Carbohydrate and Amyloid staining

Presented By
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IHC
Thank you for attending the session: Colon Cancer. This email is a reminder that to receive a contact hour certificate for the meeting you must report your attendance to NSH through the NSH Contact Hour Portal - ce.nsh.org.

Instructions for adding your hours to your account are located below. If you have any questions, please contact the NSH office, 443-535-4060.

Kind regards,

INSERT NAME

Directions for Submitting Your Hours to the NSH Contact Hour Portal

1. Login to NSH Contact Hour Portal – ce.nsh.org (do not use www prior to the web address)
   a. First Time to the Site? – complete the “Not Yet Registered” form to create your contact hour portal user account. Once you have created a user account you will be asked to complete the user profile form (your name, address etc). To complete the profile and access hours from previous events you will need your NSH Customer ID number in the first step. NSH members can find their Customer ID# on their NSH membership cards. If you don’t know your Customer ID number contact NSH at histo@nsh.org.
   b. Returning to the site? – simply log in using the “Already Registered” box on the right.

2. Once you are logged in select “Session Tracker” from the top navigation

3. Add your Contact Hours for an event
   a. Step 1: Year: Select the year in which the event was held.
   b. Step 2: Event Title: All events approved in a specific year for contact hours by NSH are available to you to in this drop down list. Select the name of the event you attended: Aurora Diagnostics Webinar Series.
   c. Step 3: Session Title: Select the workshop or webinar you attended: Colon Cancer. The number of contact hours awarded for this session is preset and cannot be edited.
   d. Step 4: Add: Click on Add and your workshop or webinar will appear in your session log listed below.

4. Print your contact hour certificate
   a. Click on the box next to each session you would like to appear on your certificate.
   b. Make sure you click all of the sessions from one event if you want them to appear on one certificate.
   c. If you want all of your hours for a specific year you will need to click on all of the sessions from that year.
Carbohydrates

- Glycogen – Liver, cardiac and skeletal muscle
- Sialomucins - Salivary glands, intestinal goblet cells, gastric lining cells
- Neutral Mucosubstance – Gastric lining cells, duodenal Brunner glands
- Acid Mucosubstances – Globlet cells of the intestine
- Hyaluronic Acid – Umbilical cord, connective tissue of dermis
- Chondroitin Sulfate – Cartilage, aorta, heart valve, umbilical cord, dermis
- Heparin Sulfate – Mast cells, aorta, cardiac connective tissue
Demonstration of Carbohydrate Complexes

- Glycogen – Periodic Acid Schiff (PAS)
- Neutral Mucosubstances – Periodic Acid Schiff (PAS), with diastase
- Acid Mucosubstances – Alcian Blue, pH 2.5 and 0.4 – The different pH’s provide differential staining of sulfated (pH 0.4) and non sulfated (pH 2.5)
- Sialomucins and Sulfated Mucosubstances – Gomori’s Aldehyde Fuchsin pH 1.7 and pH 1.0
- Acid Mucosaccharides – Colloidal Iron
- Chondromucins – Safranin O
- Heparin Sulfate – Gomori’s Aldehyde Fuchsin
Case 1

- 68 y/o female
- Material submitted: Peritoneal Biopsy
- Clinical Diagnosis: Nausea Vomiting Abd pain Abdominal Mass
- Final Diagnosis: Poorly differentiated non-small cell carcinoma (breast primary should be strongly considered)
- Pankeratin positive
- CK-7 positive
- CK-20 negative
- LCA, /CEA, Synaptophysin, Chromograin, TTF-1 (Negative)
- ER 95% positivity
- PR 10% positivity
- AB-PASH and AFB (no acid fast or fungal organisms noted)
- Mucicarmine (no definite glandular differentiation)
Case 1 H&E

Nests of pyknotic cells

Dense fibroconnective tissue

6-3-2016
Case 1 H&E ptn high
Case 1 muci control
Case 1muci ptn
Case 1 ab pas control
Case 1 abpas ptn
IHC results case 1

- Pankreaktalin +
- CK-7 +
- CK-20 –
- LCA, CEA, Synaptophysin, Chromogranin and TTF-1 all –
- ER 95% positive
- PR 10% positive
Case 2

- Material Submitted: Brain Tumor
- Clinical Diagnosis: Brain Tumor
- 53 y/o female
- Microscopic Description: Sections demonstrate irregular nests of markedly pleomorphic epitheloid cells exhibiting intermediate to large hychromatic nuclei with moderate eosinohilic cytoplasm and abundant mitotic figures. There is brisk vascularity throughout. Special stains are pending. Some glandular differentiation is suggested.
- Final Diagnosis: Brain, left frontal region, biopsies – Metastatic poorly differentiated adenocarcinoma
- Differential cytokeratin stains yield immunophenotype suggesting breast, lung, ovary, or endometrial primaries. (Positive CK -7) A TTF-1 stain fails to confirm lung primary. Glandular differentiation is evident with positive Mucicarmine. Negative immunostains include Cytokeratin 20, GFAP, S-100, HMB 45 and Mart 1.
Case 2 H&E
Case 2 H&E

Large hyperchromatic nuclei

Eosinophilic cytoplasm
Case Muci ptn

Mucicarmine positive
Case 2

- Differential cytokeratin stains yield immunophenotype suggesting breast, lung, ovary, or endometrial primaries (positive CK-7). A TTF-1 stain fails to confirm lung primary. Glandular differentiation is evident with positive Mucicarmine. Negative immunostains include CK-20, GFAP, S-100, HMB-45 and Mart-1

- Final Diagnosis: Metastatic poorly differentiated adenocarcinoma
Case 3

- 42 y/o male
- Material Submitted: Liver Biopsy
- Clinical Diagnosis: Stomach CA
- Final Diagnosis: Poorly Differentiated adenocarcinoma
- CK-7 positive
- CK-20 scattered positivity
- TTF-1 positivity to suggest a bronchogenic primary
- CEA strong positivity
- S-100 no positivity
- HMB-45 no positivity
- Mart-1 no positivity
- LCA no positivity
- C-Kit positivity to suggest a gastrointestinal stromal tumor
- SMA no positivity
- Mucicarmine no positivity
- AB-PASH no definite stainable mucin
Case 3 H&E

- Enlarged nuclei
- Prominent nucleoli
Case 3 H&E
Case 3 fe ptn
Case 3 fe control
Case 3AB-PAS ptn
Case 3  ab-pas control

Glandular cells
Case 3 retic control
Case 3  Retic ptn

Retic fiber
Case 3 tri ptn
Case 3 tri control
Case 3 mucin control
Case 3 muci ptn
Case 3 LCA control
Case 3 LCA ptn
Case 3 SMA control
Case 3 SMA ptn
Case 3 C-kit Control
Case 3 C-kit ptn
Case 3

• Final Diagnosis: Poorly differentiated adenocarcinoma (The likely primary site is stomach or pancreas. A metastatic colorectal carcinoma is less likely.)
Case 4

- 58 y/o male
- Material submitted: Mediastinum mass and 4R node
- Clinical Diagnosis: Lung Mass
- Final Diagnosis: Metastatic poorly differentiated non small-cell carcinoma
- Note: No definite glandular or squamous differentiation can be confirmed by p63, CK 5&6 and mucicarmine stains. Strong cytokeratin 7 and TTF-1 positivity with negative cytokeratin 20 support lung primary.
Case 4 H&E

Neoplastic epithelial cells
Case 4 muci ptn
Case 4 muci control
Case 4 ck 7 ptn
Case 5

- Patient: 88 y/o female
- Material Submitted: Left ankle skin biopsy
- Final Diagnosis:
  - Markedly sclerotic, focally ulcerated skin with scale crust
  - Negative for fungal elements (PAS LG)
  - Negative for amyloid deposition (Congo Red)
  - Negative for squamous cell carcinoma
Case 5
Case 5 pas Ig control

Fungal organisms
Case 5 pas Ig ptn

Epidermis
511 congo red ptn
511 congo red control
Case 5

- Final Diagnosis: Markedly sclerotic, focally ulcerated skin with scale crust
- Negative for fungal elements
- Negative for amyloid deposition
- Negative for squamous cell carcinoma
Case 6

• Ptn:51 y/o female
• Tissue submitted: esophageal Biopsy
• Clinical History: Nausea
• Post-Operative Diagnosis: Esoph. Ulcer, gastritis
• Final Diagnosis: Benign esophageal and gastric type mucosa with chronic inflammation, Fibrinoneutrophilic debris consistent with origin from the bed of an ulcer, Negative for dysplasia, Negative for specialized intestinal metaplasia and fungal organisms (AB-PASH)
Case 6 H&E

- Gastric mucosa
- Inflammatory cells
- Esophageal mucosa

3-8-2016
Case 6 pt n H&E

lymphocyte

eosinophil
Case 6 abpas control
Case 6 ptn abpas
Case 6

• Final Diagnosis:
• Benign esophageal mucosa with chronic inflammation
Case 7

- Ptn: 61 y/o male
- Tissue submitted: Esophagitis GE junction
- Final Diagnosis: Acute and chronic esophagitis with features suggestive of reflux, negative for dysplasia, Negative for fungal organisms and specialized intestinal metaplasia (ABPASH)
Case 7 abpas control
Case 7 abpas ptn
Case 7

- Final Diagnosis:
- Acute and chronic esophagitis with features suggestive of reflux, Negative for dysplasia, Negative for fungal organisms and specialized intestinal metaplasia
Case 8

- Ptn: 52 y/o female
- Clinical Diagnosis: EGD with bx, polypectomy
- Pre-operative diagnosis: N & V, abd pain
- Post-operative diagnosis: HH, gastric, polyp
- Tissue submitted: EG junction bx
- Final diagnosis: (EG junction) Benign gastric type mucosa, positive for focal specialized intestinal epithelium (AB-PAS), Negative for dysplasia, Minimal inflammation
Case 8 H&E
Case 8 ab pas
Case 8 abpas control
Case 8

- Final Diagnosis:
  - Benign gastric type mucosa
  - Positive for focal specialized intestinal epithelium
  - Negative for dysplasia
  - Minimal inflammation
PAS Reaction

- **Purpose** – Demonstration of polysaccharides, neutral mucosubstances, and basement membranes
- **Fixative** – 10% neutral-buffered formalin or Bouin solution. Blood smears should be fixed in methyl alcohol for 10 to 15 minutes
- **Control** – Kidney
- **Reagents:**
  1. Periodic Acid
  2. 1N Hydrochloric acid
  3. Potassium metabisulfite
  4. Basic Fuchsin
- **Results:** Glycogen, neutral mucosubstances, certain epithelial sulfomucins and sialomucins, colloid material of the thyroid and pars intermedia of the pituitary, basement membrane, and fungal walls show a positive PAS (bright rose) reaction
PAS Technical Notes

• The absence of staining does not necessarily mean that carbohydrate residues are absent
• Fast green counterstain – fungus
• Metabisulfite rinses after Schiff’s is recommended
• Washing in tap water very important
• Cannot use Glutaraldehyde as a fixative
• Always use a control slide
• Chromate-containing fixative not recommended
• Do not use liver as a control
• Periodic acid is recommended as the oxidizer (must be fresh)
• If the schiff’s is not colorless do not use (no not use more than twice)
• Hematoxylin is often used as the counterstain
PAS Reaction With Diastase Digestion

- **Purpose:** Demonstration of glycogen in tissue sections
- **This is a very sensitive histochemical method for glycogen. Diastase and alpha-amylase act on glycogen to depolymerize it into smaller sugar units that are washed out of the section.**
- **Fixative:** 10% neutral-buffered formalin, formalin alcohol, or absolute alcohol
- **Quality Control:** 2 controls are used one with diastase and one without diastase
PAS With Diastase Reagents

- Periodic acid
- Schiff Reagent
- Potassium metabisulfite
- Malt Diastase Solution
PAS with Diastase

Results

• Glycogen will stain bright rose red on the section labeled “without” and will be absent from the section labeled “with”
PAS with Diastase
Technical Notes

• Human saliva can be used instead of alpha-amylase
• Use only recommended temperatures and recommended times
• This procedure may not work on tissue sections fixed in picric acid-containing fixatives
• Cervix is an excellent control
Pas control
Pas with diastase
Best Carmine

- Purpose: Demonstration of glycogen
- Principle: Not as specific as the PAS method but more sensitive
- Fixative: Absolute alcohol is preferred; Carnoy and Bouin solution may also be used
Best Carmine Reagents

- Carmine Stock Solution (Carmine, Potassium carbonate, Potassium chloride, distilled water)
- Working Carmine solution (Stock carmine, 28% ammonium hydroxide, methyl alcohol)
- Differentiating solution (Absolute ethyl alcohol, methyl alcohol, distilled water)
Best Carmine Results

- Glycogen: Pink to red
- Nuclei: Blue
Best Carmine Technical Notes

• More specific than PAS
• Do not use autopsy liver as a control
• Must filter before use
• Ammonium hydroxide is a severe eye and respiratory irritant and is also corrosive and must be prepared and used in a chemical hood.
Mayer Mucicarmine Stain

• Purpose: Staining of “epithelial” mucin in tissue sections
• Principle: It stains carboxylated and sulfated mucins, but not neutral mucins.
• Fixative: 10% neutral-buffered formalin
• Control: Unautolyzed colon, small intestine or appendix
Mayer Mucicarmine Results

- Mucin: Deep rose to red
- Capsule of Cryptococcus: Deep rose to red
- Nuclei: Black
- Other tissue elements: Blue or yellow
Mayer Mucicarmine Reagents

• Mucicarmine Stock Solution (carmine, alum lake, aluminum Hydroxide, ethyl alcohol, Aluminum chloride)
• Mucicarmine Working solution (stock solution, distilled water)
• Weigert Iron Hematoxylin
• Metanil Yellow (counterstain)
Mayer Mucicarmine Technical Notes

• Do not over-stain with hematoxylin or metanil yellow
• Use Weigert Iron Hematoxylin
• Stock mucicarmine has a short shelf life
• No not use autolyzed tissue as a control
• Can be used to demonstrate Cryptococcus neoformans
• Mucins is a term used to describe the intracellular secretions of various cells, and although these secretions appear to be microscopically similar, they differ slightly in composition.
• Stock solutions should be prepared under a hood.
Mucicarmine
Mucicarmine Stain
Alcian Blue, pH 2.5

- **Purpose:** Demonstration of acid mucopolysaccharides
- **Principle:** Alcian blue is believed to form salt linkages with the acid groups of acid mucopolysaccharides
- **Fixative:** 10% neutral formalin or Bouin solution
- **Control:** A section of unautolyzed small intestine, appendix, or colon should be used as a positive control
Alcian Blue, pH 2.5
Reagents

• Acetic Acid, 3%
• Alcian Blue, 1% solution
• Nuclear-Fast Red
Alcian Blue, pH 2.5

Results

• Hyaluronic acid  Dark Blue
• Sialomucins  Dark Blue
• Background  Pink to red
• Nuclei  Red
Technical Notes

• Alcian blue certified by the Biological Stain Commission should be used
• Dye lots must be documented and Staining time adjusted if necessary
• Rinsing with acid after the alcian blue solution will help prevent nonspecific staining
• Complete hydration is critical
• Over-staining will cause nuclear staining
• Wash well after nuclear fast red
Alcian blue, pH 1.0

• Purpose: Demonstration of sulfated mucosubstances

• Principle: At a pH of 1.0 alcian blue stains only sulfated acid mucopolysaccharides and sulfated sialomucins (glycoproteins)

• Fixative: 10% neutral Buffered Formalin or bouin solution

• Quality control: A section of unautolyzed small intestine, appendix, or colon should be used as a positive control
Alcian blue, pH 1.0
Reagents and results

- 0.1 N Hydrochloric Acid solution
- 1% Alcian Blue Solution, pH 1.0

- Sulfated mucosubstances: Pale blue
- Background: Pink to red
- Nuclei: Red
Alcian Blue Hyaluronidase

• Purpose: Differentiation of epithelial and connective tissue mucins
• Principle: Glycoporteins (“epithelial” mucins) will not be digested when treated with hyaluronidase
• Fixative: 10% neutral buffered formalin
• Quality control: 2 sections of umbilical cord should be used as a control (“with” and “without”). A section of small bowel, appendix, or colon may be used as a second control to demonstrate epithelial mucins.
Alcian Blue with Hyaluronidase Reagents and Results

- 0.1 M Potassium Phosphate, Monobasic
- 0.1 M Sodium Phosphate, Dibasic
- Buffer Solution
- Hyaluronidase Digestion solution
- Alcian Blue Staining Solution

Without digestion = acid mucopolysaccharides and sialomucins: Deep blue

With digestion = mucosubstances containing hyaluronic acid and chondroitin sulfates A and C – Marked loss of staining
AB PASH
Alcian Blue-PAS-Hematoxylin

• Purpose: Differentiation between neutral and acidic mucosubstances; this procedure is used in many laboratories today for the detection of intestinal metaplasia

• Principle: Acidic mucosubstances are stained with the alcian blue technique and neutral mucosubstances are stained by the PAS reaction.

• Quality Control: Use a kidney or a mucin control, depending on the diagnostic tissue to be stained. A section of cervix containing both endocervix and ectocervix also provides a good control.
AB PASH Reagents

- Acetic Acid, 3%
- Alcian blue
- Periodic Acid
- Sodium metabisulfite
- Schiff Reagent
- Harris Hematoxylin
AB PASH RESULTS

• Exclusively acid mucosubstances  Blue

• Neutral polysaccharides  Magenta

• Certain substances will be colored by both PAS and alcian blue  Purple
Colloidal Iron
Muller-Mowry Colloidal Iron

• Purpose: Demonstration of carboxylated and sulfated mucopolysaccharides and glycoproteins
• Principle: Colloidal ferric ions are, at a low pH, absorbed principally by carboxylated and sulfated mucosubstances. The excess reagent is washed out and the classic Prussian blue reaction is used to demonstrate iron bound to the tissue.
• Quality control: A section of unautolyzed small bowel, appendix, or colon may be used as a control
Colloidal Iron Reagents

- Ferric Chloride, 29%
- Colloidal Iron (Stock solution)
- Working Colloidal Iron solution
- Potassium Ferrocyanide Solution, 2%
- Hydrochloric Acid, 2%
- Ferrocyanide-Hydrochloric Acid solution
- Acetic Acid, 12%
- Nuclear-Fast Red solution
Colloidal Iron Results

- Acid mucopolysaccharides and sialomucins: Deep Blue
- Nuclei: Pink-red
- Cytoplasm: Pink
Colloidal Iron Technical Notes

- Strongly acid mucins do not stain
- PAS stain can be used as a counterstain
- Colloidal iron is not considered specific for acid mucopolysaccharides
- Hyaluronidase digestion can be used
- Excellent for demonstration of cryptococcus neoformans
Amyloid
Amyloid

- Amyloidosis is a disease characterized by an amorphous, eosinophilic, extracellular deposit that gradually replaces cellular elements of vital organs and causes progressive loss of function and eventual death. The composition of amyloid varies between patients and between organs in the same patient.
- Carbohydrate components may or may not be present.
- A useful reaction for distinguishing the homogeneous deposits of amyloid form other homogenous deposits in gross tissue employs an iodine solution.
Four Major Patterns of amyloid distribution

1. Deposits may be found in the tongue, heart, gastrointestinal tract, skeletal and smooth muscles, nerves, skin, and carpal ligaments. Types 1 and 3 tend to have this localization pattern.

2. Deposits may involve the liver, spleen, kidneys, and adrenals, Types 2 and 4 tend to have this pattern.

3. Mixed distribution involving areas of the above two patterns

4. Localized distribution involving a single tissue or organ
Alkaline Congo Red Method

• Purpose: The demonstration of amyloid in tissues
• Principle: Green birefringence following Congo red staining is considered the most specific technique for the demonstration of amyloid.
• Fixative: alcohol or Carnoy solutions is preferred, 10% neutral-buffered formalin, Bouin solution, or Zenker solution may be used.
• NOTE: Cut paraffin sections at 8 to 10 microns
• Quality control: Sections containing amyloid must be used and prolonged storage of control slides will result in loss of stainability.
Congo Red Reagents and Results

- Stock 80% alcohol Saturated with Sodium Chloride
- Alkaline Salt solution
- Stock congo Red Staining solution
- Working congo Red Staining solution

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloid</td>
<td>Deep pink to red</td>
</tr>
<tr>
<td>Elastic tissue</td>
<td>Pale pink</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Blue</td>
</tr>
</tbody>
</table>
Congo Red Technical Notes

- Bright apple-green birefringence is exhibited with polarized light
- The thickness of the section is critical
- Differentiation is avoided
- False-positive staining may be obtained
- The sodium chloride and the high alcohol content present in the dye solvent tend to depress dye ionization
- Instructions must be followed exactly
Crystal Violet
Crystal Violet

• **Purpose:** This is a good rapid screening method for amyloid but is not as specific as the Congo red method

• **Principle:** The metachromatic staining of amyloid is because of the mucopolysaccharide content.

• **Fixative:** 10% neutral buffered formalin

• **Quality control:** A section containing amyloid that has been freshly cut
Crystal Violet reagents and results

- Stock Saturated Crystal Violet Solution
- Working Crystal Violet solution
- Modified Apathy Mounting Medium

Amyloid: Purplish Violet
Other Tissue elements: Blue
Crystal Violet Technical Notes

• Must use correct mounting media or “Bleeding will occur”
• Stained sections should be air dried
• Stain my take overnight for reaction to occur
Thioflavine T Fluorescent Method
Thioflavine T Fluorescent Method

• Purpose: The demonstration of Amyloid
• Principle: Thioflavin T is a fluorescent dye that attached to amyloid. The background nuclear fluorescence is quenched by staining with aluminum hematoxylin
• Fixative: 10% neutral buffered formalin
• Quality control: A freshly cut section containing amyloid
Thioflavine T Reagents and results

• Thioflavin T, 1%
• Acetic Acid, 1%
• Mayer Hematoxylin

Amyloid: Fluoresces yellow to yellow/green
Thioflavin T Technical Notes

- A pH of 1.4 is recommended
- Fluorescent microscope is required with the correct filters
- Sections are mounted from water
- Lipid granules, juxtaglomerular granules, and mast cells may give a yellow fluorescence, but should be differentiated easily from amyloid.
CPT Codes

• 88312- Special stain including interpretation and report (eg. Acid fast, methenamine silver, stains for organisms)
• 88313- Special stains all others (eg. Iron, trichrome, amyloid, ABPAS, PASH)
The End