Materials & Methods

M1. HS patients and control patients

HS patients, of which 10 were female, had a mean age of 41.5 years (standard deviation [SD] 10.6), a mean body mass index of 32.1 kg m^{-2} (SD 7.6), and a mean HS duration of 16.4 years (SD 10.9). Seventeen patients were current or past smokers. Disease severity was assessed according to Hurley staging: 2 patients suffered Hurley I, 14 patients suffered Hurley II, and 4 patients suffered Hurley III. The control subjects consisted of otherwise healthy patients undergoing dermatologic and/or 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.

M2. *In vivo* protein quantification of skin homogenates

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.

M3. Immunohistochemistry

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).

M4. Statistical analysis of the secondary outcome

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 both the non-parametrically continuous variables and categorical variables (detectable versus non-detectable). 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.

Results

R1. 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 3 analytes had a positive *r* (range +0.198 to +0.423) (Table S1). The top-3 upregulated 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-ɣ, 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 ($p < 0.05$).

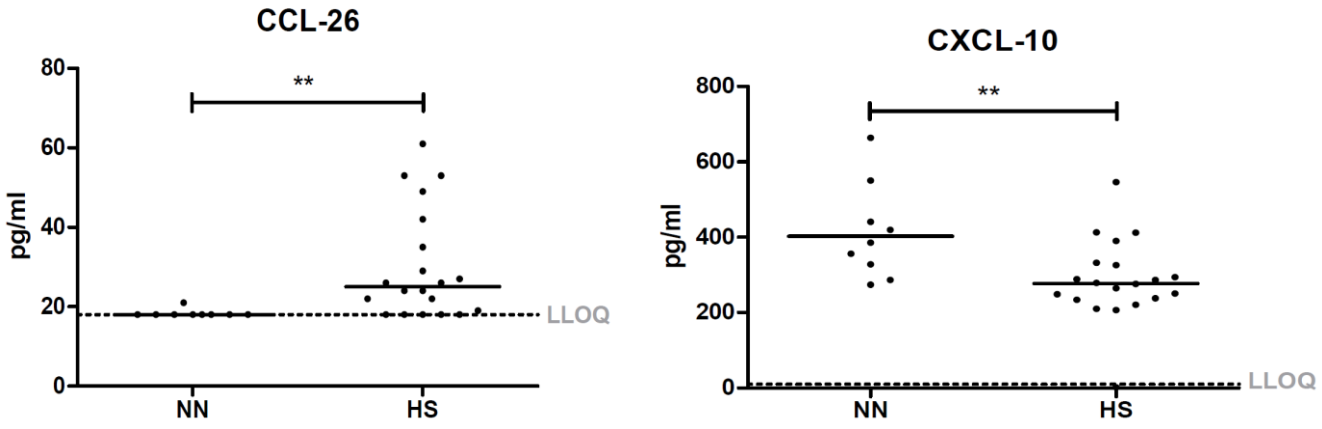
Tables & Figures

Table S1. Correlation between protein expression in plasma and skin of HS patients (n = 20). 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

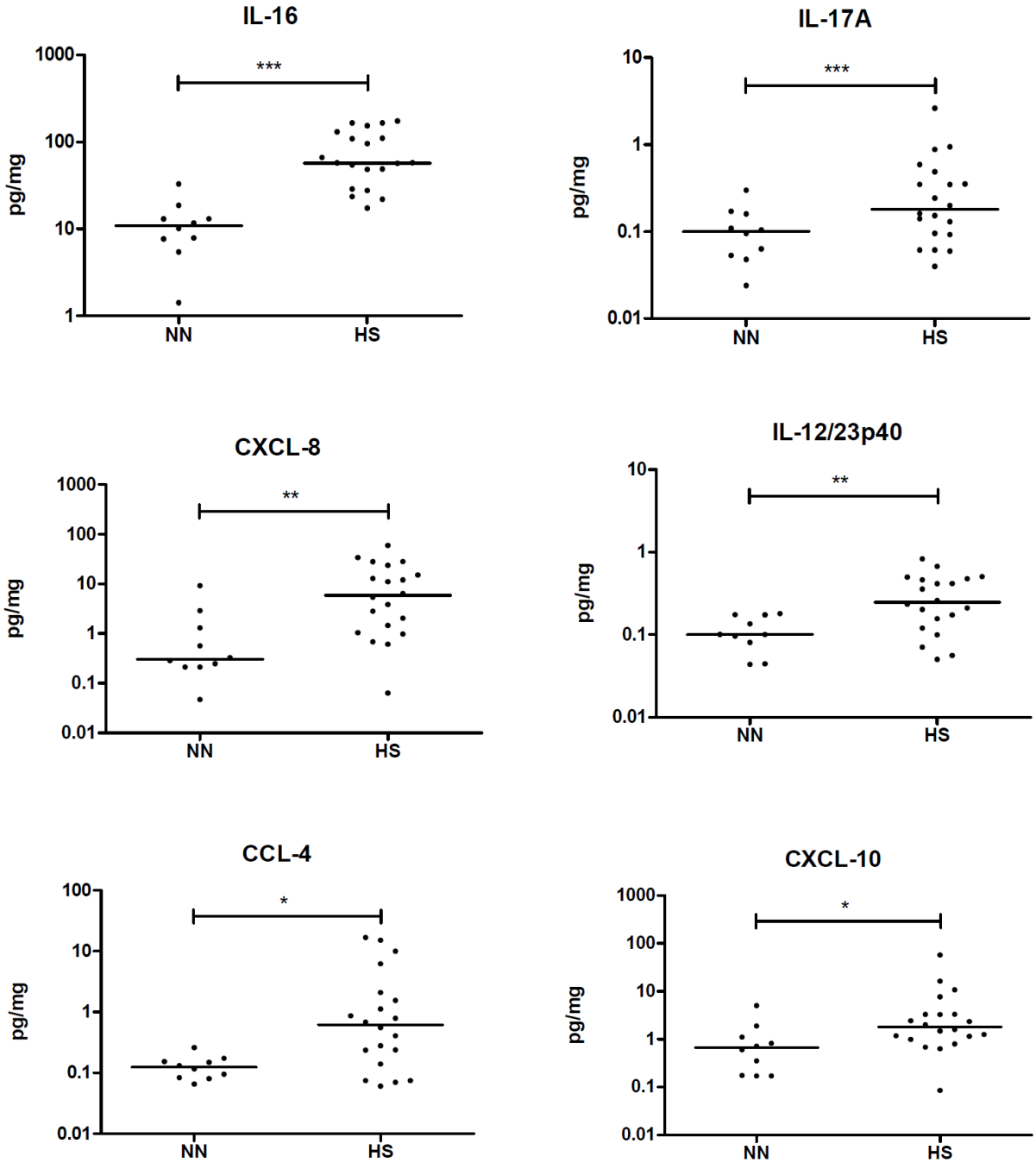
r: correlation coefficient (range -1 to +1) calculated using the Spearman rho test. Nine markers were not tested for a correlation as these markers were not detected in either skin or plasma: IL-2, IL-4, IL-5, IL-7, IL-12p70, IL-8-HA, CCL-26, VEGF, GM-CSF.

Figure S1. Significantly different levels of CCL-26 (p = 0.004) and CXCL-10 (p = 0.003) in plasma of HS patients (n = 20) in comparison with healthy control subjects (n = 10).



Horizontal bars display the median. For CXCL-10, one data point of NN is out of the y-axis range. ** $p < 0.01$. HS: hidradenitis suppurativa patients. LLOQ: lowest limit of quantification. NN: healthy controls.

Figure S2. Six significantly elevated inflammatory proteins in lesional skin of HS patients (n = 20) in comparison with healthy control subjects (n = 10): IL-16 ($p < 0.001$), IL-17A ($p < 0.001$), CXCL-8 ($p = 0.001$), IL-12/23p40 ($p = 0.007$), CCL-4 ($p = 0.011$), CXCL-10 ($p = 0.011$).



The Y-axis displays a logarithmic scale with the concentrations expressed as pg per mg dry skin tissue. Horizontal bars display the median. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. HS: hidradenitis suppurativa patients. NN: healthy controls. The lowest limit of quantification (LLOQ) was used to calculate the concentration of IL-17A in the healthy controls (0 of 10 detected).

Figure S3. Immunohistochemical detection of CCL-4, CXCL-10 and CCL-26 in HS lesional skin and healthy control. CCL-4, CXCL-10 and CCL-26 were not expressed in healthy skin (control panels). CCL-4 was localised to the inflammatory HS infiltrate, whereas CXCL-10 and CCL-26 were expressed at a higher intensity. DAB with haematoxylin counterstain. Bar inserted corresponds with 100μM.