Hidradenitis suppurativa is a chronic inflammatory dermatosis with presentations ranging from painful nodules and abscesses to draining tunnels. Using an unbiased proteomics approach, we assessed cardiovascular-, cardiometabolic-, and inflammation-related biomarkers in the serum of patients with moderate-to-severe hidradenitis suppurativa. The serum of patients with hidradenitis suppurativa clustered separately from that of healthy controls and had an upregulation of neutrophil-related markers (Cathepsin D, IL-17A, CXCL1). Patients with histologically diagnosed dermal tunnels had higher serum lipocalin-2 levels compared with those without tunnels. Consistent with this, patients with tunnels had a more neutrophilic-rich serum signature, marked by Cathepsin D, IL-17A, and IL-17D alterations. There was a significant serum–skin correlation between proteins in the serum and the corresponding mRNA expression in skin biopsies, with healthy-appearing perilesional skin demonstrating a significant correlation with neutrophil-related proteins in the serum. CSF3 mRNA levels in lesional skin significantly correlated with neutrophil-related proteins in the serum, suggesting that CSF3 in the skin may be a driver of neutrophilic inflammation. Clinical significantly correlated with the levels of lipocalin-2 and IL-17A in the serum. Using an unbiased, large-scale proteomic approach, we demonstrate that hidradenitis suppurativa is a systemic neutrophilic dermatosis, with a specific molecular signature associated with the presence of dermal tunnels.

INTRODUCTION

Hidradenitis suppurativa (HS) is a chronic inflammatory disease with an estimated prevalence of 1% (Jemec and Kimball, 2015; Jemec et al., 1996; Sabat et al., 2020). HS has a wide spectrum of clinical presentations, ranging from inflamed nodules and abscesses to interconnecting draining tunnels and late-stage fibrotic disease. Patients with HS face multiple comorbidities, including inflammatory bowel disease, depression, sexual dysfunction, and an increased risk of cardiovascular disease and metabolic syndrome (Kurek et al., 2013; Matusiak et al., 2010; Miller et al., 2014; Reddy et al., 2020; Sabat et al., 2012; van der Zee et al., 2014). Current treatment options for HS have limited efficacy (Frew et al., 2020) and are hindered by a lack of blood and serum biomarkers for assessment of inflammatory activity and therapeutic response.

Although the exact pathogenesis of HS remains unclear, recent studies have led to a paradigm shift from the traditional model of follicular occlusion as a driver of the disease to appreciating HS as a systemic inflammatory disorder with alterations involving plasma cells and B cells (Gudjonsson et al., 2020; Lowe et al., 2020); neutrophils (Byrd et al., 2019); dendritic cells (Lowe et al., 2020); macrophages (Byrd et al., 2018; Thomi et al., 2018); and multiple other proinflammatory axes, including the T helper type 17 pathway (Navrazhina et al., 2020; Wolk et al., 2011). However, most of this work has been based on histological and transcriptomic profiling of skin biopsies. Several studies have examined the serum proteome to identify potential disease biomarkers, with varying results regarding the abundance of these cytokines and proteins compared with healthy controls (Blok et al., 2016; Jiménez-Gallo et al., 2017; Vossen et al., 2019; Vossen et al., 2017). A study assessing the in-depth proteomic profile of HS serum to identify the biomarkers of disease is lacking.

Multiple studies have utilized the Olink broad proteomic panels to gain molecular insight into the disease activity in the serum of patients with inflammatory dermatoses, including atopic dermatitis (AD) ( Brunner et al., 2019, 2017), alopecia areata (Glickman et al., 2021), and psoriasis vulgaris, as well as for biomarkers of therapeutic response in psoriasis (Kim et al., 2018). In addition to an increase in inflammatory proteins, these studies have identified alterations in cardiovascular biomarkers, consistent with the systemic inflammation associated with these disorders. In this study, we aimed to evaluate protein expression in HS serum.
RESULTS
The proteomic profile of HS serum is molecularly distinct from that of healthy controls and other systemic inflammatory dermatoses

Using the Olink Proteomics platform (Uppsala, Sweden), we assessed the serum proteome of patients with Hurley II and III HS (n = 22) and body mass index (BMI)–matched healthy control individuals (n = 9) using the inflammation (92 biomarkers), cardiometabolic (92 biomarkers), cardiovascular II (92 biomarkers), and cardiovascular III (92 biomarkers) panels. Patient demographics can be found in Supplementary Table S1. Principal component analysis demonstrated that HS samples clustered separately from healthy volunteers (Figure 1a; Supplementary Figure S1). An unsupervised two-dimensional hierarchical clustering algorithm was used to group the samples on the basis of differentially expressed proteins (DEPs, defined as absolute values of [fold change] ≥ 1.2 and P ≤ 0.05) (Figure 1b). HS serum had a significant increase in neutrophil-related proteins (IL-17A, CXCL1, Cathepsin D), mediators of atherosclerosis (HGF), chemotactic cytokines and receptors (CCL5, IL-4RA), and GFs (TGF-α, HGF, HB-EGF) (Figure 1b) (Bell et al., 2018). HS exhibited elevations of ST2 protein, a biomarker of cardiovascular stress and fibrosis that is a potential predictor for outcomes in patients with heart failure (Villicorta and Maisel, 2016). This finding is consistent with the increased risk of cardiovascular complications in patients with HS (Miller et al., 2014; Reddy et al., 2020).

We then conducted an enrichment analysis of DEPs for Gene Ontology biological processes terms. Pathways that were significantly enriched in the serum of patients with HS relative to that of healthy volunteers are shown in Figure 1c, with the vertical line demonstrating a false discovery rate of 0.05. The most significantly enriched pathways were related to general immune response (positive chemotaxis, chemokine-mediated signaling pathway, lymphocyte chemotaxis, inflammatory response, immune response, signal transduction) and neutrophil-mediated inflammation (neutrophil chemotaxis, neutrophil degranulation). Because the serum contains secreted proteins, we assessed the cellular structures in which the DEPs are localized to function. Protein annotation through evolutionary relationship statistical overrepresentation test for Gene Ontology cellular component terms identified tertiary granule lumen ($P = 3.95E-05$), specific granule lumen (neutrophil associated) ($P = 5.57E-05$), and extracellular space ($P = 9.42E-13$) as the cellular locations in which HS-specific proteins functioned (Mi et al., 2019) (Figure 1d). Given that smoking may be associated with HS, we performed a sensitivity analysis to account for smoking status. There were seven proteins differentially expressed between HS smokers and nonsmokers (SLAMF7, QPCT, CHIL1, SELE, NCAM1, MB, and SERPINA7). None of these proteins were differentially expressed between patients with HS and healthy controls regardless of the smoking status.

Because we observed systemic inflammation in HS serum, we compared the HS serum proteome with previously published Olink cardiovascular and inflammation panels in AD and psoriasis vulgaris (Brunner et al., 2017) using absolute value (fold change) ≥ 1.2 and $P ≤ 0.05$ (Figure 1e). All three dermatoses had an upregulation of mediators involved in atherosclerosis (HGF) (Bell et al., 2018). Compared with AD and psoriasis, HS only had 11 unique DEPs. HS was characterized by a significant upregulation of proteins related to neutrophil chemotaxis (CXCL1) and biomarkers of cardiovascular disease (ST2), with downregulation of IL-17D. HS was more akin to psoriasis, with an upregulation of T helper type 17 pathway (IL-17A) and neutrophil-related proteins (IL-17A, Cathepsin D, CCL24). Both HS and AD had an upregulation of GF TGF-α and IL-4 immune signaling proteins (IL4-RA, SLAMF1).

LCN2 differentiates HS subtypes in serum
Lipocalin-2 (LCN2), also known as neutrophil gelatinase–associated lipocalin, has been suggested as a potential biomarker in HS, with reports identifying elevated levels of LCN2 in serum of patients with HS and palmoplantar pustular psoriasis (Wolk et al., 2018, 2017). LCN2 is a potent chemoattractant for neutrophils, promoting adhesion and extravasation of granulocytes (Schroll et al., 2012). LCN2 can also be used to measure inflammation in the context of inflammatory bowel disease (Chassaing et al., 2012; Thorsvik et al., 2017). Given the neutrophil signature detected in HS serum and the applicability of LCN2 as a biomarker of inflammatory disease, we asked whether LCN2 is elevated in our HS cohort. Unsupervised two-dimensional hierarchical clustering of all samples arranged by increasing LCN2 levels identified two HS subgroups: a subset of patients with HS with high LCN2 levels and a subset of patients with HS with low LCN2 levels, which clustered more closely with healthy controls (Figure 2a). We identified a node of the dendrogram that was associated with increasing levels of LCN2 (black box, Figure 2a). This cluster identified proteins directly proportional to LCN2 levels in the serum, including neutrophil-related proteins (AZU1, MPO, EN-RAGE, DEFA1, CEACAM8, matrix metalloproteinase 9, CXCL8) (Figure 2b and c). Phylogenetic tree clustering of all the samples demonstrated two distinct subtypes of HS on the basis of high or low LCN2 levels in the serum (Figure 2d). We then evaluated the clinical and histological parameters associated with patients in each subgroup. The majority of the patients in the LCN2-high subgroup had histologically diagnosed dermal HS tunnels (on the basis of ultrasound examination of HS skin as well as on the basis of the presence of a visible tunnel on the histological assessment of the biopsy) compared with those in the LCN2-low subgroup, in which patients did not have histologically diagnosed tunnels. Because the criteria of histologically confirmed dermal tunnels were used, it is plausible that a patient may have had a tunnel that was missed by the punch biopsy, thus explaining the two outliers in the cohort (Figure 2d).

HS patients with tunnels have a different serum proteomic profile than patients without tunnels
We then compared the DEPs in the sera of HS patients with and without histologically confirmed tunnels, with fold change relative to healthy controls shown (Figure 3a), and also conducted an enrichment analysis of the DEPs unique to
HS samples with tunnels (relative to healthy controls) using the canonical, Kyoto Encyclopedia of Genes and Genomes, Reactome, and bioCarta pathways. Serum of HS patients with tunnels had an enrichment of pathways involved in proliferation and signal transduction, extracellular matrix remodeling, and tissue development (development biology, axon guidance, pathways in cancer) (Figure 3b). There were 41 unique DEPs in tunnel samples compared with 23 proteins unique to the nontunnel samples, both relative to healthy controls (Figure 3c). There was minimal overlap between tunnel and nontunnel samples (five proteins). Smoking status did not influence the serum proteome between patients with and those without tunnels; of the seven DEPs between HS smokers and nonsmokers, only one (SERPINA7) was differentially expressed between tunnel and nontunnel HS samples.

HS samples with tunnels had a neutrophilic signature (Cathepsin D, IL-17A, IL-17D, LCN2) compared with HS samples without tunnels. Serum of HS patients with tunnels had an upregulation of cardiovascular-associated biomarkers (HGF, ST2, PGLYRP1). Given the neutrophilic signature associated with tunnels and that pus draining from tunnels is neutrophil mediated, we asked whether patients with draining or nondraining tunnels had a different serum proteome profile (Figure 3d). There was a significant difference in the levels of neutrophil-related proteins (IL-17A, LCN2, CXCL8, EN-RAGE, DEFA1, matrix metalloproteinase 9) between HS samples with draining and nondraining tunnels. In cases where there was no significant difference in the protein levels between the healthy volunteers and HS patients or between tunnel and nontunnel samples, there was a significant difference in the protein levels between samples with draining and nondraining tunnels (EN-RAGE, DEFA1, matrix metalloproteinase 9). This suggests that patients with actively draining tunnels have a different serum proteome profile. Furthermore, cardiovascular biomarker ST2 was significantly elevated in patients with draining tunnels, further linking the role of tunnels and the increased risk for cardiovascular comorbidities in HS (Figure 3d).
Figure 2. Serum LCN2 levels differentiate HS into two subgroups. (a) Unsupervised hierarchical clustering of all proteins in HS HV control serum arranged by increasing levels of LCN2. Red indicates the upregulated protein expression levels, and blue indicates the downregulated protein expression levels. The blue circle demonstrates the node of interest on the dendogram, with the black box identifying a cluster of proteins that are directly proportional to LCN2 levels. (b) Magnification of the LCN2-related cluster of proteins identifies a clear demarcation of two HS subgroups on the basis of the protein level of LCN2 in serum. (c) Pearson correlation between expression of LCN2 and other neutrophil-related proteins in HS serum. $r$ is Pearson correlation. (d) High LCN2 subgroup consists of patients with histologically diagnosed tunnels, with serum samples from this subgroup clustering separately and away from those of patients without tunnels and low LCN2 levels. HS, hidradenitis suppurativa; HV, healthy volunteer; MMP, matrix metalloproteinase.
Correlation of biomarkers suggests a skin‒blood interaction in HS
We examined lesional (LS), and healthy-appearing perilesional (PL) and nonlesional skin biopsies as previously described (Frew et al., 2019) (Figure 4a). We first asked whether there was any correlation between HS skin and serum by studying IL-6, which has been previously suggested as a biomarker in HS serum (Jiménez-Gallo et al., 2017). IL-6 protein level in the serum was significantly correlated with IL-6 mRNA in LS ($r = 0.62, P = 0.0096$), PL ($r = 0.56, P = 0.0138$), and nonlesional ($r = 0.5, P = 0.0261$) skin (Figure 4b). We focused further analysis on PL skin biopsies because the overall RNA quality was better in PL samples than in LS samples, consistent with the increased presence of neutrophils in lesions of LS skin. There was a significant correlation between the proteins involved in the IFN axis (CXCL9, CXCL11), known psoriasis-related proteins (peptidase inhibitor-3/elafin, SELP), B-cell related protein (IGLC2), neutrophil-related markers (LCN2, SERPINA5, SLAMF1), and markers associated with general inflammation (HGF, HO-1) (Figure 4c).

G-CSF or CSF3 is a major hematopoietic cytokine regulating granulopoiesis and is involved in inducing both granulocyte production and release from the bone marrow (Furze and Rankin, 2008; Semerad et al., 2002). Given the strong correlation between neutrophil-related biomarkers in the serum and skin and the neutrophilic signature associated with HS overall, we asked whether CSF3 is a possible driver of increased neutrophil activity. The mRNA levels of CSF3 were elevated in the LS and PL skin of patients with HS compared with the skin from healthy volunteers. Therefore, we asked whether there was a correlation between CSF3 levels in LS skin and neutrophil-related biomarkers in the serum (Figure 4d). Indeed, many of the neutrophil-related markers correlated with increased expression of CSF3 mRNA in the skin, suggesting that the active inflammatory lesion may be driving the recruitment of neutrophils and thus increasing the expression of neutrophil-related proteins in the serum (Figure 4d).

Levels of neutrophil-related proteins in the serum correlate with HS clinical activity
We then asked whether clinical characteristics correlated with LCN2 and IL-17A protein levels in the serum (Figure 5). Given that only patients with advanced HS (Hurley stages II and III) were included in this study, we could not correlate the serum levels of LCN2 and IL-17A with Hurley stage. However, patients with Hurley stage III were more likely to present with draining tunnels ($P = 0.0091$) and thus were more likely to have neutrophilic inflammation in the serum, consistent with the analysis in Figure 3d. Unlike the

International Hidradenitis Suppurativa Severity Score System criteria, Hurley staging does not take into account the presence of nodules, abscesses and draining tunnels in a weighted approach. International Hidradenitis Suppurativa Severity Score System, which assigns weighted points to the number of nodules, abscesses, and draining tunnels or fistulae, correlated the most with serum protein levels of LCN2 and IL-17A, suggesting its utility as a tool to quantify disease activity and clinical response in HS. Therefore, our data suggest that the International Hidradenitis Suppurativa Severity Score System score may be more representative of disease activity than Hurley staging. Furthermore, International Hidradenitis Suppurativa Severity Score System scores also correlated with other markers of general inflammation (TNF-$
\alpha$, IL-6) and biomarkers of cardiovascular risk (ST2, HGF, TIE2). This may suggest that patients with more severe HS are at an increased risk of cardiovascular disease.

DISCUSSION
This study presents a large-scale proteomic analysis of serum and skin from patients with moderate-to-severe HS. Consistent with previous reports in the skin, we identified an elevation of IL-17A in HS serum (Kelly et al., 2015; Lima et al., 2016; Navrazhina et al., 2020). We demonstrate systemic neutrophilic inflammation in HS, consistent with elevated absolute neutrophil counts in the blood (Supplementary Table S1). Unbiased analysis of samples demonstrated clustering of HS on the basis of high and low LCN2 expression in the serum, which corresponded with histologically confirmed presence or absence of epithelialized dermal tunnels, respectively. This is consistent with neutrophils and keratinocytes (likely from the epithelialized tunnels) being the source of LCN2 elevation (Wolk et al., 2017). There was a significant serum‒skin correlation of neutrophilic markers present in the PL skin, suggesting that there is an ongoing systemic inflammation even in healthy-appearing skin. Consistent with this, smaller-scale studies have shown that there is an upregulation of proinflammatory pathways even in healthy-appearing unaffected skin, further giving credence to the concept of HS as a systemic dermatosis (Navrazhina et al., 2020; Sanchez et al., 2019; van der Zee et al., 2011). Consistent with our work, previous ELISA-based analysis of HS serum has demonstrated dysregulation in pathways involving general inflammation (Blok et al., 2016), neutrophil activation (Wolk et al., 2017), complement pathway (Hofman et al., 2018), and antibody formation (Assan et al., 2020). We identified CSF3 in the skin as a potential regulator of neutrophilic inflammation in the serum. Taken together, our data suggest that HS has significant clinical and molecular heterogeneity, demonstrating that HS patients with dural tunnels have a different proteomic profile.

HS is a heterogeneous disease in its clinical presentation; however, it is unknown whether different morphological structures manifest in unique inflammatory signatures in HS skin and serum. We present a large-scale proteomic analysis demonstrating an unbiased clustering of HS disease into distinct subgroups. HS had fewer DEPs than psoriasis and AD, which could reflect a smaller body surface area affected, the compartmentalization of immune response in HS and the heterogeneity of our patient cohort likely affecting the number of statistically significant DEPs. When subdivided by the presence of tunnels, there was a higher number of DEPs than when examining the entire heterogeneous group. We demonstrate an interesting association between a morphological structure in the skin (tunnels) and serum biomarkers. Patients with draining tunnels had significantly higher levels of neutrophil-related proteins in the serum (IL-17A, MPO, LCN2, CXCL8, EN-RAGE, DEF3A, matrix metalloproteinase 9) than those with nondraining tunnels. This is consistent with
Figure 3. HS patients with tunnels have a different serum proteome profile compared to patients without tunnels. (a) Heatmap of all differentially expressed proteins (abs [FCH] ≥ 1.2, and P ≤ 0.05) between HVs and HS patients without tunnels, HVs and HS patients with tunnels, or between HS patients with and without tunnels. FCHs relative to those of HVs are shown; *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. (b) Enriched biological processes in the serum of HS patients with tunnels by canonical, KEGG, Reactome and BioCarta pathways using the XGR tool. Vertical line shows FDR = 0.05. (c) Venn Diagram of the differentially expressed proteins in HS patients with and without tunnels relative to HVs. (d) Olink expression of serum protein levels shown in Log2(Expression) for neutrophil.
the pus in draining tunnels being neutrophil mediated. Interestingly, HS patients with tunnels demonstrated an enrichment of pathways related to ECM remodeling and developmental biology. These signatures may explain the development of dermal tunnels and shift the paradigm from tunnels being an end-stage fibrotic feature of the disease to an active inflammatory structure. LCN2 is a protein secreted by granulocytes, neutrophils, and keratinocytes. TNF-α is a potent inducer of LCN2 in granulocytes, whereas TNF-α and IL-17 have been shown to induce LCN2 production in keratinocytes (Wolk et al., 2017). Consistent with this, patients with tunnels had increased levels of IL-17A and TNF-α in the serum, which provides the direct mechanistic link for the increased levels of LCN2 in patients with tunnels. Whereas some studies have shown that LCN2 is associated with obesity (Koiou et al., 2012; Mosialou et al., 2020; Wang et al., 2007) and could provide some cardiometabolic protection (Mosialou et al., 2020), a study of patients with psoriasis did not find a correlation between BMI and LCN2 levels but did report an elevation of LCN2 in the serum of psoriasis patients compared with healthy controls (Kamata et al., 2012). Similarly, we did not find a significant difference in BMI between LCN2-low and LCN2-high patients in our cohort \( P = 0.34 \). In our study, LCN2 levels in the serum are correlated with neutrophilic markers and the number of tunnels, suggesting that the strong LCN2 signature associated with the disease activity and that the presence of tunnels may supersede any differences related to the BMI. We believe that

Figure 4. There is a significant serum–skin correlation in HS. (a) LS skin was biopsied at an edge of an active inflammatory lesion. PL and NL skin biopsies were taken from healthy-appearing skin 2 cm and 10 cm from the edge of the active inflammatory lesion, respectively, and were biopsied on the same anatomical area as the LS biopsy. (b) Correlation plots of IL-6 protein serum levels and the IL-6 mRNA levels in the LS, PL, and NL skin; scatterplots are shown with estimated linear regression and 95% confidence interval; \( r \) is Pearson correlation. (c) Serum–skin correlation of serum protein levels (Log2 Olink expression) with their corresponding mRNA levels in the PL skin (Log2 mRNA expression). (d) Serum–skin correlation between mRNA levels of CSF3 in the LS skin and the levels of neutrophil-related proteins in the serum. HS, hidradenitis suppurativa; LS, lesional; NL, nonlesional; PL, perilesional.
the elevated LCN2 levels were likely derived from the high inflammatory burden of the disease rather than BMI. This highlights the role of tunnels in disease activity. Patients with draining tunnels had significantly higher levels of ST2 protein than those with nondraining tunnels, suggesting that the inflammation extends beyond the skin and may potentially affect cardiovascular health.

Furthermore, the presence of tunnels influences the time it takes to achieve Hidradenitis Suppurativa Clinical Response in the PIONEER study of adalimumab (Frew et al., 2020, 2021). Our data provide the molecular mechanism for why patients with tunnels have a different disease activity and treatment response (Frew et al., 2021). Differences in biomarkers between tunnels and nontunnel samples may define disease endotypes that could impact therapeutic choices. For example, we found higher levels of TNF in tunnel-positive individuals, and it has been shown that the presence of tunnels increases the time to the clinical response to adalimumab (Frew et al., 2021). We speculate that the high levels of TNF could require the use of high-dose TNF antibodies that may have been shown to be effective in severe cases (Ghias et al., 2020). Our study provides evidence of how morphological structures could influence the molecular profile of patients with HS. Identifying subjects with HS (potentially on the basis of the presence or absence of tunnels) can identify not just novel biomarkers specific to each disease subset but may also potentially identify effective treatment unique to each subgroup with HS.

Our findings demonstrate that the skin may be a driver of the neutrophilic inflammation in the serum as we observed a proportional correlation between CSF3 mRNA in the skin and the neutrophil-related proteins in the serum. We had previously reported an increasing gradient of IL-17 from nonlesional to LS skin (Navrazhina et al., 2020). IL-17 has been shown to stimulate granulopoiesis by inducing G-CSF (Hirai et al., 2012; Schwarzenberger et al., 2000, 1998; Xu and Cao, 2010). It is plausible that IL-17, TNF-α, and IL-6 in HS skin may stimulate G-CSF production by fibroblasts, monocytes, and endothelial cells, which in turn stimulates the release of neutrophils from the bone marrow (Kaushansky, 2006; Xu and Cao, 2010).Potentially, G-CSF could affect cutaneous characteristics as several individual case reports have reported psoriasiform cutaneous eruption in patients receiving G-CSF (Cho et al., 1998; Jang et al., 2017; Kavanaugh, 1996; Mössner et al., 2004).

Acute and chronic pain contributes significantly to the reduced QOL in patients with HS (Savage et al., 2020). In this study, we explored the correlation between clinical parameters and the levels of neutrophilic proteins in the serum. Of particular interest is the correlation between LCN2 and IL-17A and the reported pain levels in HS. The mechanisms of pain levels in HS have not been elucidated. Reports have suggested that neutrophil chemotactic leukotriene B4 as well as the migration cascade of neutrophils themselves can lead to hyperalgesia and mechanical hypernociception (Cunha et al., 2008; Levine et al., 1984). Consistent with this, animal studies have shown that both LCN2 and IL-17A are involved in mechanical hyperalgesia (Ebbinghaus et al., 2017; Jeon et al., 2013). Our data provide a plausible mechanism of how high levels of neutrophilic proteins may contribute to the pain burden in HS.

A strength of our study is that we assessed a large panel of known biomarkers in an unbiased approach. Consistent with our data, previous studies of HS serum demonstrated elevated levels of IL-17A, TNF-α, LCN2, and IL-6 (Jiménez-Gallo et al., 2017; Matusiak et al., 2017, 2009; Wolk et al., 2017). However, the clinical disease heterogeneity in HS complicates the identification of biomarkers. All of the patients in our study were either untreated or had undergone a washout period, therefore eliminating these confounders in the analysis. Furthermore, given that changes in proteomic profiles are related to changes in BMI and fat distribution, we utilized BMI-matched healthy controls (Lind et al., 2020). Importantly, rather than focusing on known disease-associated cytokines, we sought to determine previously unreported disease-associated biomarkers through the use of a large biomarker panel and a hypothesis-free approach.
The limitations of our work include a modest sample size (although comparable with those of other cohorts studied [Blok et al., 2016; Brunner et al., 2019; Wolk et al., 2017]), the use of controls who were older than the HS cohort, and the relative limitation of the Olink platform where analysis is restricted to pregrouped biomarker subsets. Future larger-scale studies are warranted to identify how other HS manifestations (abscesses vs. nodule, draining vs. nondraining tunnel) impact serum proteome. Additionally, our analysis is limited to patients with moderate and severe HS, and it would be desirable to study patients with new onset of HS or patients with mild HS in future studies. If serum biomarkers are also elevated in the early-stage disease, these biomarkers may facilitate diagnosis and decrease the 5–14 year diagnostic delay experienced by patients with HS [Jemec and Kimball, 2015]. Given the cyclical nature of HS severity, marked by debilitating flare ups and a remitting course, serum biomarkers become particularly crucial to assess disease activity and accurately diagnose as well as measure therapeutic response.

In conclusion, we demonstrate that HS is a systemic, inflammatory condition associated with neutrophil-rich signature in the serum, with a significant serum–skin correlation of neutrophilic activity. We identify a highly-inflammatory HS subgroup corresponding with increased LCN2 protein levels in the serum and histologically confirmed tunnels in the skin.

**MATERIALS AND METHODS**

**Patients**

The study was approved by the Institutional Review Board of The Rockefeller University (New York, NY), and written informed consent was obtained. In total, 22 patients with Hurley stage II (n = 15) and stage III (n = 7) and nine BMI-matched healthy controls were included in this study (Supplementary Table S1). Exclusion criteria included being diagnosed with HIV and hepatitis B or C, being currently pregnant, or breastfeeding. Patients were required to undergo a washout period of five half-lives from previous systemic treatments, including all oral antibiotics, retinoid, and biologic therapies.

**Serum protein quantification**

Samples were centrifuged after collection, and serum was stored at −80 °C. Samples were analyzed using the proteomic Olink Proseek multiplex assay. Serum (10 μl) was used for proximity extension assay, which uses a real-time PCR to detect oligonucleotide-labeled antibody probe pairs to individual targets, as previously described [Assarsson et al., 2014; Bettoli et al., 2016]. Samples were assessed using the Olink Inflammation (92 analytes), cardiovascular II (92 analytes) and cardiovascular III (92 analytes), and cardiometabolic panel (92 analytes). All of the samples met the quality control (QC) for the Olink panels with the exception of one healthy control sample that did not meet the QC for cardiovascular II and inflammation panels and was therefore excluded from the analysis within these two panels. Only samples that had detected the expression of all the proteins in the panels were included in the heatmaps.

**Skin mRNA quantification**

RNA from frozen skin biopsies was isolated using miRNeasy Mini Kit (Qiagen, Hilden, Germany), and DNA was removed using on-column DNase digestion from the RNase-free DNase Set (Qiagen). RNA sequencing was performed using NovaSeq 6000 (Illumina, San Diego, CA), and analysis was conducted as previously described [Suárez-Farinás et al., 2015; Visvanathan et al., 2019]. RT-PCR was used to assess CSF3 expression in the LS skin, and expression of CSF3 mRNA in the skin was normalized to the house-keeping gene hARP as previously described [Navrazhina et al., 2020]. Probes used were TaqMan Gene Expression Assay CSF3 (Hs00718432_g1) and hARP (AID1UPS) from Thermo Fisher Scientific (Waltham, MA).

**Statistical analysis**

Statistical analysis was performed in R language (R-project.org, R Foundation, Vienna, Austria) using publicly available Bioconductor Project packages (www.bioconductor.org; Bioconductor Core Team, Buffalo, NY). QC of Olink data was accomplished using Olink’s standard QC pipeline [Lind et al., 2015]. One healthy control sample did not pass the QC for cardiovascular II and inflammatory panel quantification and therefore was excluded from the analysis in both panels. The Olink platform presents data in Normalized Protein eXpression arbitrary Log2 scaled units. Protein expression profiles were modeled with linear models using the limma framework as previously described [Brunner et al., 2017]. This model considers disease state and tunnel status as fixed factors, whereas random effect related to the subjects was included in the model (Brunner et al., 2017; He et al., 2020). The least squared means and comparisons for protein profiles among the different groups were estimated, and hypothesis testing was performed under the general framework for linear models in the limma package. A sensitivity analysis for the smoking status was implemented, demonstrating no imputation-related departures from conclusions reached. DEPs were defined as those with absolute value of (fold change) $\geq 1.2$ and $P \leq 0.05$, consistent with previous studies (Brunner et al., 2017; He et al., 2020) Correlation between mRNA levels in the skin, protein expression in the serum, and clinical parameters were evaluated using Pearson correlations on log2-transformed expression values.

**Pathway analysis**

Gene set enrichment analysis was performed using the eXploring Genomic Relations (accessed 11/15/2020) [Fang et al., 2016] for biological processes pathways, including Kyoto Encyclopedia of Genes and Genomes [Kanehisa et al., 2010], BioCarta (Croft et al., 2014), Reactome (Croft et al., 2014), and Gene Ontology (The Gene Ontology Consortium, 2019). False discovery rate cutoff at 0.05 was used to identify significant enrichment. Statistical over-representation test was performed using Protein Annotation Through Evolutionary Relationship Gene Ontology cellular component complete analysis tool [Mi et al., 2019]. Significance was defined as a false discovery rate < 0.05.

**Data availability statement**

The datasets related to this article can be found at https://doi.org/10.17632/j7bb355tr and https://data.mendeley.com/datasets/jk7bb355tr/2 hosted at Mendeley. All other supporting data are available on written request to the corresponding author.

**ORCIDs**

Kristina Navrazhina: http://orcid.org/0000-0001-1405-2955
Sandra Garcket: http://orcid.org/0000-0002-4465-8547
Juana Gonzalez: http://orcid.org/0000-0001-7933-7017
David Grand: http://orcid.org/0000-0001-1519-7435
John W. Frew: http://orcid.org/0000-0001-5042-3632
James G. Krueger: http://orcid.org/0000-0002-3775-1778

**CONFLICT OF INTEREST**

JGK has received research support (grants paid to institution) from AbbVie, Amgen, Bristol Myers Squibb, Boehringer Ingelheim, EMD Serono,
K Navrazhina et al.
Hidradenitis Suppurativa Serum Proteome Analysis

Innovaderm, Kineta, LEO Pharma, Novan, Novartis, Parexel, Pfizer, Regeneron, and Vitae and personal fees from Abbvie, Acris Organics, Allergan, Aurigen, Biogen Idec, Boehringer Ingelheim, Escalier Biosciences, Janssen, Lilly, Novartis, Pfizer, Roche, and Valeant. JWF has conducted advisory work for Janssen, Boehringer Ingelheim, Pfizer, Kyowa Kirin, LEO Pharma, Regeneron, and UCB; participated in trials for UCB; and received research support from Ortho Dermatologics. The remaining authors state no conflict of interest.

ACKNOWLEDGMENTS
We acknowledge members of the Krueger laboratory for their thoughtful discussions throughout during project and manuscript preparation. KN was supported by a Medical Scientist Training Program grant from the National Institute of General Medical Sciences of the National Institutes of Health under award number T32GM007739 to the Tri-Institutional MD-PhD Program (Weill Cornell Medicine, The Rockefeller University, Memorial Sloan Kettering Cancer Center). JWF and JGK were supported in part by grant # UL1 TR001866 from the National Center for Advancing Translational Science, National Institutes of Health Clinical and Translational Science Award program. JWF was supported by the Shapiro-Silverberg Fund for the Advance ment of Translational Research and the Hidradenitis Suppurativa Foundation Danby Grant.

AUTHOR CONTRIBUTIONS
Conceptualization: KN, JWF, JGK; Data Curation: KN, SG; Formal Analysis: SG, KN; Funding Acquisition: JWF; JGK; Investigation: KN, JG, DG, JWF; Methodology: JG, KN, JWF, DG; Supervision: JGK; Writing - Original Draft Preparation: KN, JWF; Writing - Review and Editing: JGK

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.02.742.

REFERENCES


Lind L, Figarsa S, Sundström J, Fall T, Årnlov J, Ingelsson E. Changes in proteome profiles are related to changes in BMI and fat distribution during 10 years of aging. Obesity (Silver Spring) 2020;28:178–86.


