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TITLE: Hepatitis C Laboratory Testing

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Slide 1:

Hello, my name is Anh Dinh, I am one of the Clinical Pathology Chief Residents at Beth Israel Deaconess Medical Center and Harvard Medical School. Welcome to this Pearl of Laboratory Medicine on "Hepatitis C Virus Laboratory Testing."

Slide 2: Hepatitis C Virus Laboratory Testing - Outline

It is estimated that 2.7 to 3.9 million people in the US are living with Hepatitis C Virus, or HCV, most of whom are unaware that they are infected. HCV-related liver disease is the most common cause of liver transplants, and every year, more people die of HCV than the next 60 infections reportable to the CDC combined, including HIV, TB and hepatitis B. With new antiviral treatment most of these deaths are preventable, so it is imperative to diagnose patients and link them into care. Today, I would like to focus on the evolution of HCV laboratory testing: beginning first with the natural history of infection, discuss the clinical manifestations, the laboratory testing, and the incidence and prevalence of HCV, which has served as a framework for the CDC's recommendations for baby boomer screening.

Slide 3: Hepatitis C Virus (HCV)

HCV is a positive strand RNA virus with a genome of 9600 nucleotides which encodes a polyprotein that's cleaved into structural and nonstructural proteins, the latter of which are the targets for antiviral therapies. The RNA polymerase lacks proofreading activity, which results in millions of viruses with minor genetic changes that arise in each infected patient. At least six major genotypes exist, based on major differences in genetic structure. This genetic diversity,

therefore, increases the likelihood for chronicity, and it makes it very difficult to develop a vaccine. However, unlike HIV or HBV, HCV does not have a stable latent DNA intermediate that resides in the nucleus. It requires continuous replication in order to be maintained and sustained, which means that in theory, antiviral agents that target the replication have the ability to eradicate the virus.

Slide 4: Genome and Therapeutic Targets

Genotype 1 is the most common in the US, representing approximately 70% of cases. Here, I've shown a diagram of the genome with regions which code for non-structural proteins involved in viral replication. These proteins are the targets of the different classes of direct-acting antiviral agents: non-structural proteins 3/4A (NS3/4A) protease inhibitors, non-structural protein 5B (NS5B) polymerase inhibitors (which include nucleoside and non-nucleoside), and nonstructural proteins 5A (NS5A) inhibitors.

Slide 5: Serologic Pattern: Acute, Resolved Infection

In an acute HCV infection, liver enzymes may become elevated, as depicted here in blue, where the enzyme alanine aminotransferase (ALT) is released into the circulation as the virus damages the hepatocytes of the liver. When symptoms present, they typically appear and persist during the time when liver enzymes are elevated, which is typically 6 weeks after infection. However, most individuals do not demonstrate noticeable symptoms and are unaware of the infection unless timely laboratory testing reveals elevated transaminases. Persons infected will have detectable antibodies to HCV, or anti-HCV shown in pink, approximately 2-3 months after exposure. Virtually all infected persons will have detectable antibodies by 6 months following exposure and these antibodies will remain throughout their lifetime. HCV RNA, indicated in yellow, is detectable even before the appearance of anti-HCV antibodies, but disappears in patients who spontaneously clear the infection. Once infected with HCV, 15-25% of patients will recover from the acute infection without long-term sequelae.

Slide 6: Serologic Pattern: Chronic Infection

Unfortunately, 75%–85% of patients are unable to clear the virus on their own and it progresses to chronic infection. In these patients, the HCV RNA persists as the virus continues to replicate.

With the continued viral replication and resulting damage to the liver, serum ALT levels will fluctuate over time, as indicated again in blue.

Slide 7: Clinical Manifestations of Chronic Infection

In chronic infections, disease progression is typically silent while damage to the liver progresses. Over a 20 to 40 year period, 15 to 30% of patients develop cirrhosis, which may result in portal hypertension, esophageal varices, ascites, and/or hepatic encephalopathy. Patients with cirrhosis also have a 1-3% annual risk of developing hepatocellular carcinoma. HCV infection also has a multitude of extrahepatic manifestations, including, but not limited to, mixed cryoglobulinemia, which can result in vasculitis, purpura, and renal disease; autoimmune disease, including thyroid disease, Immune thrombocytopenic purpura, and autoimmune hemolytic anemia; insulin resistance and diabetes, porphyria cutanea tarda, and more recent studies show a possible association with Parkinson's disease.

Slide 8: Laboratory Testing

Laboratory testing for HCV begins first with a screening immunoassay for anti-HCV antibodies. Currently in the US, there are enzyme, chemiluminescence, or Point of Care immunoassays that are FDA-approved. When the screening test is positive, confirmatory testing is required to definitively diagnose an individual with HCV: previously with a recombinant immunoblot assay (RIBA) testing for added specificity of the anti-HCV antibody, and currently by molecular tests for HCV RNA, which determine patients with active viral infection.

For many of the available antiviral therapies, testing for HCV genotype and genotype subtype has become essential in the selection of the appropriate regimens, dosing, and duration of treatment.

Slide 9: Testing Algorithm (2003)

In 2003, the CDC outlined a testing algorithm, whereby if an antibody screen was positive, the specificity of this reactivity could be determined by performing the RIBA to help identify patients with non-specific reactivity. This algorithm was only designed to determine whether the antibody screen was a true positive or a biologic false positive, because antibody results with low signals, were, by and large, false positives. It was not designed to determine whether the patient had an ongoing infection (we can see that not all the arms pass through the HCV RNA).

Slide 10: Testing Algorithm (2013)

Now looking to the testing algorithm developed in 2013, the CDC made a number of changes to focus on identifying individuals with current infections. As more effective HCV treatments were developed, the focus shifted away from general identification of individuals exposed to HCV, to specifically diagnosing those who had an ongoing, treatable infection. At this point, the RIBA was discontinued, and it had been replaced with the molecular HCV RNA viral load determination. Only in individuals who had a potential infection with the previous 6 months, is a repeat antibody screen or HCV RNA viral load recommended.

While this algorithm is much simpler, it has a few limitations. First, patients with a positive antibody but a negative viral load have either non-specific reactivity on the screening assay or have a past resolved infection. With the RIBA test no longer available, these two entities cannot be distinguished. In this setting, the CDC recommends using a second manufacturer's HCV antibody assay to identify biologic false positive HCV antibody results. The algorithm is also not intended for diagnosis of an acute infection or in immunocompromised patients, where only HCV RNA may be detected and a repeat antibody screen or HCV RNA viral load is recommended. Identifying these individuals poses a difficult task for clinical care providers.

Slide 11: Incidence of HCV Infection in the US

This graph illustrates the estimated number of new HCV infections in the United States, per year. We can see that the incidence in the 1980s was about 200 to 300,000 cases per year. Before HCV was discovered, it was known as non-A, non-B hepatitis, a bloodborne virus that was common among transfusion recipients and persons who injected drugs. As a result, prevention strategies were introduced, such as indirect blood screening through surrogate markers. After the discovery of HCV in 1989, the development of serologic tests led to the improvement of blood safety. The incidence of HCV decreased with additional measures to improve patient safety, including the needlestick safety and prevention act in 2001.

Slide 12: Worldwide Prevalence of HCV (2005)

Based on the 2003 to 2010 National Health and Nutrition Examination Survey (NHANES), the incidence of HCV had dropped over the last couple of decades. However, of the estimated 3% or 130-170 million of the world's population infected with HCV, Egypt is considered to have the highest prevalence of HCV, with approximately 10% of people chronically infected. For

comparison, an estimated 1% or ~2.7 million people are infected in the US. This survey also estimated that a large percentage of people remain undiagnosed, where anywhere from 45-85% of individuals are unaware of their infection. These estimates are based on the NHANES national survey in which a representative sample of the civilian, non-institutionalized population was tested for antibodies to HCV, and if positive, HCV RNA viral load. The study excluded populations, such as the homeless or incarcerated, which are known to have an increased prevalence of HCV.

Slide 13: Risk-Based HCV Screening (1998)

The CDC's first attempt to detect infected patients was through risk-based screening, in which patients with a risk for HCV transmission and infection were tested. Risks included past or present injection drug use, having received blood transfusions or solid organ transplants before July 1992 (when screening of blood products began), having received clotting factors before 1987 (prior to viral inactivation methods), chronic hemodialysis, infants of HCV-infected mothers, needlestick injuries in healthcare workers, signs of liver disease (persistently elevated ALT), and HIV infection.

Slide 14: Treatment for HCV (2013 to present)

The goal of antiviral treatment is the achievement of virological cure, termed sustained virologic response or SVR, and defined as undetectable HCV RNA 12 to 24 weeks after treatment. Traditional treatment for HCV consisted of PEG-interferon and ribavirin, which resulted in SVR in approximately 45% of patients with genotype 1 infection and were associated with numerous difficult-to-tolerate adverse effects.

In late 2013, two direct acting antiviral agents were approved that could be used in all-oral regimens. These have been followed by additional all-oral regimens with an 8 to 24-week duration that offer cure rates of over 90% to almost all patient populations including those with HIV coinfection, decompensated cirrhosis, liver transplant, and end-stage kidney disease. Increasingly effective antiviral therapies have been developed which continue to evolve. As of June 28, 2016, the FDA has approved sofosbuvir/veltapasvir for the treatment of all six major genotypes, with clinical trial results showing 95-99% of patients attaining SVR. However, testing for HCV genotype and genotype subtype is still important since this regimen will not be used for genotype 1 infection in the US. Treatment guidelines continue to change with new

developments, and a useful resource for the up-to-date treatment guidance is available through the American Association for the study of Liver Disease (AASLD) and the Infectious Disease Society of America (IDSA).

Slide 15: CDC Birth Cohort Studies

From NHANES data collected from1988 to 1994, it was determined that there was a peak of infection in 30 to 49-year-old patients. Data collected approximately 10 years later showed a similar peak of prevalence in patients who were now ten years older. There appeared to be a fixed group of people with HCV infection that were moving through time. When the prevalence was plotted by year of birth, the peak prevalence was found to be in those people born from 1945 through 1965, a group of people culturally known as “baby boomers.” The incidence of infections observed in the 1960’s through 1980’s among young adults had now persisted as prevalent infection in older adults. In the most recent NHANES data from 2003 to 2010, baby boomers accounted for more than 80% of the total estimated population of chronically HCV-infected adults. The prevalence of HCV RNA positivity among individuals in this birth cohort is estimated at 3.4%, approximately 6-times higher than individuals born in other years and as a result, the CDC recommended HCV screening for this high-prevalence population.

Slide 16: One-time Screening for Baby Boomers

It became clear that a risk-based approach missed these individuals, who may have received blood transfusions before the introduction of screening in 1992 or have a history of other risk factors. Physicians were not asking their 60-year-old patients if they had ever injected drugs, and many of these patients did not or could not identify any risk factors for their infection, risk exposures that happened decades ago, and may not be relevant to the person’s current life circumstances. With a large population infected with HCV, many patients not knowing their status, and the availability of highly effective treatments, the CDC recommended that in addition to testing adults with risk factors for HCV, all adults born between 1945 and 1965 should receive one-time testing for HCV without prior ascertainment of HCV risk factor. This recommendation was released by the CDC in 2012, the United States Preventative Services Task Force in 2013, and the World Health Organization in 2014. Testing linked to care and treatment was anticipated to reduce the risk of HCC by 70%, liver disease by 90% and the risk of all-cause mortality by 50%.

Slide 17: Limitations

A CDC evaluation of HCV testing and reporting in eight US sites, from 2005 to 2011 revealed that of the total persons reported with a positive test for HCV infection across all sites, approximately 50% of HCV antibody-positive patients had no confirmatory HCV viral load performed. The HCV viral load is essential for identifying patients with an active infection, but the test may not be ordered or there may be difficulty obtaining a new patient sample for testing. At our institution, we have identified a streamlined approach to screening baby-boomers as well as reflexing the viral load for individuals with positive antibody screens. The process obviates the need to order the HCV RNA testing and offers a solution for individuals who are often lost to follow-up.

Unfortunately, the new antiviral regimens are expensive, and health systems have raised concerns about affordability. Some clinicians are considering the clinical benefit in relation to the potential harm with delayed treatment. That said, there are many benefits to detecting patients who are infected with HCV. They can be evaluated for the presence of chronic liver disease: if these patients have cirrhosis (and at least 25% of these baby boomers will), we can counsel on alcohol use, check for varices, liver cancer surveillance, vaccinate for hepatitis A or B virus, and advise on over-the-counter medications. The management of a cirrhotic patient, regardless of the cause, is completely different from that of a noncirrhotic patient. Without screening, these patients may never know they're infected because many are asymptomatic.

Slide 18: Summary

In summary, chronic hepatitis C is a major cause of liver disease, leading to cirrhosis, hepatocellular carcinoma, and end-stage liver disease, and ultimately, we would want a preventive vaccine. In the meantime, we've identified a population with a disproportionate prevalence of HCV: individuals born between 1945 and1965. There are many facets to ultimately eliminating a disease, but full implementation of baby boomer screening is a great first step.

Slide 19: References

Slide 20: Acknowledgments

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Hepatitis C Laboratory Testing.”