Bowman's capsule rupture links glomerular damage to tubulointerstitial inflammation in ANCA-associated glomerulonephritis

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ABSTRACT

Objective. *We have recently described the frequency of Bowman's capsule (BC) rupture in a considerable subset of patients with antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN). Interestingly, recent reports established a better performance of glomerulocentric ANCA scoring systems after adding BC rupture to these classification systems, suggesting that characteristics of this lesion are independent from glomerular lesions. Since BC rupture may link glomerular damage to tubulointerstitial lesions via direct interaction with the surrounding interstitium, we here aimed to expand our current knowledge of this distinct lesion by a systematic description of tubulointerstitial lesions analogous to the Banff classification in association with the presence of BC rupture in ANCA GN.*

Methods. *A total number of 44 kidney biopsies with confirmed renal involvement of ANCA GN were retrospectively included between 2015 till 2020 in a single-centre observational study.*

Results. *We here show that presence of BC rupture was associated with severe deterioration of kidney function at disease onset, similar to previous findings regarding long-term renal survival. Furthermore, BC rupture in ANCA GN was associated with tubulointerstitial inflammation and ultrastructural analysis revealed direct cellular exchange between Bowman's space and the interstitium, potentially contributing to the observed deterioration of kidney function and worse renal outcome in ANCA GN.*

Conclusion. *BC rupture is associated with renal outcome in ANCA GN, therefore underscoring the need for further studies with regard to the glomerular-tubulointerstitial interaction in this disease.*

Introduction

Bowman's capsule (BC) rupture was first described more than 30 years ago and we have recently described the frequency of BC rupture in a considerable subset of patients with antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN) (1). Histologically, the typical feature of AAV in the kidney is pauci-immune necrotising and crescentic ANCA GN implemented in the glomerulocentric scoring system proposed by Berden *et al*. (2). In theory, glomerular injury is an upstream process in ANCA GN and tubulointerstitial injury is commonly thought to be downstream of this glomerular injury. These events are thought to lead to tubular atrophy and fibrosis, which were finally implemented into the scoring system proposed by Brix *et al*. (3). Interestingly, recent reports established a better performance of these scoring systems after the addition of BC rupture to these classification systems, suggesting that characteristics of this lesion are independent from already described glomerular lesions, tubular atrophy or fibrosis (4). Since BC rupture may directly link glomerular damage to tubulointerstitial lesions via a direct interaction with the surrounding interstitium, we here aimed to expand our current knowledge of this distinct lesion by a systematic description of tubulointerstitial lesions in association with the presence of BC rupture in 44 kidney biopsies with confirmed ANCA GN.

Materials and methods

Study population

A total number of 44 kidney biopsies with confirmed renal involvement of ANCA GN at the University Medical Center Göttingen were retrospectively included between 2015 till 2020 (Ta-

ble I), the patient cohort has, in part, been described previously (5). All studies involving human participants were reviewed and approved by the Institutional Review Board of the University Medical Center Göttingen, Germany (protocol numbers 22/2/14 and 28/09/17). Medical records were used to obtain data on age, sex, diagnosis (GPA or MPA) and laboratory results. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (6). The worst measurement during the initial course of the disease was used to determine the severity of kidney injury.

Table I. Total patient cohort of ANCA GN.

No. of renal biopsies	44
Clinical data	
Age (IQR) – years	65.5 (54.3-74)
Female sex – no. (%)	20 (45.5)
ANCA subtype MPO/PR3 – no. (%)	21/23 (47.7/52.3)
History of vasculitis – no. (%)	5 (11.4)
Disease activity on admission	
BVAS (IQR) – points	18 (15-21)
CRP (IQR) – mg/L	64.8 (30.4-118)
Haematuria – no. (%)	43 (97.7)
Extrarenal manifestations	
Pulmonary haemorrhage – no. (%)	5 (11.4)
Skin involvement – no. (%)	7 (15.9)
Renal injury	
Serum creatinine (IQR) – μmol/L	263 (116-385)
Serum creatinine ≥500 μmol/L – no. (%)	6 (13.6)
eGFR (IQR) – mL/min/1.73 m^2	20.2 (12-51.1)
RRT – no. (%)	12 (27.3)
Indication for RRT	
Refractory volume overload – no. (%)	4 (33.3)
Symptomatic uraemic intoxication – no. (%)	6 (50)
Hyperkalemia – no. (%)	4 (33.3)
Metabolic acidosis – no. (%)	3 (25)

Median values and IQR are shown.
ANCA: anti-neutrophil cytoplasmic antibodies; BVAS: Birmingham Vasculitis Activity Score; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; GN: glomerulonephritis; IQR: interquartile range; MPO: myeloperoxidase; No.: number; PR3: proteinase 3; RRT: renal replacement therapy.

Definitions

On admission, the Birmingham Vasculitis Activity Score (BVAS) version 3 was calculated as described previously (7). Severe kidney injury was defined by requirement of renal replacement therapy (RRT) within 30 days after admission. RRT was performed intermittently (intermittent haemodialysis) as deemed necessary by the treating physicians. Indications in all cases included refractory volume overload, symptomatic uraemic intoxication (uraemic encephalopathy, anorexia, asthenia), severe hyperkalaemia and metabolic acidosis (Table I).

Renal histopathology

Two renal pathologists (SH and PS) evaluated all biopsies and were blinded to all clinical data and analyses. Within a renal biopsy specimen, each glomerulus was scored separately for the presence of BC rupture in injured glomeruli (crescentic and/or necrotic). Consequently, the percentage of glomeruli affected by BC rupture was calculated as a fraction of the total number of glomeruli in each renal biopsy. Renal biopsies were also evaluated analogous to the Banff scoring system for allograft pathology (8).

Transmission electron microscopy (TEM)

1 to 2 mm thick tissue pieces were postfixed in 3% glutaraldehyde-PBS overnight; thereafter, the slices were washed, and small blocks (2x3 mm) of cortex were cut, postfixed in OsO_4 (1% for 2 hours), and subsequently dehydrated and embedded into Epon by standard procedures. Semithin (1 µm thick) sections of each animal and, in addition, series of semithin sections of selected blocks as well as ultrathin sections of selected areas were cut on a ultracut microtome (Leica, Nussloch, Germany) using a diamond knife. Ultrathin sections were stained with uranyl acetate and lead citrate and studied with transmission electron microscopy (TEM), as previously described (9).

Statistical methods

Variables were tested for normal distribution using the Shapiro-Wilk test. Non-normally distributed continuous variables are expressed as median and interquartile range (IQR), categorical variables are presented as frequency and percentage. Statistical comparisons were not formally powered or prespecified. Spearman correlation analyses were used to analyse correlations and shown by a heatmap reflecting mean values of Spearman's ϱ, asterisks indicate $p<0.05$. Comparison of survival curves was performed with log rank (Mantel-Cox) testing. Data analyses were performed with GraphPad Prism (v. 8.4.3 for MacOS, GraphPad Software, San Diego, California, USA).

Results

We first analysed the presence of BC rupture in association with clinical parameters in AAV (Fig. 1A and Table I). The fraction of glomeruli affected by BC rupture was independent of gender, age, ANCA subtype or extrarenal AAV manifestation (Fig. 1B). These observations were further supported by a specific correlation of BC rupture with severe deterioration of kidney function at disease manifestation reflected by rise of serum creatinine and eGFR loss (Fig. 1B). Furthermore, increased fraction of glomeruli affected by BC rupture correlated with requirement of renal replacement therapy (RRT) within 30 days after admission (Fig. 1B), confirmed by survival analysis for cumulative incidence of RRT (Fig. 1C). In summary, the presence of BC rupture was associated with severe deterioration of kidney function at dis-

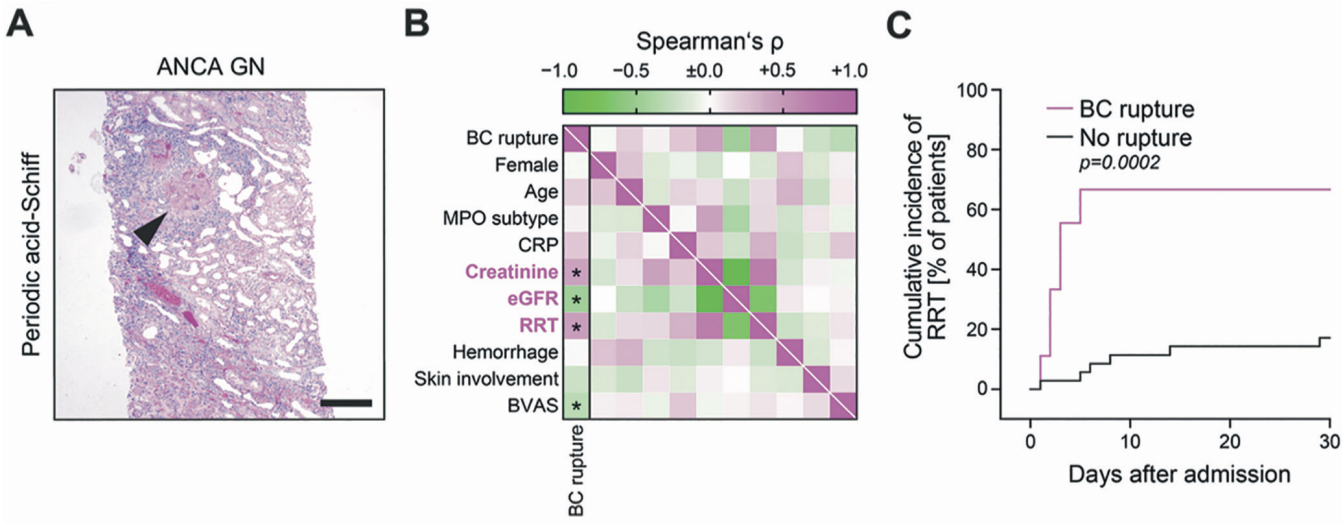

Fig. 1. BC rupture is associated with severe deterioration of kidney function at disease onset.
(**A**) Representative periodic acid-Schiff reaction-stained renal biopsy in ANCA GN with BC rupture (arrowhead, scale bar: 200 μm). (**B**) Association between the fraction of glomeruli affected by BC rupture and clinical parameters in ANCA GN is shown by a heatmap reflecting mean values of Spearman's ρ, asterisks indicate $p<0.05$. (**C**) Survival analysis of the cumulative incidence of RRT within 30 days after admission, comparison of survival curves was performed with log rank (Mantel-Cox) testing.
ANCA: anti-neutrophil cytoplasmic antibodies; BC: Bowman's capsule; BVAS: Birmingham Vasculitis Activity Score; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; GN: glomerulonephritis; MPO: myeloperoxidase; RRT: renal replacement therapy.

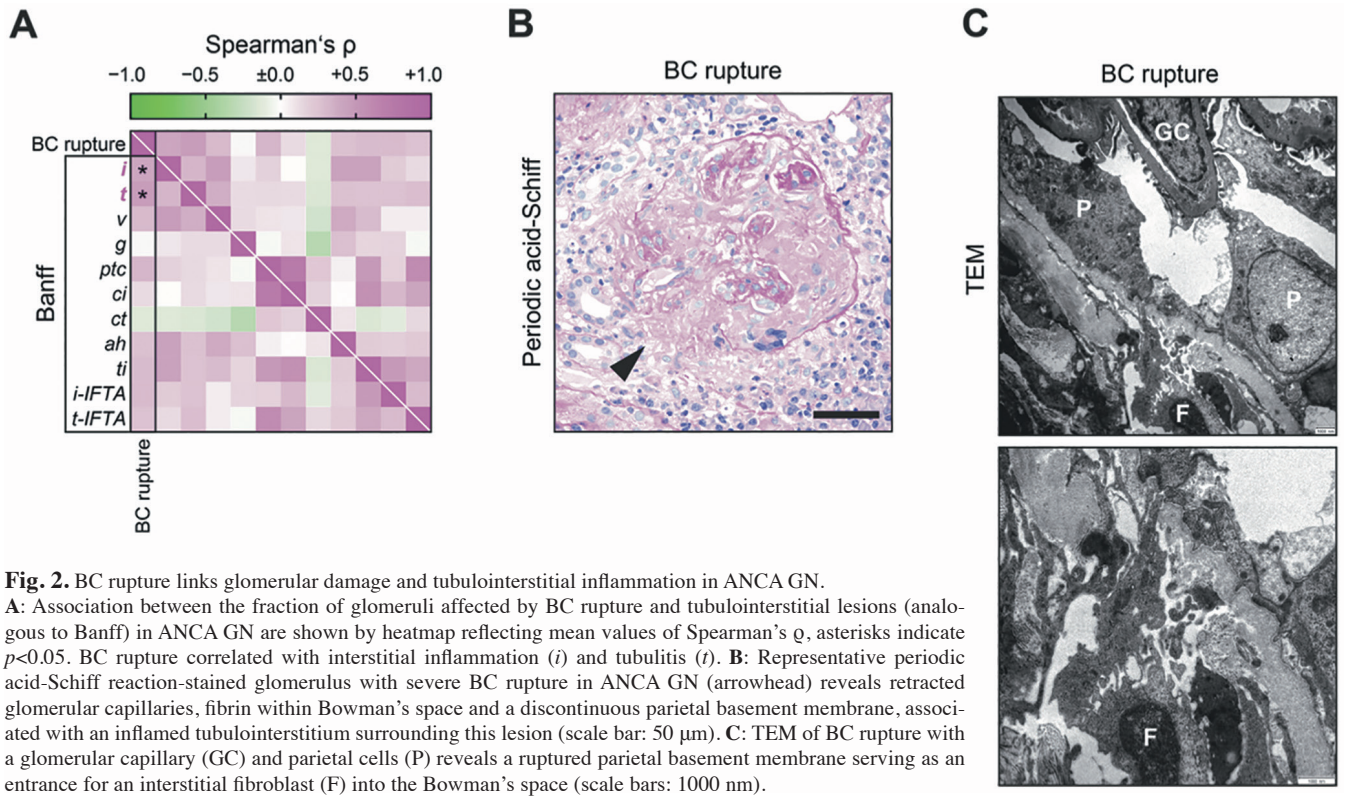

Fig. 2. BC rupture links glomerular damage and tubulointerstitial inflammation in ANCA GN.
A: Association between the fraction of glomeruli affected by BC rupture and tubulointerstitial lesions (analogous to Banff) in ANCA GN are shown by heatmap reflecting mean values of Spearman's ϱ, asterisks indicate $p<0.05$. BC rupture correlated with interstitial inflammation (*i*) and tubulitis (*t*). **B**: Representative periodic acid-Schiff reaction-stained glomerulus with severe BC rupture in ANCA GN (arrowhead) reveals retracted glomerular capillaries, fibrin within Bowman's space and a discontinuous parietal basement membrane, associated with an inflamed tubulointerstitium surrounding this lesion (scale bar: 50 μm). **C**: TEM of BC rupture with a glomerular capillary (GC) and parietal cells (P) reveals a ruptured parietal basement membrane serving as an entrance for an interstitial fibroblast (F) into the Bowman's space (scale bars: 1000 nm).
Ah: arteriolar hyalinosis; ANCA: anti-neutrophil cytoplasmic antibodies; BC: Bowman's capsule; *ci*: interstitial fibrosis; *ct*: tubular atrophy; *g*: glomerulitis; GN: glomerulonephritis; *i*: interstitial inflammation; *i-IFTA*: inflammation in IFTA; *t*: tubulitis; *ptc*: peritubular capillaritis; *ti*: total inflammation; *t-IFTA*: tubulitis in IFTA; TME: transmission electron microscopy; *v*: intimal arteritis.

ease onset, similar to previous findings regarding long-term renal survival (4). Next, we analysed tubulointerstitial lesions in association with BC rupture. By using the Banff scoring system, an increased fraction of glomeruli affected by BC rupture correlated with interstitial inflammation (*i*) and tubulitis (*t*, Fig. 2A), implicating that BC rupture links glomerular damage to tubulointerstitial nephritis. These observations were further highlighted by severe interstitial inflammation surrounding glomeruli affected by BC rupture with vasculitc injury and obious disconti-

nuity of the parietal basement membrane (Fig. 2B). Transmission electron microscopy (TEM) of BC rupture revealed ruptured parietal basement membranes serving as an entrance for interstitial cells into the Bowman's space (Fig. 2C), suggesting that BC rupture enables direct interaction of the tubulointerstial compartment with the inner part of the glomerulus and *vice versa*.

Discussion

To our knowledge, this is the first report linking severe glomerular damage and consecutive BC rupture to tubulointerstitial inflammation in ANCA GN. We here describe that an increased fraction of glomeruli affected by BC rupture was associated with severe deterioration of kidney function at disease onset, similar to previous findings regarding long-term renal survival (4). This is in line with previous reports that deterioration of kidney function at disease onset also affects long-term renal survival and relapse rate in ANCA GN (10). Furthermore, an increased fraction of glomeruli affected by BC rupture in ANCA GN was associated with tubulointerstitial inflammation, suggesting that interstitial inflammation may also have predictive value in assessing the risk for decline of kidney function in ANCA GN (11, 12). The concept of tubulointerstitial injury mediating impairment of renal function has been described more than five decades ago, showing that decline of kidney function exhibited a stronger correlation with the severity of tubulointerstitial rather than with glomerular damage (13). It has previously been proposed that the fibrous strand-strengthened basement membrane of the BC might serve as a barrier for inflammatory cell invasion into Bowman's space (14). In this context, an intact BC prevents inflammatory cells from gaining access to the glomerular space, but once BC is breached, inflammatory cells can access the glomerular space in crescentic GN with BC rupture enabling direct pathological interaction between both of these compartments. This concept has previously been confirmed experimentally and also reported in biopsies from patients with autoimmune and anti-GBM crescentic GN (15-17). For years, it has been well established that severe glomerular injury leads to the degeneration of the corresponding tubule and to the loss of this specific nephron but resulting in a limited renal injury (18). More than 20 years ago, the concept of misdirected urinary filtration was established as a mechanism for nephron degeneration in focal segmental glomerulosclerosis (FSGS) (19). Parietal epithelial cell adhesions to the glomerular tuft leads to a pathological space between the glomerular capillary lumen and the parietal basement membrane, resulting into urine accumulation between the tubular epithelial layer and its basement membrane. As a consequence, the corresponding tubule undergoes tubular atrophy and interstital fibrosis accompanied by a mild chronic inflammation. In FSGS, misplaced interstial urine is mostly still enclosed by an intact tubular basement membrane, thereby limiting extensive urinary spread into the surrounding interstitium. In contrast, BC rupture might serve as pathological gap for primary urine diffusely entering the interstitial space without any physiological barriers, thereby linking glomerular damage and tubulointerstitial inflammation and resulting in an inflammatory destruction of adjacent nephrons. This possible mechanism provides a potential inflammatory stimulus of intrarenal disease spreading, thus explaining the previously observed improvement of established ANCA scoring systems by implementation of BC rupture (2-4).

In summary, we here expand the current knowledge of BC rupture and systematically describe an association with tubulointerstitial nephritis, potentially contributing to a severe deterioration of kidney function at disease onset and worse renal outcome in ANCA GN as described previously (4). The main limitations of our study are its retrospective design in a single centre and a selection bias towards more severe cases of ANCA GN with limited information of kidney function before disease manifestation as a tertiary referral centre. Nevertheless, BC rupture has independently been described to be associated with renal outcome in ANCA GN. This underscores the need for further studies with regard to the glomerular-tubulointerstitial interaction in this disease.

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