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Kidney Biopsy Teaching Case

# Chronic Ifosfamide Toxicity: Kidney Pathology and Pathophysiology

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Ifosfamide is a nitrogen mustard alkylating agent used as both a first-line and a salvage chemotherapeutic agent in the treatment of testicular germ cell tumors, various sarcomas, carcinomas, and some lymphomas. A well-known complication of ifosfamide therapy is transient acute kidney injury. However, in a minority of patients, the reduction in kidney function is progressive and permanent, sometimes occurring long after exposure to ifosfamide. Scattered reports have described the pathologic findings in kidneys permanently affected by ifosfamide toxicity. We present the findings of an illustrative case and review the pathology and molecular mechanisms of long-term ifosfamide toxicity with implications for personalized medicine.
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***INDEX WORDS:*** Ifosfamide; tubular toxicity; chemotherapy-related injury; chronic kidney injury; drug toxicity.

## INTRODUCTION

Chemotherapeutic agents used for the treatment of malignant neoplasms frequently induce collateral kidney injury. Two widely used oxazaphosphorine nitrogen mustards, ifosfamide and its related compound cyclophosphamide, are known to cause both acute and chronic kidney injury. Ifosfamide is particularly notorious for sporadically causing irreversible kidney injury. We describe the histologic findings of chronic ifosfamide-induced kidney injury and possible pathophysiologic mechanisms underlying these changes.

## CASE REPORT

### Clinical History and Initial Laboratory Data

A 22-year-old man was first given the diagnosis at age 17 years of stage IIB classic Hodgkin lymphoma. He received multiple rounds of chemotherapy, including ABVD (adriamycin, bleomycin, vinblastine, and dacarbazine), GVD (gemcitabine, vinorelbine, and doxorubicin), and DHAP (dexamethasone, cytarabine, and cisplatin), but his disease was refractory to treatment. He underwent autologous stem cell transplantation and haploidentical allogeneic stem cell transplantation at age 18 years. Prophylaxis for graft-versus-host disease included tacrolimus, which was limited to 10 days around the time of the allogeneic stem cell transplantation.

Two years later, the patient experienced a skin rash that initially was attributed to graft-versus-host disease and empirically treated with tacrolimus for 5 days, after which tacrolimus therapy was discontinued. Trough tacrolimus levels ranged from 1.2-8.6 ng/mL during this course.

The patient's lymphoma relapsed 3 years later and failed to respond to salvage therapies with bendamustine and brentuximab vedotin. At age 21 years, he underwent salvage chemotherapy with ifosfamide (2 cycles) administered with sodium-2-mercaptoethane sulfonate (MESNA) in preparation for a donor lymphocyte infusion. After receiving the donor lymphocyte infusion, his disease relapsed, prompting plans for a third stem cell transplantation. To prepare for this, he received 3 additional cycles of ifosfamide with MESNA. After each cycle of ifosfamide, the patient's serum creatinine concentration increased, reflecting kidney function deterioration, but returned to a new (elevated) baseline (Fig 1A). Each cycle was associated with severe thrombocytopenia, and the first 2 cycles were associated with a mild elevation in serum lactate dehydrogenase level (Fig 1B). After the final cycle, his serum creatinine concentration increased progressively to 3 mg/dL (corresponding to estimated glomerular filtration rate calculated with the 4-variable MDRD [Modification of Diet in Renal Disease] Study equation of ~40 mL/min/1.73 $m^2$). This was accompanied by proteinuria quantitated by a spot urine protein-creatinine ratio of 5.5 g/g (2+ on urinalysis). An albumin-creatinine ratio of 511.8 mg/g indicated substantial nonalbumin proteinuria. These findings prompted a kidney biopsy 12 weeks after completion of this last cycle of therapy.

### Kidney Biopsy

The biopsy specimen showed renal cortex with 17-20 glomeruli, of which 3 were globally sclerosed. One glomerulus had a hilar thrombus (Fig 2A) and another had mesangiolysis (Fig 2B), both features of thrombotic microangiopathy (TMA). Focal nodular hyalinosis also was present, suggesting that the glomerular and vascular findings were attributable to calcineurin-inhibitor (tacrolimus) toxicity. Isometric tubular epithelial cytoplasmic vacuolization, a feature of acute calcineurin-inhibitor toxicity, was not present. The remaining glomeruli were unremarkable. The most prominent finding was diffuse and severe acute tubular injury (Fig 2C and D). Several tubular segments were extensively denuded of epithelial cells, and many of the surviving cells demonstrated nuclear atypia, including nucleomegaly and hyperchromasia. These atypical-appearing cells prompted consideration of possible viral infection, but immunostains did not detect the presence of polyomavirus or cytomegalovirus. In addition to the acute tubular injury, there was diffuse tubulointerstitial inflammation and interstitial edema. This active inflammatory infiltrate made it difficult to

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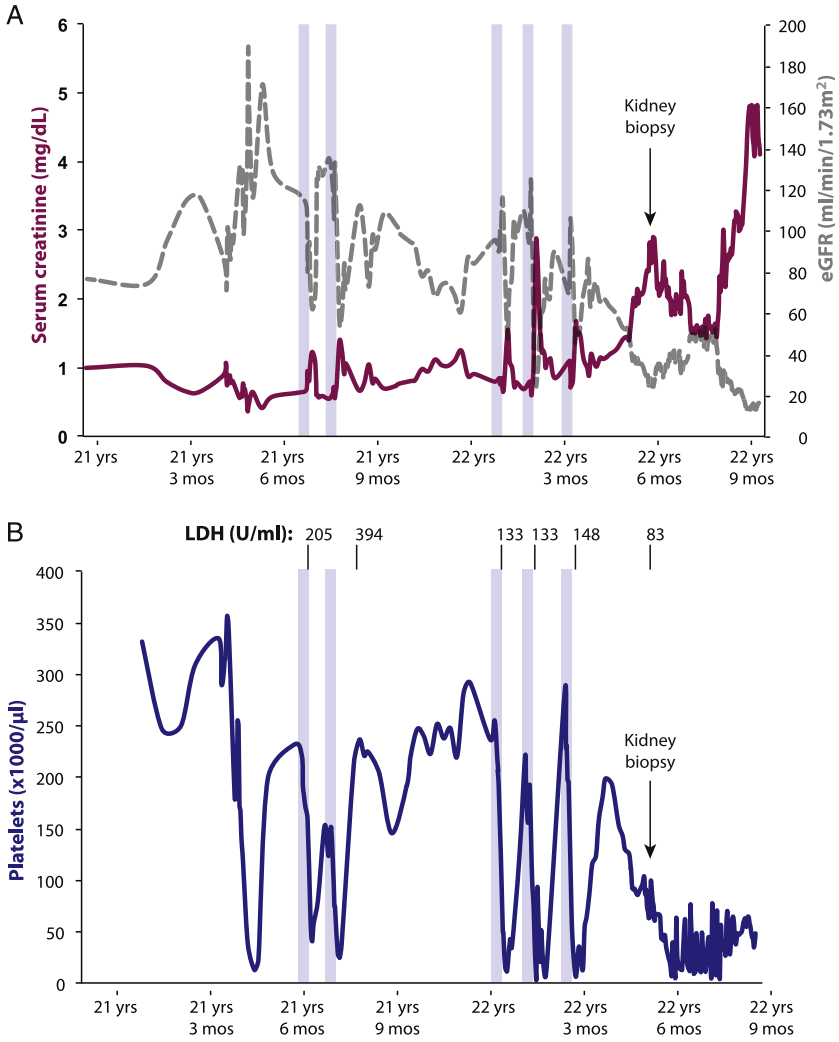


**Figure 1.** (A) Time course of the patient's decline in kidney function. The patient's serum creatinine levels are plotted as a function of age (solid line, adult reference range, 0.51-1.18 mg/dL). Estimated glomerular filtration rate is plotted along the right axis (dashed line). Vertical gray bars below the time course indicate ifosfamide treatments. (B) The patient's platelet count during the same period is plotted. Serum lactate dehydrogenase levels in U/mL are shown at indicated time points (reference range, 80-190 U/mL).

accurately assess the degree of chronic parenchymal injury. Immunofluorescence microscopy study findings were unremarkable. Electron microscopy did not show immune deposits, abnormal mitochondrial forms within tubular epithelial cells, or viral particles. Glomeruli were not present in the electron microscopic sample to evaluate for acute endothelial injury.

### Diagnosis

Based on the biopsy findings, a diagnosis of diffuse acute tubular injury with tubulointerstitial nephritis was rendered. The epithelial cytologic atypia was noted, and association of tubular injury to the patient's treatment with ifosfamide was suggested. The degree of interstitial fibrosis was deemed difficult to assess accurately due to extensive interstitial inflammation and edema. With this caveat, chronic tubulointerstitial injury was estimated to be mild. Focal TMA involving glomeruli was noted. Focal nodular arteriolar hyalinosis, a feature associated with calcineurin-inhibitor toxicity, also was described.

### Clinical Follow-up

After the biopsy, stem cell transplantation was postponed temporarily and the patient was aggressively volume resuscitated. However, this maneuver resulted in only modest improvement in kidney function, as detected by a small reduction in serum creatinine concentration. Four months later, he was restarted on tacrolimus therapy as part of preparative conditioning for stem cell transplantation, but developed fevers and septic shock. His kidney function declined precipitously, which was attributed to hypovolemic injury superimposed on chronic tubular injury and diminished kidney reserve. His serum creatinine concentration has remained highly elevated and he currently is preparing for renal replacement therapy.

## DISCUSSION

In most patients, ifosfamide-induced decreased kidney function is temporary, and kidney function appears to normalize upon cessation of therapy. However, long-term analysis of adult survivors of pediatric malignancies treated with ifosfamide has shown permanently decreased kidney function, comparable in magnitude to unilateral nephrectomy.[1] Rarely, the ifosfamide-related kidney injury is progressive, leading to end-stage kidney disease. Potential risk factors for persistent nephrotoxicity in children include high cumulative dose, younger age at presentation, and reduced kidney mass (eg, prior nephrectomy).[2-6] There are fewer data for risk factors in adults, but older age and concurrent treatment with cisplatin appear to increase the risk for persistent decreased

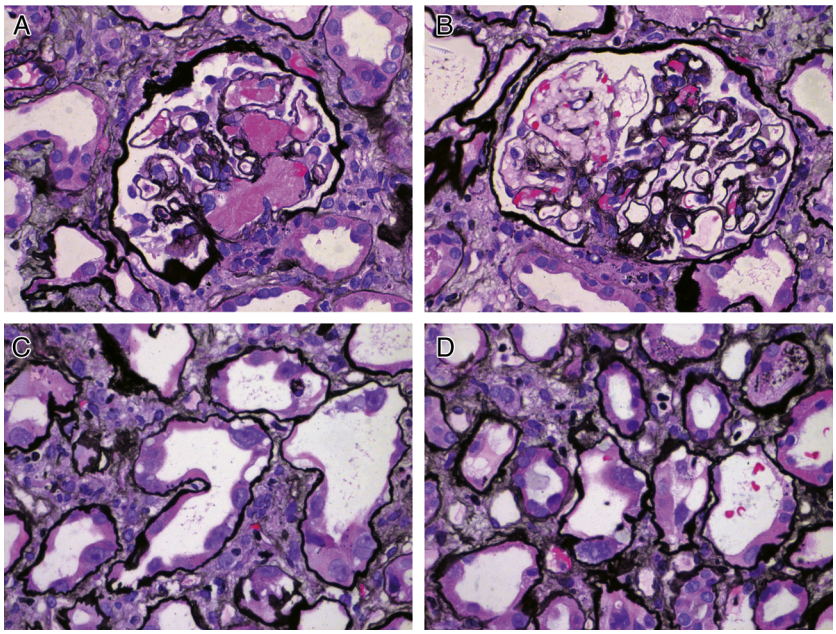


**Figure 2.** Histologic features of ifosfamide kidney toxicity. (A, B) The patient's kidney biopsy specimen shows rare glomeruli with thrombi or mesangiolysis, both features of thrombotic microangiopathy. (C, D) The tubular parenchyma shows marked attenuation with prominent reactive changes in the nuclei of the remaining epithelial cells. (A-D: Jones methenamine silver; original magnification, ×600.)

kidney function.[7] Our review of the literature of biopsy-proven decreased kidney function after ifosfamide treatments (Table 1) identifies histologic features of permanent ifosfamide-related kidney toxicity. Severe acute tubular injury is a universal feature. Several reports describe marked cytologic atypia in the surviving epithelial cells. Tubulointerstitial nephritis often is present, but tubulointerstitial fibrosis is variable. Ultrastructural features are consistent with acute tubular injury, but specific distinguishing ultrastructural features of ifosfamide toxicity have not been reported. Our survey also emphasizes the fact that permanent and severe kidney failure appears to be a sporadic complication of ifosfamide therapy without a clear relationship to cumulative dose or duration of therapy. However, differences in dosing and delivery protocols, cumulative doses, and nearly universal treatment with additional nephrotoxic chemotherapeutic agents make it difficult to predict the incidence of decreased kidney function based on ifosfamide exposure alone (Table 1).

A recent study suggests that tubular cells can transport and concentrate ifosfamide intracellularly by hOCT2 (an organic cation transporter encoded by the *SLC22A2* gene).[8] Once internalized, the epithelial cytochrome CYP3A4 converts ifosfamide into active nitrogen mustard compounds that alkylate and damage DNA and initiate cell death programs.[9-11] Tubular epithelial CYP2B6 can inactivate ifosfamide by N-dechloroethylation to release chloroacetaldehyde, a compound without significant antitumor activity, but potentially responsible for many of ifosfamide's neurotoxic and nephrotoxic side effects.[12] Tubular epithelial cells may be able to detoxify a limited quantity of chloroacetaldehyde,[13] but higher concentrations lead to depletion of intracellular glutathione[14] and adenosine triphosphate levels,[15] resulting in impaired tubular epithelial cell function and acute injury. In this way, hOCT2-mediated transport of ifosfamide into kidney tubular epithelial cells followed by in situ cytochrome-mediated modification results in the localized generation of high levels of genotoxic nitrogen mustards and tubulotoxic chloroacetaldehyde. The resultant DNA damage may manifest histologically as nuclear atypia, which has similarities to radiation-induced atypia,[16] another therapeutic modality that induces DNA damage. Some authors have likened the cytologic atypia seen in chronic ifosfamide tubular injury to karyomegalic nephropathy,[17] an entity that has been associated with mutations in the *FAN1* gene, which encodes a DNA repair enzyme.[18] Thus, an underlying genetic predisposition (via *FAN1* or other genes) may lead to the sporadic irreversible kidney injury in a subset of patients after ifosfamide therapy.

We speculate that the irreversible genotoxic damage of putative tubular epithelial progenitors[19] or surviving tubular epithelial cells[20,21] also would result in impaired regeneration, as indicated by the low mitotic activity of the remaining epithelial cells relative to the degree of epithelial injury. A previous study reported a trend toward lower mitotic activity in ifosfamide-injured tubular parenchyma.[17] Compared to a case of severe anuric acute tubular injury in which mitotic figures could easily be identified (Fig 3A, arrow), mitotic activity was not readily identified in our case (Fig 3B). Therefore, we determined the mitotic index by using the MIB-1

**Table 1.** Summary of Clinical and Pathologic Features of Cases of Biopsy-Proven Ifosfamide Kidney Toxicity

| Patient Age (y)/Sex | Malignancy | Other Nephrotoxins | Total Ifosfamide Dose | Onset of Symptoms[a] | Outcome | Pathology | Reference |
|---|---|---|---|---|---|---|---|
| 15/M | Osteosarcoma | Cisplatin, Adriamycin, methotrexate | 70 g/m$^2$ (with MESNA) | NS[b] | Death (metastatic osteosarcoma, *Candida* bronchopneumonia) | Interstitial fibrosis and tubular atrophy, regenerative changes of distal and collecting tubules | 28 |
| 15/M | Ewing sarcoma | Cisplatin | 80 g/m$^2$ (with MESNA) | 5 y | Persistent proximal tubule dysfunction | Partial loss of proximal tubular epithelial cells with denudation of basement membranes | 29 |
| 5/M | Embryonal rhabdomyosarcoma | Vincristine | 54 g/m$^2$ | 2 mo | Persistent proximal and distal tubule defect | Interstitial inflammation, hyperplastic epithelial cells | 30 |
| 5/F | Embryonal rhabdomyosarcoma | Carboplatin, epirubicin, actinomycin D, vincristine | 57 g/m$^2$ | 7 mo | Persistent tubular defects | Focal inflammation at corticomedullary junction | 30 |
| 33/M | Mixed alveolar/embryonal rhabdomyosarcoma | Vincristine, Adriamycin, cyclophosphamide, radiation (scatter) | 108 g/m$^2$ | 10 y | Progressive GFR loss; ESRD | Severe interstitial fibrosis and tubular atrophy without interstitial inflammation | 31 |
| 26/F | Mixed germ cell tumor | Cisplatin, vinblastine, bleomycin | 36 g/m$^2$ (with MESNA) | 4 y | Progressive GFR loss; ESRD | Severe interstitial fibrosis, lymphoplasmacytic infiltrate, tubular atrophy | 32 |
| 50/F | Breast carcinoma | Cyclophosphamide, methotrexate, 5-fluorouracil | 30 g/m$^2$ | 9 mo | Permanent, but stable tubular dysfunction | Marked tubular atrophy and diffuse interstitial fibrosis, mild patchy inflammatory infiltrate, partial denudation of epithelium, irregular hyperchromatic nuclei, no regeneration | 33 |
| 40/F | Breast carcinoma | Cyclophosphamide, methotrexate, 5-fluorouracil, paclitaxel | 23 g/m$^2$ | 4 mo | Permanent, but stable tubular dysfunction | Marked tubular atrophy and diffuse interstitial fibrosis, mild patchy inflammatory infiltrate, partial denudation of epithelium, irregular hyperchromatic nuclei, no regeneration | 33 |
| 47/F | Breast carcinoma | Cyclophosphamide, methotrexate, 5-fluorouracil | 20 g/m$^2$ | 3 wk | Progressive GFR loss; ESRD | Marked tubular atrophy and diffuse interstitial fibrosis, mild patchy inflammatory infiltrate, partial denudation of epithelium, irregular hyperchromatic nuclei, no regeneration | 33 |
| 49/M | Gastrointestinal stromal tumor (GIST) | Adriamycin | NS | 2 mo | Progressive GFR loss; ESRD | Flattening of epithelium, denudation, tubular atrophy | 34 |
| 15/M | Ewing sarcoma | Cyclophosphamide, vincristine, Adriamycin, dactinomycin | NS | 18 mo | Progressive GFR loss; ESRD | Karyomegalic-like features with large atypical tubular epithelial cell nuclei, flattening, severe interstitial fibrosis, tubular atrophy, mild inflammatory infiltrate | 17 |

*(Continued)*

**Table 1 (Cont'd).** Summary of Clinical and Pathologic Features of Cases of Biopsy-Proven Ifosfamide Kidney Toxicity

| Patient Age (y)/Sex | Malignancy | Other Nephrotoxins | Total Ifosfamide Dose | Onset of Symptoms[a] | Outcome | Pathology | Reference |
|---|---|---|---|---|---|---|---|
| 13/F | Ewing sarcoma | Vincristine, Adriamycin, actinomycin | NS | 4 y | Stable decrease in kidney function | Karyomegalic-like features with large atypical tubular epithelial cell nuclei, flattening, severe interstitial fibrosis, tubular atrophy, mild inflammatory infiltrate | 17 |
| 14/F | Ewing sarcoma | Vincristine, actinomycin | NS | 2 y | Stable decrease in kidney function | Karyomegalic-like features with large atypical tubular epithelial cell nuclei, flattening, severe interstitial fibrosis, tubular atrophy, mild inflammatory infiltrate | 17 |
| 62/F | Ovarian carcinoma | Cyclophosphamide, Adriamycin, cisplatin | NS (with MESNA) | 5 wk | Progressive GFR loss | Focal tubular atrophy, diffuse interstitial fibrosis, no interstitial nephritis | 35 |
| 60/M | Malignant fibrous histiocytoma (MFH) | Adriamycin, radiation (kidneys shielded) | 28 g/m$^2$ (with MESNA) | 5-6 mo | Progressive GFR loss; ESRD | Diffuse tubulointerstitial damage with degenerative/regenerative changes without significant inflammation | 36 |
| 56/M | Osteogenic sarcoma | Cisplatin, Adriamycin | 26 g/m$^2$ (with MESNA) | 2-3 mo | Progressive GFR loss; ESRD | Moderate tubulointerstitial fibrosis; prominent regenerative/degenerative changes in tubular epithelial cells; mild interstitial inflammation | 36 |
| NS/F | Hepatic mesenchymoma | Vincristine, carboplatin, epirubicin, actinomycin D | 14 g/m$^2$ (with MESNA) | NS[b] | Complete recovery | "Proximal tubule sclerosis" | 37 |
| 2/NS | Unspecified solid tumor | NS | NS (with MESNA) | NS | NS | Severe tubular atrophy and tubulointerstitial nephritis | 38 |
| 26/F | Sarcoma (type not specified) | NS | 56 g/m$^2$ (with MESNA) | NS | Partially recovered kidney function; ESRD | Extensive interstitial fibrosis and proximal tubular injury (necrosis, vacuolization, atrophy) | 39 |
| 48/M | Non-Hodgkin lymphoma | NS | 33 g/m$^2$ (with MESNA) | NS | Progressive GFR loss; ESRD | Extensive interstitial fibrosis and proximal tubular injury (necrosis, vacuolization, atrophy) | 39 |
| 22/M | Classic Hodgkin lymphoma | Adriamycin, cisplatin, tacrolimus | 58 g/m$^2$ (with MESNA) | 6 mo | Progressive GFR loss; ESRD | Acute tubular injury with marked nuclear atypia; tubulointerstitial nephritis; focal thrombotic microangiopathy involving glomeruli | [c] |

Abbreviations: ESRD, end-stage renal disease; GFR, glomerular filtration rate; MESNA, sodium-2-mercaptoethane sulfonate; NS, not specified.
[a]Time from first dose to first observation of renal symptoms.
[b]Appears to have been within a few to several weeks.
[c]Case described here.

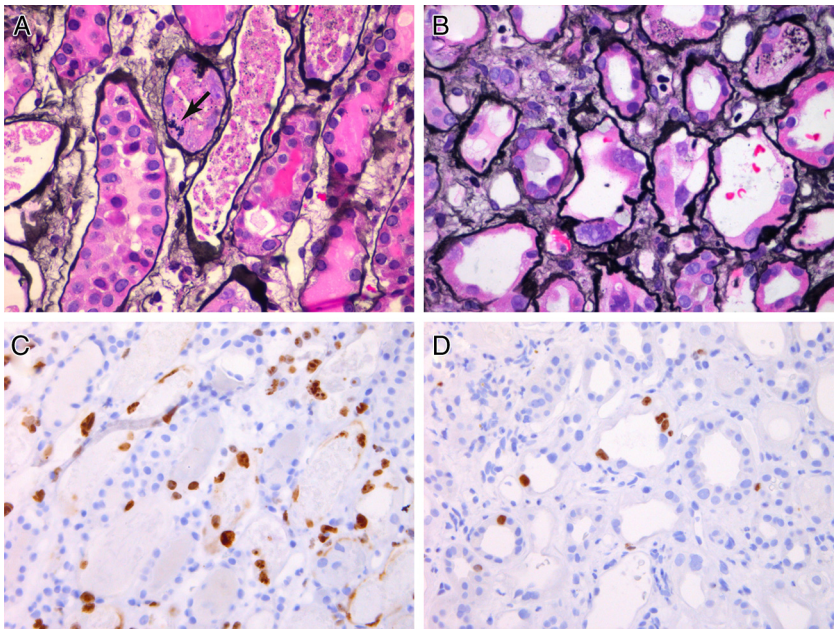


**Figure 3.** Ifosfamide kidney toxicity is associated with low tubular epithelial cell mitotic activity. (A) Mitotic figures (arrow) are identified readily in a case of acute tubular necrosis, but (B) not in the present case (A, B: Jones methenamine silver). (C) The case of acute tubular necrosis demonstrates brisk mitotic activity (mitotic index, ~24.6%) as determined by ki67 (MIB-1) immunostaining. (D) In comparison, the present case of ifosfamide-related injury has a lower mitotic index of 5.4%. (Original magnification: [A, B] ×600; [C, D] ×400.)

antibody to detect the cell-cycle marker Ki67 and enumerated more than 600 tubular epithelial nuclei in 5 high-power fields (original magnification, ×400). This revealed a mitotic index of 24.6% in the case of severe acute tubular necrosis due to an unknown cause (Fig 3C) and a mitotic index of 5.4% in our case of ifosfamide-related tubular injury (Fig 3D). One additional case of ifosfamide-related tubular toxicity in our case files (with limited clinical information) also had a low mitotic index of 2.1%. Two other cases of acute tubular necrosis due to acute liver injury/pancreatitis (ethanol overdose) and sepsis/vancomycin toxicity revealed mitotic indexes of 2.2% and 9.4% (data not shown). The specific uptake and localized generation of chloroacetaldehyde may underlie the acute tubular dysfunction seen in rodents and humans treated with ifosfamide.[22] This tubular dysfunction manifests histologically as acute tubular necrosis seen universally in this patient population (Table 1). Because the readily available histamine $H_2$ receptor antagonist cimetidine is able to competitively inhibit ifosfamide uptake by hOCT2,[8] prospective studies to test whether cimetidine can prevent against ifosfamide-induced nephrotoxicity may be clinically beneficial.

An unexpected finding in this patient was TMA focally involving 2 of the sampled glomeruli. Because glomeruli were not present in the sample submitted for electron microscopy, an ultrastructural assessment of acute endothelial injury within the glomerular capillaries could not be performed. TMA has not been reported as a complication of ifosfamide therapy. Thrombocytopenia immediately followed cycles of ifosfamide therapy in our patient (Fig 1), which is an expected outcome of chemotherapy.[23] However, lactate dehydrogenase levels were not markedly elevated at these times (except for mild elevations after the first 2 cycles), arguing against widespread systemic TMA. Anecdotal reports suggest that cyclophosphamide, which is a compound structurally related to ifosfamide, can cause TMA.[24] However, properly controlled evidence to support this clinical impression and dissociate it from malignancy-induced TMA is lacking. In fact, cyclophosphamide frequently is used to treat TMA due to lupus.[25,26] However, calcineurin inhibitors such as tacrolimus, with which the patient was treated briefly, are known to cause TMA.[27] As further evidence of chronic calcineurin-inhibitor toxicity, nodular arteriolar hyalinosis was present in our patient's biopsy specimen. However, the patient's exposure to tacrolimus was remote at the time of biopsy and other features of acute calcineurin-inhibitor toxicity (isometric tubular epithelial cytoplasmic vacuolization) were not present. Therefore, the precise cause of TMA in this patient is unclear.

Since its initial use as a chemotherapeutic agent, remarkable advances have been made in understanding the pharmacologic and pathophysiologic properties of ifosfamide. Future studies should focus on polymorphisms in metabolic and protective enzymes that may risk-stratify patients prior to treatment. Additional work is needed to develop molecular derivatives of ifosfamide that are more therapeutically effective with fewer side effects and cytoprotective therapies to limit toxicity to normal tissues while preserving antitumor effects. Regardless, patients

receiving ifosfamide should have aggressive monitoring of their kidney function, both while receiving therapy and in follow-up. The care of patients with life-threatening malignancies often is dictated by management of the tumor. However, oncologists and nephrologists should remain cognizant of the potential for permanently decreased kidney function after ifosfamide therapy, sometimes necessitating renal replacement therapy in this patient population.

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