma or serum.

Our study has several strengths including the *in parallel* assessment of inflammatory markers in skin and plasma using a 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. Lastly, the cross-sectional study design did not allow for analysis of cytokine- and chemokine modulation by anti-inflammatory therapies.

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.

REFERENCES

1 Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. *JAMA* 2017; **318**: 2019-32.

2 Dufour DN, Emtestam L, Jemec GB. Hidradenitis suppurativa: a common and burdensome, yet under-recognised, inflammatory skin disease. *Postgrad Med J* 2014; **90**: 216-21.

3 Matusiak L, Bieniek A, Szepietowski JC. Hidradenitis suppurativa markedly decreases quality of life and professional activity. *J Am Acad Dermatol* 2010; **62**: 706-8, 8 e1.

4 Vossen A, Schoenmakers A, van Straalen KR *et al.* Assessing Pruritus in Hidradenitis Suppurativa: A Cross-Sectional Study. *Am J Clin Dermatol* 2017; **18**: 687-95.

5 Matusiak L, Szczech J, Kaaz K *et al.* Clinical Characteristics of Pruritus and Pain in Patients with Hidradenitis Suppurativa. *Acta Derm Venereol* 2018; **98**: 191-4.

6 Prens E, Deckers I. Pathophysiology of hidradenitis suppurativa: An update. *J Am Acad Dermatol* 2015; **73**: S8-S11.

7 van der Zee HH, de Ruiter L, Boer J *et al.* Alterations in leucocyte subsets and histomorphology in normal-appearing perilesional skin and early and chronic hidradenitis suppurativa lesions. *Br J Dermatol* 2012; **166**: 98-106.

8 Jimenez-Gallo D, de la Varga-Martinez R, Ossorio-Garcia L *et al.* The Clinical Significance of Increased Serum Proinflammatory Cytokines, C-Reactive Protein, and Erythrocyte Sedimentation Rate in Patients with Hidradenitis Suppurativa. *Mediators Inflamm* 2017; **2017**: 2450401.

9 Hessam S, Sand M, Gambichler T *et al.* Interleukin-36 in hidradenitis suppurativa: evidence for a distinctive proinflammatory role and a key factor in the development of an inflammatory loop. *Br J Dermatol* 2018; **178**: 761-7.

10 Matusiak L, Szczech J, Bieniek A *et al.* Increased interleukin (IL)-17 serum levels in patients with hidradenitis suppurativa: Implications for treatment with anti-IL-17 agents. *J Am Acad Dermatol* 2017; **76**: 670-5.

11 van der Zee HH, Laman JD, de Ruiter L *et al.* Adalimumab (antitumour necrosis factor-alpha) treatment of hidradenitis suppurativa ameliorates skin inflammation: an in situ and ex vivo study. *Br J Dermatol* 2012; **166**: 298-305.

12 Chowdhury F, Williams A, Johnson P. Validation and comparison of two multiplex technologies, Luminex and Mesoscale Discovery, for human cytokine profiling. *J Immunol Methods* 2009; **340**: 55-64.

13 Mukaka MM. Statistics corner: A guide to appropriate use of correlation coefficient in medical research. *Malawi Med J* 2012; **24**: 69-71.

14 Kagami S, Kakinuma T, Saeki H *et al.* Significant elevation of serum levels of eotaxin-3/CCL26, but not of eotaxin-2/CCL24, in patients with atopic dermatitis: serum eotaxin-3/CCL26 levels reflect the disease activity of atopic dermatitis. *Clin Exp Immunol* 2003; **134**: 309-13.

15 Yokobayashi H, Sugaya M, Miyagaki T *et al.* Analysis of serum chemokine levels in patients with HIV-associated eosinophilic folliculitis. *J Eur Acad Dermatol Venereol* 2013; **27**: 212-6.

16 Suga H, Sugaya M, Miyagaki T *et al.* Association of nerve growth factor, chemokine (C-C motif) ligands and immunoglobulin E with pruritus in cutaneous T-cell lymphoma. *Acta Derm Venereol* 2013; **93**: 144-9.

17 Pascual JC, Garcia-Martinez FJ, Martorell A *et al.* Increased total serum IgE levels in moderate-to-severe hidradenitis suppurativa. *Br J Dermatol* 2016; **175**: 1101-2.

18 Miller IM, Ring HC, Prens EP *et al.* Leukocyte Profile in Peripheral Blood and Neutrophil-Lymphocyte Ratio in Hidradenitis Suppurativa: A Comparative Cross-Sectional Study of 462 Cases. *Dermatology* 2016; **232**: 511-9.

19 Matusiak L, Bieniek A, Szepietowski JC. Soluble interleukin-2 receptor serum level is a useful marker of hidradenitis suppurativa clinical staging. *Biomarkers* 2009; **14**: 432-7.

20 Matusiak L, Bieniek A, Szepietowski JC. Increased serum tumour necrosis factor-alpha in hidradenitis suppurativa patients: is there a basis for treatment with anti-tumour necrosis factor-alpha agents? *Acta Derm Venereol* 2009; **89**: 601-3.

21 Wieland CW, Vogl T, Ordelman A *et al.* Myeloid marker S100A8/A9 and lymphocyte marker, soluble interleukin 2 receptor: biomarkers of hidradenitis suppurativa disease activity? *Br J Dermatol* 2013; **168**: 1252-8.

22 Tsaousi A, Witte E, Witte K *et al.* MMP8 Is Increased in Lesions and Blood of Acne Inversa Patients: A Potential Link to Skin Destruction and Metabolic Alterations. *Mediators Inflamm* 2016; **2016**: 4097574.

23 Schlapbach C, Hanni T, Yawalkar N *et al.* Expression of the IL-23/Th17 pathway in lesions of hidradenitis suppurativa. *J Am Acad Dermatol* 2011; **65**: 790-8.

24 Kelly G, Hughes R, McGarry T *et al.* Dysregulated cytokine expression in lesional and nonlesional skin in hidradenitis suppurativa. *Br J Dermatol* 2015; **173**: 1431-9.

25 van der Zee HH, de Ruiter L, van den Broecke DG *et al.* Elevated levels of tumour necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-10 in hidradenitis suppurativa skin: a rationale for targeting TNF-alpha and IL-1beta. *Br J Dermatol* 2011; **164**: 1292-8.

26 Menten P, Wuyts A, Van Damme J. Macrophage inflammatory protein-1. *Cytokine Growth Factor Rev* 2002; **13**: 455-81.

27 Lee EY, Lee ZH, Song YW. CXCL10 and autoimmune diseases. *Autoimmun Rev* 2009; **8**: 379-83.

28 Wolpe SD, Davatelis G, Sherry B *et al.* Macrophages secrete a novel heparin-binding protein with inflammatory and neutrophil chemokinetic properties. *J Exp Med* 1988; **167**: 570-81.

29 Tecchio C, Cassatella MA. Neutrophil-derived chemokines on the road to immunity. *Semin Immunol* 2016; **28**: 119-28.

30 Souwer Y, Groot Kormelink T, Taanman-Kueter EW *et al.* Human TH17 cell development requires processing of dendritic cell-derived CXCL8 by neutrophil elastase. *J Allergy Clin Immunol* 2018; **141**: 2286-9 e5.

31 Pelletier M, Maggi L, Micheletti A *et al.* Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010; **115**: 335-43.

32 Kanni T, Zenker O, Habel M *et al.* Complement activation in hidradenitis suppurativa: a new pathway of pathogenesis? *Br J Dermatol* 2018; **179**: 413-9.