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Learning Objectives

1. Describe the basic anatomy of the urinary system.
2. Identify the three types of epithelial cells that line the urinary system as they appear in a urine sample.
3. Explain how the type of epithelial cell is related to its function.
4. Realize the clinical significance of increased numbers of each type of epithelial cell in the urine.
5. List the clinical conditions that cause abnormal numbers of renal tubular cells to appear in the urine.

INTRODUCTION

When a microscopic examination of urine sediment is performed, the presence or absence of epithelial cells is noted, and their type described. Squamous epithelial cells are the most common type seen, and are not abnormal in voided urine. Transitional epithelial cells and renal tubular cells may also be seen. Epithelial cell identification may be difficult but should be done correctly because large numbers may be clinically significant. The purpose of this education activity is to relate the various types of epithelial cells to their sites of origin in the urinary tract, describe their function and clinical significance, and improve correct identification.

THE RENAL SYSTEM PHYSIOLOGY

The normal urinary system is composed of a right and left kidney and ureters, the bladder, and urethra. The urine is formed in the functional units of the kidneys, the nephrons. The formed urine is collected in the pelvis of each kidney, flows to the bladder through the ureters, and collects in the bladder. It is stored in the bladder until emptied through the urethra. See Figure 1. and Figure 2 on the following pages.
Figure 1. Urinary System

Urinary System

pelvis

kidney

ureter

bladder
There are 1 – 1.2 million nephrons in an average kidney. Each nephron (Figure 3. on the following page) is composed of a tuft of blood vessels known as a glomerulus and the renal tubules. The tubules include Bowman’s capsule, the proximal convoluted tubule and the distal convoluted tubule. Several nephrons flow into a collecting duct. These combine to form a renal papilla and eventually flow into the pelvis and ureter.
Figure 3. Structure of a Nephron

Kidney

Nephron

Glomerulus

Figure 3. Illustrated by Eric F. Glassy, MD
Each kidney derives its blood supply from a renal artery off the aorta. The arteries divide into smaller and smaller vessels, finally becoming tiny afferent arterioles, which enter the glomerulus. Filtration takes place in the glomerulus. The blood leaves the glomerulus through efferent arterioles throughout the kidney; the arterioles lie adjacent to corresponding renal tubules. Reabsorption and secretion between the flowing blood and glomerular filtrate is what allows the elimination of waste, retention of nutrients, maintenance of water and acid-base balance, and hormone synthesis.

**EPITHELIAL CELLS**

**Squamous Cells**

The urinary tract is lined by epithelial cells. The most common type is the squamous epithelial cell. The squamous epithelial cells line the female urethra, bladder trigone, and distal portion of the male urethra. They also line the vagina, and are seen in greater numbers in voided specimens from females. They have little or no clinical significance and usually represent contamination. Their purpose is to provide a barrier against physical, biologic, and chemical injury.

Squamous cells are 30 – 50 µm in diameter, large and flat, with a single round or oval nucleus about the size of a red cell or small lymphocyte. The edges may be curled and the cell may be round, polygonal, or rectangular (Image 1 below).

**Image 1: Squamous Cell**

This image is from voided urine from a healthy female. The arrow points to an epithelial cell.

Source: Contributed by David Blomberg, MD
Transitional Cells

Transitional (or urothelial) cells (Image 2 below) line the urinary tract from the pelvis of the kidney to the base, or trigone, of the bladder in females and first part of the urethra in males. They are multilayered and flatten at the surface. They are 4 – 6 times the size of a red cell, spherical or polyhedral with a larger round or oval nucleus. The nucleus is located in the center or slightly off center. Transitional cells easily take on water, and may appear swollen. Stained cytocentrifuge preparations are helpful if identification is difficult.

Image 2: Transitional Cell

There were several transitional cells (arrowed) seen in this voided urine from a patient after cystoscopy. Source: Contributed by David Blomberg, MD. Used in the CAP 2007 CM-A Survey.

Transitional cells also serve as a protective barrier. They differ from squamous cells in that their plasma membranes fold in a mosaic-like fashion allowing for expansion and contraction of the urinary bladder (Image 3 on the following page).
CM-B 2007: Epithelial Cells

Image 3: Squamous Cell/Transitional Cell Split Image

Image on the left shows a squamous cell; image on the right shows a transitional cell.


Very small numbers have no clinical significance. Large clusters of sheets are seen after bladder catheterization or washing. If there are large numbers with large irregular nuclei, the pathologist should be consulted as they may represent a malignancy.

Renal Tubular Cells

Renal tubular epithelial cells (or renal epithelial cells, RTEs) are found from the proximal to distal convoluted tubules, and lining the collecting ducts. Commonly, they are elongated and polyhedral with granulated cytoplasm. They are generally 3 – 5 times the size of a red cell. There is usually a single distinct round nucleus, often eccentric. There is often one flattened side. The size of the cells increases in the more distal structures such as the collecting ducts. Again, stained cytocentrifuge preparations are helpful. Small numbers of RTEs are not significant. If more than 15 renal tubular epithelial cells are seen per 10 high power fields, there is likely active renal disease or injury, according to G.B. Schumann. RTEs are the most clinically important of the epithelial cells and are seen in acute tubular necrosis, with drug or metal toxicity, viral infections involving the kidney, and renal transplant rejection. Fever, toxins, inflammation, infections, and neoplasms increase the rate of shedding of RTEs. Images 4, 5, and 6 on the following pages illustrate renal tubular epithelial cells.
Image 4: Renal Tubular Epithelial Cell

Arrows point to a renal tubular epithelial cell.

Image 5: Renal Tubular Epithelial Cells

Arrows point to a clump of tubular epithelial cells.
Image 6: Renal Tubular Epithelial Cells

There were approximately 10 RTEs per 10 high power fields in this patient with acute tubular necrosis. 
*Source: Images 4, 5, and 6 contributed by David Blomberg, MD.*

RTEs are involved in the formation of urine as it flows through the nephrons. The proximal tubules are lined by RTEs with prominent brush borders (microvilli) and may appear striated under the microscope. The brush border becomes less prominent in the more distal RTEs. RTEs absorb and secrete, and play a role in concentrating and forming urine.

*Oval Fat Bodies*

In the condition known as lipiduria, fat is found in the urine. This occurs in the nephrotic syndrome, advanced diabetes, lupus, and ethylene glycol or mercury poisoning. Oval fat bodies are renal tubular epithelial cells filled with fat droplets. These are very refractile droplets that vary in size. The fat can be stained with Sudan or oil red 0. Cholesterol esters are visible as a Maltese cross pattern with polarized light (Image 7 on the following page).
Positive for oval fat bodies.

Source: Contributed by David Blomberg, MD. Used in the CAP 2004 CM-C Survey.

Hemosiderin

RTEs may also contain hemosiderin (Image 8 on the following page). This is a form of iron, which is seen in the urine 2 – 3 days after acute hemolysis. Conditions such as paroxysmal nocturnal hemoglobinuria and other causes of intravascular hemolysis would result in hemosiderin granule formation. The granules are coarse, yellow-brown, and similar to amorphous urates. The granules stain blue with Prussian blue. A wet staining procedure, the Rous test, may be used, or a cytocentrifuge preparation may be stained.
Positive for hemosiderin

*Source: Contributed by Robert W. Novak, MD.*

Viral inclusions may be seen in rubella, herpes, and cytomegalovirus infections.

**Summary**

The epithelial cells lining the urinary tract are shed into the urine. Evaluation of these cells is an important part of the microscopic examination of urinary sediment. Squamous cells are not clinically significant, but transitional cells in large numbers should be evaluated for uniformity and normal nuclear features. If abnormal, they may indicate a malignancy. Renal tubular epithelial cells in increased numbers are always clinically significant. Additional abnormalities such as lipid or hemosiderin inclusions should be evaluated. The information gathered from an expert urine sediment examination is essential for accurate diagnosis.
References

Roberta L. Zimmerman, MD, FCAP: Roberta L. Zimmerman, MD, is the Clinical Laboratory Director and Pathologist for Grand Itasca Clinic and Hospital in Grand Rapids, Minnesota. She also serves as Laboratory Director and consulting pathologist for three Northern Minnesota hospitals, provides coroner’s autopsies for Itasca and Koochiching Counties and is a member of the Hematology and Clinical Microscopy Resource Committee for the CAP. She has a special interest in clinical pathology, urinalysis in particular.
Learning Objectives:
1. Describe the tests that are usually performed in the clinical laboratory for the immediate evaluation of a patient with possible acute bacterial meningitis.
2. Indicate how positive and negative likelihood ratios are calculated and how these likelihood ratios can be combined to support or refute a diagnosis using results from a battery of tests.
3. Discuss the process of developing prediction rules and how these can be used to provide a rationale for clinical management of patients based on initial laboratory data.

INTRODUCTION

Acute bacterial meningitis is a medical emergency requiring prompt diagnosis and rapid initiation of appropriate therapy. Outcome is clearly improved by prompt and appropriate therapy but acute bacterial meningitis is still associated with significant mortality and morbidity.\(^1\) There are many aspects of the diagnosis and management of acute bacterial meningitis that are undergoing intensive evaluation and change. The introduction of conjugate vaccines for the classic causes of bacterial meningitis (\textit{Haemophilus influenzae}, \textit{Streptococcus pneumoniae} and \textit{Neisseria meningitidis}) is changing the epidemiology of bacterial meningitis.\(^1\) The need for neuroimaging studies prior to the performance of a lumbar puncture and the value of steroid therapy in conjunction with antibiotic therapy are both the subject of debate and guideline development.\(^2\) The changing susceptibility of organisms, especially \textit{Streptococcus pneumoniae}, has lead to a change in initial empiric antibiotic therapy and the requirements for evaluation isolates in the microbiology laboratory.\(^2\) These clinical aspects are beyond the scope of this presentation; the interested laboratorian is referred to the articles listed in the references. The purpose of this education activity is to discuss the initial laboratory testing of blood and spinal fluid in patients suspected of bacterial meningitis and how analysis of these test’s performance characteristics can aid in making or excluding a presumptive diagnosis (culture results are required for a definitive diagnosis) of acute bacterial meningitis.

Clinical Case Study A

Ms. Bactimen, a 40-year-old woman presents to the emergency department with a 72-hour history of high fever, lethargy, and for the past 24 hours, confusion. She has been previously healthy and denies any medications. Physical examination shows a stiff neck and discomfort with straight leg-raising. A neurologic examination demonstrates no evidence of a localized lesion and examination of the optic fundus shows a sharp disc with no evidence of increased intracranial pressure. Acute bacterial meningitis is a major concern.
The following laboratory tests on blood and cerebral spinal fluid (CSF) are essential to evaluate the possibility of bacterial meningitis:

- Complete blood cell count
- Blood culture
- CSF cell chamber count
- Cytocentrifuge preparation of CSF stained with Wright stain for differential count
- Cytocentrifuge preparation of CSF for Gram stain
- CSF protein and glucose levels
- CSF testing for bacterial antigen
- CSF culture

All of the tests listed above are useful in evaluating a patient with possible bacterial meningitis. The only test that is absolutely essential to defining bacterial meningitis is the CSF culture. An elevated white count with neutrophilia and immature neutrophils present is certainly often present in patients with meningitis, but in compromised patients (the very young or very old, patients on chemotherapy, or patients with marrow compromise) it may not be found. Elevated white count with neutrophilia is also seen in many conditions other than bacterial meningitis; many are infectious (e.g., sepsis) but many are not (e.g., trauma). Thrombocytopenia is commonly associated with meningococcal meningitis but it can also be present in a number of infectious conditions without evidence of CSF infection. A blood culture is needed to detect bacteremia. Bacteremic patients are at risk for meningitis (via hematogenous seeding) and many patients with meningitis may have bacteremia, but bacteremia is not an essential indicator of meningitis.

Analysis of CSF cell counts, white cell differentials, Gram stains, glucose, and protein are useful in predicting that meningitis will be proven by culture. Several important technical points need to be considered before predictive measures using these determinations can be discussed. As noted with the blood white count, CSF white cell counts in patients who are neutropenic (due to chemotherapy or marrow compromise) are not going to be predictively useful. This reasoning would also apply to a patient who has an abnormal blood glucose value; the ratio of the CSF to serum glucose is much more useful than an absolute value. Traditional chamber counts of CSF are appropriate for defining total red cell count and total white cell count, but Wright stains for differential counts and Gram stains should be performed on slides prepared by cytocentrifugation. Cytocentrifuge preparations provide a superior sampling and are more sensitive and accurate than chamber differentials and drop/smear preparations for Gram stain. Blood contaminated spinal fluid renders cellular and chemical predictors of bacterial meningitis much less useful. Glucose and protein values are no longer reflective of CSF and mathematical corrections of white cell count based on red cell counts are not valid and may lead to erroneous interpretations. CSF antigen tests have only a secondary role to play in predicting bacterial meningitis. They only detect specific bacteria, they appear to provide no added value when the cellular and chemical factors are not predictive of meningitis, and they do not appear to be significantly more sensitive than high quality cytocentrifuge slides prepared for Gram stains.
Predictive values can be developed for test results. Patients with and without a specific condition can undergo laboratory tests of various parameters and the results of this testing can be used to define a sensitivity (patients with positive test results who have the disease/total patients with the disease) and specificity (patients with a normal result who do not have the disease/total patients without the disease). Sensitivity and specificity data can then be combined to produce positive likelihood ratios (+LR, which apply when tests are positive) and negative likelihood ratios (-LR, which applies when tests are negative). Likelihood ratios are defined as follows:

\[ +LR = \frac{\text{Sensitivity}}{1-\text{Specificity}} \]
\[ -LR = \frac{1-\text{Sensitivity}}{\text{Specificity}} \]

The higher a +LR is, the more likely the patient has the condition tested for, the lower the –LR the less likely it becomes. If the results of multiple tests are obtained, the appropriate likelihood ratios can are multiplied together to obtain the overall likelihood for that pattern of results in the condition of interest. An illustrative example can be found below:

Disease X is often diagnosed using tests A, B, and C. The +LR for A = 4; B = 7; and C = 6. The –LR for A = .2, B = .3, and C = .1. The patient results show a positive result for test A and C and a negative result for test B.

What is the overall likelihood ratio for the disease based on the results obtained?

Answer: The overall likelihood ratio is 7.2 \( \{\text{overall LR} = 4(\text{+LR}) \times 0.3(-\text{LR}) \times 6(\text{+LR})\} \).

Based on a review of large studies of laboratory findings in patients with bacterial meningitis, the following likelihood ratios have been established for the common predictive tests above.

<table>
<thead>
<tr>
<th>CSF Test</th>
<th>+ LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF WBC &gt; 500/μL</td>
<td>15</td>
<td>0.30</td>
</tr>
<tr>
<td>CSF/Blood glucose ratio &lt; 0.4</td>
<td>18</td>
<td>0.31</td>
</tr>
<tr>
<td>CSF protein &gt; 45 mg/dL</td>
<td>1.1</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Clinical Case Study A, cont’d
Ms. Bactimen has a CSF white cell count of 637/μL, a CSF/Blood glucose ratio of 0.21 and a CSF protein of 43 mg/dL.

Based on these results has there been an increase in likelihood that the patient has bacterial meningitis?

Answer: The patient’s results indicate an increased likelihood of bacterial meningitis. The +LR is used for CSF white cell count and CSF/Blood glucose and the –LR for CSF protein. The result is:

Overall LR = 15 x 18 x 0.9 = 243

If one has a good idea of what the initial clinical probability for the condition is, (e.g., 20% of patients who present with Ms. Bactimen’s clinical findings are proven to have bacterial meningitis) then the nomogram provided below (Figure 1.), which uses the clinical probability and the overall likelihood ratio of the testing can be used to arrive at the probability of the condition after testing.10 In this case, using the nomogram, the post testing probability of meningitis goes to >85%.

Figure 1. Nomogram Depicting the Probability of a Condition After Testing.
CSF levels of lactate may also be of value in the diagnosis of bacterial meningitis. Studies of CSF lactate indicate that using a CSF lactate level of $>27$ mg/dL ($>3$ mmol/L) has a sensitivity of 60% and a specificity of 80%.

Estimate the $+LR$ and $-LR$ of a CSF lactate. Might lactate be a more useful test than CSF protein?

Answer: The $+LR = 0.6/1-0.8 = 3$ and the $-LR = 1-0.6/0.8 = 0.5$. It does appear that based on the magnitude of the likelihood ratios that CSF lactate might be a more useful test (also more difficult) to include in the predictive tests.

Clinical Case Study B

A 3-month-old male infant presents to the emergency department with high fever and irritability. On examination, there is no evidence of a localized infection (e.g., otitis, pneumonia) and no rash. His neurologic examination is normal and there is no history of seizures. No one else in the family is ill. An evaluation is ordered that includes a complete blood count, urinalysis and urine culture, blood culture, and a spinal tap to obtain cerebrospinal fluid for culture and Gram stain, cell count and differential, protein, and glucose.

Is there a way, using the data available while the child is still in the emergency department, to determine if the child is low risk for bacterial meningitis?

Answer: The question asks if there is a validated prediction rule that can be used to categorize the patient’s risk. The development of prediction rules is an important part of evidence-based medicine. Prediction rules are developed using large numbers of individuals evaluated for a particular condition by a standardized approach. A proportion of the population is used to develop the rule and then the rest of the population is used to validate the rule that is developed. Some rules attempt to be quite precise and involve complex calculations. For this patient a mathematical rule developed by Hoen\textsuperscript{11} would say:

Probability of Bacterial Meningitis $= 1/(1+e^L)$
Where $L = (0.52 \times \#$ of months since August 1) – (12.76 x CSF/blood glucose ratio) x (CSF Neutrophils x $10^6/L)^{0.333} – (2.71 \times$ age) + 7.79.
This type of analysis obviously requires a computer program to do the calculation, but if fully validated, it would be useful in making medical decisions. Many prediction rules are criterion based and involve arriving at a score that then provides the clinician with guidance. A prediction rule that has been validated by the Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics states that in a child (1 month to 19 years) who has CSF pleocytosis (>10 cells/μL) and has not had antibiotics, the child can be considered very low risk for bacterial meningitis if **ALL** of the following are present:

- CSF Gram stain is negative
- CSF absolute neutrophil count is <1000 cells/μL
- CSF protein is <80 mg/dL
- Blood absolute neutrophil count is <10,000 cells/μL
- There is no history of a seizure before or at the time of presentation

This type of approach can be applied in a checklist fashion to provide clinical guidance and clearly relies heavily on laboratory evaluation.
References

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