No Evident Systemic Terminal Complement Pathway Activation in Hidradenitis Suppurativa

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TO THE EDITOR

In hidradenitis suppurativa (HS) lesional skin, activation of both innate and adaptive immunity has been described, characterized by upregulation of a multitude of cytokines and complement components (van der Zee et al., 2011). Higher levels of C5a and soluble C5b-9 (sC5b-9) in the plasma of patients with HS have been reported, indicating systemic late-phase complement pathway activation in HS (Kanni et al., 2018). This formed the basis for two (ongoing) clinical trials—NCT03001622 and NCT03487276—targeting C5a and C5aR1 in patients with HS. In addition, deposition of complement components C1q, C3b, and C4d and enhanced expression of complement receptors and complement pathway genes have been found in HS lesions (Blok et al., 2016; Gudjonsson et al., 2020; Hoffman et al., 2018). The finding of systemic complement activation in HS is remarkable, because elevation of other markers of systemic complement activation, such as CRP and IL-6, is rare in HS. We therefore evaluated systemic complement activation in the plasma of 76 patients with HS and that of 10 controls (five males, five females) (Table 1) by quantifying the levels of C3, C3d, C5a, and sC5b-9.

Data and blood samples were collected after obtaining written informed consent from patients in the HiScreen Registry and Biobank at the Department of Dermatology of the Erasmus University Medical Center (Rotterdam, The Netherlands). The Institutional Review Board (Erasmus University Medical Center) approved the HiScreen Registry and Biobank (MEC-2016-246). C3d was measured by sandwich ELISA as previously described (Peakman et al., 1987). Quantitative antigenic assay for C3 was performed by radial immunodiffusion technique with monospecific antisera (Fijen et al., 1996). Concentrations of sC5b-9 were determined by ELISA as described earlier (Damman et al., 2011). C5a in plasma was quantified using a commercially available ELISA kit (HK349, Hycult, Uden, The Netherlands). See Supplementary Materials and Methods for details on data collection, reference values, and statistical analysis.

The median plasma C3 concentrations for patients with HS and for controls were within the normal range (Table 1 and Figure 1). The median concentrations of C3 were statistically higher among patients with Hurley stage I than among controls ($P = 0.031$) and those with Hurley stage II ($P = 0.024$). A cluster of 15 patients was identified with C3 concentrations below the normal range (Figure 1). These patients were more frequently of Hurley stage II, but no other significant associations were found. Compared with controls, median C3d levels were significantly increased for each Hurley stage ($P < 0.001$; Hurley stage II, $P < 0.001$; Hurley stage III, $P = 0.041$; Table 1). C3d concentrations among patients with Hurley stage I were significantly higher than those among patients with Hurley stage II ($P < 0.001$) and those with Hurley stage III ($P = 0.042$). The C3d/C3 ratios were significantly higher in each Hurley stage relative to those in the controls. All concentrations of sC5b-9 and C5a were in the low to normal range and were comparable for controls and patients with HS. There were no significant differences in the concentrations of complement components between sexes or smoking status. The median CRP concentration was significantly higher in patients with Hurley stage III than in those with Hurley stage I ($P = 0.04$). The CRP levels significantly correlated with concentrations of C3, C3d, and C3d/C3 ratio ($P = 0.002$; $P = 0.001$, and $P = 0.019$, respectively; Table 1).

Our study reveals that circulating levels of C5a and sC5b-9 in patients with HS are not significantly increased relative to those in controls. Although concentrations of C3 and C3d as well as those of C3d/C3 ratio were increased in patients with HS, these were all within normal range, except for C3d. However, plasma C3d levels are highly dependent on the concentration of the C3 parent molecule. Therefore, to accurately interpret the C3d levels, C3d should always be additionally reported as a C3d/C3 ratio (Mollnes et al., 2007). This ratio is a more robust marker of complement activation because C3d has a half-life of hours in contrast to C5a and sC5b-9, which have half-lives of minutes. C3d profoundly influences acquired immune responses through the C3d receptor (CD21) expressed on B lymphocytes and follicular dendritic cells (Merle et al., 2015). Hence, C3d could contribute to B-cell activation in HS and could potentially aid in the production of pathogenic (auto)antibodies (Byrd et al., 2019; Gudjonsson et al., 2020).

In contrast to the study by Kanni et al. (2018), we did not identify elevated systemic levels of C5a in patients with HS. C5a is difficult to detect in vivo because it is biologically highly active and binds rapidly to C5a receptors (Oppermann and Götze, 1994; Wagner and Hugli, 1984). However, if an increase in C5a was masked by this rapid binding, increased concentrations of sC5b-9 could serve as a proxy for elevated levels of C5a. Yet, we did not find increased levels of sC5b-9. Remarkably, plasma levels of C5a and sC5b-9 in controls were 4–10 times higher than those found in our study (Kanni et al., 2018). One other study

Abbreviations: HS, hidradenitis suppurativa; sC5b-9, soluble C5b-9

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Table 1. Patient Characteristics and Complement Levels

Patient Characteristics and Measurements	Total Population with HS (n = 76)		Hurley Stage I (n = 23)		Hurley Stage II (n = 37)		Hurley Stage III (n = 16)		Healthy Controls (n = 10)	
Patient characteristics										
Sex										
Female, n (%)	45	(59.2)	15	(65.2)	21	(56.8)	9	(56.3)	5	(50.0)
Male, n (%)	31	(40.8)	8	(34.8)	16	(43.2)	7	(43.8)	5	(50.0)
Age, mean (±SD)	40.3	(11.5)	39.1	(13.3)	39.8	(10.6)	43.4	(11.0)	69.6	(13.6)
Age of onset, median (IQR)	20.0	(15.0–29.8)	22.0	(14.0–33.0)	20.0	(16.0–28.5)	17.5	(15.0–40.3)		
Disease duration, y, median (IQR)	15.5	(7.3–25.8)	15.0	(4.0–22.0)	15.0	(9.5–26.0)	20.5	(10.3–27.5)		
BMI, median (IQR)	29.5	(24.9–35.9)	31.1	(25.4–37.9)	28.2	(24.9–35.0)	29.6	(24.2–38.3)		
Missing, n	6		5		1		0			
Smoking status										
Current or ex-smoker, n (%)	53	(69.7)	14	(60.9)	29	(78.4)	10	(62.5)		
Never smoked, n (%)	23	(30.3)	9	(39.1)	8	(21.6)	6	(37.5)		
IHS4										
Mild (≤3)	26	(52.0)	15	(75.0)	9	(45.0)	2	(20.0)		
Moderate (4–10)	14	(28.0)	5	(25.0)	7	(35.0)	2	(20.0)		
Severe (≥11)	10	(20.0)	0		4	(20.0)	6	(60.0)		
Missing, n	26		3		17		6			
Measurements										
CRP (mg/l), median (IQR)	6.0	(3.2–11.5)	4.7	(1.4–6.5)	5.6	(3.1–9.8)	16.6	(3.3–31.9)		
C3 (mg/ml), median (IQR)	1.4	(1.2–1.6)	1.4	(1.2–1.7)	1.3	(0.6–1.5)	1.4	(0.7–1.7)	1.3	(1.2–1.4)
C3d (µg/ml), median (IQR)	5.2	(2.8–8.1)	7.0	(5.8–9.8)	3.7	(2.4–6.3)	3.8	(2.7–7.6)	2.3	(1.7–3.0)
C3d/C3 ratio, median (IQR)	4.4	(3.1–7.1)	5.2	(4.0–7.1)	4.4	(2.1–6.5)	4.3	(2.4–10.4)	1.7	(1.2–2.6)
sC5b-9 (ng/ml), median (IQR)	58.0	(43.0–96.6)	58.0	(45.0–81.0)	61.0	(40.5–134.8)	44.1	(33.8–90.2)	59.7	(30.2–190.3)
C5a (ng/ml), median (IQR)	1.8	(1.3–2.3)	1.9	(1.6–2.3)	1.8	(1.1–2.3)	1.8	(0.8–2.2)	2.0	(1.7–3.2)

Abbreviations: BMI, body mass index, HS, hidradenitis suppurativa; IHS4, International Hidradenitis Suppurativa Severity Score System; IQR, interquartile range; sC5b-9, soluble C5b-9.

reported low to normal levels of C5a and C5b-9 in patients with HS (Li et al., 2020). These substantial differences are likely caused by the use of different ELISA systems. Therefore, comparing ELISA results of complement levels should be done with caution because of the different properties of the capture and detection antibodies used. Cross-reactivity with nonactivated but neoepitope-expressing C5 native protein, artificially increasing the levels of C5a, should be ruled out (Nilsson et al., 2017). The ELISA kit used in our study recognizes a neoepitope on C5a desArg not present in the native C5 component, preventing such cross-reactivity.

The finding of complement deposition in HS lesions with enhanced expression of complement receptors and complement pathway genes without systemic spillover is puzzling (Blok et al., 2016; Gudjonsson et al., 2020; Hoffman et al., 2018). It is still unclear whether local complement activation also comprises terminal pathway activation and, if so, why it remains strictly compartmentalized in inflamed HS skin. Therefore, further in-depth research on in situ complement activation, in combination with functional studies, is warranted.

In conclusion, our results do not support a prominent role for systemic complement activation in the pathogenesis of HS. The absence of evident systemic and in situ terminal pathway activation argues against the potential benefits of therapeutic intervention in the C5a–C5aR1 axis, the therapeutic target of two (ongoing) clinical trials in HS.

Data availability statement

Datasets related to this article are available on request.

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CONFLICT OF INTEREST

EPP declares grant from AbbVie paid to Erasmus University Medical Center and advisory board membership. BH declares fees from Janssen-Cilag (advisory boards, educational grants, consultations, investigator initiative studies), AbbVie (advisory boards, educational grants, consultations, investigator initiative studies), Novartis Pharma (advisory boards, consultations, investigator initiative studies), UCB Pharma (advisory boards, consultations), Leo Pharma (consultations), Solenne B.V. (investigator initiative studies), Celgene (consultations, investigator initiative studies), Akari Therapeutics (consultations, investigator initiative studies), Philips (consultation), Roche (consultation), Regeneron (consultation), and Sanofi (consultation), for which fees were paid to the institution. The remaining authors state no conflict of interest.

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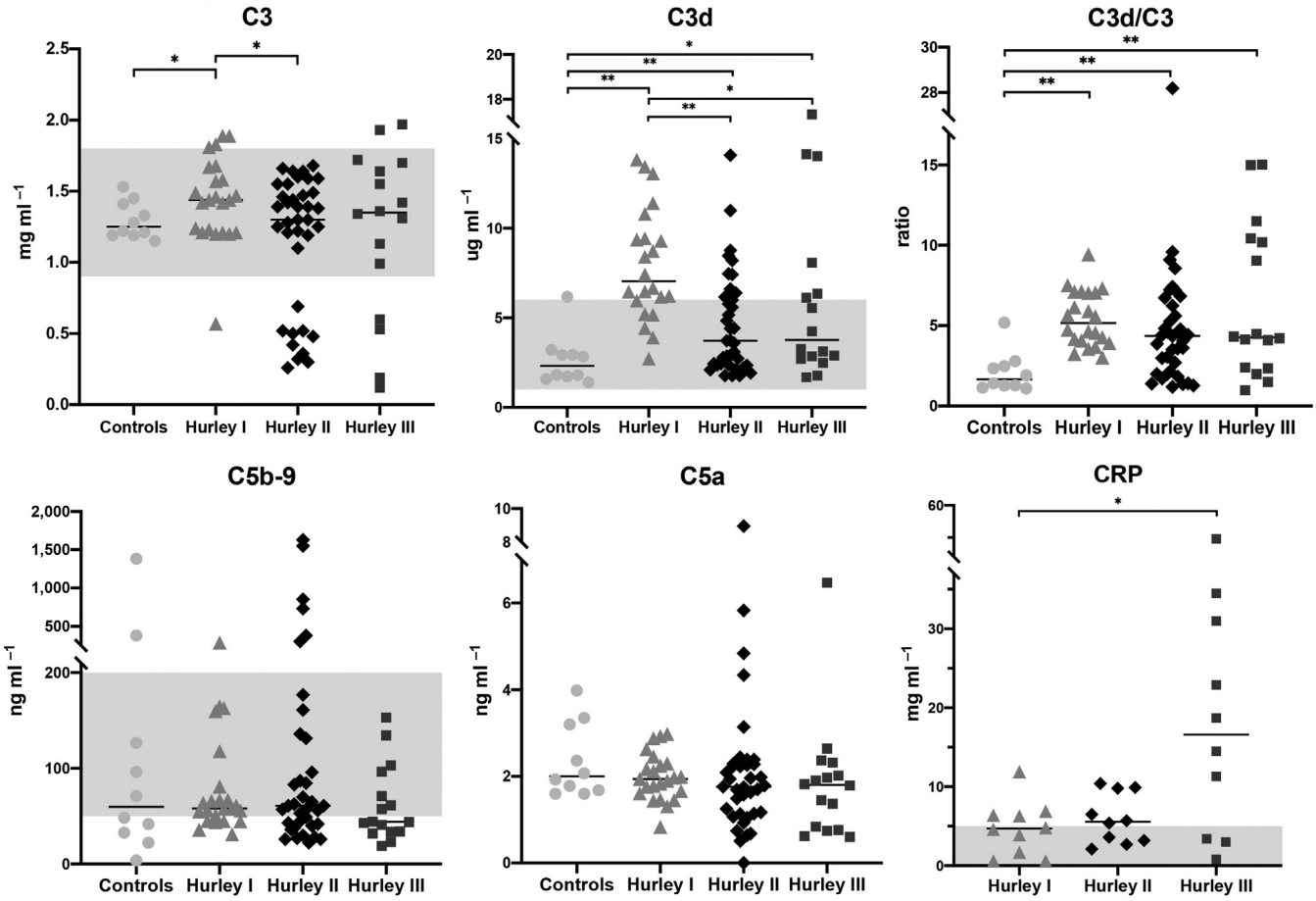

Figure 1. Distribution of complement levels in plasma. Concentrations of C3, C3d, C3d/C3 ratio, C5b-9, and C5a per Hurley stage (n = 76 patients with HS, n = 10 controls). The C3d/C3 ratio was calculated by dividing the C3d values (in μg/ml) by the C3 levels (in mg/ml). Reference values were based on a normal distribution of a historical cohort of 33 healthy volunteers for C3d and 16 healthy volunteers for sC5b-9. The reference interval is defined as the mean plus three SDs of 33 healthy controls for C3d and 16 healthy volunteers for sC5b-9. $*P < 0.05$, $**P < 0.01$ (Mann–Whitney *U* test). HS, hidradenitis suppurativa; sC5b-9, soluble C5b-9.

Registry and Biobank are financially supported by AbbVie (The Netherlands). The collection of blood and patient data used in this study was approved by the Institutional Review Board of the Erasmus University Medical Center (Rotterdam, The Netherlands) (MEC-2016-246/MEC-2013-337).

AUTHOR CONTRIBUTIONS

Conceptualization: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Data Curation: LMP, CBA, KRVS, MAJS, EPP, JD; Formal Analysis: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, JD; Funding Acquisition: MAJS, EPP, BH, JD; Investigation: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Methodology: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Project Administration: LMP, CBA, KRVS, MAJS, EPP, BH, JD; Resources: LMP, CBA, KRVS, MAJS, EPP, BH, JD; Supervision: MAJS, EPP, JD; Validation: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Visualization: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Writing - Original Draft Preparation: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD; Writing - Review and Editing: LMP, CBA, KRVS, HHVDZ, MAJS, JDL, EPP, BH, JD

Disclaimer

AbbVie (Hoofddorp, The Netherlands) had no role in the design or conduct of the study, the interpretation of the data, or the decision to submit the manuscript for publication.

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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.2021.03.037.

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GLP-1 Analogs and SGLT2 Inhibitors Do Not Increase Risk of Bullous Pemphigoid

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TO THE EDITOR

The association of bullous pemphigoid (BP) and dipeptidyl peptidase-4 inhibitors (DPP-4is) used for diabetes mellitus (DM) has recently attracted special interest in the field of BP research. (Nishie and Tasanen, 2019; Varpuluoma et al., 2018a). However, other diabetes drugs have been studied to a lesser extent in this context. In our previous case-control study of 3,397 patients with BP diagnosed in Finland between 1997 and 2013, we did not find increased risk of BP associated with oral DM drugs other than DPP-4is (Varpuluoma et al., 2018b). However, owing to the limited number of either cases or controls on newer drugs, we were unable to study all the DM drugs in the Finnish market. This study aims to analyze the association of DM drugs and BP, especially focusing on the newer regimens.

This is a retrospective case-control study of patients with BP older than 40 years diagnosed in Finland between 1 January 1997 and 31 December 2018. Data on patient records were obtained from the Finnish Care Register for Health Care. Patients were selected by a diagnosis of BP (International Classification of Diseases-10 code L12.0). The control population was composed of patients diagnosed with basal cell carcinoma and matched by age, sex, and year of diagnosis (within 2 years) in a 1:4 ratio. Data on purchased DM drugs for the 2 years immediately preceding diagnosis were obtained from the drug reimbursement register of the database of the Social Insurance Institution of Finland (Supplementary Table S1). Associations between DM drug usage and BP were evaluated using a conditional logistic regression model. Results were adjusted with DM, several neurological diseases, and use of aldosterone antagonists, anticholinergics, antipsychotics, and dopaminergic drugs that have been associated with increased risk for BP (Liu et al., 2020). Because several new DM drugs were approved for use in Finland during the study period, for these drugs, we included only cases and controls diagnosed after the approval. Methods and databases are described in detail in our previous studies (Varpuluoma et al., 2018a, 2018b).

Table 1. Characteristics of Bullous Pemphigoid Cases and Basal Cell Carcinoma Controls Retrieved from the Finnish Care Register for Health Care

Characteristic	Cases n = 5,079 (%)	Controls n = 19,663 (%)[1]
Female	2,968 (58.44)	11,523 (58.60)
Male	2,111 (41.56)	8,140 (41.40)
Age, y, mean (range)	77.6 (40–104)	77.7 (40–104)

[1]Matched by age, sex, and year of diagnosis in a 1:4 ratio. Owing to the lack of data in the drug imbursement register, 581 cases had fewer than the intended 4 controls.

Abbreviations: BP, bullous pemphigoid; DM, diabetes mellitus; DPP-4i, dipeptidyl peptidase-4 inhibitor

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SUPPLEMENTARY MATERIALS AND METHODS

Study population

Data were collected from patients participating in the HiScreen Registry and Biobank at the Department of Dermatology of the Erasmus University Medical Center (Rotterdam, The Netherlands). The HiScreen Registry and Biobank were approved by the Institutional Review Board of the Erasmus University Medical Center (MEC-2016-246). This registry collects data on age, sex, body mass index, smoking status, and disease severity (Hurley stage, abscess and nodule counts, and International Hidradenitis Suppurativa Severity Score System). Routine blood samples were collected at the first visit in 8-ml vacuum EDTA tubes (BD Vacutainer, Plymouth, UK) under aseptic conditions. Directly after collection, blood was centrifuged to separate the plasma, which was subsequently aliquoted and stored at −80 °C until further analysis.

Complement and CRP assays

Concentrations of the complement components C3, C3d, and C5a and the membrane attack complex soluble C5b-9 were measured in the laboratory of the Department of Nephrology, University Medical Center Groningen (Groningen, The Netherlands). The C3d/C3 ratio was determined by dividing the C3d values in μg/ml by the C3 concentration in mg/ml. Soluble C5b-9 concentrations were determined by in-house sandwich ELISA as described earlier (Damman et al., 2011). Reference values were based on a normal distribution of a historical cohort of 33 healthy volunteers for C3d and 16 healthy volunteers for soluble C5b-9. Blood samples of this cohort was collected, processed, and analyzed identically to the blood of controls and patients with hidradenitis suppurativa in this study. All values within three SDs from the mean of these historical control populations were deemed within the normal range. CRP concentrations were measured in a subgroup of patients with hidradenitis suppurativa (Hurley stage I, n = 10; Hurley stage II, n = 10; Hurley stage III, n = 10) in the general laboratory of the Erasmus University Medical Center (Rotterdam, The Netherlands) by turbidimetric analysis, with a cut-off value of 5 mg/l.

Statistical analysis

Patient characteristics are presented as numbers (percentages) for categorical variables and as mean ± SD or median (interquartile range) where appropriate for continuous variables. Concentrations of C3, C3d, C5a, and soluble C5b-9 and CRP are expressed as median (interquartile range). Normality was assessed using the Kolmogorov–Smirnov test. Differences in plasma levels between Hurley stages, sex, body mass index category, and smoking status were assessed using univariate logistic regression analysis. All statistical tests were two-sided, and $P \leq 0.05$ was considered statistically significant. Statistical analysis was performed using IBM Statistical Package for the Social Sciences Statistics for Windows, version 25, 2017 (IBM, Armonk, NY).

SUPPLEMENTARY REFERENCE

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