Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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Title: Alpha 1-antitrypsin

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Slide 1:
Title slide

Slide 2:
Alpha 1-antitrypsin (AAT) is located on the long arm of chromosome 14 and is a member of the Serpin protease family. These proteases are the principle inhibitors of intra cellular and extra cellular proteolytic pathways, making them critical for physiological homeostasis. Other famous members of the Serpin family include antithrombin, which is involved in coagulation; C-1 inhibitor, which helps to regulate the complement cascade; and plasmin inhibitor to regulate fibrinolysis.

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The mechanism of AAT anti-protease activity can be visualized using these crystal structures of AAT and neutrophil elastase. Contrary to what the name implies, AAT is more responsible for inhibiting the protease neotrophil elastase than it is for inhibiting trypsin. AAT is shown here on top, the smaller molecule in blue and purple, and below it is the crystal structure of neutrophil elastase. The active site of neutrophil elastase is depicted in yellow. Neutrophil elastase initially binds to AAT as a substrate and attempts to cleave it. Instead, a covalent bond is formed between the two molecules and AAT executes protease activity - cleaving the active site of neutrophil elastase, and completely displacing it to the opposite side of the molecule. The effect is irreversible for both proteins, which is why AAT is sometimes referred to as a suicide inhibitor.

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The physiological significance of this protease inhibition is shown here. In a normal individual, AAT is synthesized in the liver and diffuses to the lungs. In the lungs, it acts as a shield against neutrophil elastase. Neutrophil elastase is an important protease secreted by neutrophils to combat any exogenous microbes that may find their way into the pleural space. However, this protease also has promiscuous activity for the connective tissue of the lungs, and thus has the potential for deleterious effects. AAT protects the lungs from the proteolytic activity of neutrophil elastase.

In a person with an AAT deficiency, the lungs remain vulnerable to degradation by neutophil elastase, eventually leading to extreme pulmonary damage resulting in emphysema and chronic obstructive pulmonary disease in the 4th or 5th decades of life.

In addition, certain mutations in AAT aggregate in hepatocytes leading to inclusion bodies and hepatocellular death. These aggregates can cause juvenile hepatitis, cirrhosis, and hepatocellular carcinoma. In fact, mutations in AAT are the most common cause of neonatal liver disease.

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AAT deficiency is inherited in an autosomal co dominant fashion, meaning that two different versions of the gene may be actively expressed and both versions contribute to the genetic trait. Prevalence of deficiency is ~1/2000 to 1/5000. The gene that encodes AAT, SERPINA1 is highly polymorphic; there are over 100 variants documented in the literature. Many of these mutations have no effect on physiological protein concentration or function. However, some of these variations have damaging physiological consequences.

The most common AAT variants are the M,Z, and S alleles. M is considered wild type or normal. Both S and Z are deficiency alleles. People who are homozygous for the Z allele are at the highest risk for both lung and liver disease.

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AAT deficiency is largely dependent on the laboratory for diagnosis. Many years ago, AAT deficiency was diagnosed by looking for low alpha 1 globulin levels on serum protein electrophoresis.
Today, there are 3 main ways that AAT deficiency – or sufficiency – is measured in the clinical lab: assessing the total AAT concentration using immunoturbidimetric assays, evaluating the presence of common alleles using cost and time efficient genetic testing, and finally, having the ability to identify both common and rare alleles using isoelectric focusing electrophoresis with immunofixation.

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Total AAT concentration is measured using immunoturbidimetric techniques. In this technique, antibodies specific to AAT are added to the serum specimen. The antibodies will form aggregates with AAT leading to a specimen with increased turbidity. Immunoturbidimetry measures the absorbance of light by the sample. The concentration of AAT will therefore be directly proportional to the amount of absorbed light.

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There are several genetic methods to detect deficiency alleles. Some, such as gene sequencing, will detect all mutations. Rapid genetic methods only detect the most common deficiency alleles, but have been utilized for their high throughput capacity and cost efficiency. In general, rapid genetic analysis is accomplished using melt curve analysis in a LightCycler instrument. This technique utilizes fluorescence resonance energy transfer, or FRET. In this technique, you have two probes that are annealed to the target sequence. The system is excited with a particular wavelength that excites one of the probes.

This probe will emit a wavelength that will excite the flurophore on the second probe. The fluorescence emitted by the second probe is measured. This energy exchange is proximity dependent. Thus, if the probes are denatured, the fluorescent emmitance will cease.

Here, I'm showing an example of the probes binding to two different alleles. The upper allele is the wildtype, and the probes are a perfect match. The lower allele is a variant that has a mutation which causes a mismatch between the probe and the target. This lowers the affinity of the probe for the target. Thus, when heat is added to the specimen, if there is mutation under the probe binding the fluorescence will be lost at a lower temperature compared to the wildtype sequence.

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This is an example of what the data from melt curve analysis might look like. These probes were designed to detect the region of the gene where the S mutation is located. The X axis is temperature and the y axis is fluorescence intensity. The pink line is the no template control –this should give no signal. The indigo line is the wildtype control. The green line is the S heterozygous control. The blue line is the unknown patient . From this analysis, you can infer that the patient does not carry an S allele. Please note that this does not mean that the patient is wildtype. It just means that they do not carry an S allele.

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AAT phenotypes are interpreted using isoelectric focusing electrophoresis, which is a technique used to separate similar proteins by their pI or pH at which they are electrically neutral using thin layer polyacrilamide gels. This is also known as the isoelectic point of a protein. The gels use an ampholyte buffer to create a linear pH gradient that increases in pH from the anode to the cathode. The proteins will migrate towards the anode if they are negatively charged until they reach the pH where they become neutral; if they are positively charged, they will migrate towards the cathode until they reach their neutral pH. This animation depicts proteins with very different pI's of 4, 7, and 9, but isoelectric focusing has much more precise resolution than this. The gradient used for focusing AAT variants is 4.2-4.9. To detect the proteins, AAT antisera is added that is conjugated to peroxidase. The antiserum binds AAT. Substrate is added to react with the peroxidase and produce a color change that stains the gel.

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This is an example of what an AAT isoelectric focusing electrophoresis gel looks like. Each variant produces multiple bands, which is thought to be a result of post translational modifications. In the first lane we have the MM variant, or what is generally thought of as the wildtype. The blue arrows point to lanes that have samples with patients who are heterogygous or homozygous for the Z allele. The Z allele is distinguished by the presence of cathodal bands. The orange arrows point to lanes that have patients heterozygous for the S allele, which is mainly distinguished by its unique band.

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Algorithms have been published to help clinicians order the proper tests. If a patient is suspected of having an AAT deficiency, the first tests that should be ordered are total AAT concentration and genotyping. This will identify S and Z hetero and homozygotes. If the total AAT and the genotype results are concordant – for example, a total AAT of 89 mg/dL (which is just below the lower end of the reference interval) corresponding to a Z heterozygote, the results should be reported. However, if the results are discrepant – for example a total AAT of 89 mg/dL corresponding to a non-S non-Z patient, the lab should perform phenotyping for the detection of rare alleles.

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In summary, AAT is an important protease inhibitor that protects the lungs from degradation by neutrophil elastase. Diagnosis of AAT deficiency is largely dependent on the clinical laboratory, and this is accomplished by an amalgam of biochemical and genetic techniques.

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References