TITLE: Liver Function Tests

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Slide 1: Title Slide
Hello, my name is Dina Greene. I am an Assistant Professor at the University of Washington in Seattle. Welcome to this Pearl of Laboratory Medicine on "Liver Function Tests."

Slide 2: Primary Liver Functions
The liver is a complex organ with multiple functions related to nutrient breakdown and storage, detoxification, and excretion. Additionally, the liver maintains intimate associations with multiple other vital organs. It is a conduit for the pancreas, GI, and spleen to exchange nutrients, hormones, and other important molecules with the rest of the body. The liver's biochemical functions rely on its supply of oxygen rich blood from the circulatory system and nutrients from the GI system.

Slide 3: The Case
We will review the major liver markers using a case that was published in the August 2009 issue of *Clinical Chemistry*. This case is a 66-year-old female retired schoolteacher who presented with chest pain and shortness of breath.

Slide 4: Patient Summary
She has no past medical history of liver disease, has no obvious abnormalities, is on no prescription medication, the whites of her eyes have no yellowing, and she has a modest amount of daily alcohol intake.

Slide 5: Initial Laboratory Tests
Her lab tests are remarkable for an elevated aspartate amino transferase or AST ~10 times the upper limit of the reference range.

Slide 6: Hepatocyte Damage Releases Intracellular Enzymes
The aminotransferases are very important for detecting acute liver disease. As hepatocytes die due to apoptosis, necrosis, or other mechanisms, the cellular contents are released into the circulation. The aminotransferase enzymes, ALT and AST, are indicators of liver cell death and can be used to answer the questions: What is the extent of hepatic damage or how many liver cells died in the last 24 hours?

Slide 7: Aspartate Aminotransferase (AST)

AST is not specific to the liver and is also found in the kidney and cardiac and skeletal muscle. In general, sex specific reference ranges are applied because the baseline concentration in males is greater than in females. AST can be elevated in a variety of liver and non-liver related conditions. Liver associated conditions include hepatitis, hemochromatosis, Wilson's disease, and non-alcoholic fatty liver disease. The latter is one of the most common reasons for mildly elevated AST.

Slide 8: Quantifying AST

AST is measured in the laboratory using a coupled enzymatic reaction. In this reaction diagram, and in all of the reactions I'll show in this talk, the reaction components colored purple are included in reagents; the components in black are produced in reaction; and pink are from the patient sample.

Slide 9: Quantifying AST

The decrease in Abs @ 340 as NADH is oxidized to NAD+ can be directly correlated to the AST activity in the sample.

Slide 10: Quantifying AST

It's also important to note that AST requires pyridoxal 5 phosphate (P5P or vitamin B6) as a cofactor. Manufacturers have different supplementation practices in their AST assays, so if a patient is vitamin B6 deficient it can lead to false negative AST results in some commercially available assays.

Slide 11:

Her AST was 544, which is ~12x the upper limit of the reference interval. But, if this were liver disease, we would expect additional markers to be elevated.

Slide 12: Alanine Aminotransferase (ALT)

Alanine amino transferase or ALT is also a transaminase and is considered to be the most important test for detecting acute and chronic liver injury. It is not specific for the liver, but is found there at higher concentrations and has a longer half-life relative to AST, leading to higher systemic concentrations for the same amount of liver injury.

Slide 13: Alanine Aminotransferase (ALT)

This patient had an ALT concentration within the normal reference interval.

Slide 14: Quantifying ALT

Similar to AST quantification, ALT is measured using a coupled enzymatic assay that detects an absorbance decrease at 340nm as reaction products are reduced. ALT also requires P5P as a cofactor.

Slide 15: Alkaline Phosphatase

Alkaline phosphatase (or ALP) is another enzyme commonly measured when liver dysfunction is suspected. ALP is present along the canalicular membrane, where it is weakly anchored through a post-translational lipid modification. When the flow of bile is obstructed, small pieces of membrane are released, and with it, ALP is released into the circulation. ALP is not specific to the liver – it has isoforms in the bone and placenta. But, if the liver is determined to be the source of ALP, it generally points to an obstructive etiology.

Slide 16: Quantifying Alkaline Phosphatase

ALP is quantified using a colorimetric reaction where the dephosphorylation of an ALP-specific substrate leads to a yellow color change that is monitored spectrophotometrically.

Slide 17: Gamma glutamyltransferase

Gamma glutamyltransferase or GGT is also an indicator of obstruction. While GGT is a less specific marker for obstruction compared to ALP, it is very specific for the liver and can be helpful to distinguish between liver and non-liver ALP elevations. A caveat to GGT measurements is that it is chronically elevated with prolonged alcohol use, which can complicate result interpretation.

Slide 18: Quantifying GGT

Similar to ALP quantification, GGT is measured using a colorimetric assay that relies on the color change of a GGT-specific substrate.

Slide 19: ALP & GGT results

Both the ALP and the GGT results in this patient were within the normal reference interval.

Slide 20: Bilirubin

Bilirubin, is a byproduct of heme metabolism that undergoes hepatic clearance. There are two main forms of bilirubin that we quantify in the laboratory – unconjugated and conjugated. Unconjugated bilirubin is a hydrophobic molecule. The liver gluceronidates unconjugated bilirubin to produce conjugated bilirubin, which is more water-soluble and therefore, easier to excrete.

The two species of bilirubin are differentially elevated in serum depending on the underlying pathology.

Slide 21: Bilirubin Quantification

While there are some exceptions, most chemistry analyzers quantify bilirubin using a diazo-based reagent either with or without an accelerator. Unconjugated bilirubin is albumin bound and therefore, doesn't readily react with the diazo reagent. In contrast, conjugated bilirubin is water-soluble and is not albumin bound and reacts quickly. To enhance the reaction of unconjugated bilirubin with the diazo reagent, an accelerator, such as caffeine, is added to the reaction to facilitate the release of unconjugated bilirubin from albumin, allowing for its quantification. These differences in reacting abilities allows for the differentiation between elevations in total bilirubin that are due to conjugated vs. unconjugated species.

Slide 22: Pathologies associated with bilirubin elevation

The species of bilirubin elevated is helpful for diagnosing various pathologies. Unconjugated bilirubin is most often elevated when there is intravascular hemolysis, liver failure, hypertension, or due to genetic causes, pathologies where bilirubin import into the liver is disrupted. In contrast, "direct" or conjugated bilirubin is elevated in acute hepatitis, biliary obstruction, or due to genetic causes, pathologies where bilirubin can enter the liver, but its proper excretion is inhibited.

Slide 23: Patient's Bilirubin Results

In this case, both the patient's total and direct bilirubin were within the normal range.

Slide 24: Albumin

Albumin is important because it actually measures liver function – meaning the liver's capacity to synthesize important macromolecules. Since the half-life of albumin is 21 days, low albumin concentrations as a result of liver pathology indicates that the liver hasn't had enough reserve to continue its physiological roles for several weeks. Decreased albumin can be found in other pathological states like inflammation and therefore, concentrations should be interpreted in conjunction with other liver markers and the clinical picture. In this case, the patient's albumin was within the normal reference interval.

Slide 25: Albumin Quantification

Albumin is quantified using a dye-binding assay. Bromocresol reacts with albumin to form a green complex, which can be measured spectrophotometrically.

Slide 26: Additional laboratory tests

Additional laboratory tests did not reveal any abnormalities.

Slide 27: Additional laboratory tests

More laboratory tests and imaging studies did not indicate any abnormalities. One aside: if anyone is interested in learning more about Alpha-1 antitrypsin, an earlier Pearl was focused exclusively on this protein (available at www.traineecouncil.org).

Slide 28: Tests not ordered

The liver is also responsible for synthesizing the vitamin K-dependent coagulation factors. If the liver reserve is depleted and liver function is inadequate, these factors will not be made and the patient will have an elevated prothrombin time (or PT). An elevated PT is not specific to liver disease and must be evaluated in the context of the clinical picture and other laboratory results. A PT was not mentioned in this case.

It is also surprising that they did not order a troponin in this patient, considering her presentation included chest pain and shortness of breath.

Slide 29:
Quoting the case, "…without signs or symptoms of liver disease, the patient was advised to discontinue alcohol consumption, and the clinical laboratory was contacted for additional studies."

Slide 30:
The clinical lab decided to look for the presence of a macroenzyme.

Slide 31: Macroenzymes
Macroenzymes are high molecular weight forms of a biomarker measured by the laboratory. The most common types are antibody-bound. It is unknown why these auto-antibodies are produced, but they are thought to be non-pathogenic. Most macroenzymes will cause false positive results, but false negative results have been documented. The elevations observed in patients with a macroenzyme are not necessarily indicative of organ dysfunction, but reflect the decreased clearance of the high molecular variant. Macroenzymes have been documented for many of the commonly measured enzymes; examples include amylase, AST, and ALP. High molecular weight variants of non-enzyme proteins have also been documented and include TSH and troponin. The biggest risk imposed by macroenzymes is the proceeding medical workup that can ensue when the physician sees a reproducible elevation in a marker of organ dysfunction.

Slide 32: Confirming the AST elevation was due to a macroenzyme
There are a few methods that can be used to identify a macroenzyme. In this case, the group incubated the specimen with protein A and protein G coated beads. Protein A and G are produced by certain bacteria and bind to the constant region of most human antibodies. This data shows that when you incubate the patient specimen, but not a control specimen, with the protein A or G coated beads, very little AST activity is recovered, indicating that most of the AST in this specimen is antibody bound. In contrast, the ALT in this patient's (and the control) sample shows complete recovery after incubation with the beads, indicating the ALT is not antibody bound.

Slide 33: What's Next?
The patient and the physician should be educated about these results and the patient should be warned that AST cannot be used to monitor her liver function. They should not be alarmed that this result is pathological, and the patient can likely continue to enjoy her 2oz of spirits without worrying about her liver.

Slide 34: Conclusion
In conclusion, liver function tests include the quantification of a variety of enzymes and proteins. Interpretation of liver pathology should involve the combined assessment of these markers and interferences should be investigated if a falsely elevated or decreased result is suspected.

Slide 35: References

Slide 36: Disclosures

Slide 37: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Liver Function Tests.”