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Can Renal Acute Tubular Necrosis Be Differentiated from Autolysis at Autopsy?*

ABSTRACT: We investigate the morphological characteristics that may differentiate between ischemic acute tubular necrosis (ATN) and autolysis in postmortem samples. Renal tissue from 57 postmortem cases with an antemortem diagnosis of ATN and 57 age-/sex-matched control cases were examined for 10 morphological characteristics: epithelial proliferation (Ki-67 immunoperoxidase positivity), fibrin thrombi, tubular epithelial whorls, mitoses, casts, autolysis, tubulorrhexis, epithelial flattening, interstitial inflammation, and interstitial expansion. Tubular epithelial whorls were found in 16 ATN cases and were absent in controls. These findings suggest that specific morphological criteria may distinguish ischemic ATN from autolysis. Diagnoses of ATN may be confirmed using these combined criteria as contributing to cause of death and/or to ascertain previously undiagnosed cases of ATN postmortem.

KEYWORDS: forensic science, renal acute tubular necrosis, tubular epithelial whorl, Ki-67 immunoperoxidase, autopsy

Acute tubular necrosis (ATN) is the most common cause of acute renal failure (ARF) and accounts for 50% of all cases of ARF in hospitalized patients and >75% of critical care cases (1–4). ARF affects 5% of hospitalized patients and has a high mortality rate of 50% (5). Those patients who survive ATN generally have a good prognosis for renal recovery (6).

ATN is a clinicopathologic condition characterized morphologically by destruction of tubular epithelial cells and clinically by acute reduction or failure of renal function (3). The kidneys are said to undergo four clinical phases during ATN: initiation, continued hypoxia and inflammatory response, maintenance, and recovery (7). ATN can arise from a variety of ischemic and nephrotoxic conditions, or a combination of both (8), but is potentially reversible because of the reparative and regenerative capabilities of the renal tubular epithelium. Ischemic ATN can result from any disturbance that impairs the blood flow to the kidney including dehydration, hypovolemia, hypotension, and shock. The reduced blood flow results in a series of microvascular events in the glomerulus and medulla including increased vasoconstriction, decreased vasodilation, endothelial and vascular smooth muscle cell structural damage, and leukocyte to endothelial adhesion contributing to vascular obstruction, leukocyte activation, and inflammation. The effects on the tubular epithelial cells themselves include cytoskeletal breakdown, loss of polarity, apoptosis and necrosis, desquamation of epithelial cells from the basement membrane and subsequent tubular obstruction, and backleak of filtrate through the exposed basement membrane (9). The reduced oxygenation and subsequent necrosis and apoptosis of the cells result in morphological changes and functional abnormalities of the kidney (10). The epithelial cells that ''slough off'' from underlying tissue block the kidney nephrons (11), resulting in decreased glomerular filtration rate, decreased urine output, and renal failure (4).

If the precipitating cause of the ischemia is removed or resolved, the tubular epithelial cells may regenerate and full function will be restored to the kidneys. The surviving epithelial cells dedifferentiate and proliferate in order to restore cells to the denuded areas of basement membrane and then differentiate in order to restore function to the affected nephrons (12).

The typical histological features of tubular epithelial cells in ATN include both reversible and irreversible injury and evidence of regeneration. Reversible injury includes cellular swelling and vacuolization, loss of brush border, cell membrane blebbing, loss of polarity (exhibited by loss of microvilli), desquamation, tubulorrhexis (disruption of the epithelial basement membrane), presence of luminal casts, dilation of Bowman's space with retraction of the glomerular tuft, prominence of juxtaglomerular apparatus, interstitial inflammation, and interstitial edema (3,7,10,13). Irreversible lethal injury includes apoptosis and necrosis, although frankly necrotic cells are not commonly seen (7). Regeneration of tubular epithelium is exhibited by the presence of mitotic figures and flattened epithelial cells with hyperchromatic nuclei (3). The regenerative actions of the epithelium can be visualized immunohistochemically using the cell proliferative marker Ki-67 (14,15).

Histologically, ATN exhibits similar features to those seen in autolysis occurring postmortem and poses difficulties in differentiating between the two processes during postmortem examinations (13). It is a commonly held view amongst autopsy pathologists that it is not possible to diagnose ATN at autopsy because of the problems of autolysis. We propose to establish diagnostic criteria to distinguish between the two processes of ischemic ATN and autolysis using a variety of cellular visualization techniques and morphological characteristics.

Methods

Selection of Cases

Cases of clinically diagnosed ATN over a 5-year period between 2001 and 2005 were identified in the Department of Forensic

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Medicine's autopsy database. All identified cases had undergone a full autopsy, including microscopy of all major organs, as part of the investigation into the death by the New South Wales State Coroner. Hospital medical records of these cases were then retrieved in order to confirm, either clinically and/or by biochemical parameters, the diagnosis of ATN. Since there is no "gold standard" for the diagnosis of ATN (6), the criteria deemed acceptable for an antemortem diagnosis of ischemic ATN were: clinical diagnosis of ATN documented in the patient's clinical record and/or clinical diagnosis of ARF with oliguria (urinary output <400 mL/day) or anuria, increasing serum levels of urea and creatinine, and absence of exposure to nephrotoxins. Fifty-seven cases met the requirements for inclusion in the study.

An equivalent number of age- and sex-matched control subjects (57 cases), who had died suddenly as a result of self-inflicted hanging but were otherwise healthy, were identified. Cases from both the ATN and control group were deidentified and examined in a blinded fashion.

Preparation of Tissue and Staining

Archival renal tissue from the coronial autopsies was retrieved for use in the study. Specimens were formalin fixed, embedded in paraffin wax, serially cut at 4 μm, and routinely stained (16) with hematoxylin and eosin (H&E), Martius scarlet blue (MSB), Masson's trichrome, and Ki-67 immunoperoxidase (Dako, Glostrup, Denmark).

Morphometric Analysis

All tissue sections were examined using light microscopy and an eyepiece graticule (Olympus Corporation, Tokyo, Japan) with 10×10 equidistant squares. Each specimen was examined along a transect from outer cortex to inner medulla.

Cells undergoing cell proliferation were assessed using Ki-67 immunoperoxidase stain. Tubular epithelial cells exhibiting dark anti-Ki-67 staining were manually counted in 20 consecutive fields along the transect at high-power (40× objective).

The presence of fibrin thrombi in glomeruli was determined using MSB stained tissues and the identification of mitotic figures, tubular casts, and tubular epithelial whorls was performed using H&E stained tissues. Each morphological characteristic was manually counted in either 20 consecutive fields at high-power or in 10 consecutive fields at medium-power (10× objective).

Tissue sections, stained with H&E, were examined for the presence of autolysis, tubulorrhexis, tubular epithelial flattening, and interstitial inflammation. Interstitial expansion, representing either antemortem fibrosis or proteinaceous fluid accumulation associated with decompositional change was assessed qualitatively using Masson's trichrome stain. Twenty consecutive fields at high-power from each tissue section were examined for the presence or absence of each characteristic. The scores were then summed and expressed as a proportion of one for each tissue sample with a maximum score of one indicating every field examined showed some degree of the characteristic being assessed.

Statistical Analysis

All results are expressed as mean ± standard deviation. Differences between groups were determined by two sample *t*-test and used two-tailed distribution. A p-value < 0.05 was considered to be statistically significant.

Results

Renal tissue histological characteristics were analyzed in postmortem samples from 57 individuals (aged 19–88, mean age 57.54) who had an antemortem diagnosis of ischemic ATN. The age- and sex-matched control group samples ranged in age from 19 to 89, mean age 57.49. Each group consisted of 25 females and 32 males. The postmortem interval, in hours, for the ATN cases ranged from 8.25 to 185 (mean 47.7 h) and for the control group ranged from 9 to 240 (mean 36.8 h). There was no statistical difference in postmortem interval between the two groups ($p = 0.104$). Cases in both groups exhibited microscopic changes consistent with nephrosclerosis, pyelonephritis, or diabetic nephropathy (15 ATN cases, 26.3% and 10 control cases, 17.5%). These pre-existing renal conditions, however, did not contribute to cause of death in either group.

Both left and right kidneys from each case were weighed, in grams, as part of the postmortem process. Left kidneys from the ATN group ranged from 70 to 1740 g (mean weight 206.1 g) and those from the control group ranged from 80 to 240 g (mean weight 146.7 g). The largest ATN case kidney weighing 1740 g was the combined weight of a kidney and an unspecified retroperitoneal mass. Disregarding this case, the left kidneys from the ATN group ranged from 70 to 360 g (mean weight 178.7 g). ATN group right kidneys ranged from 60 to 320 g (mean weight 171.9 g) and those from the control group ranged from 80 to 240 g (mean weight 138.4 g). Statistical analysis revealed significant differences between both left ($p = 0.046$ with largest kidney, $p = 0.002$ without largest kidney) and right ($p = 0.001$) kidneys.

TABLE 1—*Morphological characteristics examined by light microscopy.*

Characteristic	ATN, $n = 57$ (mean [SD] [range])	Control, $n = 57$ (mean [SD] [range])	p-Value
Proliferating cells, Ki-67 40× objective	19.5 (29) (0–120)	5 (9.2) (0–47)	0.001
Mitotic figures, H&E 40× objective	0.053 (0.225) (0–1)	0.070 (0.258) (0–1)	NS (0.700)
Glomeruli containing fibrin thrombi, MSB 10× objective	0.004 (0.015) (0–0.077)	0.011 (0.040) (0–0.200)	NS (0.199)
Tubular epithelial whorls, H&E 40× objective	0.32 (1.31) (0–9)	0	NS (0.057)
Tubular epithelial whorls, H&E 10× objective	1.93 (5.15) (0–31)	0	0.005
Tubular epithelial casts, H&E 40× objective	0.65 (1.85) (0–13)	0.79 (5.44) (0–41)	NS (0.854)
Tubular epithelial casts, H&E 10× objective	5.3 (14.2) (0–88)	3.3 (14.0) (0–100)	NS (0.445)
Degree of tubulorrhexis, H&E 40× objective	0.030 (0.082) (0–0.562)	0.007 (0.025) (0–0.150)	0.041
Degree of interstitial expansion, H&E and MT 40× objective	0.533 (0.412) (0–1)	0.195 (0.312) (0–1)	<0.001
Degree of autolysis, H&E 40× objective	0.828 (0.297) (0.05–1.00)	0.865 (0.249) (0.05–1.00)	NS (0.471)
Degree of epithelial flattening, H&E 40× objective	0.001 (0.132) (0–0.10)	0.001 (0.009) (0–0.05)	NS (1.000)
Degree of interstitial inflammation, H&E 40× objective	0.170 (0.243) (0–0.95)	0.136 (0.172) (0–0.70)	NS (0.388)

SD, standard deviation; NS, non-significant; MSB, Martius scarlet blue; MT, Masson's trichrome; H&E, hematoxylin and eosin.

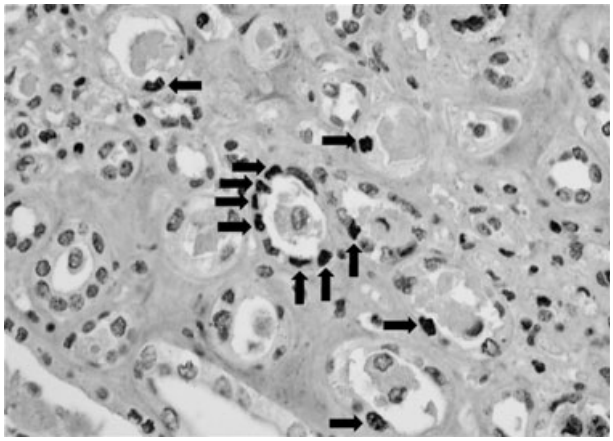

FIG. 1—*Proliferating tubular epithelium. Ki-67 expression in nuclei of tubular epithelial cells (arrows) (40× objective).*

Table 1 summarizes the findings of the morphometric analysis of the 10 characteristics examined in each of the 114 renal samples. Forty-five (78.9%) ATN cases showed at least one proliferating tubular epithelial cell (Fig. 1), as visualized by Ki-67 positivity, and a maximum of 120 proliferating cells per transect. Thirty-five (61.4%) control cases also showed some proliferating tubular epithelial cells but only to a total maximum of 47. Statistical analysis revealed a significant difference in the total number of proliferating cells per transect ($p = 0.001$).

Sixteen (28.1%) ATN cases demonstrated the presence of tubular epithelial whorls (Figs. 2 and 3), which were absent from all the control cases. Statistical analysis revealed a significant difference in total number of tubular epithelial whorls when examined at medium-power ($p = 0.005$).

Seventeen (29.8%) ATN cases exhibited the presence of tubulorrhexis (Fig. 4) compared with only five (8.8%) control cases that demonstrated disruption of the tubular epithelial basement membrane. Forty-four (77.2%) tissue sections exhibiting ischemic ATN also demonstrated the presence of interstitial expansion. Less than half of the control samples (47.4%) exhibited interstitial expansion. The presence of tubulorrhexis ($p = 0.041$) and interstitial expansion ($p < 0.001$) were both shown to statistically differ between the ATN and control renal tissue samples.

Mitotic figures, glomeruli containing fibrin thrombi, and tubular epithelial flattening were relatively uncommon findings and were not shown to statistically differ between the two groups. Tubular epithelial casts, presence of autolysis (Fig. 5), and presence of interstitial inflammation, although quite common findings in both groups, were also shown not to statistically differ between the ATN and control groups.

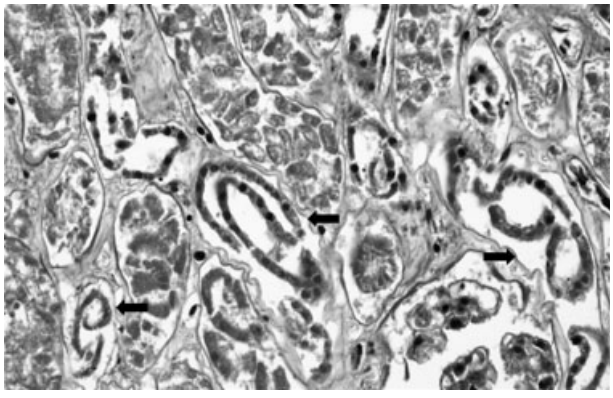

FIG. 2—*Tubular epithelial whorls (arrows) (H&E 10× objective).*

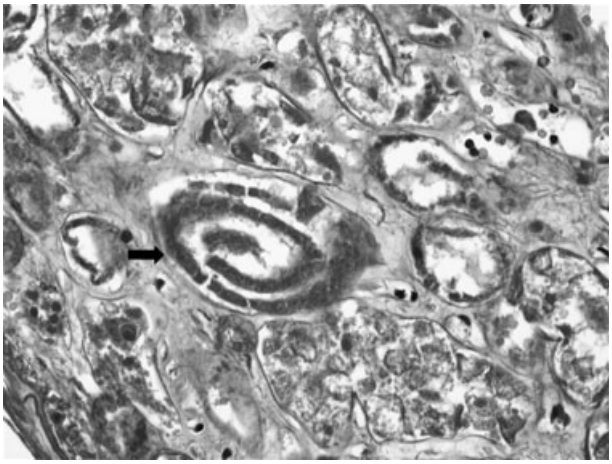

FIG. 3—*Tubular epithelial whorl (arrow) (H&E 40× objective).*

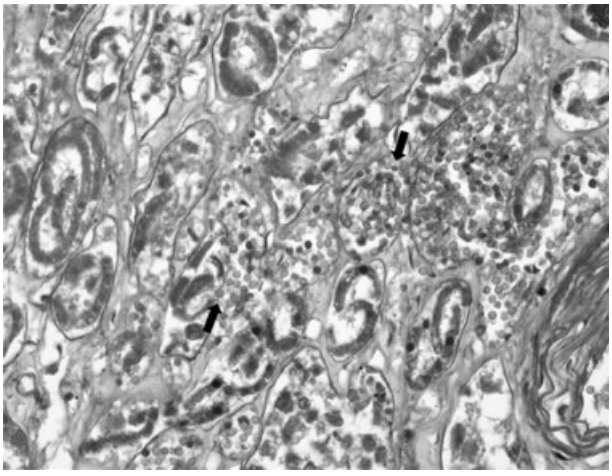

FIG. 4—*Tubulorrhexis (arrows) (H&E 10× objective).*

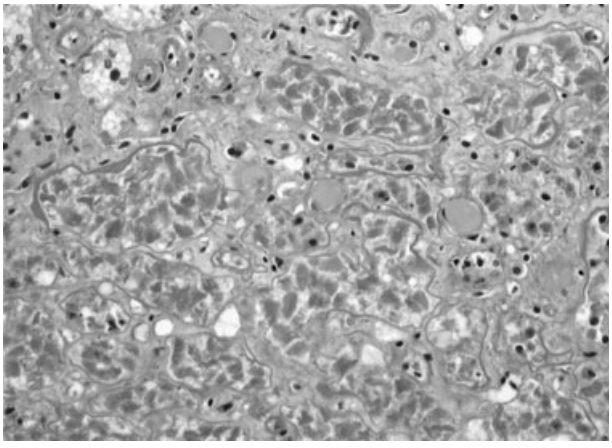

FIG. 5—*Autolysis of tubules (H&E 10× objective).*

The sensitivity and specificity of the four characteristics that were shown to be statistically different were calculated. The sensitivity of the presence of tubular epithelial whorls at medium-power magnification in ATN was 28.07% and had a specificity of 100%. The presence of tubulorrhexis was also highly specific at 91.22% but not sensitive (29.82%). Proliferating tubular epithelial cells and the presence of interstitial expansion were both moderately sensitive in tissue exhibiting ATN, 78.94% and 77.19%, respectively, but had low specificity, 38.59% and 52.63%.

Discussion

One of the problems in the diagnosis of ischemic ATN in postmortem samples is the presence of autolysis. Autolysis is believed to frequently prevent or hinder the diagnosis of ATN in postmortem examinations because of similar architectural and nuclear features such as swelling and vacuolization, and nuclear fragmentation.

Morphological criteria diagnostic of ATN were first described by Oliver et al. in a study examining human, canine, and rabbit kidneys having undergone either ischemic or nephrotoxic insults. The morphological features included the presence of necrotic cells, tubular regeneration, mitotic figures, loss of cell brush border, desquamation, tubular casts, interstitial inflammation, and tubular dilation (1). A number of the features though, including tubular desquamation and tubular casts, are also found in autolysis and cannot be used to differentiate between the two processes during autopsy.

We propose the use of a novel morphological feature, the tubular epithelial whorl, in addition to those already described histological features that were shown to statistically differ between ischemic ATN and control renal tissue samples (tubulorrhexis, proliferating cells, and interstitial expansion), to be used in the diagnosis of ATN in postmortem examinations.

The epithelial whorl consists of a coil of epithelium contained within the renal tubule. The presence of tubular epithelial whorls, although not very sensitive, is highly specific (100%) to renal tissue exhibiting ischemic ATN in this study. Tubulorrhexis, a disruption of the tubular epithelial basement membrane, was also shown to be highly specific (91.22%) but insensitive as a morphological criterion for ATN. The number of tubular epithelial proliferating cells, as visualized by Ki-67 immunoperoxidase positivity and demonstrating the reparative capability of tubular epithelium following an ischemic insult, was shown to be a sensitive but nonspecific criterion for ATN. A mean of 19.5 proliferating cells was present in 78.9% of examined ATN cases. In control renal tissue, there was only a mean of five proliferating tubular epithelial cells present per section examined. The presence of apparent interstitial expansion was similarly found to be sensitive but nonspecific. The greater degree of interstitial expansion exhibited by ATN renal tissue may account for the greater weight exhibited by both the right and left kidneys of the ATN cases, as measured during the postmortem examination.

The remaining five characteristics (fibrin thrombi in glomeruli, tubular epithelial mitotic figures, tubular casts, tubular epithelial cell flattening, and interstitial inflammation) were shown not to significantly differ between the two groups and thus would not be useful in detecting ATN in postmortem renal samples. Statistical analysis also revealed that the presence of autolysis was not significantly different between the ATN and control groups ($p = 0.471$). Both groups showed a similar degree and range of autolysis affecting each tissue section.

In the current study, we confirmed that tubular epithelium exhibiting proliferating tubular epithelial cells, especially when present in large numbers, tubulorrhexis, and interstitial expansion are histological features that help distinguish ATN from autolysis in postmortem samples.

The present study also showed that tubular epithelial whorls appeared exclusively in renal samples exhibiting ATN and were absent from all control samples. Thus, number of proliferating tubular epithelial cells and tubular epithelial whorls might be used as reliable criteria in the differential diagnosis of acute tubular necrosis from autolysis at autopsy.

References

1. Oliver J, MacDowell M, Tracy A. The pathogenesis of acute renal failure associated with traumatic and toxic injury. Renal ischemia, nephrotoxic damage and the ischemuric episode. J Clin Invest 1951;30:1307–439.
2. Brady HR, Clarkson MR, Lieberthal W. Acute renal failure. In: Brenner BM, Levine SA, editors. Brenner & Rector's the kidney. Philadelphia: Saunders, 2004;1215–92.
3. Kumar V, Abbas AK, Fausto N, editors. Robbins and Cotran pathologic basis of disease, 7th edn. Pennsylvania: Elsevier, 2005;969–71.
4. Gill N, Nally JV, Fatica RA. Renal failure secondary to acute tubular necrosis. Chest 2005;128:2847–63.
5. Alkhunaizi AM, Schrier RW. Management of acute renal failure: new perspectives. Am J Kidney Dis 1996;28:315–28.
6. Esson ML, Schrier RW. Diagnosis and treatment of acute tubular necrosis. Ann Intern Med 2002;137:744–52.
7. Lameire N. The pathophysiology of acute renal failure. Crit Care Clin 2005;21:197–210.
8. Santos WJQ, Zanetta DMT, Pires AC, Lobo SMA, Lima EQ, Burdman EA. Patients with ischaemic, mixed and nephrotoxic acute tubular necrosis in the intensive care unit—a homogeneous population? Crit Care 2006;10:R68.
9. Bonventre JV, Weinberg JM. Recent advances in the pathophysiology of ischemic acute renal failure. J Am Soc Nephrol 2003;14:2199–210.
10. Solez K, Morel-Maroger L, Sraer JD. The morphology of ''acute tubular necrosis'' in man: analysis of 57 renal biopsies and a comparison with the glycerol model. Medicine 1979;58:362–76.
11. Guyton AC, Hall JE. Textbook of medical physiology. 11th edn. Pennsylvania: Elsevier, 2006.
12. Bonventre JV. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. J Am Soc Nephrol 2003;14(Suppl.):55–61.
13. Salinas-Madrigal L, Sotelo-Avila C. Morphologic diagnosis of acute tubular necrosis (ATN) by autofluorescence. Am J Kidney Dis 1986;7:84–7.
14. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984;133:1710.
15. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182:311–22.
16. Bancroft JD, Stevens A, editors. Theory and practice of histological techniques. 3rd edn. Edinburgh: Churchill Livingstone, 1990.

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