Biomarkers of tissue turnover and systemic inflammation are associated with disease severity and activity in patients with hidradenitis suppurativa

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Title Page

Title: Biomarkers of tissue turnover and systemic inflammation are associated with disease severity and activity in patients with hidradenitis suppurativa

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Short title: Biomarkers of severity in hidradenitis suppurativa
To the Editor

Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition of the follicular epithelium occurring predominantly in the axillae, inframammary folds, groins and buttocks (Gansevoort et al. 2011). Dependent on the disease severity, various pharmacologic and surgical interventions to prevent and treat new lesions, as well as remove existing inflammatory nodules, abscesses, and sinus tracts, can be initiated. Biomarkers reflecting disease severity and activity of HS could potentially aid the need for better tools to diagnose HS as well as determine treatment effects (Blok et al. 2016). Here both imaging and blood-based techniques have been proposed. For imaging, ultrasound (US) is the most well-characterized method, while other methods such as medical infrared thermography (MIT) and optical coherence tomography (OCT) are emerging methods which demonstrate higher degree of accuracy compared to US (Grand et al. 2019).

The extracellular matrix (ECM) of the skin results in generation of protein fragments released into the blood, which can be quantified as blood-based biomarkers. Such biomarkers reflect scarring and fibrosis of the skin and could be potential emerging targets for HS (Dengjel et al. 2020; Sand et al. 2018). Collagens are the most abundant ECM proteins of the body, where type I, III, IV, VI and VII are the most well described in the skin. Type I and III collagen are primarily found in the dermis, type IV collagen is located in the basement membrane by the junction of epidermis and dermis, type VI collagen is located throughout the dermis, while type VII collagen is known as an anchoring fibril, stabilizing the lower part of the dermo-epidermal basement membrane to the underlying dermis (Dobrota et al. 2021; Sand et al. 2018). In addition to collagens, the interfilament protein vimentin plays a role in macrophage and fibroblast differentiation and keratinocyte differentiation in wound healing (Mortensen et al. 2015), while S100A9, a subunit of calprotectin (S100A8/A9),
contributes to the epithelial cell function including keratinocyte differentiation (Iotzova-Weiss et al. 2015).

We hypothesized that serological biomarkers of ECM remodelling linked to chronic inflammation are upregulated in patients diagnosed with HS compared to healthy subjects and associated with disease severity and activity, and systemic inflammation.

Serum samples were collected from a previously well-characterized cohort of 331 consecutive, newly referred patients with HS attending a tertiary referral center (Department of Dermatology, Bispebjerg Hospital, Copenhagen, Denmark) from 2018-2020 (Nielsen et al. 2021). Written informed consent was obtained. Patients were grouped based on their disease severity defined by Hurley stages; mild = Hurley I (n=148), moderate = Hurley II (n=145), and severe = Hurley III (n=38) and information on clinical characteristics was obtained by examination and interview. Disease activity was measured by Hidradenitis Suppurativa Score (HSS) (Sartorius et al. 2009). The study was approved by the Scientific Ethics Committee of the Capital Region of Denmark, Hillerød, Denmark in compliance with the Helsinki Declaration of 1975 (H-21003878). Serum samples from healthy donors were acquired by BioIVT (London, UK). We examined the competitive ELISA biomarkers C1M, C3M, C4M, C5M, C7M, VICM, CPa9-HNE, C4G, PRO-C3, PRO-C4 and PRO-C6 (Supplementary Table 1). All biomarkers are validated for human serum. The inter- and intra-assay coefficients of variation are >15% and 10% respectively for all assays.

A total of 331 patients with HS (228 women and 93 men) with a mean age of 39.98 years (SD=13.93) were included, together with 16 healthy donors (10 women and 6 men) with a mean age of 35.19 years (SD=7.43) as reference (Table 1).

C1M was significantly higher in patients with Hurley stage II and III compared to stage I (p=0.043 and <0.0001 respectively), and between Hurley stage II and III (p<0.0001). C3M and C4M were
significantly elevated in patients with Hurley stage II and III, compared to Hurley stage I (p=0.014 and 0.001; p=0.0001 and p<0.0001, respectively). No significant differences were detected for C5M. C7M, was significantly elevated in Hurley stage III compared to Hurley stage I and II (p=0.0003 and p=0.0042 respectively). The macrophage activity biomarker, VICM, was significantly higher in patients with Hurley stage II and III compared to stage I (p=0.0167 and p<0.0001 respectively), and between Hurley stage II and III (p=0.0152). The human neutrophil activity biomarker, CPa9-HNE, was significantly higher in Hurley stage III compared to Hurley stage I (p=0.0019). No significant differences were detected between Hurley stages for the biomarkers C4G, PRO-C3, PRO-C4 and PRO-C6 (Figure 1A-K). Spearman’s correlations were performed. Correlations between Rho=0.2-0.4 were considered weak, Rho=0.4-0.6 were considered moderate, while Rho=0.6-0.8 were considered strong correlations. C1M, C3M, C4M, C7M, CPa9-HNE and PRO-C4 were moderately-strongly related to the HSS score (r=0.414, p<0.0001 to r=0.233, p<0.0001, respectively. Supplementary Figure 1). C1M, C3M and PRO-C4 all showed weak positive correlations (r=0.122, p=0.026 to r=0.138, p=0.012), while PRO-C3 presented a weak negative correlation to number of boils in the past month (r=-0.204, p=0.0002, Supplementary Figure 2).

We evaluated a biomarker panel of ECM turnover and systemic inflammation in a large, well-characterized cohort of HS patients with aim to identify potential blood-based biomarkers for HS. There is a need for HS biomarkers to help understand the pathogenesis and to monitor the disease progression. This need is crucial for use in clinical trials, but also to advance knowledge of the biological complexity behind HS. The evaluated biomarkers in this study represent proteins located in dermis, epidermis, and the epidermal basement membrane, reflecting the individual skin compartments remodeled during HS progression. A balanced ECM is necessary to preserve skin integrity and tissue homeostasis (Dengjel et al. 2020; Nisar et al. 2019). Using biomarkers which can detect a local skin manifestation of a systemic disease in circulation, may identify disease
phenotypes (Martorell et al. 2020). Of particular interest are the findings of biomarkers associated with disease severity and disease activity. Inflammatory biomarkers, such as ESR, neutrophils, and CRP, have previously been proposed as biomarkers of disease severity and efficacy of treatment, but with mixed results (Mekkes and Bos 2008; Van Rappard and Mekkes 2012). While it may be difficult to relate serum levels of these inflammatory markers to HS, it may be the burden of both inflammatory and fibrotic factors which contribute to the skin tissue turnover in HS. Measuring end products of tissue destruction, which are the downstream effect of the inflammatory signals, may facilitate better monitoring of tissue turnover.

In summary, this is, to our knowledge, the first study to evaluate novel ECM biomarkers and systemic inflammation in patients with HS. The findings indicate an association between biomarkers of tissue turnover and disease severity and activity. Such biomarkers may be utilized to assess patients’ eligibility to targeted treatments and fill the medical need for biomarkers in clinical management and trials.
DATA AVAILABILITY STATEMENT

All data from this study are included in this article. Data are kept on file and are not publicly available due to privacy data legislation. Requests can be directed to the corresponding author.

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CONFLICT OF INTEREST STATEMENT

SHN, MAK and ACBJ are full-time employees at Nordic Bioscience A/S. Nordic Bioscience is a privately-owned, small–medium size enterprise (SME) partly focused on the development of biomarkers. None of the authors received fees, bonuses or other benefits for the work described in the manuscript. SHN, MAK and ACBJ hold stocks in Nordic Bioscience A/S.

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AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization: SHN, SSG, ACBJ, SFT; Data Curation: SFT; Formal Analysis: SHN, SSG, ACBJ, MK, SFT; Investigation: SHN, SSG, YY, AHRJ, VWN, KG, SFT; Methodology: SHN, SFT; Resources: SHN, SSH, YY, AHRJ, VWN, MK, KG, ACBJ, SFT; Supervision: ACBJ, SFT; Visualization: SHN, SSG, ACBJ, SFT; Writing Original Draft Preparation: SHN; Writing – Review and Editing: SHN, SSG, YY, AHRJ, VWN, MK, KG, ACBJ, SFT.
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**TABLES**

Table 1. Patient characteristics in relation to Hurley stage.

|                        | Healthy controls | Hurley stage 1 n=148 | Hurley stage 2 n=145 | Hurley stage 3 n=38 | Total HS n=331 | P-value |
|------------------------|------------------|-----------------------|----------------------|---------------------|---------------|---------|
| **Age, years, mean (SD)** | 35.19 (7.43)     | 37.15 (13.73)         | 40.88 (13.16)        | 49.62 (15.15)       | 39.98 (13.93) | 0.002   |
| **Age at onset of HS, mean (SD)** | - (11.48)       | 24.25 (11.48)         | 24.32 (11.15)        | 28.38 (17.57)       | 24.75 (12.22) | 0.785   |
| **Duration of HS, mean (SD)** | - (11.41)       | 9.22 (12.12)          | 16.69 (12.00)        | 21.24 (13.00)       | 15.52 (12.19) | <0.00   |
| **Sex, n (%)** | | | | | | 1 |
| Females | 10 (63) | 113 (76) | 103 (71) | 13 (34) | 228 (69) | <0.00 |
| Males | 6 (37) | 35 (24) | 42 (29) | 25 (66) | 93 (31) | 0.01 |
| **BMI, mean (SD), kg/m²** | - (6.60) | 28.79 (6.70) | 29.77 (6.71) | 27.83 (6.71) | 29.11 (6.70) | 0.157 |
| **Smoking, n (%)** | | | | | | 0.034 |
| Never | - | 41 (28) | 29 (20) | 1 (3) | 71 (22) | |
| Former | - | 29 (20) | 35 (24) | 9 (24) | 73 (22) | |
| Current | - | 77 (52) | 81 (56) | 28 (73) | 186 (56) | |
|                             | -        | 1.62 (1.93) | 3.13 (3.68) | 2.57 (3.75) | 2.39 (3.11) | 0.0003  |
|-----------------------------|----------|-------------|-------------|-------------|-------------|---------|
| Boils in the past month,   | -        | 6.22 (2.62) | 6.61 (2.55) | 8.45 (2.19) | 6.65 (2.62) | <0.00 1 |
| mean (SD)                   |          |             |             |             |             |         |
| Pain (VAS), mean (SD)       | -        | 7.80 (5.90) | 19.86 (13.61)| 56.00 (27.19)| 18.62 (19.83)| <0.00 1 |
| HSS, mean (SD)              |          |             |             |             |             |         |
| Systolic blood pressure     |          |             |             |             |             |         |
| Diastolic blood pressure    |          |             |             |             |             |         |
| Previously diagnosed        |          |             |             |             |             |         |
| comorbidities               |          |             |             |             |             |         |
| Hypertension, n (%)         | -        | 17 (11.49%) | 13 (8.97%)  | 7 (18.42%)  | 37 (11.18%) | 0.255   |
| Diabetes, n (%)             | -        | 5 (3.38%)   | 9 (6.21%)   | 9 (23.68%)  | 23 (6.95%)  | 0.0001  |
| Arthritis, n (%)            | -        | 11 (7.43%)  | 13 (8.97%)  | 5 (13.16%)  | 29 (8.76%)  | 0.534   |
| Inflammatory Bowel Disease, | -        | 8 (5.41%)   | 15 (10.34%) | 2 (5.26%)   | 25 (7.55%)  | 0.237   |
| n (%)                       |          |             |             |             |             |         |
| Asthma, n (%)               | -        | 6 (4.05%)   | 5 (3.45%)   | 0 (0%)      | 11 (3.32%)  | 0.459   |
| COPD, n (%) |  | 1 (0.68%) | 3 (2.07%) | 3 (9.68%) | 7 (2.11%) | 0.022 |
|-------------|-------------|-----------|-----------|-----------|-----------|-------|
| **Current treatment, n (%)** | | | | | | |
| Topical azelaic acid | | 18 (12.16) | 11 (7.59) | 3 (7.89) | 32 (9.67) | 0.386 |
| Topical clindamycin | | 29 (19.59) | 17 (11.72) | 5 (13.16) | 51 (15.41) | 0.162 |
| Oral tetracyclines | | 7 (4.73) | 11 (7.59) | 7 (18.42) | 25 (7.55) | 0.017 |
| Oral clindamycin + rifampicin | | 2 (1.35) | 3 (2.07) | 1 (2.63) | 6 (1.81) | 0.830 |
| Biologics (anti-TNF) | | 1 (0.68) | 7 (4.83) | 0 (0%) | 8 (2.42) | 0.041 |
| **Inflammatory blood biomarkers** | | | | | | |
| CRP, mg/L | | 3.52 (3.72) | 5.88 (7.48) | 20.13 | 6.46 | <0.00 |
| ESR, mm/h | | 10.30 | 14.18 | (27.40) | (11.85) | 01 |
| | | (8.50) | (14.08) | 31.51 | 14.46 | |
|                | NLR              | Hemoglobin (10^9/L) | Leucocytes (10^9/L) | Basophils (10^9/L) | Eosinophils (10^9/L) | Lymphocytes (10^9/L) | Monocytes (10^9/L) | Neutrophils (10^9/L) | NLR | Hemoglobin | Leucocytes | Basophils | Eosinophils | Lymphocytes | Monocytes | Neutrophils | NLR | Hemoglobin | Leucocytes | Basophils | Eosinophils | Lymphocytes | Monocytes | Neutrophils |
|----------------|------------------|--------------------|---------------------|--------------------|---------------------|---------------------|---------------------|---------------------|-----|------------|------------|-----------|-------------|----------------|------------|----------------|-----|------------|------------|-----------|-------------|----------------|------------|---------------|-----|------------|------------|-----------|-------------|----------------|------------|---------------|
|                | -                | 2.29 (0.93)        | 2.37 (0.96)         | 3.46 (1.69)        | 2.46 (1.12)        | <0.00               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Hemoglobin    | -                | 8.66 (0.77)        | 8.74 (0.92)         | 8.75 (1.12)        | 8.70 (0.88)        | <0.00               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Leucocytes    | -                | 7.47 (2.23)        | 8.67 (2.96)         | 10.82              | 8.38 (2.86)        | 0.307               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Basophils     | -                | 0.05 (0.03)        | 0.05 (0.03)         | 0.07 (0.03)        | 0.05 (0.03)        | <0.00               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Eosinophils   | -                | 0.19 (0.19)        | 0.20 (0.15)         | 0.23 (0.18)        | 0.20 (0.17)        | 0.004               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Lymphocytes   | -                | 2.13 (0.71)        | 2.38 (0.82)         | 2.29 (0.63)        | 2.26 (0.76)        | <0.00               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Monocytes     | -                | 0.55 (0.15)        | 0.62 (0.24)         | 0.74 (2.84)        | 0.61 (0.22)        | 0.127               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
| Neutrophils   | -                | 4.58 (1.65)        | 5.34 (2.24)         | 5.24 (2.25)        | 0.004              | <0.00               | 0.1                  | 0.1                 | 0.13| 0.86       | 0.74       | 0.67      | 0.05        | 0.05          | <0.00      | 0.05          | <0.00| 0.1         | <0.00      | 0.05      | <0.00        | <0.00         | 0.1      | <0.00        |
|                        | eGFR mL min⁻¹ 1.73m² | Blood glucose, mean (SD), mmol/L | Triglycerides, mmol/L | LDL, mmol/L | HDL, mmol/L | ALAT (U/L) | ALP (U/L) |
|------------------------|----------------------|----------------------------------|----------------------|-------------|-------------|------------|-----------|
|                        | -                    | 86.64 (10.88)                    | -                    | 2.55 (0.84) | 1.45 (0.54) | 22.56 (10.55) | 71.31 (27.46) |
|                        |                      | 85.99 (10.72)                    | 2.59 (0.84)          | 1.50 (0.54) | 1.30 (0.36) | 27.12 (19.50) | 76.08 (74.00) |
|                        |                      | 88.32 (11.58)                    | 2.18 (0.87)          | 1.32 (0.51) | 1.32 (0.51) | 20.32 (10.79) | 91.00 (28.47) |
|                        |                      | 86.35 (10.86)                    | 2.52 (0.85)          | 1.37 (0.47) | 1.37 (0.47) | 24.29 (15.31) | 75.95 (27.03) |
|                        |                      | 0.323                            | 0.0001               | 0.318       | 0.015       | 0.047      | <0.00 01 |

Categorical variables are written as number (percentage), while continuous variables are mean (standard deviation). Statistical differences between the clinical and patient demographics were calculated by a Kruskal-Wallis test. BMI, body mass index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HS, hidradenitis suppurativa; NLR, neutrophil-to-lymphocyte ratio; eGFR, estimated glomerular filtration rate; HSS, hidradenitis suppurativa score; COPD; Chronic obstructive pulmonary disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALAT, alanine aminotransferase; ALP, alkaline phosphatase.
Figure Legends

**Figure 1. Levels of extracellular matrix biomarkers measured in serum from HS patients in Hurley stage I (n=148), Hurley stage II (n=145) and Hurley stage III (n=38).** A-E) Serum levels measuring degradation of type I collagen (C1M), type III collagen (C3M), type IV collagen (C4M), type V collagen (C5M) and type VII collagen (C7M), F) Serum levels of citrullinated degradation of vimentin (macrophage activity), G) Serum levels of calprotectin (S100A9) degraded by human neutrophil elastase (Neutrophil activity), H) Serum levels of type IV collagen degraded by granzyme B (T-cell activity), I) Serum levels of type III collagen formation, J) Serum levels of type IV collagen turnover, 7S domain, and K) Serum levels of type VI collagen formation. Dotted lines represent the IQR of the healthy subjects included as reference. Statistical differences were calculated using an ANCOVA corrected for age and gender. Significance threshold was set at \( p<0.05 \), and data are presented as mean with 95% CI. Significance levels: \(*p<0.05\), **\( p\leq 0.01\), ***\( p\leq 0.001\), ****\( p<0.0001\).
Type I collagen degradation

Type III collagen degradation

Type IV collagen degradation

Type V collagen degradation

Type VII collagen degradation

Citrullinated vimentin degradation (Macrophage activity)

Calprotectin degradation (Neutrophil activity)

Type IV collagen degradation (T-cell activity)

Type III collagen formation

Type IV collagen formation

Type VI collagen formation

- Hurley Stage I
- Hurley Stage II
- Hurley Stage III
**Supplementary Table 1. Description of measured biomarkers.**

| Biomarker  | Description                                                                 | Reference                      |
|------------|-----------------------------------------------------------------------------|--------------------------------|
| C1M        | Neo-epitope of MMP-2, -9, -13 mediated degradation of type I collagen (alpha 1 chain) | (Leeming et al. 2011)          |
| C3M        | Neo-epitope of MMP-9 mediated degradation of type III collagen                | (Vassiliadis et al. 2011)      |
| C4M        | Neo-epitope of MMP-2, -9, -12 mediated degradation of type IV collagen (alpha 1 chain) | (Sand et al. 2013)            |
| C5M        | Neo-epitope of MMP-2,9 mediated degradation of type V collagen                | (Veidal et al. 2012)          |
| C7M        | Neo-epitope of type MMP-13 mediated degradation of type VII collagen         | (Sand et al. 2018)            |
| VICM       | Neo-epitope of MMP-2, 8 and trypsin mediated degradation of citrullinated vimentin | (Vassiliadis et al. 2012)     |
| Cpa9-HNE   | Neo-epitope of human neutrophil elastase (HNE) mediated degradation of calprotectin (S100A9) | (Mortensen et al. 2022)       |
| C4G        | Neo-epitope of granzyme B mediated degradation of type IV collagen            | (Jensen et al. 2020)          |
| PRO-C3     | Released N-terminal pro-peptide of type III collagen                        | (Nielsen et al. 2013)         |
| PRO-C4     | Internal epitope in the 7S domain of type IV collagen                       | (Leeming et al. 2012)         |
| PRO-C6     | C-terminal of released C5 domain of type VI collagen α3 chain (endotrophin) | (Sun et al. 2015)             |
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Supplementary Figures

Supplementary Figure 1. Association between biomarker levels, Hurley stages and HSS illustrated by scatter plots. A-E) Serum levels measuring degradation of type I collagen (C1M), type III collagen (C3M), type IV collagen (C4M), type V collagen (C5M) and type VII collagen (C7M), F) Serum levels of citrullinated degradation of vimentin (macrophage activity), G) Serum levels of calprotectin (S100A9) degraded by human neutrophil elastase (Neutrophil activity), H) Serum levels of type IV collagen degraded by granzyme B (T-cell activity), I) Serum levels of type III collagen formation, J) Serum levels of type IV collagen turnover, 7S domain, and K) Serum levels of type VI collagen formation. Grey circles = Hurley stage I, Green triangles = Hurley stage II and Blue squares = Hurley stage III.

Supplementary Figure 2. Heatmap showing Spearman’s correlations between the measured biomarkers, clinical and biochemical parameters; Age, body mass index (BMI), estimated glomerular filtration rate (eGFR), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), neutrophils and hidradenitis suppurativa score (HSS). Spearman rank correlation was used to determine correlations. Significance levels: *<0.05; **p≤0.01, ***p≤0.001.