Dermal C4d Deposition and Neutrophil Alignment Along the Dermal–Epidermal Junction as a Diagnostic Adjunct for Hypocomplementemic Urticarial Vasculitis (Anti-C1q Vasculitis) and Underlying Systemic Disease

Jeffrey Damman, MD, PhD, Antien L. Mooyaart, MD, PhD,* Marc A.J. Seelen, MD, PhD,† and Martijn B.A. van Doorn, MD, PhD‡*

Abstract: Urticarial vasculitis (UV) is a clinicopathologic entity characterized by persistent urticarial lesions with biopsy features of vasculitis. Currently, only certain *clinical* features such as arthralgia and serum complement concentrations are used to identify UV patients at risk for an underlying systemic disease. Hypocomplementemic urticarial vasculitis (HUV) is in contrast to normocomplementemic urticarial vasculitis (NUV), strongly associated with underlying systemic disease, especially systemic lupus erythematosus (SLE). The aim of this study was to find specific *histopathological* features associated with HUV and underlying systemic disease in UV. In addition, the use of complement C4d deposition in skin biopsies was evaluated as a diagnostic adjunct for HUV- and UV-associated systemic disease. In this retrospective study, the clinical, histopathological, and immunohistological (C4d) features of 43 patients with UV were compared between HUV and NUV and analyzed for association with UV-associated systemic disease. Eight of 43 patients with UV (19%) had hypocomplementemia. Patients with HUV showed a significantly higher number of perivascular neutrophils and lower number of eosinophils compared to NUV. Of all histopathological features, alignment of neutrophils along the dermal–epidermal junction (DEJ) and dermal granular C4d deposition were found to be strongly associated with HUV and underlying SLE. This study shows that both the alignment of neutrophils along the DEJ and dermal C4d deposition are strongly associated with HUV and SLE. Therefore, these (immuno)histopathological features can be used as an easy diagnostic adjunct for early detection of underlying systemic disease in UV.

Key Words: urticarial vasculitis, complement, hypocomplementemia, systemic lupus erythematosus, C4d, neutrophils

(*Am J Dermatopathol* 2020;42:399–406)

INTRODUCTION

Urticarial vasculitis (UV) is a clinicopathologic entity characterized by persistent urticarial lesions with biopsy features of vasculitis. Clinically, UV is characterized by the presence of papules or plaques with erythematous borders and central clearing (urticarial wheals) by definition persisting for more than 24 hours at the same location. The wheals are usually accompanied by itch, tenderness, and/or burning pain and tend to resolve with purpura or hyperpigmentation. The disease is relatively rare with an estimated prevalence of 5% of patients with chronic urticaria, mostly occurring in women.[1] Patients suffering from UV often present with accompanying symptoms such as angioedema, arthralgia, fever, (epi)scleritis, or uveitis. UV is regarded as a type III hypersensitivity reaction in which (peri)vascular deposits of immune complexes and complement fragments can be found in the vessel wall by immunofluorescence.[2] It has recently been shown that in more than half of the patients with UV, autoantibodies against C1q can be detected.[3] Anti-C1q antibodies bind to the collagenous region of C1q and activate the classic pathway of the complement system. This is reflected in the serum by decreased circulating levels of classical pathway components C1q and C4 as well as C3.[4] Therefore, UV can be divided into normocomplementemic UV (NUV) and hypocomplementemic UV (HUV) depending on serum complement levels. The latter is now defined as anti-C1q vasculitis in the 2012 Revised International Chapel Hill consensus Conference Nomenclature of Vasculitides.[5] NUV is mostly idiopathic but can also be triggered by certain drugs, sun exposure, and infections such as hepatitis. By contrast, HUV is associated with more severe disease and can indicate the presence of an underlying systemic disease, in particular systemic lupus erythematosus (SLE) or hypocomplementemic urticarial vasculitis syndrome (HUVS). HUVS is characterized by urticaria with hypocomplementemia, arthralgia/arthritis, glomerulonephritis, recurrent abdominal pain, and obstructive lung disease. In all patients with HUVS, anti-C1q antibodies can be detected.[1,6–8]

Histopathological examination showed classic features of small vessel leucocytoclastic vasculitis including endothelialitis, karyorrhexis, erythrocyte extravasation, and fibrinoid necrosis. However, in practice, these features are only found in a minority of patients with UV. Depending on the

From the *Department of Pathology, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands; †Department of Nephrology, University Medical Center Groningen, Groningen, the Netherlands; and ‡Department of Dermatology, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands.

The authors declare no conflicts of interest.

Correspondence: Jeffrey Damman, MD, PhD, Department of Pathology, Erasmus Medical Center Rotterdam, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands (e-mail: j.damman@erasmusmc.nl).

timing of the biopsy, histopathological features can vary from urticarial inflammation, with a cell poor perivascular and interstitial infiltrate of eosinophils and/or neutrophils, to more lymphocytic inflammation. In addition, subtle features of vascular damage are seen such as endothelialitis and (varying degrees of) erythrocyte extravasation and karyorrhexis. In contrast to classic leucocytoclastic vasculitis, fibrinoid necrosis is only present in the minority of cases.[1,2,6,7,9]

At this moment, only certain *clinical* features and serum measurements are used to identify UV patients at risk for an underlying systemic disease. These features include among others arthralgia, fever, serum complement consumption, and antinuclear antibody (ANA) detection. The aim of this study was to find specific *histopathological* features associated with HUV and underlying systemic disease. In parallel with serum complement consumption in HUV, we investigated the use of complement C4d deposition in the skin biopsy as a diagnostic adjunct for HUV- and UV-associated systemic disease. The results of our study could potentially aid in an earlier diagnosis of HUV- and/or UV-associated systemic disease.

METHODS

Patient Selection

In this retrospective study, 43 patients were included who underwent a skin biopsy between 2010 and 2018 in the Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands. Patients were included when both the clinical and histopathological criteria for UV were met and serum complement concentrations were measured at the time of biopsy. Demographics and clinical features were retrieved from the medical records (Table 1). Clinical criteria for UV were as follows: patients should present with urticarial wheals persisting for >24 hours on the same location. Histopathological criteria for UV were as follows: vasculopathy in the presence of lymphocytes, neutrophils, and/or eosinophils accompanied by varying degrees of erythrocyte extravasation and karyorrhexis. Vasculopathy was defined as the presence inflammatory cells under the endothelium with endothelial cell swelling and vessel wall disruption. Fibrinoid necrosis was not required. A final diagnosis of SLE was made based on the Systemic Lupus International Collaborating Clinics (SLICC) criteria.

Serum Measurements

Serum complement C1q, C3, and C4 were measured by radial immunodiffusion. ANA were detected by indirect immunofluorescence on HEp-2 cells. ANA values of 1:80 or higher were considered positive. Anti C1q antibody concentration in the patient with HUVS was measured by ELISA (Sanquin, Amsterdam, the Netherlands). Patients were divided into NUV and HUV groups based on serum complement concentrations of C3 and C4. Hypocomplementemia was defined as C3 of less than 0.9 g/L (normal 0.9–1.8 g/L) or C4 less than 0.1 g/L (normal 0.1–0.4 g/L). Low C1q was defined as a level below 0.12 g/L (normal 0.12–0.25 g/L).

Histopathological Features and Immunohistochemistry

Formalin-fixed paraffin-embedded tissue sections were stained with hematoxylin and eosin for light microscopic evaluation. Alcian blue staining was performed for evaluation of dermal mucin deposition. Immunohistochemistry was performed for C4d using a monoclonal antibody (SP91, Cell marque, dilution 1:75). Histopathological features were scored by 2 independent dermatopathologists (J.D. and A.M.) by routine light microscopy. The inflammatory infiltrate was scored on a nominal scale of 0–3: none (0), mild (1), moderate (2), and profound (3). All other histopathological parameters were scored dichotomously (yes/no).

Statistics

To compare demographics between the NUV and HUV groups, the Mann–Whitney *U* test was performed for continuous variables and the Fisher exact test for categorical variables on a nominal scale. For comparison between the histopathological features of NUV and HUV, the Fisher exact test was used for categorical variables on a nominal scale, whereas χ^2 was used for categorical variables on an ordinal scale. Statistical analysis was performed using SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.), and 2-sided *P*-values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Demographics and Clinical Features

In general, the majority of patients suffering from UV were middle-aged women with a median age of 46 years, mostly presenting with urticarial wheals on the upper legs (Table 1). A total number of 8 patients had low complement concentrations (HUV), whereas 35 patients were normocomplementemic (NUV). Although a higher number of cases with purpura, arthralgia, and angioedema was found in HUV compared with the NUV group, this difference was not statistically significant. No appreciable difference was observed between both baseline characteristics (age and gender) as well as other clinical features between the NUV and HUV group. Complement C3, C4, and C1q concentrations were (per definition) significantly lower in the HUV group compared with NUV. Serum anti-C1q antibody level in the patient with HUVS was strongly elevated to 271 AU/mL (normal <0.70). ANA positivity was found more frequently in the HUV group compared with NUV (75% vs. 28%, $P = 0.036$). Positive ANA values of patient with HUV (n = 6) were, respectively, 1:80, 1:160, 1:640, 1:1280, 1:2560, and 1:5120. Positive ANA values of NUV patients (n = 9) were, respectively, 1:80 (×2), 1:160 (×3), 1:320, 1:1280 (×2), and 1:2560. Regarding the underlying cause of UV, SLE was found to be significantly higher in HUV (50%) compared with NUV (3%), $P = 0.003$.

TABLE 1. Demographics and Disease Characteristics

Clinical Features	NUV (n = 35)	HUV (n = 8)	*P*
Age	46 (31–58)	46 (34–61)	0.791†
Female	27/35 (77)	6/8 (75)	1.0*
Biopsy location			0.618‡
Hand/wrist	2 (6)	0 (0)	
Arm	6 (17)	1 (13)	
Shoulder	4 (11)	0 (0)	
Abdomen	2 (6)	1 (13)	
Upper leg	12 (34)	3 (38)	
Lower leg	6 (17)	2 (25)	
Knee	1 (3)	0 (0)	
Foot	1 (3)	0 (0)	
Back	0 (0)	1 (13)	
Unknown	1 (3)	0 (0)	
Wheals >24 h	35/35 (100)	8/8 (100)	N.A.
Residual hyperpigmentation	17/20 (85)	3/3 (100)	0.644*
Purpura	6/27 (22)	3/6 (50)	0.123*
Itch	28/33 (85)	6/8 (75)	0.606*
Pain or burning sensation	23/31 (74)	5/7 (71)	1.0*
Arthralgia	13/33 (39)	4/6 (67)	0.374*
Fever	8/32 (25)	2/6 (33)	0.644*
Angioedema	13/32 (41)	5/7 (71)	0.215*
Laboratory measurements			
Complement C3 (g/L)	1.3 (1.13–1.46) (34/35)	0.49 (0.32–0.61) (8/8)	<0.001†
Complement C4 (g/L)	0.25 (0.31–0.26) (32/35)	0.08 (0.04–0.13) (7/8)	<0.001†
Complement C1q (g/L)	0.22 (0.21–0.26) (23/35)	0.1 (3/8)	0.005†
ANA	9/32 (28)	6/8 (75)	0.036†
Associated condition			
Systemic lupus erythematosus	1 (3)	4 (50)	0.003*
HUVS	0 (0)	1 (13)	
Idiopathic retroperitoneal fibrosis, B-cell clone	0 (0)	1 (13)	
Relapsing polychondritis	1 (3)	1 (13)	
Infectious	1 (3)	0	
FMF	1 (3)	0	
Drugs	1 (3)	0	
ANCA-negative GPA	1 (3)	0	
Crohn disease, arthritis	1 (3)	0	
Idiopathic	28 (80)	1 (13)	

Age data is expressed as median (interquartile range), all other data (except for laboratory measurements) are expressed as positive numbers (%). All *P*-values are 2-sided.
*Fisher exact test.
†Mann–Whitney test.
‡χ^2 test.
ANCA, antineutrophilic cytoplasmic autoantibody; FMF, Familial Mediterranean Fever; GPA, granulomatosis with polyangiitis; N.A., not applicable.

Histopathological Features

In general, in UV, the predominant histopathological pattern was a mild-to-moderate superficial and deep perivascular and interstitial lymphohistiocytic infiltrate admixed with neutrophils and/or eosinophils in varying number and accompanied by karyorrhexis, erythrocyte extravasation, and in the minority of cases with fibrinoid necrosis (Fig. 1 and Table 2). In almost all cases, vasculopathy was present with lymphocytes and histiocytes in varying number admixed with neutrophils and eosinophils. Although the infiltrate was also found to be located deeper in the dermis, in most cases, the infiltrate was "top heavy" and surrounded the papillary dermal capillaries and postcapillary venules of the superficial vascular plexus. In about one-third of cases, the infiltrate also extended in the superficial subcutaneous fat. Additional features were eosinophilic degranulation, neutrophil alignment along the dermal–epidermal junction (DEJ; Fig. 1), neutrophil epitheliotropism, papillary edema, mucin deposition, and basophilic degeneration.

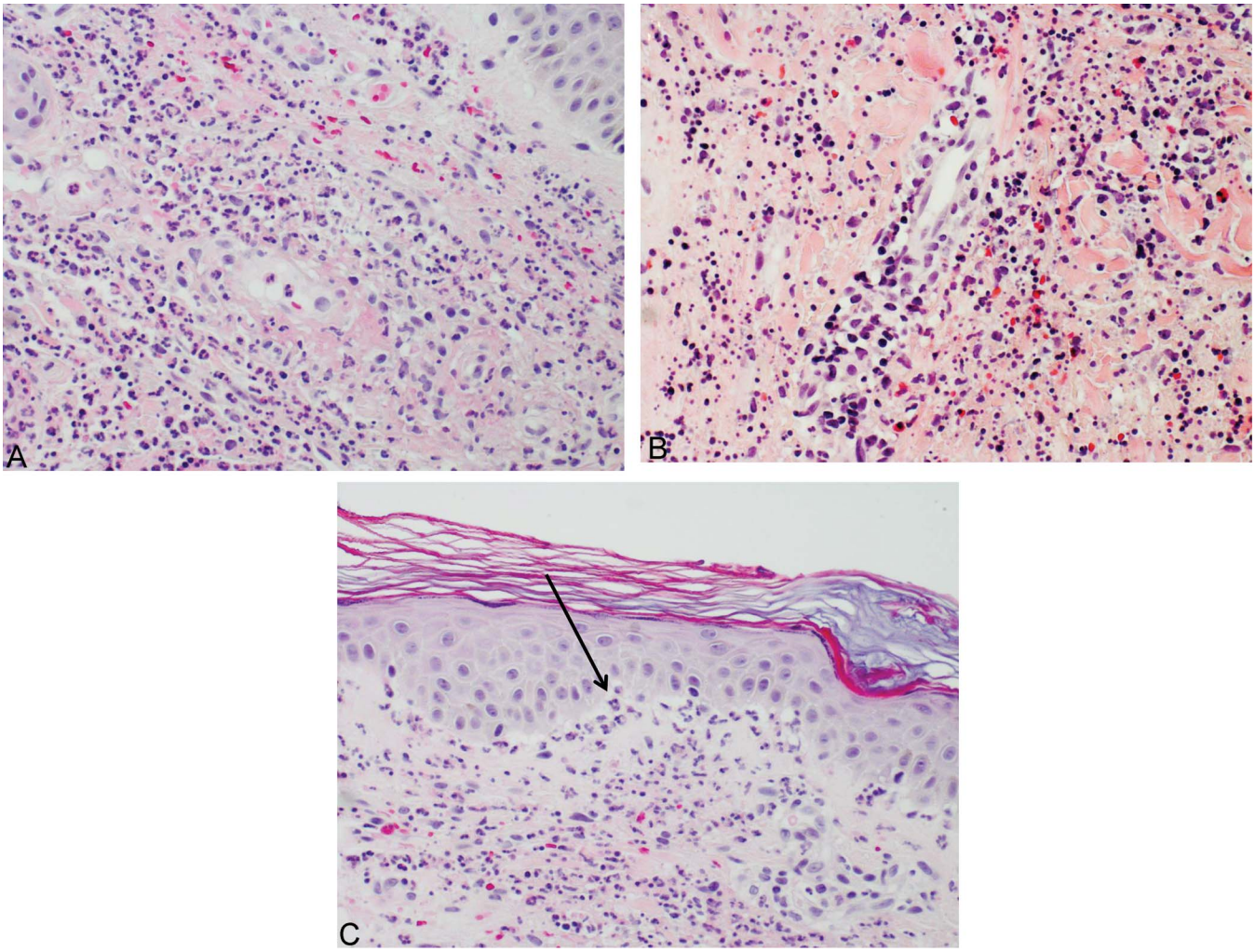

FIGURE 1. A, A case of neutrophilic urticarial vasculitis with extensive erythrocyte extravasation. A different case of NUV (B) shows an example of lymphocytic vasculopathy with influx of lymphocytes and neutrophils under the endothelium surrounded by nuclear dust and extravasated erythrocytes. C, A case of HUV with alignment of neutrophils along the DEJ (arrows).

In HUV, the perivascular and interstitial neutrophil infiltration was more extensive compared with NUV, reaching statistical significance in the perivascular component ($P = 0.047$, Fig. 1). Also, in the vasculopathic component, the neutrophil count was higher in HUV versus NUV, but this was not statistically significant ($P = 0.083$). In HUV, the perivascular and interstitial eosinophilic infiltrate was less extensive compared with NUV, reaching statistical significance for the interstitial component ($P = 0.035$). The degree of erythrocyte extravasation and nuclear dust was higher in HUV versus NUV, of which nuclear dust reached statistical significance ($P = 0.049$). The strongest association of this study was the finding of neutrophil alignment along the DEJ, which was found in 75% of the HUV cases compared with 17% in NUV cases (Fig. 1). All other histopathological features were similar for the HUV and NUV groups. None of our patients showed features of interface dermatitis.

C4d Analysis

Dermal C4d deposition was significantly higher in a granular pattern on and between the perivascular infiltrate in HUV versus NUV (Figs. 2A, B, $P < 0.001$). Although the number of vessels with linear vascular and sometimes granular C4d deposition was higher in HUV versus NUV, this difference was not statistically significant (Figs. 2C, D, $P = 0.091$). Also, the intensity of vascular C4d deposition was similar between both groups ($P = 0.540$). None of our patients showed a granular C4d deposition along the basement membrane.

We also investigated the direct association of neutrophil alignment along the DEJ and C4d positivity with SLE and compared this in terms of sensitivity, specificity, and positive and negative predictive values (Table 3) We found that hypocomplementemia in UV is most strongly associated with SLE. The sensitivity of HUV for SLE is 80%, and the specificity is increased from

TABLE 2. Histopathological Features in NUV and HUV

	NUV (n = 35)	HUV (n = 8)	*P*
Infiltrate architecture			
Superficial perivascular	34 (97)	8 (100)	1.0*
Deep perivascular	33 (94)	7 (88)	0.470*
Superficial interstitial	33 (94)	7 (88)	0.470*
Deep interstitial	30 (86)	6 (75)	0.587*
Subcutaneous fat (if present)	No: 5 (14) Yes: 13 (37)	No: 3 (38) Yes: 3 (38)	0.259†
Perivascular			
Total:			
Mild	10 (29)	1 (13)	0.639†
Moderate	21 (60)	6 (75)	
Profound	4 (11)	1 (13)	
Lymphocytes:			
None	1 (3)	0 (0)	0.578†
Mild	17 (49)	6 (75)	
Moderate	16 (46)	2 (25)	
Profound	1 (3)	0 (0)	
Histiocytes:			
None	2 (6)	0 (0)	0.752†
Mild	23 (66)	6 (75)	
Moderate	10 (29)	2 (25)	
Profound	0 (0)	0 (0)	
Neutrophils:			
None	1 (3)	0 (0)	0.047†
Mild	22 (63)	1 (13)	
Moderate	9 (26)	6 (75)	
Profound	3 (9)	1 (13)	
Eosinophils			
None	4 (11)	1 (13)	0.193†
Mild	23 (66)	5 (63)	
Moderate	8 (23)	1 (13)	
Profound	0 (0)	1 (13)	
Interstitial			
Total:			
None	2 (6)	1 (13)	0.182†
Mild	23 (66)	2 (25)	
Moderate	9 (26)	4 (50)	
Profound	1 (3)	1 (13)	
Lymphocytes:			
None	4 (11)	2 (25)	0.443†
Mild	29 (83)	5 (63)	
Moderate	2 (6)	1 (13)	
Profound	0 (0)	0 (0)	
Histiocytes:			
None	2 (6)	2 (25)	0.103†
Mild	32 (91)	5 (63)	
Moderate	1 (3)	1 (13)	
Profound	0 (0)	0 (0)	
Neutrophils:			
None	6 (17)	1 (13)	0.108†
Mild	22 (63)	2 (25)	
Moderate	6 (17)	4 (50)	
Profound	1 (3)	1 (13)	
Eosinophils:			
None	7 (20)	4 (50)	0.035†
Mild	24 (69)	3 (37)	
Moderate	4 (11)	0 (0)	
Profound	0 (0)	1 (13)	
Vasculopathy			
Total	33 (94)	8 (100)	1.0 *
Lymphocytes	30 (91)	8 (100)	1.0 *
Histiocytes	29 (88)	7 (88)	1.0 *
Neutrophils	22 (67)	8 (100)	0.083*
Eosinophils	15 (46)	4 (50)	1.0*
Vessels			
Fibrinoid necrosis	4 (11)	2 (25)	0.308*
Erythrocyte extravasation:			
None	7 (20)	0 (0)	0.074†
Mild	19 (54)	6 (75)	
Moderate	9 (26)	1 (13)	
Profound	0 (0)	1 (13)	
Additional features			
Eosinophilic degranulation:			
None	9 (26)	3 (38)	0.479†
Mild	21 (60)	5 (63)	
Moderate	5 (14)	0 (0)	
Profound	0 (0)	0 (0)	
Nuclear dust			
None	0 (0)	0 (0)	0.049†
Mild	25 (71)	2 (25)	
Moderate	8 (23)	5 (63)	
Profound	2 (6)	1 (13)	
Neutrophil epitheliotropism	3 (9)	2 (25)	0.228*
Neutrophils along the DEJ	6 (17)	7 (88)	<0.001*
Papillary edema	4 (11)	1 (13)	1.0*
Mucin deposition (alcian blue):			0.904†
None	16/31 (52)	4/8 (50)	
Mild	12/31 (39)	3/8 (38)	
Moderate	2/31 (7)	1/8 (13)	
Profound	1/31 (3)	0/8 (0)	
Basophilic degeneration	12 (34)	5 (63)	0.230*
C4d positivity			
Dermal granular	4/32 (13)	7/8 (88)	<0.001*
Vessel number:			0.091†
None	8/32 (25)	1/8 (13)	
1%–5%	16/32 (50)	2/8 (25)	
5%–25%	6/32 (19)	2/8 (25)	
>25%	2/32 (6)	3/8 (38)	
Vessel intensity:			0.540†
None	8/32 (25)	1/8 (13)	
Mild	12/32 (38)	3/8 (38)	
Moderate	9/32 (29)	4/8 (50)	
Profound	3/32 (9)	0/8 (0)	

All data are expressed as positive numbers (%). All *P*-values are 2-sided.
*Fisher exact test.
†χ^2 test.

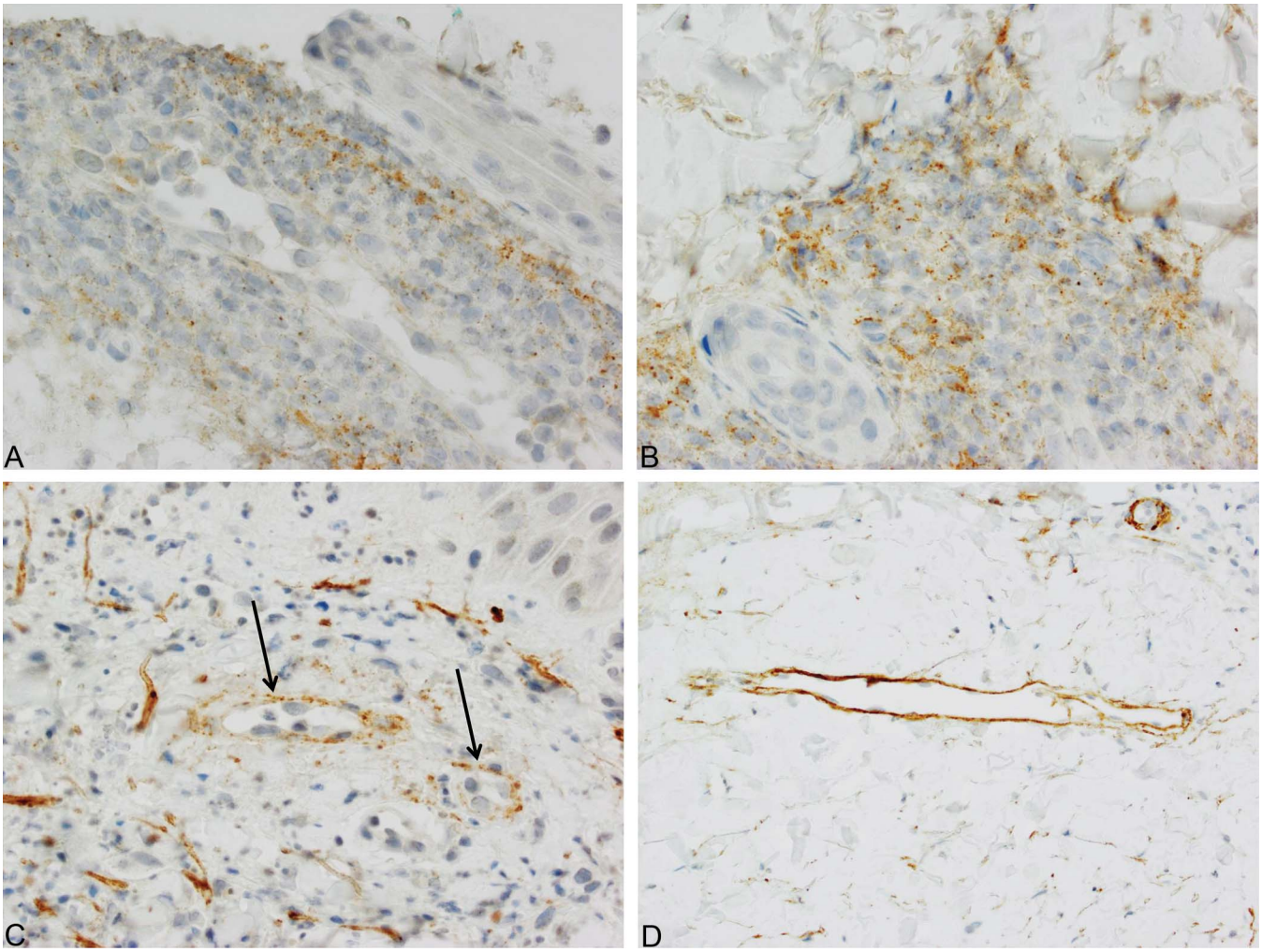

FIGURE 2. Granular dermal C4d deposition in HUV in a patient with underlying systemic lupus erythematosus (A and B). C4d is deposited in a granular fashion on and between the perivascular neutrophilic infiltrate in HUV, reflecting classic complement activation after immune complex deposition. Granular vascular endothelial C4d deposition (C, arrows) and strong continuous C4d deposition (D) in postcapillary venules in a patient with HUV.

89% to 95% when combined with ANA values. Both neutrophils along the DEJ and C4d show lower specificity, also when combined with HUV and/or ANA values (data not shown). Analysis for C4d was performed only for 4 of 5 SLE patients because in one case, there was not enough material left for proper C4d staining.

DISCUSSION

UV is a rare disease in which patients present with persistent urticarial lesions at the same location with biopsy features of vasculitis. UV can be idiopathic, infection- or drug-induced, or a manifestation of an underlying systemic disease such as SLE, Sjögren syndrome, or malignancy. At this moment, only few clinical features such as arthralgia and fever as well as serum complement measurements are used to identify UV patients at risk for an underlying systemic disease. HUV is in contrast to NUV, strongly associated with underlying systemic disease, especially SLE. In addition to clinical signs of HUV, the aim of this study was to find histopathological features associated with HUV- or UV-associated systemic disease. Besides, we also investigated the use of complement C4d deposition in the skin biopsy as a diagnostic adjunct. The main finding of our study is that both alignment of neutrophils along the DEJ and dermal C4d deposition are strongly associated with HUV and SLE.

Clinically, UV can be distinguished from chronic spontaneous urticaria by the presence of unprovoked urticarial wheals that persist for >24 hours at the same site and have a tendency to heal with residual purpura and/or hyperpigmentation. UV is regarded as a type III hypersensitivity reaction in which immune complexes are deposited in the skin followed by activation of the classic pathway of complement. Subsequently, C4 and C3 are activated leading to the release of C5a and ultimately to the formation of the membrane attack complex C5b-9.[2,10] C5a has strong chemotactic

TABLE 3. Sensitivity, Specificity, and Positive and Negative Predictive Values of Circulating and (immuno)histopathological Markers in UV for SLE

	No SLE (38)	SLE (5)	*P*	Sens	Spec	PPV	NPV
Hypocomplementemia	4/38 (11)	4/5 (80)	0.003	80	89	50	97
ANA	10/38 (29)	5/5 (100)	0.005	100	74	50	100
Neutrophil DEJ	9/38 (24)	4/5 (80)	0.024	80	76	31	97
C4d	8/38 (22)	3/4 (75)	0.056	75	79	27	97
Hypocomplementemia ANA	2/38 (5)	4/5 (80)	0.001	80	95	67	97

ANA, antinuclear antibodies; NPV, negative predictive value; PPV, positive predictive value; sens, sensitivity; spec, specificity.

properties for neutrophils but can also act on mast cells to release histamine, chemokines, cytokines, and other vasodilatory substances.[10] Due to the increased vascular permeability, both vascular and extravascular immune complex deposition is permitted. Both immunoglobulins and C3 have been found by immunofluorescence on vascular endothelium as well as granular deposition in the basement membrane zone in the case of underlying SLE. Based on the level of complement activation, clinical features can differ from NUV only showing urticarial lesions, HUV showing urticarial lesions more frequently associated with systemic involvement, and HUVS which is associated with severe systemic disease and multiorgan involvement. It is likely that both the level and nature of circulating antibodies directly influence the level of complement activation and severity of the disease. Antibodies can be directed against autologous antigens (autoantibodies against self-antigens) or exogenous antigens (eg, infective agents and drugs). C1q autoantibodies against the collagen region of C1q can be found in more than 50% of patients with HUV and in 100% of HUVS. A large part of this can be explained by the high number of SLE patients in this group. This raises the question whether SLE and HUV(S) could represent different expressions of the same disease.

The current study is the most extensive histopathological study on UV including many additional histopathological features known to be associated with systemic disease. Similar to previous studies, we found that a neutrophil-predominant dermal infiltrate is associated with HUV.[6,7,9,11,12] In addition to previous publications, we also scored a substantial number of additional histopathological features. In our study, the strongest histopathological feature associated with HUV is the presence of neutrophils along the DEJ and local dermal C4d deposition. Besides association with HUV, neutrophil DEJ and dermal C4d deposition were also directly associated with SLE (Table 3). As markers for SLE, hypocomplementemia alone or combined with ANA levels shows superior sensitivity and specificity over histopathological features, also when these features were added to both parameters. However, irrespective of serum measurement, our findings can prompt the dermatopathologist when faced with these (immuno)histopathological features to suggest an association with an underlying systemic disease in UV. Alignment of neutrophils along the DEJ has been described for bullous SLE and more recently in neutrophilic urticarial dermatosis and so-called "autoimmunity-related neutrophilic dermatosis."[13,14] In contrast to the previous entities, we are the first to report this histopathological clue in UV. Moreover, our findings further strengthen the hypothesis that SLE and HUV represent different expression of the same disease.[15,16]

In SLE-associated HUV, immunoglobulins and C3 can be found in a granular fashion in the (peri)vascular endothelium and along the basement membrane (lupus-band) by immunofluorescence.[16] The presence of immunoglobulins along the basement membrane can likely explain our findings of neutrophils along the DEJ. Our study also shows vascular but predominantly perivascular granular C4d deposition on and between the infiltrate, reflecting immune-complex–mediated classic complement activation. Immunoglobulins are known to have a relatively short half-life on the endothelium and are only found in a minority of cases by direct immunofluorescence in UV. C4d is a split product of C4 and thereby reflects prior antibody-mediated activation of the classic complement pathway. The detection of C4d has been extensively used as a marker of antibody-mediated rejection after kidney transplantation. C4d itself is biologically inactive, but has a long half-life and therefore serves as an excellent marker of prior or ongoing complement activation. At this moment, it is the only antibody against a complement component that is routinely used on formalin-fixed paraffin-embedded tissue and can therefore easily be used in clinical practice instead of direct immunofluorescence on frozen tissue. It allows clinicians to proceed directly to serological investigation of an underlying systemic disease.

In summary, the current study shows that both the alignment of neutrophils along the DEJ and dermal C4d deposition are strongly associated with HUV and SLE. Therefore, these (immuno)histopathological features can be used as an easy diagnostic adjunct for early detection of underlying systemic disease in UV.

REFERENCES

1. Davis MD and Brewer JD, Urticarial vasculitis and hypocomplementemic urticarial vasculitis syndrome. *Immunol Allergy Clin North Am.* 2004;24:183–213, vi.
2. Wisnieski JJ. Urticarial vasculitis. *Curr Opin Rheumatol.* 2000;12:24–31.
3. Jachiet M, Flageul B, Deroux A, et al. The clinical spectrum and therapeutic management of hypocomplementemic urticarial vasculitis: data from a French nationwide study of fifty-seven patients. *Arthritis Rheumatol.* 2015;67:527–534.

4. Antes U, Heinz HP, Loos M. Evidence for the presence of autoantibodies to the collagen-like portion of C1q in systemic lupus erythematosus. *Arthritis Rheum.* 1988;31:457–464.
5. Sunderkotter CH, Zelger B, Chen KR, et al. Nomenclature of cutaneous vasculitis: dermatologic addendum to the 2012 revised International Chapel Hill consensus conference nomenclature of vasculitides. *Arthritis Rheumatol.* 2018;70:171–184.
6. Mehregan DR, Hall MJ, Gibson LE. Urticarial vasculitis: a histopathologic and clinical review of 72 cases. *J Am Acad Dermatol.* 1992;26: 441–448.
7. Sanchez NP, Winkelmann RK, Schroeter AL, et al. The clinical and histopathologic spectrums of urticarial vasculitis: study of forty cases. *J Am Acad Dermatol.* 1982;7:599–605.
8. Wisnieski JJ, Baer AN, Christensen J, et al. Hypocomplementemic urticarial vasculitis syndrome. Clinical and serologic findings in 18 patients. *Medicine (Baltimore).* 1995;74:24–41.
9. Lee JS, Loh TH, Seow SC, et al. Prolonged urticaria with purpura: the spectrum of clinical and histopathologic features in a prospective series of 22 patients exhibiting the clinical features of urticarial vasculitis. *J Am Acad Dermatol.* 2007;56:994–1005.
10. Giang J, Seelen MAJ, van Doorn MBA, et al. Complement activation in inflammatory skin diseases. *Front Immunol.* 2018;9:639.
11. Dincy CV, George R, Jacob M, et al. Clinicopathologic profile of normocomplementemic and hypocomplementemic urticarial vasculitis: a study from South India. *J Eur Acad Dermatol Venereol.* 2008;22:789–794.
12. Soter NA, Mihm MC, Gigli I, et al. Two distinct cellular patterns in cutaneous necrotizing angiitis. *J Invest Dermatol.* 1976;66:344–350.
13. Saeb-Lima M, Charli-Joseph Y, Rodríguez-Acosta ED, et al. Autoimmunity-related neutrophilic dermatosis: a newly described entity that is not exclusive of systemic lupus erythematosus. *Am J Dermatopathol.* 2013;35:655–660.
14. Broekaert SM, Böer-Auer A, Kerl K, et al. Neutrophilic epitheliotropism is a histopathological clue to neutrophilic urticarial dermatosis. *Am J Dermatopathol.* 2016;38:39–49.
15. Matarredona J, Sendagorta E, Rocamora A, et al. Systemic lupus erythematosus appearing as an urticarial vasculitis. *Int J Dermatol.* 1986;25: 446–448.
16. Davis MD, Daoud MS, Kirby B, et al. Clinicopathologic correlation of hypocomplementemic and normocomplementemic urticarial vasculitis. *J Am Acad Dermatol.* 1998;38:899–905.