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Chromogranin A Tubulopathy: Differing Histopathologic Patterns of Acute Tubular Injury in the Setting of Neuroendocrine Neoplasms

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Introduction: Neoplasms of neuroendocrine derivation or differentiation may express specific peptides, some of which are capable of producing clinical symptomatology and others used as biomarkers: one such peptide being chromogranin A (CGA). Herein, we describe histopathologic changes present in kidney specimens from patients with such neoplasms, and illustrate 2 patterns of acute tubular injury (ATI) attributable to CGA.

Methods: Eleven patients with a history of a neoplasm of neuroendocrine derivation or differentiation and having histopathologic sampling of the kidney were retrospectively identified, 3 of whom had ATI with either engorgement of the proximal tubular epithelium by resorbed material or tubular cast formation.

Results: Two patterns of ATI were observed. One characterized by acutely injured proximal tubular cells engorged with resorption granules that expressed CGA via immunoperoxidase staining. Another pattern was characterized by intraluminal tubular cast material associated with ATI that did not exhibit restriction of immunoglobulin light chains (LCs), but immunoperoxidase staining for CGA revealed that the cast material was composed of the neuroendocrine-associated peptide. The level of serum CGA does not appear to necessarily equate to developing either of these 2 patterns of ATI.

Conclusions: Patients with a neoplasm of neuroendocrine derivation or differentiation may develop ATI, and in certain cases may be secondary to CGA renal tubular deposition.

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Neuroendocrine cells and neoplasms of neuroendocrine derivation or differentiation are capable of expressing and secreting peptides. In the setting of neoplasms of neuroendocrine derivation or differentiation, the peptides can be used for diagnostic identification, classification, and as biomarkers of therapeutic efficacy.[1,2] Variable from case-to-case and somewhat dependent on the tissue of derivation, peptide(s) elaborated can be attributed with symptomatology, subsequently deeming the neoplasm as being "functional" (e.g., insulin with an insulinoma, cortisol with Cushing syndrome). Chromogranin A (CGA) is one such peptide, and has been used as a biomarker for numerous neuroendocrine neoplasms derived from a variety of tissue origins.[2] Serum CGA also can be detected at basal levels in patients without an underlying neuroendocrine neoplasm, and is elevated in the setting of decreased glomerular filtration.[3,4]

The description of kidney injury in the setting of neoplasms of neuroendocrine derivation or differentiation is lacking. Most described cases are without a histopathologic correlation, as a kidney biopsy was not performed, and decreased kidney function has been attributed to hemodynamic effects imparted by secreted, functional peptides.[5,6] In this article, we illustrate how CGA can specifically engorge and collect intracellularly within proximal tubules or precipitate and form intraluminal tubular casts resulting in clinically evident kidney injury.

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METHODS

A retrospective review of the electronic pathology database at the Brigham and Women's Hospital was conducted for which non-neoplastic kidney tissue was collected and that the patient had a history of a neoplasm with neuroendocrine derivation or differentiation. The electronic medical record was used to provide clinical, laboratory, and radiologic data. In total, we identified 17 such cases from between 2003 and 2018, which included 6 non-neoplastic kidney biopsies, 10 autopsies, and 1 nephrectomy. Secondary to extensive autolysis that obfuscated confident evaluation of the tissue, the kidney tissue from 6 of the autopsy cases was not included, leaving 11 total cases to evaluate. All neoplasms expressed chromogranin A by immunoperoxidase staining.

Irrespective of the procedure used for obtaining the kidney tissue (autopsy, nephrectomy, or biopsy), all cases had tissue fixed in formalin and embedded in paraffin: sections were evaluated by light microscopy using hematoxylin-eosin, periodic acid–Schiff, Jones silver, and trichrome stains. All kidney biopsies had fresh tissue triaged and frozen for immunofluorescence microscopy as well as tissue provided for electron microscopic (EM) evaluation. Immunofluorescence staining was performed on paraffin sections after protease digestion from 1 kidney biopsy (case 1) and 1 autopsy kidney (case 2). EM was performed on kidney tissue retrieved from the paraffin tissue block from 1 autopsy kidney (case 2).

In addition to the conventional stains performed on paraffin sections for light microscopic examination of kidney tissue, immunoperoxidase staining for CGA (Thermo Fisher Scientific Lab Vision, Fremont, CA), gastrin (Leica Biosystems, Newcastle, UK), glucagon (gift from Dr. Arthur Like), insulin (Linco Research Inc., St Charles, MO), and somatostatin (gift from Dr. Arthur Like) was also performed. Unfortunately, the paraffin block for case 3 could not be retrieved from storage and immunoperoxidase staining for the aforementioned peptides was not performed.

The study was approved by the Institutional Review Board at our institution. A P value of <0.05 was considered statistically significant. Statistical analysis was completed using JMP (SAS Institute, Cary, NC).

RESULTS

Demographics and Clinical Characteristics

The 11 patients had a median age of 67 years (interquartile range [IQR] 57 to 73 years old). There were 7 male and 4 female patients, all of which were white. At the time of tissue collection, the patients had a median serum CGA of 1273 ng/ml ($n = 9$, IQR 411.5–8748 ng/ml), serum creatinine of 3.48 mg/dl ($n = 11$, IQR 1.56–4.66 mg/dl), blood urea nitrogen 51.5 mg/dl ($n = 10$, IQR 32.5–51.5 mg/dl), serum albumin of 3.0 g/dl ($n = 9$, IQR 2.25–3.5 g/dl), and urine protein-to-creatinine ratio of 4.25 mg/mg ($n = 6$, IQR 0.4–8.6).

The approaches to clinical management for each of the 10 patients who had a diagnosis of a neoplasm of neuroendocrine derivation or differentiation *before* sampling of the kidney differed secondary to disease burden, overall performance status, and/or other comorbidities (Table 1). Two patients were managed with both chemotherapy and surgery with either combined pancreatectomy/splenectomy or a tumor debulking with colon/mesenteric resection. Two patients received chemotherapy plus a somatostatin analog, 2 patients received chemotherapy alone, and 2 patients had received a somatostatin analog alone.

The median interval from primary diagnosis of the neoplasm to the time of kidney tissue sampling was 13 months for all patients ($n = 11$, IQR 3–24 months), with 1 patient having the primary diagnosis concurrently determined at the time the kidney was sampled. All patients had radiologic or tissue-sampled proven evidence of metastatic disease, with variance in the tissue sites involved (see Table 1). Of these 11 patients in the series, 2 patients (case 10) were still alive at the time of this article's preparation.

Histopathologic Findings, With a Focus on Tubular Changes

Briefly, aside from cases 1 to 3, which are described in detail (*vide infra*), cases 4 to 11 showed either the absence or generally mild and focal proximal tubular intracytoplasmic and brush border resorption granules reactive by immunoperoxidase staining for CGA, and never revealed significant expression of gastrin, glucagon, insulin, or somatostatin. CGA reactivity was limited in its localization to intraluminal tubular casts (when present) and proximal tubular intracytoplasmic granules, and not expressed by other segments of the tubular nephron, glomerular, interstitial, or vascular cell types.

Seven cases revealed evidence of ATI, 3 of which were associated with either intracellular engorgement of proximal tubules by CGA-positive granules (case 1) or intraluminal tubular cast formation (case 2 with CGA-positive casts, and case 3 for which the paraffin tissue block was unavailable for immunoperoxidase staining). The 4 remaining cases with evidence of ATI showed no significant CGA deposition (cases 7–9, and 11), and were seen in the setting of a diffusely proliferative IgA nephropathy, new-onset minimal change disease, an acute and chronic thrombotic angiopathy, and a patient with preceding gastroenteritis with

Table 1. Clinical and laboratory characteristics and patient management

Case	Primary neoplasm	Site(s) of metastasis	Neoplasm-directed therapy prior to kidney sampling	Serum creatinine (mg/dl)	Serum CGA (ng/ml)
Case 1	Pancreatic neuroendocrine neoplasm	Liver	Temozolomide and octreotide	5.05	8819
Case 2	Uterine carcinosarcoma with neuroendocrine and rhabdomyosarcomatous differentiation	Liver, omentum, peritoneum, left ureter, colon, diaphragm	Carboplatin	3.93	8677
Case 3	Neuroendocrine neoplasm, likely colonic primary	Liver	Irinotecan, 5-FU, surgical debulking	7.4	343
Case 4	Pancreatic neuroendocrine neoplasm	Liver, lungs, heart, small intestine, vertebrae, abdominal and thoracic LN	Bevacizumab, temozolomide, sunitinib, capecitabine	1.67	1273
Case 5	Pancreatic neuroendocrine neoplasm	Liver	Chemotherapy (unspecified in records)	3.34	Not performed
Case 6	Pancreatic neuroendocrine neoplasm	Liver, celiac LN, sacrum, brain	Octreotide	1.4	62800
Case 7	Pancreatic neuroendocrine neoplasm	Liver	Temozolomide, pancreatectomy, splenectomy	4.66	4540
Case 8	Neuroendocrine neoplasm, unknown primary	Liver, mediastinum, skull, omentum, sacrum, left femur, left humerus	Everolimus, octreotide	4.45	874
Case 9	Neuroendocrine neoplasm, unknown primary	Right kidney	No treatment prior to kidney sampling	1.56	Not performed
Case 10	Ileocecal neuroendocrine neoplasm	Mesenteric LN	Lanreotide	0.88	473
Case 11	Duodenal neuroendocrine neoplasm	Liver	Chemotherapy (unspecified in records)	3.48	350

CGA, chromogranin A; 5-FU, 5-fluorouracil; LN, lymph node.

vomiting and diarrhea: 3 with glomerular processes that can be associated with concurrent ATI and the last with hypovolemia. Although case 3 had a diffuse proliferative immune complex–mediated glomerulonephritis present, it was relatively mild with regard to the glomerular inflammation present and the ATI was predominately in association with the many intraluminal casts.

Three cases (cases 4, 6, and 10) revealed mild and focal positive CGA staining within occasional proximal tubular resorption granules and the brush border, but without evidence of ATI, so it is likely that relatively low levels of tubular resorption of CGA do not impart acute tubular epithelial injury. Case 5 showed no evidence of ATI and also did not reveal CGA reactivity in the tissue by immunoperoxidase staining.

Significant glomerular, interstitial, and vascular histopathologic features for all 11 cases are reviewed in Table 2.

Detailed Findings From Cases With ATI Either With Increased Proximal Tubular Resorption of CGA or Intraluminal Tubular Cast Formation

Case 1

A 74-year-old white man with metastatic pancreatic neuroendocrine tumor presented with progressively rising creatinine from the time of diagnosis 5 months prior. The patient had received 1 cycle of temozolomide, and after radiologic studies showed somatostatin receptor expression, was started on octreotide. Laboratory testing noted at admission serum creatinine of 5.05 mg/dl (was 1.49 mg/dl at time of initial diagnosis of malignancy), estimated glomerular filtration rate of 11 ml/min per 1.73 m^2, blood urea nitrogen of 70 mg/dl, serum albumin of 3.4 g/dl, urine protein-to-creatinine ratio of 0.48 mg/mg, microalbumin-to-creatinine ratio of 59 mg/g, serum CGA of 8819 ng/ml, uric acid of 14 mg/dl, and a complete blood count showed a normocytic anemia, thrombocytopenia, and a relative neutrophilia. The difference between the urine protein-to-creatinine ratio and microalbumin-to-creatinine ratio was proposed to be in part filtered CGA; however, without measured urine levels, this was only speculative. Normal/negative serum protein electrophoresis and immunofixation, serum-free LC ratio, serum C3 and C4, antinuclear antibodies, antinuclear cytoplasmic antibodies, and anti–double-stranded DNA. Lower extremity edema (1+) noted on examination. Medical history was notable for hypertension, hyperlipidemia, coronary artery disease, gout, being a former smoker, and was under surveillance for low-grade prostatic adenocarcinoma.

The kidney biopsy consisted of cortex, with 6 of 19 glomeruli globally sclerosed (Figure 1). The glomeruli showed mild mesangial expansion by extracellular matrix. Many tubules contained protein reabsorption granules in the epithelial cells, with focal loss of the proximal tubular brush border. The proximal tubular intracytoplasmic granules were periodic acid–Schiff positive, brightly eosinophilic on hematoxylin-eosin stain, and red by trichrome stain. By immunoperoxidase staining, the tubular granules positively expressed CGA, and were negative for insulin, glucagon, somatostatin, and gastrin. Few mitoses and apoptotic bodies are seen in

Table 2. Histopathologic findings

Case	Tubular changes by LM	CGA expression by IHC	Other histopathologic findings	Etiology for ATI if present
Case 1	Engorged proximal tubules with occluded lumina, and ATI	Positive in the engorging, intracellular proximal tubular resorption granules	Few tubular calcium oxalates, a mild/inactive IgA-dominant glomerulopathy, 20%–30% IFTA, moderate vascular sclerosis	Engorgement of proximal tubules with intracytoplasmic CGA
Case 2	ATI associated with luminal casts	Positive in the medullary predominate luminal casts	10% IFTA, moderate vascular sclerosis	Luminal casts composed of CGA
Case 3	ATI associated with luminal casts	IHC not performed	Diffuse proliferative IC mediated glomerulonephritis, 20% IFTA, mild/moderate vascular sclerosis	Luminal casts suggested to be composed of CGA
Case 4	No significant acute changes	Positive very focally within proximal tubular resorption granules and the BB	Adaptive GS, 25% IFTA, mild vascular sclerosis	No ATI
Case 5	No significant acute changes	Negative	Nodular mesangial expansion, 30%–40% IFTA, moderate vascular sclerosis	No ATI
Case 6	No significant acute changes	Positive, very focally within proximal tubular resorption granules and the BB	Mild mesangial expansion, 10%–15% IFTA, moderate vascular sclerosis	No ATI
Case 7	ATI with focal necrosis	Negative	Diffuse proliferative IgAN, several tubular calcium oxalates, 50% IFTA, severe vascular sclerosis	Diffuse proliferative IgAN
Case 8	ATI with focal necrosis	Positive, within occasional proximal tubular resorption granules and the BB	MCD, 10% IFTA, severe vascular sclerosis	New-onset MCD and the nephrotic syndrome
Case 9	ATI with focal necrosis	Positive, within occasional proximal tubular resorption granules and the BB	Acute/chronic thrombotic angiopathy, inactive IgAN, 10%–20% IFTA, severe vascular sclerosis	Acute/chronic thrombotic angiopathy
Case 10	No significant acute changes	Positive, within occasional proximal tubular resorption granules and the BB	<5% IFTA, moderate vascular sclerosis	No ATI
Case 11	ATI with focal necrosis	Negative	Diabetic glomerulosclerosis, 70% IFTA, moderate vascular sclerosis	Hypovolemia in setting of a gastroenteritis with vomiting and diarrhea

ATI, acute tubular injury; BB, brush border; CGA, chromogranin A; GS, glomerulosclerosis; IC, immune complex; IFTA, interstitial fibrosis and tubular atrophy; IgAN, IgA nephropathy; IHC, immunohistochemistry; LM, light microscopy; MCD, minimal change disease.

tubular epithelial cells. Twenty percent to 30% of the kidney cortex showed interstitial fibrosis and tubular atrophy, and arteries/arterioles showed moderate sclerosis. Congo red staining was negative. Frozen sections of the sample submitted for immunofluorescence studies showed fine granular reactivity for IgA (1+), IgM (trace), C3 (trace), kappa LC (trace), and lambda LC (trace) both along the glomerular capillaries and in the mesangium. Tubules contained few intraluminal casts reactive for polyclonal IgA. There was no difference in reactivity between kappa and lambda LC in the tissue. Paraffin sections were pretreated with protease solutions for antigen retrieval and immunofluorescence studies showed similar findings as those performed on frozen tissue. EM examination revealed glomerular visceral epithelial cells with mild segmental effacement of their foot processes. The glomerular basement membranes were unremarkable. The mesangium contained isolated finely granular electron-dense deposits. Most proximal tubules were engorged by vacuoles containing electron-dense material, some with ring-shaped structure. The kidney biopsy was diagnosed as ATI associated with extensive proximal tubular resorption granules immunoreactive for CGA in the setting of a known neuroendocrine neoplasm, few tubules containing calcium oxalate crystals, a mild IgA-dominant immune complex–mediated glomerulopathy without evidence of current inflammatory activity, and moderate chronic changes of the parenchyma.

The patient was started on hemodialysis, and was managed with allopurinol and colchicine for the elevated uric acid considered secondary to the neoplasm. Unfortunately, the patient's clinical status declined and the patient died 8 days after the kidney biopsy was performed.

Case 2

A 76-year-old white woman was admitted after having been diagnosed 3 months prior with metastatic uterine carcinosarcoma with pleural and peritoneal effusions, acute kidney failure, respiratory distress, metabolic acidosis, and *Enterococcus faecalis* bacteremia. Before admission, the patient had received 1 cycle of carboplatin. Laboratory testing noted at admission serum creatinine of 3.93 mg/dl (was 1.62 mg/dl at time of initial diagnosis of malignancy), estimated glomerular filtration rate of 12 ml/min per 1.73 m^2, blood urea nitrogen of 78 mg/dl, serum albumin of 2.0 g/dl, 3+ proteinuria, and serum CGA of 8677 ng/ml. Unfortunately, the patient's clinical status did not improve and the patient died approximately a week after admission. An autopsy was performed and revealed metastatic

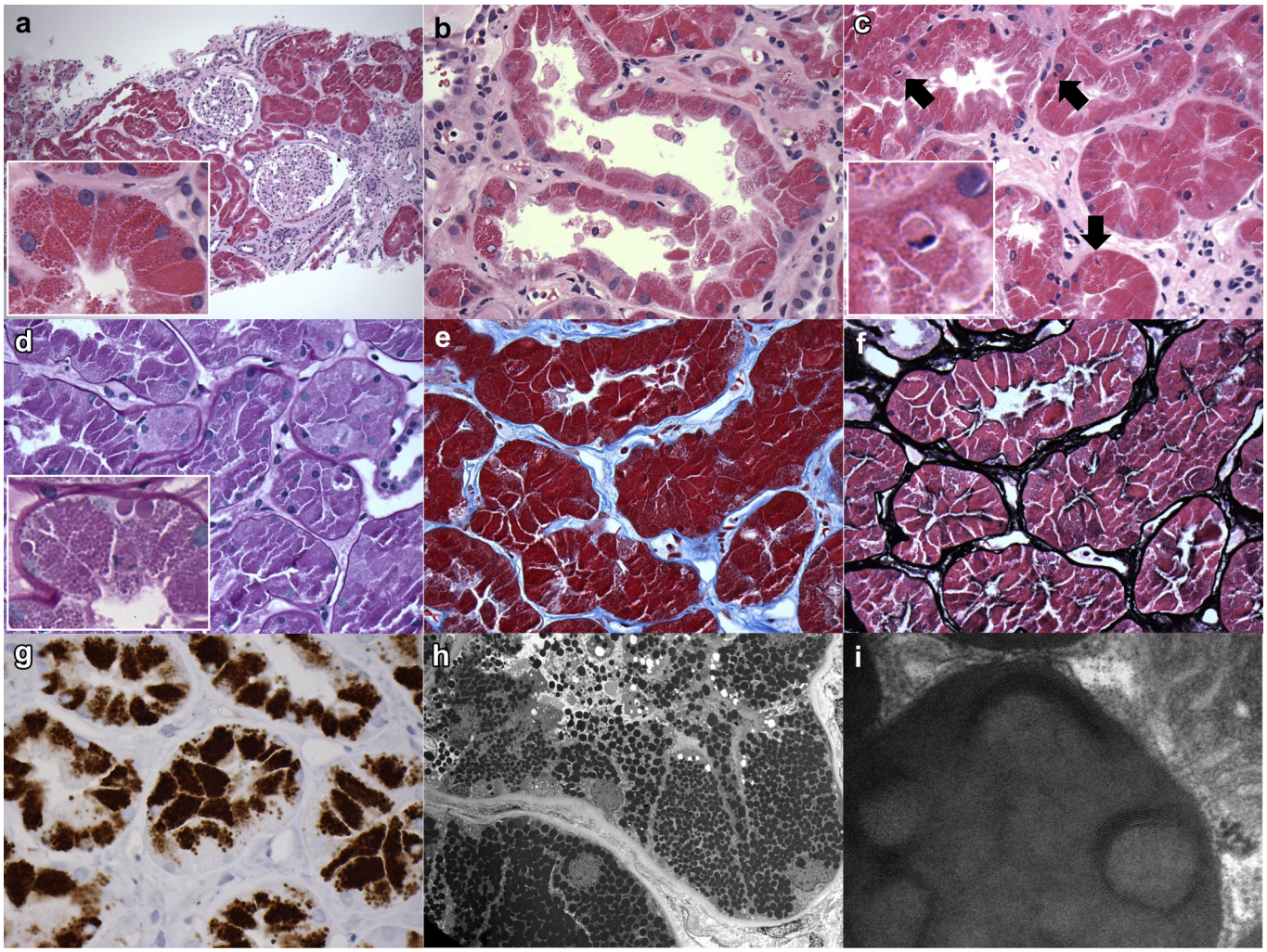

Figure 1. Kidney biopsy findings from case 1. By light microscopy, proximal tubules (and not more distal tubular segments) are engorged with resorption granules that are eosinophilic by hematoxylin and eosin staining (a–c), are positive by periodic acid–Schiff staining (d), red by trichrome stain (e), and do not stain with Jones silver stain (f). Proximal tubules exhibit acute tubular injury with flattening of the tubular epithelium and focal loss of the brush border (b), and with regenerative changes in the form of occasional apoptotic nuclei (c, arrows pointing to apoptotic bodies). The intracytoplasmic granules are reactive for chromogranin A by immunoperoxidase staining (g), and negative for gastrin, glucagon, insulin, and somatostatin expression (these negative immunoperoxidase studies are not shown). By electron microscopy, the proximal tubules contain numerous intracytoplasmic lysosomes containing electron-dense material (h), some with ring-shaped structure (i). Original magnification: (a) ×100 (inset, ×600), (b–f) ×400 (c inset, ×400; d inset, ×600), (g) ×600, (h) ×1500, and (i) ×120,000.

uterine carcinosarcoma with neuroendocrine and rhabdomyosarcomatous differentiation.

At the time of the autopsy, kidney tissue was submitted for formalin fixation and paraffin embedding; however, no tissue was originally submitted for EM and fresh material was not reserved for immunofluorescence microscopy. The kidneys sampled at the time of autopsy revealed glomeruli with no significant histopathologic changes by light microscopy (Figure 2a–f). Frequent distal tubules contained intraluminal casts, mostly in the medulla and rarely present in the cortex. By immunoperoxidase staining the tubular casts positively expressed CGA, and did not significantly show expression of insulin, glucagon, somatostatin, or gastrin. There was 10% cortical interstitial fibrosis and tubular atrophy, and moderate arterial/arteriolar sclerosis. Paraffin sections were pretreated with protease solutions for antigen retrieval and incubated with antibodies specific for the heavy chains of IgG, IgA, and IgM, and for kappa and lambda LC, with no tissue deposits observed and no difference in reactivity between kappa and lambda LC in the tissue. EM was performed on tissue retrieved from the paraffin block and reprocessed for ultrastructural examination. Tubules contained intraluminal granular debris. The kidney was diagnosed as a cast nephropathy composed of CGA reactive material and lacking dominance of either kappa or lambda LC, and mild chronic changes of the parenchyma.

Case 3

A 62-year-old white man who was status-post resection of a colonic/mesenteric mass determined to be a

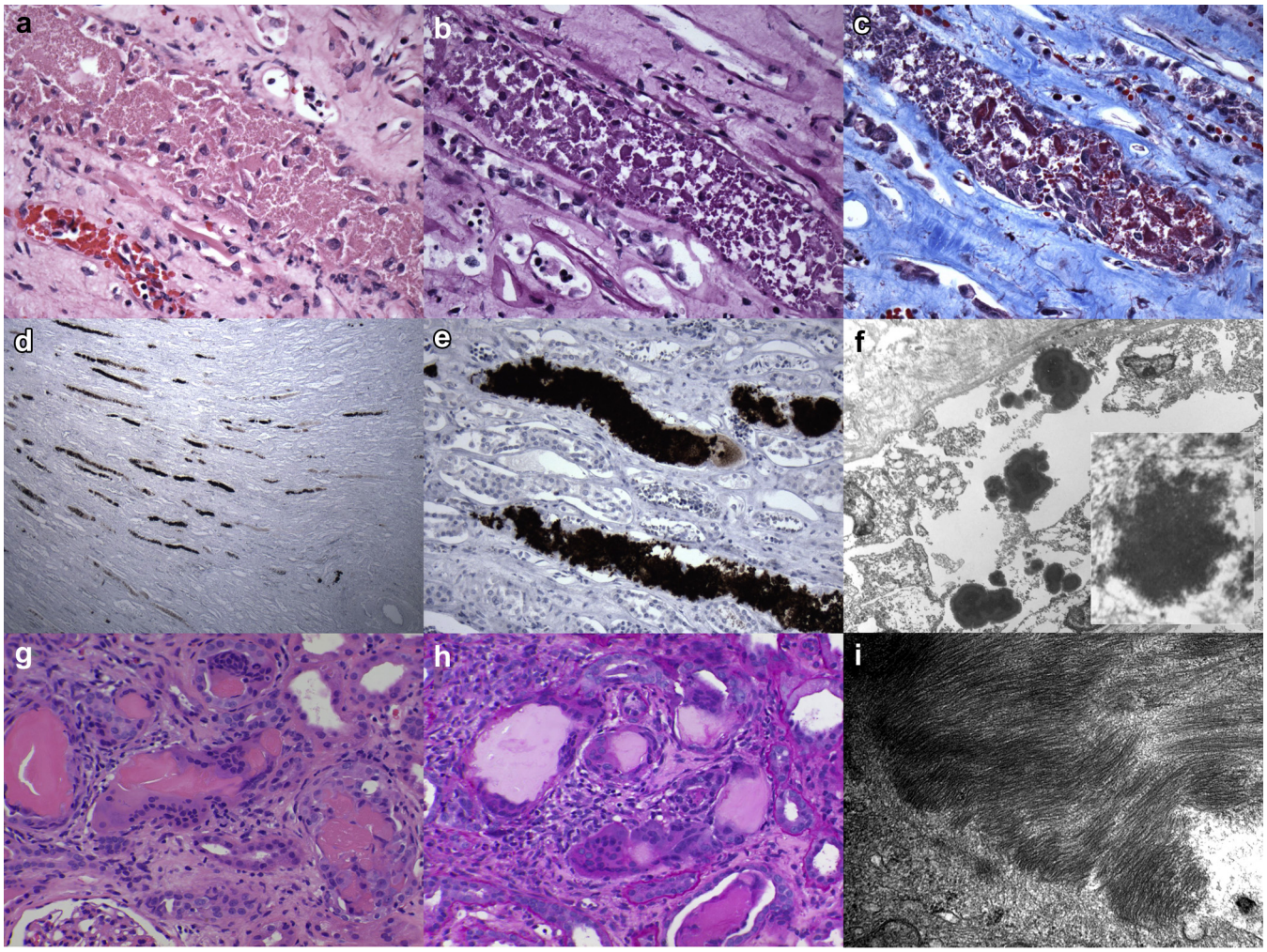

Figure 2. Kidney biopsy from case 2 (a–f). By light microscopy, tubules contain predominately granular intratubular casts (a) that are positive by periodic acid–Schiff (PAS) staining (b) and stain red by trichrome stain (c). The intraluminal casts are reactive for chromogranin A by immunoperoxidase staining (d,e) and are found almost exclusively within distal tubular elements of the kidney medulla. By electron microscopy (EM), the intraluminal casts are composed of granular material (f). Kidney biopsy from case 3 (g–i). By light microscopy, tubules contain intraluminal casts that elicit an inflammatory response that includes multinucleated giant cells (g). The casts are negative by PAS staining (h), and some appear fractured. By EM, the intraluminal casts are composed of fibrillary material and organized in parallel curvilinear bundles (i). Original magnification: (a–c,g,h) ×400, (d) ×20, (e) ×200, (f) ×2500 (f inset, ×25,000), and (i) ×50,000.

neuroendocrine neoplasm 2 years prior, presented with metastatic disease to the liver and acute kidney injury. Laboratory testing noted at admission included serum creatinine of 7.4 mg/dl (from 1.9 mg/dl 3 weeks prior), estimated glomerular filtration rate of 8 ml/min per 1.73 m^2, blood urea nitrogen of 59 mg/dl, serum albumin of 3.0 g/dl, urine protein-to-creatinine ratio of 4.3 mg/mg, and serum CGA of 343 ng/ml. Normal/negative serum and urine protein electrophoresis and immunofixation, serum C3 and C4, antinuclear antibodies, antinuclear cytoplasmic antibodies, and anti–glomerular basement membrane.

The kidney biopsy consisted of cortex, with 19 nonsclerosed glomeruli (Figure 2g–i). Bowman's space was occasionally dilated. The glomeruli showed mild mesangial expansion by matrix and cellular elements, and mild but diffuse endocapillary hypercellularity with neutrophils. Several tubules contained casts that stained negative periodic acid–Schiff, many of which were fractured in appearance and surrounded by mononuclear inflammatory and multinucleated giant cells. Tubules were somewhat distended and with flattened epithelium. The interstitium contained moderate mixed inflammation, with 20% cortical interstitial fibrosis and tubular atrophy, and mild arterial/arteriolar sclerosis. Unfortunately the paraffin block could not be retrieved and therefore immunoperoxidase staining for CGA, insulin, glucagon, somatostatin, and gastrin could not be performed. Immunofluorescence microscopy performed on frozen tissue showed diffuse granular deposition of IgG (3+), C3 (4+), and kappa LC (2+) and lambda LC (2+) in the glomeruli, and the intratubular casts were negative for kappa and lambda LC reactivity. EM showed many scattered subepithelial

glomerular capillary wall electron-dense deposits, and few intramembranous, subendothelial, and mesangial deposits. Tubules contained casts composed of fibrillary material (diameter ~10 nm) organized into parallel curvilinear bundles. The kidney biopsy was diagnosed as a cast nephropathy composed of material lacking dominance of either kappa or lambda LC in the setting of a known neuroendocrine neoplasm, a diffuse proliferative immune complex–mediated glomerulonephritis, and mild chronic changes of the parenchyma.

Based on the kidney biopsy and the consideration that the tubular casts were secondary to material elaborated by the neuroendocrine neoplasm (as opposed to a lymphoma or plasma cell neoplasm), the patient was started on 5-fluorouracil and irinotecan, as well as 60 mg prednisone daily. Kidney function continued to deteriorate and hemodialysis was commenced. Four days later, the patient was discharged with a serum creatinine of 5.2 mg/dl for continued care (including hemodialysis) at another institution. Although the patient was not seen further at our institution, the patient was noted to have died 10 years after the kidney biopsy.

DISCUSSION

We have illustrated 11 cases for which histopathologic examination of kidney tissue was performed for patients with neoplasms of neuroendocrine derivation or differentiation, and described 2 cases in which ATI was associated with CGA. A third case (case 3) also revealed intraluminal tubular cast formation that did not exhibit immunoglobulin LC restriction, but can only be speculated to be composed of material elaborated by the patient's neuroendocrine neoplasm, as immunoperoxidase staining for neuroendocrine specific peptides could not be performed (similar to the case report of Butani and Ducore).[7] Two general patterns of tubular injury were observed: tubular cast formation and proximal tubular engorgement and injury, mediated by CGA intraluminal precipitation and cast formation or excessive CGA uptake, respectively.

CGA is a member of a family of acidic glycoproteins, expressed and secreted by endocrine and neuroendocrine cells, and certain neoplasms derived from such tissues as well as separately derived neoplasms with neuroendocrine differentiation are capable of expressing and secreting the peptide.[8] CGA is filtered by the glomerulus and can be detected in urine of both patients with an underlying neuroendocrine neoplasm, as well as basal secretion in healthy subjects.[9,10] Based on its amino acid sequence, the peptide has an expected molecular weight of 48 kDa; however, posttranslational modifications and a relatively high content of acidic amino acids (imparting a negative charge) likely reduce the migration rate on sodium dodecyl sulfate gel electrophoresis and explain the observed molecular weight of 70 kDa.[11–13] CGA is likely internalized by proximal tubular cells via megalin and cubilin-mediated endocytosis, such as seen in the setting of free immunoglobulin LC and other filtered low molecular weight proteins, which fuse with lysosomes to be hydrolyzed and their amino acid components returned to circulation across the basolateral membranes.[14–17] Although the anionic charge of CGA could to some degree impede tubular resorption (as cationic proteins are more readily resorbed by the tubular epithelium), there is nonetheless proximal tubular uptake of the peptide and marked uptake as seen in case 1.[18,19] If megalin is involved in the uptake of CGA, variable intracellular signaling cascades and subsequent downstream effects could be mediated by this scavenger receptor.[20] A low molecular weight molecule that has been shown to be associated with ATI is lysozyme (although it is cationic) in the setting of chronic myelomonocytic leukemia.[21]

Direct cellular toxicity imparted by CGA has not been described in the literature. In case 1, it is evident that the proximal tubular epithelial cells are selectively engorged with CGA, and with morphologic evidence of cellular injury with variably flattened epithelium, focal loss of the brush border, and prominent apoptotic bodies and rare mitotic figures. No CGA expression was seen in other tubular epithelium or podocytes, even though the latter is known to be capable of protein resorption via megalin and cubilin.[22] Unfortunately, urine testing at the time did not include evaluation of analytes that would illustrate proximal tubular dysfunction and Fanconi syndrome. The engorged proximal tubular epithelium also appeared to encroach significantly on the tubular lumen, thereby also potentiating tubular injury and reduced renal filtration by an obstructive mechanism. The findings suggest a threshold above which the intracellular concentration of CGA becomes injurious and toxic to the proximal tubular epithelium. The exact mechanism(s) driving these changes in this setting only can be speculated and extrapolated from our knowledge of injury mediated by other similar proteins, with further studies required.

Intraluminal cast formation and resultant tubular epithelial injury mediated by immunoglobulin LC (LC or myeloma cast nephropathy), bile, myoglobin, and hemoglobin have been previously described. Intraluminal casts are considered to impart direct toxic injury to the adjacent epithelium, and in certain circumstances with significant distal nephron accumulation obstruction augment tubular injury and further retard kidney function. Immunoglobulin LC casts by light microscopy are negative to periodic acid–Schiff,

sometimes with a fractured appearance, and may elicit an immediate inflammatory response that can include multinucleated giant cells. The restricted expression of a given immunoglobulin LC is proven via immunohistochemistry (using generally immunofluorescence microscopy, but immunoperoxidase staining can also be used). Bile casts by light microscopy are occasionally yellow-green in color and highlighted by Hall stain, and do not show immunoglobulin LC restriction with immunohistochemical techniques. In LC, bile, myoglobin, or hemoglobin cast nephropathy, one would not expect the casts to express CGA (or any other neuroendocrine-associated peptide). Likewise, the casts formed in the setting of a neoplasm with neuroendocrine derivation or differentiation would not exhibit immunoglobulin LC restriction, would be Hall stain negative, and would not express myoglobin or hemoglobin by immunohistochemical staining.

The intraluminal conditions that promote CGA cast formation are potentially similar to those that augment other cast nephropathies. Immunoglobulin LC can bind to uromodulin to promote intraluminal aggregation, with additional augmenting factors including reduced pH, dehydration, hypercalcemia, diuretics (furosemide increases tubular sodium chloride), and nonsteroidal inflammatory agents.[23] CGA, with an isoelectric point of 4.5 to 5.0, would be suspected to follow a similar pattern as LC cast formation in the more distal and acidic portions of the nephron, and is congruent with the distribution of casts seen in case 2.[12,24] The significant intratubular inflammatory reaction to the casts identified in case 3 precludes definitive determination of the exact portion of the tubular nephron involved; however, illustrates that the cast material is capable of such a reaction. Bile casts are considered to develop in the setting of longstanding elevated serum total and direct bilirubin levels, and are thought to contribute to kidney dysfunction by way of generation of oxygen free radicals and obstructive mechanisms.[25,26] Similarly, oxidative stress and lipid peroxidation mediated by heme proteins, whether in the setting of rhabdomyolysis or hemolysis, can result in tubular epithelial injury.[27,28] CGA has not been characterized as being able to facilitate free radical production or lipid peroxidation. It is unclear from our few cases by what exact mechanism(s) intraluminal tubular casts are formed, what factors mediate their formation, and how beyond potential obstructive mechanisms they would prove directly injurious to tubular epithelium.

Although the size of the presented series of patients is relatively small, certain observations can be made from the collected data. All 9 patients for whom serum CGA levels were available were above the reference range of the assay (<93 ng/ml); however, only 3 patients exhibited the 2 described patterns of ATI, likely in part attributable to CGA. The 3 cases (cases 1–3) that had either ATI via intracellular engorgement of proximal tubules or intraluminal cast formation had serum CGA levels that overlapped, with the remaining 6 cases that did not exhibit these specific forms of tubular injury, and there is no statistical difference between these 2 groups on comparison ($P = 0.61$). At least from these data points, the serum CGA level alone does not appear to predict significant proximal tubular engorgement or cast formation.

In summary, we have illustrated 2 patterns of ATI mediated by CGA in the setting of patients having an underlying neoplasm of neuroendocrine derivation or differentiation. As such, clinical teams managing patients with such neoplasms should consider the role of CGA in mediating acute kidney injury. Further studies are required to determine the factors that impart tubular injury when there is significant intracellular accumulation, as well as factors that increase the propensity for CGA casts to form.

DISCLOSURE

All authors declared no competing interests.

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