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PRESENTER: Inherited Disorders of the Urea Cycle

Slide 1:

Hello, my name is <**Van Leung Pineda**>. I am the <**Section Director for Clinical Chemistry and POC at Children's Healthcare of Atlanta and adjunct Faculty at Emory University School of Medicine**>. Welcome to this Pearl of Laboratory Medicine on "**Inherited Disorders of the Urea Cycle**."

Slide 2: The Urea Cycle

The Urea cycle primarily occurs in the liver and is important for removal of nitrogenous waste. The role of the cycle is to convert nitrogenous waste from the toxic form of ammonia to urea, which can be readily excreted in the urine. The source of ammonia in the liver for the urea cycle comes from primarily nitrogen containing amino acids glutamine and alanine, which tend to be elevated in patients with urea cycle defects. The urea cycle is a source of arginine production as well as production of the non-proteinogenic amino acid citrulline and ornithine. The cycle depends on enzymes and amino acid transporters and takes place in both the cytosolic and mitochondrial compartments of the cell.

Slide 3: The Urea Cycle: Enzymes & Transporters

The cycle starts in the mitochondria when glutamate and acetyl-CoA are converted to N-acetylglutamate by the n-acetylglutamate synthase enzyme, or NAGS for short and labelled number 1. N-acetylglutamate activates the enzyme carbamoyl-phosphate synthase (or CPS), number 2. CPS in turn catalyzes the conversion of ammonia, ATP and bicarbonate to

carbamoyl phosphate. Carbamoyl phosphate can be converted to orotic acid. Orotic acid is an important diagnostic marker for the distal urea cycle defects. Alternatively, ornithine transcarbamoylase (OTC), third step, catalyzes the transfer of the phosphate group from carbamoyl phosphate to ornithine to produce citrulline. Citrulline then enters the cytosol from the mitochondria. In the cytosol, citrulline combines with aspartate, which is shuttled from the mitochondria by the transporter citrin, and with the action of the enzyme arginosuccinate synthase, in the fourth step, arginosuccinate is made. The next step, number 5, is the cleavage of arginosuccinate to arginine and fumarate by the enzyme argininosuccinate lyase. Arginine is then hydrolyzed into ornithine and urea by arginase in the sixth and final step. Urea is then excreted, and ornithine is transported back to the mitochondria by the amino acid transporter ORNT1 to start another cycle. Urea cycle disorders arise from defects in the 6 catalytic enzymes indicated in blue boxes or the two transporters: citrin and ORNT1.

Slide 4: Disorders of the Urea Cycle

The incidence of Urea Cycle Disorders, or UCDs, in the US is estimated to be 1 in 8200 births. The calculated overall average birth prevalence of UCDs is approximated to be 1 in 35,000, with two-thirds having symptoms in the neonatal period. The mortality rate is 24% in neonatal cases, and 11% in later onset cases. Most mutations that cause UCDs are inherited in an autosomal recessive pattern. Therefore, the risk of recurrence is 25% for parents of an affected patient. The exception is ornithine trascarbamoylase or OTC deficiency, enzyme 3 in the previous slide, which is X-linked inherited; and where about 15% of female carriers can develop symptoms that require medical intervention.

Slide 5: Disorders of the Urea Cycle II

The key feature of UCDs' clinical presentation is increased ammonia. However, the absence of hyperammonemia should not rule out the suspicion of UCDs. Mild hyperammonemia can be seen with citrullinemia type II and arginase deficiency. Hyperammonemia can be triggered by metabolic stress, such as a protein load or infection induced catabolism. The majority of cases present in the neonatal period, but some will manifest symptoms later, even into adulthood. This heterogeneity is dictated by the severity of the mutation, some cases may retain partial enzymatic function. Median age of presentation for patients outside of the newborn stage is 2 years; however about 20% of cases presented over 12 years old.

Slide 6: Disorders of the Urea Cycle III

This slide lists the individual UCDs. I have bolded here the short terms for each disease, as they are a mouthful to pronounce. The ones in black are enzymatic deficiencies and are considered the core UCDs, and the ones in blue are amino acid transporter defects. The affected genes are shown next to each deficiency in italics. For example, in number 2, the disorder where the enzyme Carbamoyl Phosphate Synthase is affected will be referred to as CPS, and is caused by mutations in the *CPS*1 gene. Similarly, in number 3 OTC is a deficiency in the Ornithine Transcarbamoylase enzyme due to mutations in the *OTC* gene, and so on. The other disorders are NAGS, citrullinemias I and II, ASA, Arginase deficiency and HHH syndrome.

Slide 7: The Urea Cycle Disorders

This slide is a graphical representation of what steps are affected in the individual disorders. For example, at number 2, CPS deficiency affects the conversion of ammonia and N-acetylglutamate to carbamoyl phosphate here on the lower left of the slide, and ASA, number 5 on the upper right affects the conversion of arginosuccinate to arginine. The main effect seen is usually an accumulation of the substrate and/or a decrease of the product for the affected step in the cycle.

Slide 8: Symptoms and Presentation

Common symptoms for UCDs will reflect the toxic accumulation of glutamine and ammonia. Symptoms can be divided into neurological and gastrointestinal. Symptoms are most severe in the newborn period. Patients are born healthy but become ill in a short time, usually after feeding. Signs can start as lethargy, irritability, difficulty maintaining normal temperature, poor feeding and vomiting. This presentation can often be confused with sepsis. It is common in this period to have hyperventilation induced respiratory alkalosis. This is caused by cerebral edema due to ammonia accumulation. Patient status can deteriorate rapidly. Infant onset cases are less acute and more irregular. Symptoms include cyclical vomiting, lethargy, anorexia, developmental delay and failure to thrive. There can be behavioral problems as well. Enlarged livers due to deficient arginine can be encountered. These symptoms can often be confused with milk protein intolerance or reflux. In later onset, presentations are more chronic and episodic. Illness tends to occur after protein ingestion or a stressor due to a catabolic state such as infection. Due to the postprandial symptoms, patients tend to prefer a vegetarian low protein diet. These patients can be a challenge to diagnose, because if the episodes are missed, test results may show up normal. Later stage patients usually have partial enzymatic deficiencies, for example female OTC carriers.

Slide 9: Symptoms and Presentation II

The individual disorders have some characteristic symptoms and signs. Patients with arginase deficiency present in later infancy to preschool years with delayed physical and intellectual growth. They can also have severe spasticity affecting the lower extremities. HHH syndrome patients have universal delays in physical and intellectual development. Children with citrullinemia II can have cholestasis and fibrosis, developmental delay, hypoproteinemia and hypoglycemia. Citrullinemia type II adults can have regular episodes of elevated ammonia and psychiatric symptoms. In general, acute encephalopathic events can occur at any time, and can be the first telltale sign of an UCD, especially for those with late onset disease.

Slide 10: Laboratory Tests

For laboratory results, elevated ammonia, greater than 100 to 150 micromole/L, is a hallmark of UCDs. Hyperammonemia in the context of normoglycemia and normal anion gap is a strong indicator of UCDs. The biochemical genetic tests of blood amino acids and urine organic acids can be important in differentiating which specific UCD is present. The turnaround time can also be faster than molecular tests. The biochemical genetic tests are first line along with ammonia and can distinguish if the hyperammonemia etiology is UCDs or other genetic cause, such as certain organic acidemias or fatty acid oxidation defects. Lactic acid can help rule out disorders of pyruvate metabolism. DNA tests can serve as confirmation. However, due to their longer turnaround times, treatment should not wait for their results. For DNA testing, knowledge of the method limitations is important. For example, some next generation sequencing tests may miss exon deletions, so a negative result should be followed up with another test, if UCDs are still suspected. The most severe UCDs are usually diagnosed before newborn screening results are available, and not all UCDs can be detected. ASA and Citrullinemia I are part of the screen in all 50 states in the U.S, by detecting elevated concentrations of arginosuccinic acid and citrulline. OTC, CPS1, Citrullinemia II, Arginase deficiency and HHH syndrome are part of some states panels, but not all.

Slide 11: Ammonia Testing

Due to ammonia's key role in considering UCDs, I would like to cover some points that can affect result interpretation. Specimen collection timing is important with episodic cases, because if the window is missed, so can the diagnosis. Heparinized plasma from stasis free veins is the preferred collection, but arterial is acceptable. Delay in processing can lead to false elevations of ammonia due to red blood cell degradation of amino acids. The sample should be placed on ice and sent to the laboratory immediately, as icing slows this degradation. The plasma is to be separated from cells and tested within 20 to 30 minutes. If unable to analyze promptly, freezing can stabilize the analyte. Rejection criteria include hemolyzed, lipemic or gross icteric specimens, room temperature or delayed specimens as these can all cause interferences and false elevations. Reporting units can be misinterpreted. This is key in cases when the patient

has been transferred from another facility. We have experienced cases where the patient's initial ammonia was performed at a different laboratory with different reporting units that were overlooked and caused confusion and delayed care.

Slide 12: Example: OTC deficiency

Abnormal results can be explained by the defects encountered in each of the specific disorders. To illustrate this I have 2 examples. First, in the most common UCD, OTC deficiency, the enzyme that mediates ornithine conversion to citrulline is deficient, therefore an accumulation of the OTC substrates ammonia and glutamate is observed, as are alanine and glutamine. Orotic acid is increased due to the backup, whereas citrulline, arginine and urea are decreased. As the defect is early in the cycle, ammonia concentrations are higher than in later defects in the cycle.
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Slide 13: Example: Arginase deficiency

In the second example, arginase deficiency, the conversion of arginine to ornithine and urea is affected. Results would show a large increase in arginine concentration and decreased urea. Because the enzymatic defect is later in the cycle, ammonia can actually be normal, or elevated but not to the concentrations seen in earlier defects of the cycle like CPS deficiency.

Slide 14: Laboratory Abnormalities

In this table, I have summarized some laboratory abnormalities seen in the specific disorders for reference. The first column identifies the specific disorder. The second column indicates expected results for ammonia, which is not elevated in all disorders. Ammonia concentrations can vary depending on the disorder and the timing of collection. In the third column, are expected results for urea, there is a decrease in the majority, as it is the end product. The fourth column indicates amino acid results that can help distinguish one disease from another. For example, arginase deficiency will show a large increase in arginine concentration. The last column shows the expected results for organic acids with specific emphasis on the concentrations of orotic acid. Decreases in orotic acid are difficult to detect due to the limit in the analytical sensitivity of the methods.

Slide 15: Treatment

Treatment can be acute or extended. Treatment is initiated as soon as a UCD is suspected and is concurrent with the diagnostic process. The primary goal is to reduce ammonia concentrations. Acute management consists of: 1) removing ammonia by pharmacologic scavenging and/or hemodialysis 2) reversing the catabolic step by caloric supplementation and nitrogen restriction and 3) reducing the risk of neurologic damage. Fluid management can

provide caloric supplementation re-establish hydration and urine output, but has to be monitored closely to prevent worsening cerebral edema. Except in arginase deficiency, arginine can be supplied to prevent catabolism and to push the urea cycle forward. Pharmacologic scavenging of ammonia is usually accomplished by using sodium phenylacetate and sodium benzoate, aka Ammonul, which activates an alternative pathway for nitrogen excretion. Overall prognosis depends on the patient's age, severity of mutation and the patient's condition at diagnosis. Prompt diagnosis and treatment can influence the extent of neurological damage, especially in the neonatal stage, which have the worst prognosis even with survival.

Slide 16: Treatment II

For extended management the goal is to prevent hyperammonemic episodes by a controlled diet. Nutritional control is assessed for adequate intake. Diet management is a dynamic process throughout the life of the patient. Specific treatment differs depending on the specific disorder, which can include liver transplantation. Laboratory tests are important for the initial workup and for monitoring therapy. Protein catabolism due to viral illness is a high risk in infants, so measures to avoid this should be taken.

Slide 17: Summary

To summarize, the urea cycle is the metabolic detoxification pathway that eliminates ammonia. Inherited mutations can result in enzymatic or amino acid transport deficiencies that cause UCDs. Ammonia testing has limitations that can affect test interpretation, and biochemical genetic tests play an important role in the diagnosis of the specific disorder as well as in monitoring treatment.

Slide 18: References

Here are my selected references

Slide 19: Disclosures

And my disclosures

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Inherited Disorders of the Urea Cycle**."