Preventing Drug Side Effects While Treating Cancer: Clues from a Patient’s DNA and Urine

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Today’s Objectives

• Understand how the cancer drug cisplatin damages the kidneys.
• Recognize the importance of kidney health in long-term cancer patient outcomes.
• Discuss biomarkers in a patient’s urine that help to test for cisplatin kidney toxicity.
• Understand how a patient’s DNA can help determine susceptibility to cisplatin kidney toxicity.
Clinical Case

Aaron is a 32-year-old patient who was diagnosed with testicular cancer two months ago and has been receiving two chemotherapy drugs (etoposide and cisplatin) every two weeks.

After the first couple of rounds of chemotherapy, Aaron lost his hair, vomited for two to three days, and became fatigued. His past medical history includes type II diabetes and obesity (BMI > 30), which he has been working to reverse through diet modifications. Aaron denies the use of tobacco products, illicit drugs, or alcohol.

Clinical Case

Aaron comes to the hospital today complaining of urine that is pinkish-red in color, back pain, and swelling in his ankles. His fatigue has worsened.

The doctors order a number of urine and blood tests to determine the cause of Aaron’s signs and symptoms.
Which of Aaron’s Laboratory Values Are Abnormal?

<table>
<thead>
<tr>
<th>Laboratory Tests</th>
<th>Aaron’s Values</th>
<th>Normal Range</th>
<th>Purpose of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>15</td>
<td>5–35</td>
<td>Liver Function</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>22</td>
<td>5–40</td>
<td>Liver Function</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dL)</td>
<td>2.4</td>
<td>0.6–1.2</td>
<td>Kidney Function</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dL)</td>
<td>38</td>
<td>7–20</td>
<td>Kidney Function</td>
</tr>
<tr>
<td>Creatine kinase (U/L)</td>
<td>45</td>
<td>22–198</td>
<td>Muscle Damage</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>225</td>
<td>120–199</td>
<td>Nutrition</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>220</td>
<td>80–140</td>
<td>Nutrition</td>
</tr>
<tr>
<td>Bicarbonate (mg/dl)</td>
<td>24</td>
<td>22–28</td>
<td>Electrolytes</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4</td>
<td>3.5–5</td>
<td>Electrolytes</td>
</tr>
<tr>
<td>White Blood Cells (#/uL)</td>
<td>2000</td>
<td>4800–10800</td>
<td>Immune System</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12</td>
<td>14–18</td>
<td>Blood System</td>
</tr>
</tbody>
</table>

How Would You Explain Aaron’s Abnormal Labs?

Kidney Function  
Cholesterol  
Glucose  
White Blood Cells  
Hemoglobin
What Signs and Symptoms Suggest Aaron Has Kidney Damage? Why?

- Blood in urine
- Back pain
- Swelling in legs
- Fatigue

What Do the Kidneys Do?

- Balance the body’s fluids and pH
- Remove waste and chemicals from the body
- Produce hormones that regulate blood pressure
- Activate vitamin D for bone health
- Control the production of red blood cells
What Risk Factors Do You Think That Aaron Has for Kidney Damage?

- Diabetes—high glucose
- Obesity
- Medications

Discovery of Cisplatin (Platinol®)
Kidneys under the Microscope

Spot the differences
- Cell loss—white areas
- Protein accumulation—hot pink

What Happens Inside the Cell?

Labile sites for ligand exchange

Crosslinks and DNA Repair

Stress to Cell Components

APOPTOSIS, NECROSIS, and INFLAMMATION
Implications of Cisplatin Nephrotoxicity

How can we prevent the long-term effects of cisplatin toxicity?

Earlier and more sensitive detection of toxicity

Biomarkers of Toxicity
Spectrum of Kidney Toxicity

Urine Biomarkers of Cisplatin Toxicity

Prospective Study
- 57 patients
- Newly diagnosed solid tumors
- Early rounds of cisplatin-containing chemotherapy
- Test novel biomarkers of toxicity
Candidate Biomarkers

**Proximal Tubule**
- KIM-1
- Clusterin
- Calbindin
- β2MG
- NAG
- Osteopontin
- Cystatin C
- IL-18
- NGAL
- Albumin

**Distal Tubule**
- Osteopontin
- Clusterin
- GSTα
- NGAL
- Calbindin

Patient Demographics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, SD)</td>
<td>56.6 ± 13.0 years</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>50% male</td>
</tr>
<tr>
<td>BMI (mean, SD)</td>
<td>27.1 ± 6.2 kg</td>
</tr>
<tr>
<td>Cisplatin Dose (mean, SD)</td>
<td>61.7 ± 23.1 mg/m²</td>
</tr>
</tbody>
</table>

### Clinical Laboratory Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Mean ± SD</th>
<th>After Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg²⁺ (mEq/L)</td>
<td>1.7 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>0.220</td>
</tr>
<tr>
<td>Na⁺ (mEq/L)</td>
<td>137 ± 2.4</td>
<td>135 ± 3.5</td>
<td>0.001</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>4 ± 0.5</td>
<td>4 ± 0.7</td>
<td>0.712</td>
</tr>
<tr>
<td>Ca²⁺ (mEq/L)</td>
<td>9.6 ± 2.3</td>
<td>9.2 ± 2.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>HCO₃⁻ (mEq/L)</td>
<td>25 ± 2.1</td>
<td>25 ± 2.5</td>
<td>0.196</td>
</tr>
<tr>
<td>Cl⁻ (mEq/L)</td>
<td>104 ± 3.3</td>
<td>103 ± 5.1</td>
<td>0.026</td>
</tr>
</tbody>
</table>

#### Kidney Function Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Mean ± SD</th>
<th>After Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCr (mg/dL)</td>
<td>0.82 ± 0.2</td>
<td>0.86 ± 0.2</td>
<td>0.330</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>14.1 ± 5.4</td>
<td>14.5 ± 6.1</td>
<td>0.529</td>
</tr>
<tr>
<td>eGFR (mL/min)</td>
<td>89.4 ± 24.8</td>
<td>90.6 ± 24.7</td>
<td>0.878</td>
</tr>
<tr>
<td>Urine Albumin/Cr (mg/mg)</td>
<td>29.9 ± 40.6</td>
<td>50.7 ± 95.2</td>
<td>0.092</td>
</tr>
</tbody>
</table>

*a13 ± 7 days post cisplatin infusion
bDay 3 post cisplatin infusion


### Biomarker Levels in Patient Urine

<table>
<thead>
<tr>
<th>Biomarker (ng/mL)</th>
<th>Baseline (mean ± SD)</th>
<th>Day 3 (mean ± SD)</th>
<th>P</th>
<th>Day 10 (mean ± SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIM-1</td>
<td>0.203 ± 0.293</td>
<td>0.332 ± 0.524</td>
<td>0.024</td>
<td>0.575 ± 0.711</td>
<td>0.002</td>
</tr>
<tr>
<td>Calbindin</td>
<td>52.50 ± 83.59</td>
<td>60.63 ± 65.96</td>
<td>0.094</td>
<td>434.4 ± 856.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clusterin</td>
<td>57.44 ± 211.9</td>
<td>40.39 ± 101.0</td>
<td>0.043</td>
<td>108.2 ± 422.7</td>
<td>0.005</td>
</tr>
<tr>
<td>GST-pi</td>
<td>27.40 ± 74.07</td>
<td>33.43 ± 93.49</td>
<td>0.221</td>
<td>43.76 ± 88.96</td>
<td>0.011</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.089 ± 0.223</td>
<td>0.042 ± 0.067</td>
<td>0.158</td>
<td>0.126 ± 0.189</td>
<td>0.061</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.507 ± 1.154</td>
<td>0.381 ± 0.549</td>
<td>0.330</td>
<td>0.838 ± 1.124</td>
<td>0.003</td>
</tr>
<tr>
<td>Albumin (mg/mL)</td>
<td>10.84 ± 13.86</td>
<td>15.92 ± 15.28</td>
<td>0.028</td>
<td>21.68 ± 24.08</td>
<td>0.008</td>
</tr>
<tr>
<td>β2M</td>
<td>136.9 ± 245.0</td>
<td>452.1±422.2</td>
<td>&lt;0.0001</td>
<td>188.1 ± 262.3</td>
<td>0.026</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>32.91 ± 45.72</td>
<td>60.91 ± 119.1</td>
<td>0.015</td>
<td>57.71 ± 102.1</td>
<td>0.024</td>
</tr>
<tr>
<td>NGAL</td>
<td>43.45 ± 80.59</td>
<td>42.03 ± 59.39</td>
<td>0.346</td>
<td>51.77 ± 75.99</td>
<td>0.168</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>1235 ± 1504</td>
<td>1240 ± 1597</td>
<td>0.784</td>
<td>2630 ± 3854</td>
<td>0.055</td>
</tr>
<tr>
<td>TFF3</td>
<td>755.9 ± 931.4</td>
<td>1282 ± 1187</td>
<td>0.001</td>
<td>1531 ± 1505</td>
<td>0.002</td>
</tr>
</tbody>
</table>
**Beta$_2$ Microglobulin**

- 12-kd protein that associates with MHC Class 1 complex
- Filtered by the glomerulus and reabsorbed by PT cells
- Prior preclinical and clinical studies have observed enhanced $\beta_2$ microglobulin excretion in urine after cisplatin treatment

![Beta2 Microglobulin Graph](image)


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**Kidney Injury Molecule-1 (KIM-1)**

- Localized to the apical membrane
- Promotes clearance of dead cells
- Ectodomain shed in response to kidney injury
- Inducible by cisplatin

![Kidney Injury Molecule-1 Graph](image)

Clinical Promise of Urinary Protein Biomarkers

- Earlier Detection of Kidney Injury
- More Sensitive Detection of Kidney Injury
- Noninvasive
- Reflect Direct Tissue Damage
- May Allow for Identification of Novel Risk Factors for Drug-Induced Kidney Injury

How Do Chemicals Travel within the Kidneys?

Blood → Filtrate

Reabsorption

Drug

Transporter

Secretion

Blood → Proximal Tubule Cells

Filtrate/Urine
Cisplatin Secretion and Kidney Targeting

Entry  OCT2
Exit   MATE1
       MRP2

When Transporters Do Not Work—What Happens to Toxicity in the Kidneys?

If OCT2 is not working, would that increase or decrease toxicity in the kidneys?

Decrease

If MATE1 and MRP2 are not working, would that increase or decrease toxicity in the kidneys?

Increase
Factors That Can Influence Transporter Function

Diseases

Drug Interactions

Genetics

Predicting Patient Drug Responses

Manufacturer Recommended Dose of Drug

No or low efficacy

Efficacy with no toxicity

Efficacy with (unpredicted) toxicity

- 20–40% of patients benefit from an approved drug
- 70–80% of drug candidates fail in clinical trials
- Drugs have been removed from the market due to toxicities

*Can we use a person’s genetic code to predict their reaction to a drug?*
Genetics in Cancer

Tumor DNA
Pick the most responsive drugs

Normal DNA
Determine how the body will influence the drugs that are selected

Genetic Variants or Polymorphisms

• Difference in the DNA sequence compared with a reference or wild-type sequence (+/+)

• Polymorphisms are variations in the DNA sequence that associate with populations
  • More common than mutations - >1% of the population
  • Can have one (+/-) or two copies (-/-) of the variant gene

<table>
<thead>
<tr>
<th>Wild-Type Sequence</th>
<th>Polymorphism Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGTTGCA</td>
<td>AGTTCGA</td>
</tr>
</tbody>
</table>
Transporter Genetics and Kidney Injury Risk

Consequences of Genetic Changes

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Loss-of-Function</th>
<th>Gain-of-Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT2</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>MATE1</td>
<td>↑ (Gain)</td>
<td>↓ (Loss)</td>
</tr>
<tr>
<td>MRP2</td>
<td>↑ (Gain)</td>
<td>↓ (Loss)</td>
</tr>
</tbody>
</table>

Would a genetic change resulting in loss or gain of function in each transporter increase or decrease risk of toxicity?

Uptake/Entry Transporter Genetics

Efflux/Exit Transporter Genetics


Precision Medicine

Use the most effective drug against a tumor’s genetics
-- and --
use the safest drug based on a patient’s genetics
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