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Vancomycin-Associated Tubular Casts and Vancomycin Nephrotoxicity

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Introduction: Vancomycin nephrotoxicity is frequent and may be due to drug-induced acute tubular necrosis (ATN) or tubulointerstitial nephritis (TIN). Vancomycin-associated tubular cast (VTC) was recently described and may represent a novel cause of vancomycin nephrotoxicity. However, much is still unknown about VTC.

Materials and Methods: Thirty-seven kidney biopsy specimens from patients who were treated with vancomycin and developed acute kidney injury (AKI) were found among a total of 4673 biopsy samples between 2010 and 2019. These biopsy specimens were subjected to light microscopy, immunofluorescence, electron microscopy, and immunolocalization for vancomycin, uromodulin, myoglobin, tubular segment–specific markers, and examined for VTCs. The findings were correlated with the clinical course.

Results: VTCs displayed precipitated vancomycin casts in a background of uromodulin; the casts were limited to the distal tubules, and always associated with a background of more diffuse renal injury (ATN or TIN). The diagnosis of vancomycin nephrotoxicity was made in in 28 of 37 patients. VTC was noted in 25 of 28 biopsy samples from patients diagnosed with vancomycin nephrotoxicity and in one of nine biopsy samples from patients without this diagnosis. Vancomycin nephrotoxicity was diagnosed in 25 of 26 patients whose biopsy specimens showed VTC, but in only 3 of 11 patients without VTC in the biopsy samples.

Conclusions: VTC displays a characteristic morphologic profile amenable to ready recognition in biopsy specimens. It results from coprecipitation of vancomycin and uromodulin. It facilitates the biopsy diagnosis of vancomycin nephrotoxicity. It may have a nephrotoxic effect superimposing on and independent from the ATN or interstitial nephritis in the pathogenesis of vancomycin nephrotoxicity.

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Staphylococcal infection resistant to conventional antibiotics including methicillin is increasingly frequent and represents one of the most severe types of infection.[1-4] Consensus guidelines from the Infectious Diseases Society of America indicate that vancomycin is the drug of choice in this situation.[5] In fact, vancomycin is the most frequently prescribed antibiotic in the hospital setting for up to 35% of hospitalized patients with infection.[2]

One of the more frequent side effects of vancomycin is nephrotoxicity, manifesting clinically as AKI, developing *de novo* or against a background of chronic kidney injury (CKI). Vancomycin nephrotoxicity (VN) has been reported in up to 25% of patients, promoted by several risk factors including high doses, elevated serum drug concentration, concomitant nephrotoxic drugs, and pre-existing systemic conditions such as obesity, CKI, diabetes, liver disease, and immunosuppression.[1-4] Many of these risk factors are also known risk factors for infection. These considerations clearly magnify the profound clinical implication of VN.

VN is often diagnosed by clinical and laboratory findings, followed by discontinuation of the drug, often resulting in improvement of renal function, thus confirming the diagnosis. However, renal biopsy may

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be indicated if the clinical diagnosis is equivocal, or in the presence of other conditions that by themselves can cause AKI.[6] In patients treated with vancomycin who develop AKI, it can be attributed to vancomycin in only 59% of cases.[7] A definite diagnosis is imperative for the decision to continue with vancomycin or replace it with other antibiotics.

Although VN has been well known for the past 50 years, the renal changes associated with it is still poorly defined, perhaps reflecting limited opportunities for renal biopsies. The lesions, reported in less than 31 renal biopsy specimens, mostly in single case studies, include TIN or ATN, developing individually or often in combination.[6] These structural changes are thought to be the cause of AKI. In 2017, renal tubular casts, which are composed of vancomycin and termed VTCs, were first described in association with VN.[8] This novel finding expands the spectrum of vancomycin-associated renal changes and may be of pathogenetic implication. However, the original study focuses on the fundamental nature of VTCs in a single renal biopsy specimen, with additional immunohistochemical detection of VTCs in eight more biopsy samples, thus leaving unanswered several aspects of VTCs including morphologic spectrum, frequency, and clinical/pathogenetic significance. The current study aims to comprehensively address some of these considerations.

MATERIALS AND METHODS

Records of renal biopsy specimens from the Houston Methodist Hospital System between 2010 and 2019 were reviewed to identify those from patients treated with vancomycin around the time of renal biopsy. Thirty-seven such biopsy samples were found among a total of 4673 biopsy specimens. The main indication for the biopsy was AKI associated with vancomycin treatment, developing *de novo* or on a background of CKI.

Each of these biopsy specimens were subjected to light microscopy (LM) (using hematoxylin and eosin, periodic acid-Schiff, trichrome, and silver methenamine stains) and immunofluorescence (for immunoglobulins [Ig] G, A, and M; complement components C3, C4, and C1q; and kappa and lambda light chains). To correlate the variable LM morphology of the tubular casts with the deposition of vancomycin and uromodulin in them, each of these biopsy specimens were submitted for antivancomycin mouse monoclonal antibody (Abbot, 6E44-21, Hospital Tenon, France; dilution 1:1000) and anti-uromodulin antibody (11911–1-AP, Proteintech, Rosemont, Illinois, USA, dilution; 1:50) as described in the original study.[8] Immunostain for myoglobin was also performed for these biopsy specimens.

To determine the location of VTCs in various tubular segments, tissue sections were submitted to immunostaining for the renal cell carcinoma marker (Vector Laboratories, Burlingame, California, USA; dilution 1:10), a specific marker for proximal tubules, and kidney-specific cadherin (Zymed Laboratories, San Francisco, California, USA; dilution 1:75), a specific marker for thick Henle loops and distal convoluted tubules.[9,10] These studies were all performed on serial tissue sections consecutive to those for routine LM, an approach that enables an integrative interpretation of the morphologic findings. Five renal biopsy specimens from patients without vancomycin treatment, displaying a variety of tubular casts including hyaline, uromodulin, light chain, calcium phosphate, or calcium oxalate casts, were also submitted to immunostaining to confirm the specificity of vancomycin immunolocalization.

Electron microscopy (EM) was studied for each biopsy both retrospectively and prospectively. Preexisting thick sections and archived electron micrographs in each biopsy were reviewed in search for VTCs which should display characteristic perhaps pathognomonic features (see below). As expected, this approach revealed only a few cases with VTCs, reflecting the traditional glomerulocentric focus of routine EM study, as well as a lack of awareness of VTCs during initial EM study. Subsequently, for each of the biopsy specimens, thick sections were prepared for all archival resin tissue blocks, which were carefully examined in search for the tubular casts with features suggestive of VTCs, matching them with the typical VTCs in the LM and immunostained sections of the same biopsy. These casts were then submitted to meticulous EM examination.

To assess the significance of VTCs, the clinical course of the patients was reviewed to determine whether the AKI was due to vancomycin treatment and how it is related to the presence of VTCs in the corresponding renal biopsy specimens.

RESULTS

Renal Biopsy Findings

The current study focuses on a comprehensive morphologic characterization of VTCs. The renal biopsy findings will be described in detail in a comprehensive clinicopathologic study. Only the findings that are deemed relevant to the focus of this study are described. Briefly, among the 37 biopsy specimens, there was acute or acute-on-chronic TIN (n = 3), ATN

Table 1. Demographic data and pathologic findings of acute kidney injury patients who received vancomycin (N = 37)

Demographic data			
Age, range, yrs		23 to 84	
Age, mean, yrs		54	
Female:male ratio		15:12	
Pathologic findings[a]			
Acute or chronic TIN		3 (8.1)	
ATN		5 (13.5)	
Both ATN and TIN		25 (67.6)	
IFTA		4 (10.8)	
VN, 28 (75.7)		No VN, 9 (24.3)	
VTCs	No VTCs	VTCs	No VTCs
n = 25 of 28 (89.3)	n = 3 of 28 (10.7)	n = 1 of 9 (11.1)	n = 8 of 9 (88.9)

ATN, acute tubular necrosis; IFTA, interstitial fibrosis and tubular atrophy; TIN, tubulointerstitial nephritis; VN, vancomycin nephrotoxicity; VTCs, vancomycin-associated tubular casts.
[a]Pathologic findings are expressed as n (%).

(n = 5), both TIN and ATN (n = 25), and interstitial fibrosis/tubular atrophy (n = 4) (Table 1). Other renal lesions were also frequent including diabetic nephropathy (n = 14), arterionephrosclerosis (n = 16), IgA nephropathy (n = 2), postinfectious glomerulonephritis (n = 1), cholesterol embolism (n = 1), myoglobin cast nephropathy (n = 2), acute pyelonephritis (n = 1), and focal segmental glomerulosclerosis (n = 1).

LM Morphology of VTCs

Among 37 renal biopsy specimens from 37 patients with vancomycin treatment around the time of renal biopsy, tubular casts with LM changes characteristic for or at least suggestive of vancomycin deposition (*i.e.*, VTCs) were noted in 26 biopsy specimens. Twenty biopsy samples that reveal VTCs are associated with the background of significant tubulointerstitial injury (n = 3 of 5 ATN; n = 1 of 3 TIN; n = 22 of 25 ATN + TIN) (Table 1, Figure 1a and b)

Several distinctive but related forms of VTCs, probably representing a continuum of formation, were identified. Most VTCs were composed of microparticles of variable sizes, ranging from finely granular to larger faint globules with peripheral condensation and a paler center. These structures formed interconnected aggregates of variable sizes, often occupying a portion of tubular lumen, but could be uniform and filled the entire lumen, creating a finely granular appearance on hematoxylin and eosin stain (Figure 2a). VTCs were faintly periodic acid-Schiff–positive (Figure 2b), displayed a variegated appearance on silver and trichrome stains (Figure 2c and d), and in thick section appeared as aggregated granules with focal purple glassy material consistent with uromodulin (Figure 2e). Some VTCs had a significant element of the larger globules, imparting a partially bubble appearance, best appreciated on periodic acid-Schiff stain (Figure 2b). Few VTCs were composed of polymorphous globules, with focal central clearing, forming connecting aggregates (Figure 2g and h), or sizable solid individual spherical globules (Figure 2j and k). These structures were often seen against a background of pale bluish material representing uromodulin. Lamellation, often not obvious by routine stains, could be obvious in thick section (Figure 2l). Rare VTCs displayed a bubble appearance, but were strongly immunopositive for vancomycin (Figure 3a and b). Some VTCs seemed to be composed entirely of uromodulin with a characteristic appearance (homogeneous pale blue on hematoxylin and eosin stain, strongly periodic acid-Schiff–positive, filling tubular lumens) (Figure 3c). However, in most of these casts, embedded within the uromodulin background, were particles which were not appreciated in routine LM, but displayed typical features of vancomycin, as revealed by immunolocalization, thick sections (Figure 3c, d, e, and 3f), and EM (see below).

VTCs were frequently associated with necrotic (probably tubular) cells (Figure 3g) or less so with intact or degenerated inflammatory cells including neutrophils. The cells of the tubular profiles that housed the VTCs often displayed acute injury including flattening, loss of differentiation, cytoplasmic vacuolization, and cell membrane disintegration (Figures 2a, g, and 3g) Most VTCs were not associated with peritubular inflammation or increased peritubular interstitial fibrosis. There was no difference in the severity or frequency of VTC in biopsy specimens with TIN, ATN, or both.

Immunolocalization of Vancomycin, Uromodulin, and Myoglobin

Immunostain successfully showed vancomycin in the biopsy tissue. The immunopositivity is noted in 34 of 37 biopsy specimens. In the 26 biopsy specimens with VTCs, the staining was localized to all LM forms of VTCs in each biopsy (Figures 1f, h, k, 3b, e, and 4b, and e) with or without additional irregular patchy staining of tubular lumens. In eight biopsy samples without VTCs, there was irregular patchy staining of tubular lumens even without tubular cast formation. In the remaining three biopsy samples, there was neither staining nor VTCs. In some positive biopsy specimens, there was also focal cytoplasmic staining of tubular cells from different tubular segments including perhaps proximal tubules. Tubular casts in the five control biopsy samples were negative.

Uromodulin was immunopositive for tubular casts in 31 biopsy specimens. Uromodulin casts were often

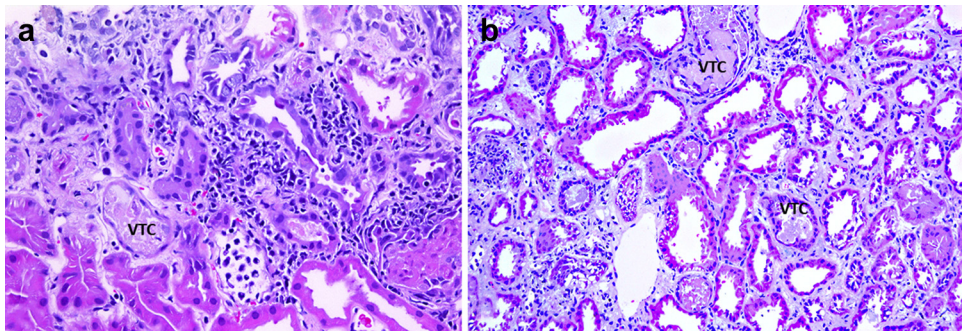

Figure 1. The background changes include acute tubulointerstitial nephritis (a), or acute tubular necrosis with a minor interstitial inflammation (b). Vancomycin-associated tubular casts (VTCs) are noted in each biopsy (hematoxylin and eosin stain; original magnification ×200 for both a and b).

numerous, widespread, and in both cortex and medulla. Uromodulin often co-existed with vancomycin in the same casts (Figures 3e, f, and 4c, and f), but it could appear in isolation. These two patterns were often observed in the same biopsy sample. In the six immunonegative cases, VTCs were not seen.

Immunostain for myoglobin was noted in few tubular casts in two biopsy specimens, both of which also displayed VTCs, independent of the myoglobin casts.

Localization of VTCs in Tubular Segments

VTCs were noted in the cortical and/or corticomedullary junction in almost all biopsy specimens, but also in the medulla in four biopsy samples, in a biopsy cohort composed of cortex in only 23 biopsy specimens, in the medulla in only 1 sample, and both in 13 samples. The nature of the tubular segments housing VTCs was somewhat difficult to determine because they are associated with severe acute injury. However,

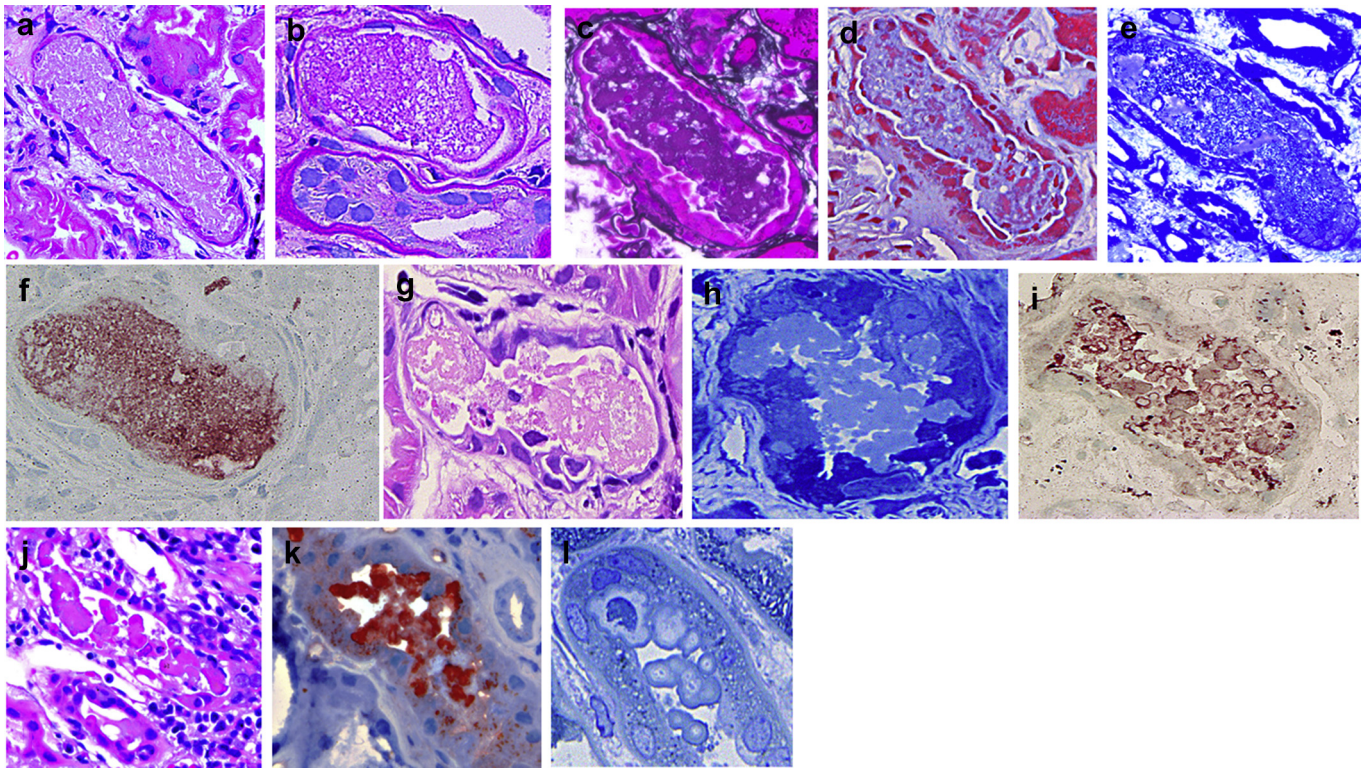

Figure 2. Morphologic spectrum of vancomycin-associated tubular casts (VTCs). (a) The tubular cast is composed of variable-size granular particles. (b) It is faintly periodic acid-Schiff (PAS) positive with a "bubble" appearance. (c,d) It displays a variegated appearance. It appears in thick section as aggregated granules with focal purple glassy material consistent with uromodulin (e) and is positive for vancomycin (f). This cast is composed of polymorphous globules with focal central clearing (g). (h,i) This appearance is also noted in the thick section and vancomycin stain. (j,k) This cast is composed of vancomycin-positive solid spherical globules, associated with necrotic cells. (l) Some globules display peripheral lamellation. (Hematoxylin and eosin stain used for a, g, and j; periodic acid-Schiff stain used for b; silver stain used for c; trichrome stain used for d, toluidine blue stain used for e, h, and l; and anti-vancomycin antibody used for f,i and K. Original magnification ×400 for all panels.)

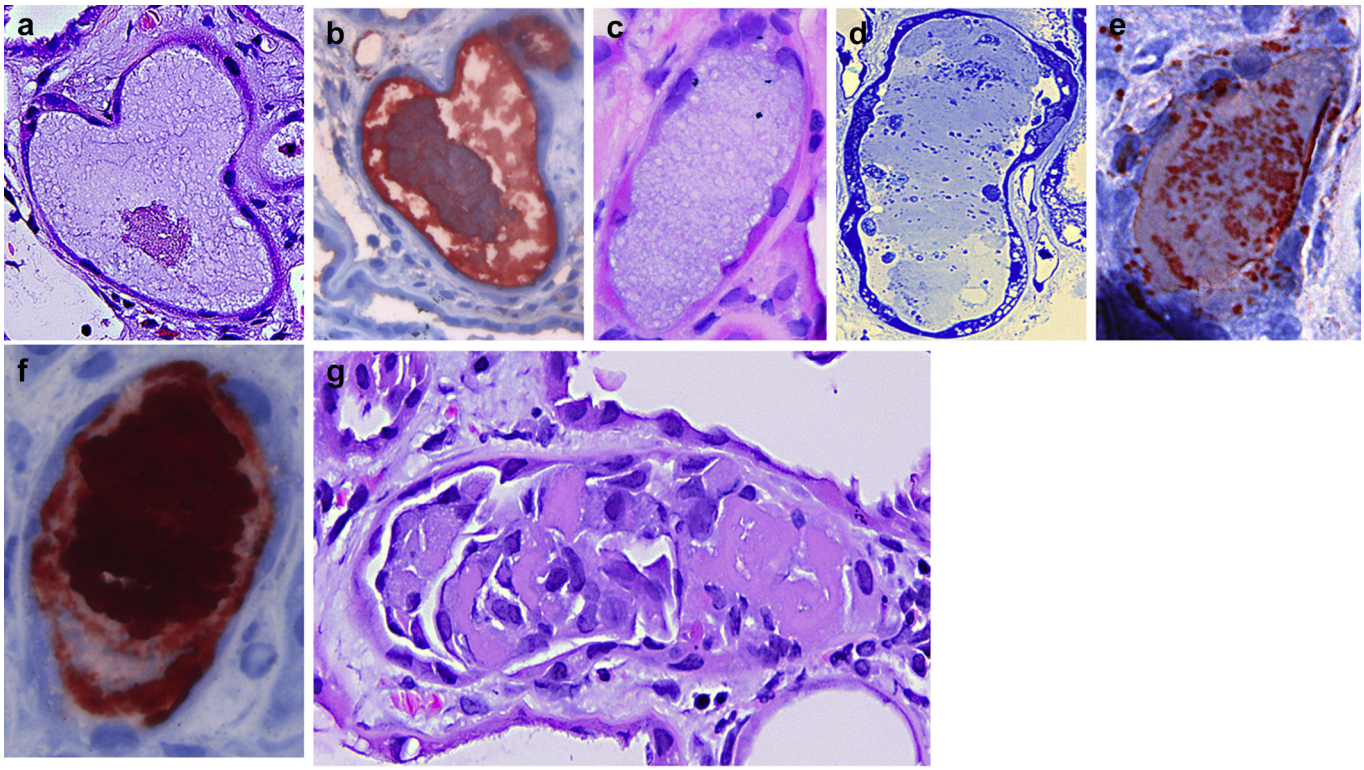

Figure 3. Morphologic spectrum of vancomycin-associated tubular casts (VTCs). This cast displays a bubble appearance with a central amorphous area (a), and is positive for vancomycin (b). This cast has an appearance suggestive of uromodulin cast (homogeneous bluish tinge by hematoxylin and eosin stain) (c); however, in thick section it displays dark granular material, representing vancomycin crystals against a background of uromodulin (d). This cast is positive for vancomycin in granular pattern (e), and positive for uromodulin in diffuse pattern (f). This cast is associated with necrotic cells (g). (Hematoxylin and eosin stain used for a, c, and g; toluidine blue stain used for d; anti-vancomycin antibody used for b and e; and anti-uromodulin antibody used for f. Original magnification ×400 for all panels.)

immunostain for tubular segment-specific markers (renal cell carcinoma Marker and kidney-specific cadherin) showed that most of the VTCs were localized to the distal nephron segments, especially the thick Henle loops and the distal convoluted tubules (Figure 5a, b, c, and d), a finding that was corroborated by EM (Figures 5e, f, g, and 7a, and c).

EM

Tubular casts with features matching with the VTCs in LM and immunostained tissue sections were identified and submitted to EM. VTCs displaying distinctive characteristics were identified in 21 biopsy specimens. Among the remaining 16 negative biopsy specimens, VTCs were not seen in the thick sections of any, but were noted in the corresponding LM sections in six biopsy samples.

The EM findings corroborated and further clarify the LM findings. Vancomycin deposits appeared perhaps initially as variably electron-dense ill-defined round particles or short tubular structures, with a diameter of less than 10 nm, forming separated or interconnected aggregates of different sizes, variable density, and irregular contour, partially occupying tubular lumens (Figure 6a). In other tubular profiles, the deposits appeared as individual or aggregated/connected spherules with well-defined smooth contours, and circumferential peripheral condensation (Figures 6b, c, and 7a, b, and c). In some of these globules, additional layers of similar condensation were noted, imparting an orderly concentric lamellation which eventually occupied the entire globules (Figure 8a-f). These structural forms may represent different phases in a continuous process of crystallization. Most of these forms were observed in every biopsy, and often noted in a single tubular cast (Figures 6b, c, and 7a). Most of these structures were associated with electron pale fibrillary structures typical for uromodulin (Figures 5c, 6a and b, 7c, and 8c). In fact, EM showed that some casts that seem to be composed entirely of uromodulin showed scattered structures with characteristic morphology of vancomycin (Figures 5f and 6c). These structures were frequently enmeshed with necrotic cell debris (Figure 7b and c). The vast majority of tubular segments with VTCs showed features of distal convoluted tubules or thick Henle loop, populated by tubular cells with often severe necrotic/degenerative changes (Figures 5a, b, c, and 7a and c).

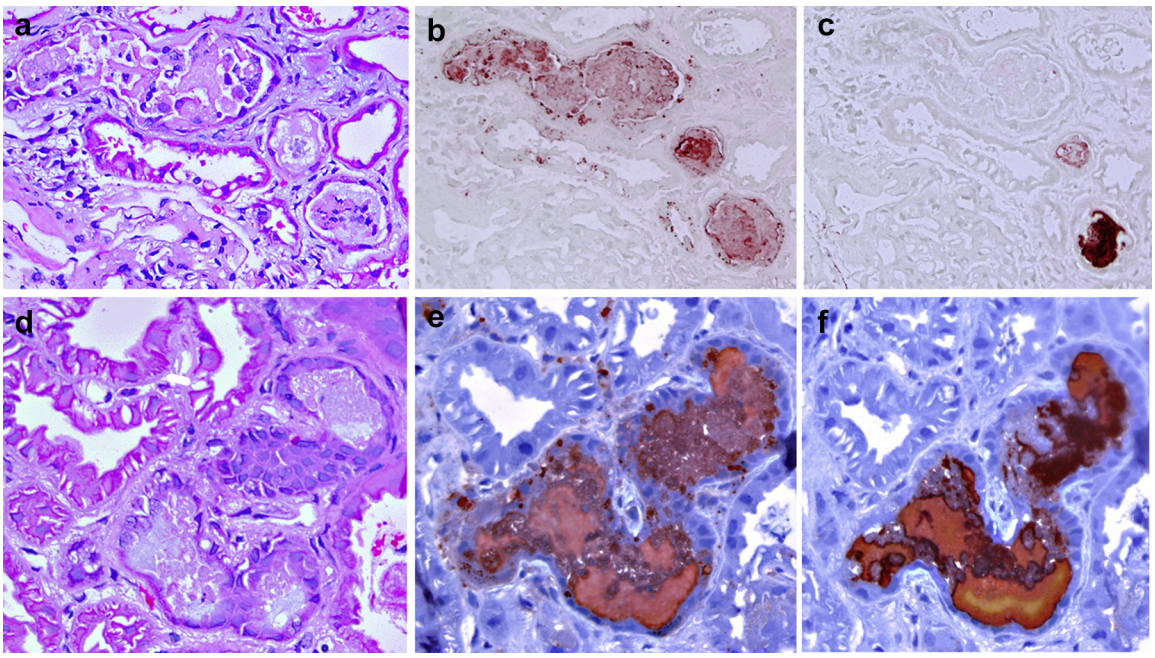

Figure 4. Immunolocalization of vancomycin and uromodulin. In the upper row, hematoxylin and eosin (HE) stain shows casts with necrotic cells (a). The casts are positive for vancomycin (b), but are negative for uromodulin (c). In the lower row, HE stain shows casts which have morphology of both vancomycin and uromodulin (d). The casts are positive for vancomycin (e) and uromodulin (f). (HE stain used for a and d; anti-vancomycin antibody used for b and e; and anti-uromodulin antibody used for c and f. Original magnification ×400 for all panels.)

Clinicopathologic Correlation

Comprehensive description of the clinical course will be detailed in another study. For the current study, the main clinical endpoint is a diagnosis of VN, for a correlation with VTCs.

In short, the clinical information for the 37 patients included age between 23 and 84 years (mean, 54 years). The female:male ratio was 15:12 (Table 1). There are frequent comorbidity conditions including diabetes mellitus (n = 16), hypertension (n = 12), cardiovascular diseases (n = 7), hypothyroidism (n = 4), marked obesity (n = 2), and HIV infection (n = 2). The trough vancomycin blood levels were between 13 and 45 mg/l (mean, 25 mg/l). The patients had infection sites which required vancomycin including skin (n = 14), bone (n = 6), joint (n = 2), heart (n = 2), lung (n = 4), bone (n = 6) and blood (n = 2). Twenty-one patients used vancomycin together with other antibiotics. All patients developed AKI 1 to 3 days after treatment, many of whom required dialysis.

The diagnosis of VN was made in 28 of 37 patients, reflecting the characteristic LM findings followed by an improvement of renal function after discontinuation of vancomycin. In the remaining nine patients, these criteria were not met and the clinicopathologic diagnoses included ATN, TIN, or interstitial fibrosis and tubular atrophy of undetermined etiology, associated with arterionephrosclerosis and or diabetic nephropathy in each case.

VTCs were noted in 25 of 28 biopsy specimens from patients with VN. VTCs were not seen in eight of nine biopsy samples from patients without VN, even in the presence of immunopositive vancomycin in tubular lumens in each of them. Conversely, VN was diagnosed in 25 of 26 patients whose biopsy specimens showed VTCs, but in only 3 of 11 patients without VTCs in the biopsy specimens.

DISCUSSION

Before the recent advent of VTCs, allusion had been made to its existence. Among the 31 single-case reported biopsy specimens from patients on vancomycin, descriptions reminiscent of VTCs were noted in a few cases and were shown in at least one report.[11] In 2017, the seminal study by Luque *et al.*[8] first clearly documented VTCs by LM, immunohistochemistry, immuno-EM, and mass spectrometry in one biopsy; further showed VTCs in another eight biopsy specimens from patients treated with vancomycin; and boldly proposed a novel pathogenetic role for VTCs.

The current study represents a comprehensive evaluation of VTCs. It shows for the first time that VTCs display an LM spectrum of distinctive morphology, perhaps reflecting different stages of precipitation and crystallization of vancomycin in tubular lumens, resulting in tubular casts. Recognizing these casts in renal biopsy specimens should not be difficult. Many VTCs display a typical LM

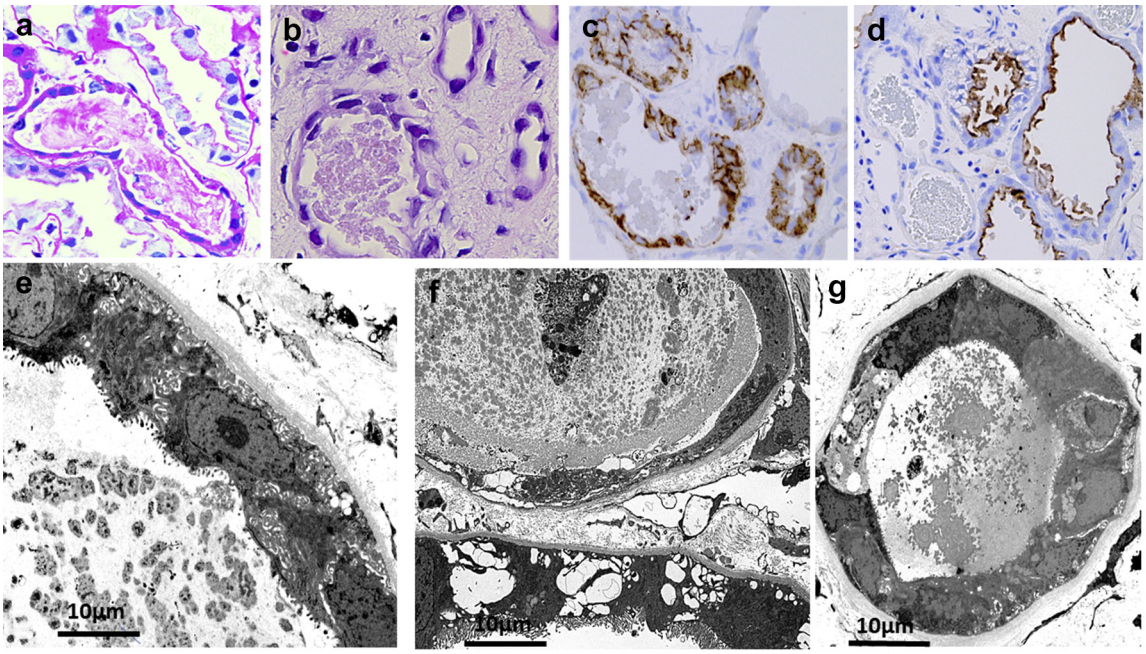

Figure 5. Vancomycin-associated tubular casts (VTCs) in tubular segment. The cast is in a damaged tubule which is adjacent to an intact proximal tubule (a). The cast is in a damaged tubule (b) which is highlighted by kidney-specific cadherin; therefore, it is distal convoluted tubule (c). Renal cell carcinoma (RCC) is negative in the tubule which contains VTCs, but it is positive in adjacent tubules (d). In electron microscopic study, VTCs are noted in distal convoluted tubule with basolateral infolding and short villi (e). VTCs and necrotic cells are present in a damaged tubule, perhaps a collecting duct, which is adjacent to a damaged proximal tubule (f). VTCs are present in a tubule, probably thick Henle loop. (Periodic acid-Schiff stain used for a; hematoxylin and eosin stain used for b; kidney-specific cadherin for c; RCC marker used for d; and electron microscopy used for e, f, and g. Original magnification ×400 for a, b, c, and d; original magnification ×8000 for e, f, and g.)

morphology, virtually diagnostic for vancomycin exposure, reflecting perhaps a predominant vancomycin component. Some VTCs, being composed mostly of uromodulin and a submicroscopic amount of vancomycin, may escape LM scrutiny, but can still be identified by immunolocalization, or EM. Because VTCs of different forms are often present in a single biopsy, the diagnostic utility of the more characteristic form of VTCs is obvious. The presence of these casts in renal biopsy specimens may be the first clue of vancomycin treatment, a clinical event not being aware of even by the attending nephrologist, but confirmed by chart review. At least three such cases have been encountered since the completion of the current study. Necrotic/apoptotic cells, sometime prominent, are often mixed with VTCs, a nonspecific finding, albeit of diagnostic cue, because such association is unusual for other types of tubular casts.

Myoglobin may simulate VTCs, a distinction best achieved by immunohistochemical staining. The concurrent presence of VTCs and myoglobin casts is noted in only two biopsy samples in our study.

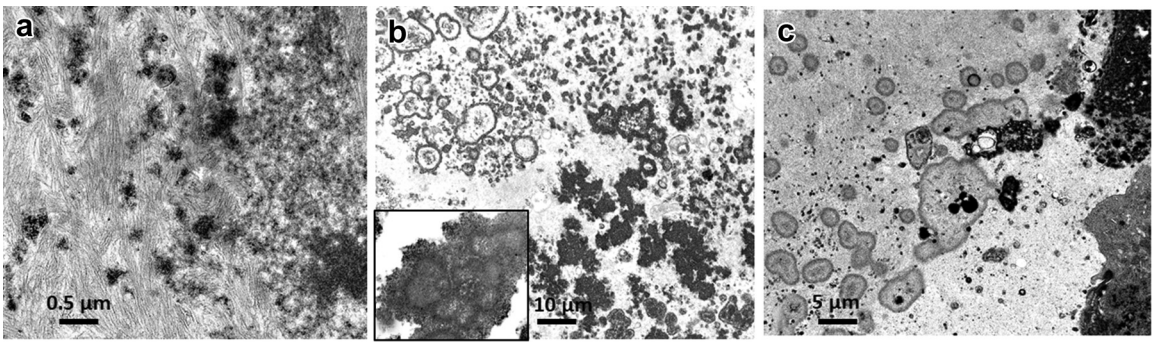

Figure 6. Electron microscopic morphology of vancomycin-associated tubular casts (VTCs). VTCs appear as electron-dense particle clusters of variable densities, in a background of fibrillary structures typical for uromodulin (a). VTCs are composed of electron-dense aggregates with internal lamina structure (b, inset). Other components include spherules with peripheral condensation and central clearing. Transitional forms are also noted (b). VTCs appear as spherules embedded within a uromodulin background (c). (Original magnification ×28,000 for a; ×12,000 and ×28,000 (inset) for b; and ×16,000 for c.)

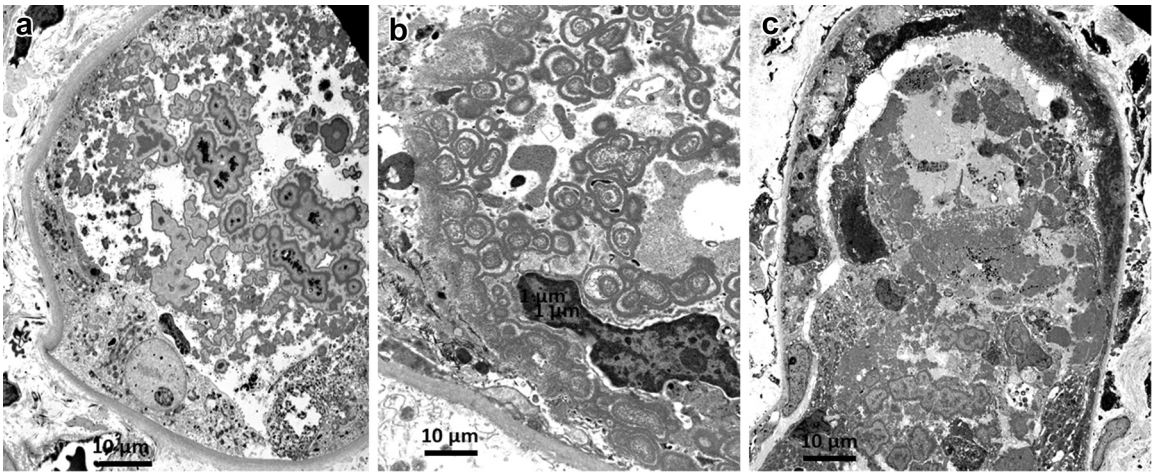

Figure 7. Electron microscopic morphology of vancomycin-associated tubular casts (VTCs). VTCs appear as polymorphic spherules of variable sizes, shapes, and structures, with overlapping features suggesting different phases of crystallization (a). VTCs appear as dense aggregates of spherules, enmeshing a necrotic cell (b). VTCs appear as compacted spherules associated with pale material typical for uromodulin and necrotic cells, occupying the entire tubular lumen (c). (Original magnification ×12,000 for a and c; and ×16,000 for b.)

Ultrastructural study of myoglobin tubular cast is limited because these casts can be readily and accurately identified by immunohistochemistry, circumventing additional diagnostic studies such as EM. Review of published studies indicates that, by EM, myoglobin casts in human renal biopsy specimens appear as homogeneous highly electron-dense, globular, or amorphous structures. Some also display a less-dense peripheral rim without substructure.[12-15] In fact, an early study in rats using infused exogenous myoglobin as a tracer for evaluation of tubular transport revealed the same ultrastructural features.[16] These ultrastructural features are distinctively different from that of vancomycin-associated tubular cast.

Although a plethora of molecules/substances can precipitate in tubular lumens and appear as casts, to the best out our knowledge, they do not share the LM morphology with VTCs.[17] Some patients in the current

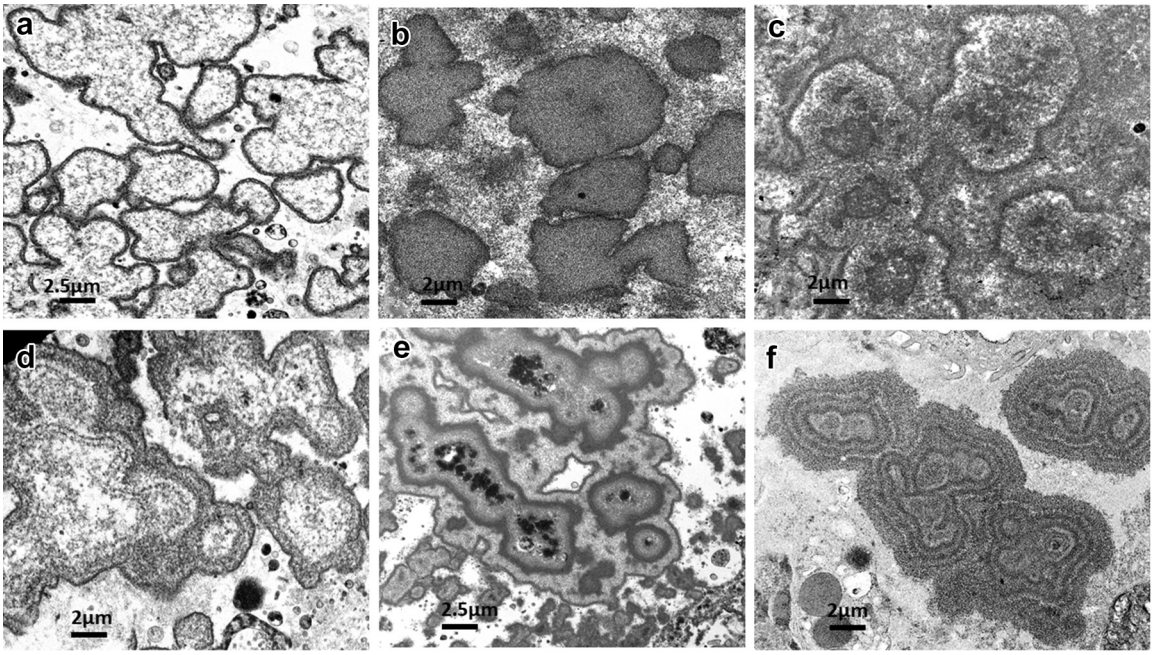

Figure 8. Electron microscopic morphology of vancomycin-associated tubular casts (VTCs). VTCs appear as aggregated, irregularly shaped, interconnected, or single spherules with single-layered electron dense envelope (a). A single-layered electron-dense envelop with uniform granular center (b). A single-layered electron-dense envelope with a variegated center in a fibrillary background (c). A multilayered electron-dense envelope, with variegated center (d). A multilayered envelope with variable electron densities (e) which are composed of concentric laminar structures (f). Transitional forms are frequent and several forms are noted in a same biopsy or the same tubular cast. (Original magnification ×28,000 for all panels.)

study were treated with other types of antibiotics including piperacillin/tazobactam, azithromycin, doxycycline, and cefepime, some of which may be nephrotoxic. However, their tubular precipitation as tubular casts to our knowledge has not been described.[18-20]

Immunohistochemical study shows vancomycin in the majority (34 of 37, 92%) of renal biopsy specimens from patients treated with vancomycin. The immunopositivity noted in 26 biopsy specimens that showed VTCs corroborates that VTCs are composed at least in part of vancomycin. It further confirms that many VTCs that appear as uromodulin casts by LM due to a predominant component of uromodulin indeed contain vancomycin. In contrast, the observation that immunopositivity can be localized to tubular lumen without VTCs recognizable by LM, as noted in eight biopsy specimens, suggests that vancomycin precipitation without forming cast does occur.

The EM study provides insights into the histogenesis of VTCs. The findings suggest a continuous process of vancomycin tubular lumen accumulation, starting from an initial stage with few scattered nanoparticles precipitated within an abundant background of uromodulin, followed by progressive vancomycin crystallization, "crowding out" the uromodulin component. Indeed, structures reflecting these changes are frequently found in the same biopsy and even in a single cast. These findings correlate well with various LM forms of VTCs, reflecting variable contribution by vancomycin and uromodulin. The EM findings also carry diagnostic weight. Although VTCs often appear characteristic by LM, confirmation may be called upon. In the absence of immunohistochemical testing for vancomycin, as expected in the usual laboratory workup, EM would be welcome. Our study shows that the ultrastructural morphology of VTCs, especially in its well-developed forms, is very characteristic. To our knowledge, this morphology is unique, with no other reported types of tubular cast displaying comparable features.

Our study, integrating LM, immunohistochemistry, and EM, substantiates the coprecipitation of vancomycin and uromodulin. This coprecipitation is almost constant and seems to proceed in an orderly manner, further suggesting that uromodulin is a prerequisite for vancomycin precipitation/crystallization. Uromodulin, previously known as Tamm-Horsfall protein, is produced exclusively in kidney in large amounts, by thick Henle loops and distal convoluted tubules, and excreted in urine. Its physiologic function is multifactorial, including renal water and electrolyte metabolism, blood pressure control, and protection from urinary tract infection and stone formation.[21,22] Along the pathway of CKI regardless of cause, uromodulin accumulates in the lumen of tubules — mostly the distal nephron segments, by itself, or in combination with other proteinaceous or cellular elements — and appears as tubular casts on microscopic examination. This tubular lumen accumulation of uromodulin perhaps reflects single nephron injury, obstructing the usual physiologic washout of uromodulin into urine. The mechanism for the vancomycin/uromodulin coprecipitation is not clear but perhaps follows the general principle of crystallization.[23] This process may involve binding of specific structural motifs of uromodulin and vancomycin. In pertinence, structural motifs of uromodulin that enable itself to polymerize forming orderly structures as appeared in EM have been identified.[24] It is also possible that the obstructive effect of uromodulin tubular accumulation raises the local concentration of vancomycin, leading to a supersaturation environment amenable to crystallization. Pertinent to these considerations is which tubular segments would house VTCs. VTCs are often associated with marked tubular cell injury, preventing accurate identification of the affected tubular segments by LM. However, the application of tubular segment-specific immunohistochemical markers suggest that most if not all VTCs are localized to the distal nephron segments, including thick Henle loops and distal convoluted tubules. Retrospective EM study exhaustively searching for VTCs and their home further supports this preferential localization. Regardless of the underlying mechanism, this coprecipitation, with its significance pathogenetic magnitude, may represent an "actionable" landmark in the pathway of VN.

The clinical significance of VTCs is addressed in this study. Although all renal biopsy specimens from patients with AKI associated with vancomycin treatment display ATN, TIN, and interstitial fibrosis and tubular atrophy in various combinations, AKI most probably due to vancomycin is finally substantiated in only 28 of 37 (75%) of these patients, compared with a reported rate of 59% in studies without kidney biopsy specimens.[7] VTCs may help identify patients with VN and are shown to be nephrotoxic because VTCs have been noted in the majority (25 of 28, 89%) of cases. The pathogenetics of VTCs are also supported by the observation that VTCs are not found in almost all (8 of 9, 89%) biopsy specimens from those patients without VN, despite tubular exposure to vancomycin as suggested by immunohistochemical localization of this drug in each biopsy. The presence of VTCs in absence of clinical VN may indicate that creatinine may not be a good diagnostic test to determine such subclinical cases. The presence of vancomycin in tubular cells by

immunohistochemistry might indicate vancomycin tubulopathy.

The pathogenesis of VTCs is still unknown. It is inconclusive whether VTCs are pathogenetic or just an "innocent bystander." Two pathogenetic pathways of VN have been described. Vancomycin, a highly hydrophilic glycopeptide, is metabolized in liver (<5%), but mostly follows the glomerular filtrate and is secreted unchanged in urine, along with minimal reabsorption and metabolism by proximal tubular cells.[25,26] Nephrotoxicity may involve overload tubular cell uptake, promoted by many known risk factors for nephrotoxicity including pre-existing CKI, leading to oxidative stress, generation of reactive oxidative radicals, mitochondrial injury, and finally cell death. This pathogenetic pathway has been substantiated in several observational and interventional models in rodents and cell cultures.[25,26] It portends human relevance because changes often labelled "ATN" are often seen in renal biopsy specimens from patients with VN.[6] VTCs were not described in any of these models.[25,26] Implication of distal tubular segment injury was noted in a single early study describing elevated level of dimethyl amine, a distal nephron segment marker, in some patients treated with "aminoglycoside and/or glycopeptide."[27] The other mechanism invokes an "allergic" reaction, supported perhaps only by the observation of acute TIN with a significant eosinophil infiltrate in a few renal biopsy specimens from patients with VN.[6]

Findings from the current study suggest that VTCs do have a pathogenetic role in addition to and independent from the known pathogenesis of VN. It is hypothesized that VN develops against the background of known risk factors through the known pathogenetic pathways. The initial insult creates *de novo* or accentuates pre-existing renal injury, including single nephron obstruction. This leads to uromodulin casts, increased local concentration of vancomycin, precipitation/crystallization of vancomycin, and localized necrosis/damage of tubular epithelial cells, all feeding into a self-perpetuating positive feedback loop of local tubular injury, superimposing on the more diffuse injury related to the background ATN and/or TIN. Observations in support of this hypothesis include (1) "Traditional" ATN and or TIN is noted in all biopsy samples from patients with VN; (2) VTCs are noted in almost all of these biopsy specimens, but not in biopsy specimens from those without VN; (3) Vancomycin may be immunohistochemically localized in tubular lumen but not forming VTCs, and VN does not develop in most such cases; (4) VTCs almost exclusively localize to distal nephron segments, which are not usually affected during the traditional nephrotoxic type ATN; and (5) Severe and frequent tubular cell necrosis are closely associated with VTC.

CONCLUSIONS

VTCs display a characteristic morphologic profile amenable to ready recognition in biopsy specimens. VTCs result from coprecipitation of vancomycin and uromodulin. VCTs facilitate the biopsy diagnosis of VN. VCT may have a nephrotoxic effect superimposing on and independent from the ATN or interstitial nephritis in the pathogenesis of VN.

DISCLOSURE

All the authors declared no competing interests.

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