The Clinical Laboratory’s Role in the Evaluation of Gastrointestinal Disease

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Disorders of the Mouth

- Many conditions lead to oral ulcers
  - Herpes
  - Pemphigoid
  - Pemphigus
  - Eruptive lichen planus
  - Friction ulcers

- Biopsy most helpful for lesions of uncertain etiology; immunofluorescence may be necessary.

- For herpes: non-Gyn cytopathology, serological testing, culture

- Accuracy of some tests depend on disease stage
Oral Herpes Detection

- Viral culture = reference method for Herpes; can detect herpes type 1 and 2 infections
  - Drawback: requires excellent specimen for virus to grow
- Non-gyn cytopathology (Tzanck smear)
  - Drawback: false negatives
- HerpeSelect®: IgM and IgG (typing on the IgG)
  - Serum specimen
  - Drawback: may not reveal active infection

Oral Cancer and HPV Testing

- Oral cancer associated with
  - HPV infection
  - Smoking
  - Alcohol use
- Non-gyn cytopathology traditionally offered
- HPV tests used for cervical cancer detection are also used for oral neoplasias; however, test not FDA approved for this use
- OraRisk™ HPV test introduced March 2010

Disorders of the Esophagus and Stomach
Esophagus or Stomach

- **Dyspepsia**: most common symptom in patients with gastritis
- **Dysphagia** (difficulty swallowing) associated with variety of abnormalities; esophageal abnormality is a likely cause.
- **Odynophagia** (pain on swallowing) may be attributed to esophageal abnormality.

Esophageal Disorders

- Gastroesophageal reflux disease (GERD)
- Barrett’s esophagus
- Esophageal cancer

*Clinical laboratory has little to offer – biopsy is mainstay of diagnosis*

Food Allergy

- Eosinophils are part of innate immune system and serve important functions in helminth infection. Eosinophilic disorders exist throughout the GI tract and may be associated with atopy/allergy
- Food allergy testing is considered for patients with dysphagia. Eosinophilic esophagitis may be eventual diagnosis.
Food Allergies

- Abnormal response to food protein affects
  - 6-8% of young adults
  - 3-4% of adults in the U.S.
- Most common food allergens in children
  - Milk
  - Eggs
  - Peanuts

- Most common food allergens in adults
  - Tree nuts
  - Fish
  - Shellfish
- Other common food allergens
  - Soy
  - Wheat

Types of Food Allergies

- IgE-mediated
  - Confirmed with skin tests or immunoassay tests
- Non-IgE-mediated (celiac, proctitis, enterocolitis)
- Mixed disorders usually associated with eosinophils
Food Allergy Testing

- In vivo skin prick test
  - High NPV (≥95%) but low PPV (≤50%)
  - Useful as initial test to rule out IgE-mediated reaction to specific food
  - Percutaneous preferred as intracutaneous has more false-positives


Food Allergy Testing

- In vitro (serum) tests
  - Radioimmunoassay procedures (radioallergosorbent test [RAST]) no longer used
  - IgE-specific immunoassay replaced RAST
    - ~ same clinical sensitivity and specificity as skin prick tests
    - Same clinical application
    - Especially useful in patients with hx of life-threatening reaction or with atopic dermatitis or dermatographism
    - Pediatric and adult panels typically offered


Celiac Disease

- Immune response to gluten in genetically susceptible people
  - Non-IgE-mediated allergy to wheat, rye, barley, oats

- Characteristics
  - Partial to complete villous atrophy of small intestine
  - Crypt hyperplasia
  - Lymphocytic infiltration of epithelium and lamina propria

Celiac Disease Perspectives

- 60% of children and 41% of adults diagnosed during study were asymptomatic
- 21% of patients with positive anti-endomysial antibody test could not receive biopsy due to physician refusal to perform procedure or insurance company to pay for it
- Only 35% of newly diagnosed patients had chronic diarrhea, dispelling myth that diarrhea must be present to diagnose celiac disease


Celiac Disease Perspectives

- Celiac disease affects 2.2 – 3.0 million Americans
- Type 1 diabetes affects 1,177,500 people, 6% (70,650) of whom have celiac disease
- 610,000 women in U.S. experience unexplained infertility; 6% (36,600) might never learn that celiac disease is the cause
- 350,000 people in U.S. have Down syndrome; 12% (42,000) of whom have celiac disease
- Average length of time for symptomatic person to be diagnosed with celiac disease in U.S. is 11 y; this dramatically increases individual’s risk of developing autoimmune disorders, neurological problems, osteoporosis, and even cancer.


“Minés” of Celiac Disease Found Among...

- Relative
  - short stature, anemia, fatigue, hypertransaminasemia
  - autoimmune disorders, Down syndrome, IgA deficiency, neuropathies, osteoporosis, infertility
  - blood donors, students, general population

- Patients with Associated diseases
  - “Healthy” groups
Common Celiac Disease Symptoms – Weight Loss and Fluid Retention

- Weight loss = direct result of inadequate carbohydrate, protein, and fat absorption
- Weight loss may not always occur
  - Enormous appetite compensates for reduced absorption of nutrients
  - Masked by fluid retention in advanced malnutrition
    - Reduced protein absorption → low blood protein levels → fluid leaks into tissues (edema) especially ankles and feet

http://www.webmd.com/digestive-disorders/celiac-disease/celiac-disease--symptoms

Common Celiac Disease Symptoms

- Anemia – lack of vitamin B₁₂ and iron absorption
- Osteoporosis – lack of vitamin D and calcium absorption
- Easy bruising – lack of vitamin K absorption
- Peripheral neuropathy (nerve damage) – vitamin B₁₂ and thiamine deficiencies → poor balance, muscle weakness, numbness and tingling in arms and legs

http://www.webmd.com/digestive-disorders/celiac-disease/celiac-disease--symptoms

Dermatitis Herpetiformis

- Erythematous macule > urticarial papule > tense vesicles
- Severe pruritus
- Symmetric distribution
- 90% no GI symptoms
- 75% villous atrophy
- Gluten sensitive
tG in skin
- 2% of CD have DH

Image from Wikimedia Commons.
Celiac Disease Diagnosis

- Presumptive diagnosis
  - Symptoms
  - Positive serology
  - Biopsy of small intestine
- Confirmation
  - Resolution of symptoms following initiation of gluten-free diet

Serological Tests for Celiac Disease

- Role of serological tests
  - Identify symptomatic individuals who need a biopsy
  - Screen asymptomatic at-risk individuals
  - Provide additional evidence to support the diagnosis
  - Monitor dietary compliance

Antibody Isotypes

- IgM
- IgG
- IgD
- IgA: the GI antibody
Sensitivity and Specificity – Definition

- Measures for assessing accuracy of test results
- **Sensitivity** = probability disease is detected when present
  - Percentage of patients with the disease who test positive
- **Specificity** = probability disease is excluded when absent
  - Percentage of patients without the disease who test negative

Celiac Disease – Adult Serologic Tests

- **Primary tests**
  - Tissue transglutaminase (tTG) IgA
  - Endomysial (EMA) IgA
  - Total serum IgA
  - Tissue transglutaminase (tTG) IgG
  - Gliadin (AGA) IgA
  - Gliadin (AGA) IgG
- **Additional tests**
  - HLA typing for Celiac disease: HLA DQ2 and DQ8
  - Actin IgA
  - Reticulin antibody (IgG, IgA)

Celiac Disease – Pediatric Serologic Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Clinical Use</th>
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<tbody>
<tr>
<td>tTG (IgA)</td>
<td>First-line diagnostic marker; monitor adherence to gluten-free diet</td>
</tr>
<tr>
<td>EMA (IgA)</td>
<td>Confirm positive tTG IgA; also use when tTG IgA negative, but clinical suspicion remains high; monitor adherence to gluten-free diet</td>
</tr>
<tr>
<td>Total serum IgA</td>
<td>Detect IgA deficiency</td>
</tr>
<tr>
<td>tTG (IgG)</td>
<td>Use for patients with IgA deficiency</td>
</tr>
<tr>
<td>AGA (IgA)</td>
<td>Use for patients w/ suggestive histopathology and negative tTG and EMA</td>
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</table>
**Tissue Transglutaminase (tTG)**

- IgA-based antibody against tissue transglutaminase (celiac disease autoantigen)

- **Advantages**
  - High sensitivity and specificity (human TTG)
  - Non-operator dependent (ELISA)
  - Relatively cheap

- **Disadvantages**
  - False negative in young children
  - False negative in IgA deficiency
  - Possibly less specific than EMA

**Endomysial Antibody – EMA**

- Antibodies against outer layer of the smooth muscle of esophagus
- Highly specific for celiac disease
- Confirms positive tTG IgA test results
- Can be used to monitor patient adherence to gluten-free diet

**Selective IgA Deficiency**

- Prevalent in general population (1 in 300)
- Prevalence in CD patients: 10-15 times that in general population (ie, 2% – 3%)
- IgA deficient individuals mostly healthy

- Symptoms
  - Sinopulmonary infections
  - Allergies
  - Autoimmune disorders, especially CD

- Testing recommendations
  - Total IgA levels on all suspected CD patients
  - Biopsy all IgA deficient patients (TBD: IgA level?)
  - Gluten free diet: IgG and IgA levels of serological marker return to normal
Gliadin Antibodies

- IgG and IgA antibodies to gluten protein
- Advantages: relatively cheap and easy to perform
- Disadvantages: poor sensitivity and specificity; NIH Consensus Statement on celiac disease against gliadin antibody testing
- Deamidated vastly improved specificity and sensitivity
- Many pediatric gastroenterologists claim gliadin antibody testing is required for pediatric population; literature from Europe supports this.

Actin Antibodies and Intestinal Atrophy

- N, 383
- PPV, 99%
- NPV, 88%


HLA Typing for Celiac Disease – HLA DQ2 and DQ8

- Use when biopsy and serology results indeterminate; family studies may be appropriate
- Stratifies patient into low- or high-risk of celiac disease
  - >97% of people with celiac disease test positive for DQ2 or DQ8
  - Low risk when both are negative
- Not diagnostic: >40% of general population are positive
Dyspepsia (Peptic Ulcer)

- **AGA definition:** chronic or recurrent pain or discomfort centered in upper abdomen (separate from heartburn)
- Patients with alarm features referred for endoscopy
  - Age older than 55 years with new onset
  - Family history of upper GI cancer
  - Unintended weight loss
  - Gastrointestinal bleeding
  - Progressive dysphagia
  - Odynophagia
  - Unexplained iron-deficiency anemia

Stomach: *H pylori* Gastric Mucosa

*H pylori – the Risks*

- Causative agent for peptic ulcers
- Increased risk of lymphoma, including MALT lymphoma
- Increased risk for adenocarcinoma
- Increased risk for atrophic gastritis
- Despite associations above, asymptomatic patients not screened for *H pylori*

**AGA recommends *H pylori* test and treat strategy for patients with dyspepsia and no alarm features**
The Approach in Japan Is Different

- Gastric carcinoma most common cancer in Japan
- ~97% of all Japanese gastric carcinomas associated with \textit{H pylori} infection
- Multi-center study showed importance of eradicating \textit{H pylori} as means of decreasing gastric carcinoma incidence
- Asymptomatic patients treated in Japan
- Japanese findings not replicated in other parts of the world

\textit{H pylori} Tests

- Serologic tests
  - Not recommended by AGA
  - Does not indicate active infection
- Stool antigen test recommended
- Urea breath test recommended
  - $^{13}\text{C}$-urea metabolized by \textit{H pylori}-associated urease

Atrophic Gastritis – Are there any markers that can predict risk of developing gastric adenocarcinoma?

- Consensus guidelines?
- An estimation of the risk for gastric adenocarcinoma would be helpful.
- With multiple biopsy specimens, this can be achieved.
- Does the clinical pathology laboratory offer much help in this matter?
Can serum gastrin levels and CagA antibodies help predict risk of gastric adenocarcinoma?

- Very difficult topic: different terminologies to describe various forms of gastritis, ethnic and genetic differences throughout world populations
- In a few multivariant studies, only serum gastrin and antibodies to CagA predict risk of developing gastric adenocarcinoma.
- No consensus group recommendations for assessing risk of cancer in H pylori-positive patients

Disorders of the Colon and Rectum

IBS – Functional Gastrointestinal Disorder

- Nerve endings in bowel lining unusually sensitive; hence, “irritable”
- Syndrome (group of symptoms), not a disease
  - Chronic abdominal pain – discomfort
  - Diarrhea and constipation – alternating bouts of both
- Other functional disorders
  - Fibromyalgia
  - Chronic fatigue syndrome
IBS versus IBD

- Mucosal inflammation with shedding of leukocytes into large intestines may offer diagnostic insights
- Leukocytes in stool sample = traditional test
- Lactoferrin test available

IBS, irritable bowel syndrome; IBD, inflammatory bowel disease.

Lactoferrin

- Differentiate IBS from active IBD and healthy people
  - Sensitivity 86%
  - Specificity 100%
- Differentiate IBS from active Crohn’s disease and healthy people
  - Sensitivity 85%
  - Specificity 100%

Am J Gastroenterol. 2003;98:1309-1314

Colorectal Cancer

- Screening (most important issue)
- When should a patient be evaluated for hereditary non-polyposis colorectal cancer syndrome?
- What about molecular testing for colon cancer patients?
Colorectal Cancer Survival – Highly Dependent on Stage at Diagnosis…


…However, Screening Compliance Is Very Poor

Cancer screening compliance in people 50 years of age

American Cancer Society, Surveillance Research.

CRC Frequency

Sporadic (65% to 85%)
Rare genetic syndromes (2%)
HNPCC (5%)
FAP (1%)
Unknown or possibly genetic (10% to 30%)
Screening with InSure® FIT™

Stool-based immunochemical test for colorectal cancer screening

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<thead>
<tr>
<th></th>
<th>GFOBT</th>
<th>InSure FIT</th>
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</thead>
<tbody>
<tr>
<td>Dietary restrictions prior to sample collection</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>54%</td>
<td>88%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98%</td>
<td>97%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>40%</td>
<td>42%</td>
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HNPCC Testing: Whom to Test?

Individuals who meet any of Bethesda criteria and in whom familial mutation unknown

- CRC diagnosed at age <50 years
- Presence of synchronous CRC (multiple CRCs ≤6 mo after initial tumor removal), metachronous CRC (CRC recurrence >6 mo after initial tumor removal), or other HNPCC-associated tumors
- CRC with MSI-H histology diagnosed at age <60 years
- CRC in ≥1 first-degree relative with HNPCC-related tumor with 1 of the cancers diagnosed at age <50 years
- CRC diagnosed in ≥2 first- or second-degree relatives with HNPCC-related tumors

Pharmacogenomic Assays Used in CRC

<table>
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<tr>
<th>Test</th>
<th>Clinical Indication</th>
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<tbody>
<tr>
<td>UGT1A1 gene polymorphism</td>
<td>Predict irinotecan toxicity</td>
</tr>
<tr>
<td>DPD gene mutation analysis</td>
<td>Predict toxicity to pyrimidine-based chemotherapeutic agents (5-FU, capecitabine)</td>
</tr>
<tr>
<td>VEGF mutation analysis</td>
<td>Predict survival and hypertension in patients treated with VEGF antagonists (bevacizumab)</td>
</tr>
<tr>
<td>KRAS, PI3KCA, BRAF, NRAS gene mutations</td>
<td>Determine potential for response to treatment with EGFR antagonists (cetuximab, panitumumab)</td>
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Infectious Diarrhea

- Occurs primarily during winter months
- Usually short-lived (1 to 3 days) and caused by virus
- Stool culture
  - Appropriate when there is systemic illness (e.g., fever) and bloody stool
- Causative bacteria
  - *Salmonella*
  - *Shigella*
  - *Campylobacter*
  - Shiga-toxin producing *E. coli*
- Fecal testing for leukocytes or lactoferrin

New Aspect to Infectious Colitis

- CDC recommendation (Oct 2009) – all stool samples submitted for routine testing from patients with acute community-acquired diarrhea be cultured for *E. coli* O157:H7 and simultaneously tested for Shiga toxins to detect non-O157 STEC
- Culture needed for public health reasons
- Assaying both offers more sensitivity
- Identifies STEC for immediate therapy

STEC, Shiga toxin-producing *Escherichia coli*.

Anal Pap Smear and HPV Testing

- At this time, no CDC recommendation
- May be suitable for high-risk patients
- New York State HIV-infected population guidelines
  - Men who have sex with men
  - Any patient with a history of anogenital condyloma
  - Women with a histopathologic abnormality of vulva or cervix
Anal Pap Smear and HPV Testing

Available Tests

- Anal Pap smear
- Anal Pap smear + HPV high risk DNA (Hybrid Capture II out-of-the-vial method)
- HPV high- and low-risk DNA (Hybrid Capture II) – swab/brush sample
- Biopsy for confirmation

Questions

1. Which serologic test is essential when evaluating a patient for celiac disease.
   A. Total Ig A
   B. Anti H. Pylori antibody
   C. Anti Nuclear antibody
   D. Ig G Gliadin antibody
   E. Ig E Gliadin antibody

Questions

2. HPV DNA testing (high risk) can be ordered on an anal specimen.
   a. True
   b. False
Questions

3. There are CDC guidelines for anal pap smears.
   A. True
   B. False

Questions

4. Food allergy testing can be used in patients with dysphagia.
   A. True
   B. False

5. In the US, we have a well developed H. pylori screening program for asymptomatic patients.
   A. True
   B. False
<table>
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<tr>
<th>Answers</th>
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<tbody>
<tr>
<td>1. A</td>
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<tr>
<td>2. True</td>
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<tr>
<td>3. False</td>
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<tr>
<td>4. True</td>
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<tr>
<td>5. False</td>
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