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TITLE: Inflammatory Bowel Disease

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Slide 1:

Hello, my name is **Melissa Snyder**. I am an **Associate Professor of Laboratory Medicine and Pathology at the Mayo Clinic**. Welcome to this Pearl of Laboratory Medicine on "**Inflammatory Bowel Disease**."

Slide 2:

Inflammatory bowel disease, or IBD, is a chronic inflammatory disease that affects various portions of the gastrointestinal system. Although the exact etiology of IBD is unclear, the pathogenesis of the disease involves a combination of environmental exposures and genetic risk factors. IBD includes 2 specific diseases – ulcerative colitis and Crohn's disease. In ulcerative colitis, the inflammation primarily occurs in the colon or rectum. In Crohn's disease, the inflammatory process can affect almost any portion of the GI system. There are also differences between the 2 diseases in terms of the damage associated with the inflammatory process. In ulcerative colitis, a pattern of diffuse inflammation is generally observed, with involvement of the mucosal layer only, leading to mostly shallow erosions. For Crohn's disease, more discontinuous inflammation is seen that is transmural in nature, which could lead to deep fissures that extend through the GI wall.

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Patients with ulcerative colitis and Crohn's disease present with similar clinical symptoms. Both diseases lead to chronic diarrhea as one of the primary symptoms, although with ulcerative colitis, this diarrhea is generally very urgent in nature. In addition, patients with ulcerative colitis tend to have more blood in their stools, which can lead to fairly significant anemia. In Crohn's disease, weight loss is more of a significant symptom. This weight loss is due partly to

malabsorption, but limited food intake is a contributing factor as well. Pain is another common feature of both ulcerative colitis and Crohn's disease. Patients with ulcerative colitis may experience pain that is abdominal or rectal in nature. In comparison, patients with Crohn's disease usually complain of intermittent pain, which is associated with obstructions within the narrowed lumen of the patient's GI system. Finally, patients with ulcerative colitis and Crohn's disease often endorse some systemic symptoms, including fever and fatigue. Interestingly, patients with either condition may also experience arthralgias.

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The pathogenesis of IBD reflects a complex interaction of multiple risk factors, which is a common characteristic of autoimmune diseases. These risk factors include a number of genetic and environmental components. Interestingly, it appears that both the genetic and environmental risk factors are related to alterations in the GI microbiota, and how the immune system responds to these changes.

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With regard to the genetics of IBD, more than 300 genes have been identified that confer some level of risk for ulcerative colitis, Crohn's disease, or both, although it is estimated that this only accounts for about 25% of the overall genetic risk. As a specific example, certain mutations in the NOD2 gene lead to increased risk for Crohn's disease. There is a gene dosage effect, with heterozygotes and homozygotes showing a 2 to 4-fold and 15 to 40-fold increased risk, respectively. However, these genetic influences are only risk factors. It is estimated that at least a certain percentage of individuals with these NOD2 mutations will never develop Crohn's disease in their lifetime. Similarly, 20 to 30% of patients with Crohn's disease do not have known NOD2 mutations.

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This same complexity is evident in the environmental exposures associated with IBD. Various studies have demonstrated a link between antibiotic use in children and risk for IBD, which is attributed to changes in the GI microbiome caused by the medication. Industrialization has also been linked to IBD, although the exact exposures, which could include pollution, changes in diet, and improved sanitation, are still debated. In contrast, infants who were breastfed tend to have lower prevalence of IBD compared to formula-fed infants. In addition, smoking as an adult, current or past, has consistently been shown to have a protective effect against IBD, although the mechanism behind this is not clear.

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Overall, IBD is considered to be a disease of the developed world. The prevalences for ulcerative colitis and Crohn's disease shown on this slide are estimates based on studies in North America and Western Europe. The peak of age of onset for IBD tends to be between 15 and 25 years of age, with not much difference between males and females. Both ulcerative

colitis and Crohn's disease appear to have increased standardized mortality ratios compared to the general population, which is slightly more apparent for Crohn's disease. This may be attributed, at least in part, to an increased risk of colorectal cancer. The risk for colorectal malignancy increases as the time from diagnosis progresses, although this is likely influenced by other risk factors and characteristics of the patient's IBD course.

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The diagnostic evaluation for IBD usually begins with a review of both gastrointestinal and systemic symptoms. A thorough family history is also important, as first-degree relatives of patients with IBD are at a significantly increased risk of developing the disease themselves. If IBD is suspected, the next part of the diagnostic evaluation is endoscopy and biopsy. Endoscopy allows the physician to determine if inflammation is present and its localization. This visual examination is the first step to determining not only if the patient has IBD, but whether the inflammation is more consistent with ulcerative colitis or Crohn's disease. During the endoscopy, tissue is usually obtained for biopsy analysis, which is used to identify specific features that may be more consistent with ulcerative colitis or Crohn's disease. There is also some laboratory testing available. Serum serology testing can be performed, which might include assessing for the presence of antibodies consistent with or specific for IBD. Stool testing might also be useful. This testing is generally used to determine if any infectious agents are present and to assess for the presence of inflammation specific to the gastrointestinal system. In the next few slides, we will look more in depth at the laboratory testing available for IBD.

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The serology testing for IBD includes pANCA and anti-Saccharomyces cerevisiae antibodies. "ANCA" stands for anti-neutrophil cytoplasmic antibody; the "p" refers to the observation that the staining pattern is perinuclear. This testing is performed by indirect immunofluorescence on ethanol-fixed neutrophils. There are 2 general classifications of pANCA staining – atypical and typical. Atypical staining is described as non-homogeneous staining of the rim around the nucleus of the neutrophils and is associated with IBD. The specific antibody to which the atypical pANCA binds is not known, although multiple candidates have been considered. In contrast, the typical pANCA pattern is described as perinuclear staining with extension into the nucleus that displays as graduated shading. This pattern is associated with small-vessel vasculitis and results from the antibody binding to the antigen myeloperoxidase.

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The other serologic markers for IBD are the anti-Saccharomyces cerevisiae antibodies. These antibodies are specific for a cell-wall mannan found in yeast and are referred to collectively as anti-glycan antibodies. These anti-Saccharomyces cerevisiae antibodies are only one of many anti-glycan antibodies found in patients with IBD, and are possibly a reflection of the immune response against the GI microbiota. In fact, patients with IBD can develop antibodies against a

variety of microbial proteins, including OmpC (the outer-membrane porin C in E. coli) and CBir1 and A4-Fla2, both of which are bacterial flagellin proteins.

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The bulk of serologic testing for IBD still centers around pANCA and Saccharomyces cerevisiae antibodies. Positivity for pANCA in the absence of Saccharomyces cerevisiae antibodies is considered to be consistent with ulcerative colitis, while positivity for the yeast antibodies in the absence of a pANCA is more consistent with Crohn's disease. Unfortunately, the observed pattern is not always this clear cut. Approximately 15% of patients with ulcerative colitis may be positive for Saccharomyces antibodies, and a similar percentage of patients with Crohn's disease may be pANCA positive.

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A meta-analysis addressed the utility of pANCA and the Saccharomyces antibodies for the diagnosis of IBD, ulcerative colitis, and Crohn's disease. The specificity of the testing for IBD and for the 2 specific diseases were all above 90%. However, sensitivity was the primary issue, which was only on the order of 50 to 60%. These data indicate that close to half of the patients with these conditions would not be positive for these serologic tests.

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This limited sensitivity has led most professional organizations to the conclusion that serology testing has very limited utility for the diagnostic evaluation of suspected IBD, since a negative result cannot be used reliably to exclude the diagnosis. Instead, most organizations suggest that pANCA and Saccharomyces cerevisiae antibody testing only be used in the sub-set of patients who are diagnosed with IBD, but for whom the physician is unable to distinguish between ulcerative colitis and Crohn's disease. In general, this may be the case for up to 10% of individuals who are diagnosed with IBD.

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Given the limitations of serology testing, the question becomes is there other laboratory testing that can provide the needed sensitivity for diagnostic evaluation? This is where stool testing could play a role. Inflammation within the gastrointestinal system is associated with the presence of neutrophils. These neutrophils release their cellular proteins, which accumulate in the fecal material that ultimately is excreted from the body. Two of these neutrophil proteins are calprotectin and lactoferrin. Calprotectin is a calcium-binding protein, and is one of the most abundant proteins in the neutrophil. Lactoferrin is an iron-binding glycoprotein with bactericidal activity.

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Neutrophils will migrate to the gastrointestinal system as part of any innate immune response, which can occur in IBD as well as in other conditions including celiac disease, infection, colorectal cancer, and even the use of NSAIDs. So, we must view both calprotectin and lactoferrin as non-specific markers of inflammation that is occurring within the gastrointestinal system. If this is the case how can it be useful as a diagnostic tool? When used as one of the initial diagnostic tests, a negative result can rule-out an inflammatory etiology of the GI symptoms. In contrast, an elevated calprotectin or lactoferrin suggests the presence of GI inflammation, although further diagnostic evaluation is needed to determine the cause.

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To summarize this Pearl of Laboratory Medicine, ulcerative colitis and Crohn's disease are chronic inflammatory diseases that can affect many different areas of the gastrointestinal system. Both diseases arise from complex interactions between genetic risk factors and environmental exposures. Establishing a diagnosis for ulcerative colitis and Crohn's disease can be challenging. Many of the symptoms, which may be somewhat nonspecific, overlap between the 2 diseases, and establishing the diagnosis still relies heavily on the clinical evaluation and endoscopy.

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The laboratory testing for IBD can be divided into fecal inflammatory markers and antibody serology. Fecal calprotectin and lactoferrin are appropriate for assessing for the presence of gastrointestinal inflammation. Based on the sensitivity of these markers, a normal result can be used to exclude an inflammatory gastrointestinal condition. In contrast, an elevated result only indicates the presence of gastrointestinal inflammation, and further diagnostic evaluation is needed to establish the cause of the immune response. Saccharomyces cerevisiae antibodies and pANCA serology are not useful for establishing a diagnosis of IBD due to limited sensitivity. However, his testing may be helpful in distinguishing between ulcerative colitis and Crohn's disease in the small sub-set of patients for whom a specific diagnosis is unclear.

Slide 18: References

Slide 19: Disclosures

Slide 20: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Inflammatory Bowel Disease"**