Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
www.traineecouncil.org

TITLE: Hepatitis E

PRESENTER: Meredith Pittman, MD

Slide 1:
Hello, my name is Meredith Pittman. I am a Pathology resident at Washington University in St. Louis. Welcome to this Pearl of Laboratory Medicine on "Hepatitis E."

Slide 2:
Hepatitis E was first recognized as a new virus during outbreaks in India during the 1950s. Healthcare workers and epidemiologists called this new virus "Enterically-transmitted, non-A, non-B Hepatitis." The seasonality of the outbreaks, occurring more often during monsoons, provided a clue that contaminated water was a major source of disease transmission. It wasn't until 1983 that a researcher was able to isolate viral particles from stool, and then in 1990, the virus was successfully cloned and sequenced.

Hepatitis E virus (HEV) has a unique genomic structure making it the only member of the family *Hepeviridae*, and the only member of the genus *Hepevirus*. It is a small, non-enveloped positive sense RNA virus that targets hepatocytes and replicates in their cytoplasm. The RNA genome has 3 overlapping open reading frames.

Slide 3:
Open reading frame 1 (ORF1) encodes structural proteins required for viral replication and protein processing, including an RNA helicase and an RNA polymerase.

Next in sequence is ORF3, which encodes a small protein that has significant variability between strains, and its specific function is unknown. *In vitro* studies suggest that it is important in promoting cell survival. The protein is expressed on the virion surface, so now it is being incorporated into serologic assays for hepatitis E.

Finally, ORF2 encodes the viral capsid which initially is synthesized as a 660 amino acid monomer that goes on to dimerize and then form units of 10. These decamers are thought to be the antigen that stimulates our immune system when exposed to the virus, and, importantly for us in the lab, a 'deactivated' version of the capsid still forms the basis of our serologic tests.

Slide 4:
There are 4 mammalian genotypes of Hepatitis E, Genotypes 1 through 4. Interestingly, they all represent the same serotype, making detection somewhat simpler. You can really break down the Hepatitis E infection into 2 patterns that split along geographic and genotypic lines.

Let's start with Genotypes 1 and 2. These genotypes are human viruses that are endemic to parts of Africa, Asia, and Central and South America. In fact, in some of these regions, Hepatitis E represents the majority of acute hepatitis, more than even Hepatitis A. As previously mentioned, ingestion of contaminated water is the primary source of viral transmission. Hepatitis E is a special concern for pregnant women, who are 8 times more likely to become infected than age-matched controls, and up to 50% of infected pregnant women go on to develop fulminant hepatic failure.

Genotypes 3 and 4 are quite a bit different. These are zoonotic viruses found in swine and game in the United States, Western Europe, and parts of China and Japan. Humans become accidental hosts when ingesting the virus from undercooked meat, primarily. The prevalence varies by region, and the incidence is difficult to study as the human infection may be subclinical and often appropriate testing is not performed. Importantly, pregnant patients do not appear to be at increased risk of infection or hepatic failure with these genotypes.

Interestingly, not all patients who have a previous infection with Hepatitis E will remain immune, meaning that antibodies can be undetectable, and patients can become re-infected.

Slide 5:
So how do we test for Hepatitis E? To answer that question, let's first look at the timeline of illness. Exposure with infection occurs about 2 weeks before any clinical signs become apparent. Although a patient's ALT and AST may begin to rise at 2 weeks, it may be 6 weeks before the patient feels "sick." Antibodies to HEV, both IgG and IgM, start to rise together at 2 weeks, peaking about the time patients are feeling ill. IgM should disappear by 3 months, while IgG will persist for months to years. HEV viremia generally peaks early and may be undetectable by the time a patient presents to the doctor.

Slide 6:
With current methods, we can test blood using an enzyme immunoassay to detect IgG and IgM and nucleic acid amplification testing to detect HEV RNA. Remember that IgM positive results have a high frequency of being false positive. Best practice is to test for IgG and IgM simultaneously. Both antibodies should be positive in a true infection.

Slide 7:
Why do we even need to test for Hepatitis E, especially in non-endemic areas where infections are predominantly Genotypes 3 and 4? One population where the diagnosis is critical is in immunosuppressed patients. Solid-organ transplant recipients and patients with AIDS can develop chronic Hepatitis E. As with other forms of chronic hepatitis, Hepatitis E can progress to cirrhosis in these patients. Additionally, chronic infection has implications for graft survival in liver transplant patients. Once diagnosed, decreasing a patient's level of immune suppression may allow for clearance of the virus with possible prevention of further liver damage.

Another area where recognition is important is in patients who may otherwise be diagnosed with drug-induced liver injury (DILI). Although drug-induced liver injury is one of the major causes of acute liver failure, and also one of the main reasons some drugs are not brought to market, the diagnosis is one of exclusion. If diagnosed incorrectly, patients may be taken off of drugs that they need. Currently, ruling out hepatitis E is not part of the algorithm for diagnosing drug-induced liver injury, but recent reports recommend all patients be tested for Hep E before a diagnosis of DILI is given.

Slide 8:
As mentioned, enzyme immunoassays can be used to test for antibodies to Hepatitis E in the blood. The basis for that testing comes from early studies showing that there was cross-reactivity among HEV virions from different geographic regions, and therefore genotypes.

It has also been shown that recombinant peptides from both ORF2 and ORF3 from a Mexican strain of HEV were able to react with sera collected from outbreaks in Pakistan, Russia, and Somalia, so again, different genotypes.

These findings provided evidence for common epitopes, facilitating the development of immunoassays that broadly react with antibodies to different HEV strains.

Slide 9:
In the United States, ELISA is the only method clinically available for Hepatitis E diagnosis. There are two major issues with the available testing. One is a lack of standardization, and another is inherent difficulty when testing for a disease in a low prevalence setting.

Slide 10:
First of all, let's talk about lack of standardization. As of right now, none of the available immunoassays are FDA-approved, meaning that laboratories use a mixture of commercially-produced and lab-developed assays. This has huge implications for inter-laboratory reproducibility of results, either clinically or in a study setting, as there is little or no standardization. Labs may use different antigens in their assays, different positive cut-off points, etc.

Slide 11:
A recent study from the CDC comparing 5 immunoassays illustrates the wide variation in sensitivities and specificities of ELISA testing for IgM when testing serum from patients with known hepatitis E versus patients with Hepatitis A, B, or C. As you can see, sensitivities ranged from 72-98%, and specificities from 78-95%, showing that reproducibility of this testing has not been optimized.

Just for argument's sake, let's assume that your lab uses the best test available, which would be test number 5 on this slide, with 98% sensitivity and 95% specificity, as we move on to our next problem, which is low prevalence.

Slide 12:
Being generous, and assuming that 10% of your acute hepatitis cases are actually because of Hepatitis E, your very sensitive and specific assay has only a 68% positive predictive value. Still, the negative predictive value of 99% can be very helpful to your clinician, especially in some of the situations we've previously discussed, such as diagnosing drug-induced liver injury.

Slide 13:
Finally, PCR-based methods can be used to look for the presence of HEV RNA in the blood and sometimes in stool. Sensitivities and specificities of these tests also vary, but they are thought to be the test of choice in immunocompromised individuals who may not mount an appropriate antibody response, or who may need monitoring for viral clearance during a chronic hepatitis E infection. Many immunocompetent patients will no longer be viremic by the time of presentation, so concurrent IgG and IgM testing are an important part of the diagnostic algorithm.

Slide 14:
In summary, Hepatitis E is an important cause of acute hepatitis worldwide, with millions of cases each year. In the United States and Western Europe, infection with genotype 3 predominates. Immunocompromised patients may progress to chronic hepatitis E which can lead to long-term liver damage. In immunocompetent patients, Hepatitis E is ideally ruled out before a diagnosis of drug-induced liver injury is made. Current clinical testing in the US is by immunoassay only, which needs improved standardization. Because of false positives with IgM, the IgG /IgM antibodies should always be ordered as a pair. And finally, PCR testing, when available, may be useful in your immunocompromised patient population.

Slide 15: References

Slide 16: Disclosures

Slide 17: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on "Hepatitis E." I am Meredith Pittman.