Special Stains, Immunohistochemistry and Genomic Testing of the Liver:

A Practical Guide for the Practicing Pathologist

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Overview

• No conflicts of Interest
Overview

• Up-front stains (ordered automatically)
• Stains in medical liver biopsies, general guidelines
• Stains in tumor biopsies, general guidelines
• Molecular tests
Up-Front Stains

- Most common stains ordered automatically on medical liver biopsies:
  - H&E
  - Iron
  - PASD
  - PAS
  - Reticulin
  - Trichrome (or Sirius Red)
Up-Front Stains

• All of these stains are useful,
Up-Front Stains

- All of these stains are useful, but
  **They are not equally useful**
Up-Front Stains

• All of these stains are useful, but

  They are not equally useful

• One example, many centers manage very well without:
  – PAS
  – Reticulin
Up-Front Stains

• Second example, sometimes iron and PASD stains are non-contributory
  – such as post transplant liver biopsies

• There are no validated, published guidelines for which stains to order automatically

• The stains used in any center are based on local preferences and history
Up-Front Stains

Next:

some general observations
Up-Front Stains

• H&E: Needed, all agree
Up-Front Stains

• H&E: Helpful in **all** biopsies
Up-Front Stains

• H&E: Helpful in all biopsies
• Trichrome: Helpful in nearly all biopsies
Up-Front Stains

• H&E: Helpful in all biopsies

• Trichrome: Helpful in almost all biopsies
  – One could argue a Trichrome stain is less useful when the H&E shows established cirrhosis
Up-Front Stains

• H&E: Helpful in all biopsies
• Trichrome: Helpful in almost all biopsies
• Iron stain: Helpful in most biopsies
Up-Front Stains

• H&E: Helpful in **all** biopsies
• Trichrome: Helpful in **almost all** biopsies
• Iron stain: Helpful in **most** biopsies
  – An alternative approach:
    • Order iron stains on biopsies with visible pigment visible on H&E, or abnormal serum Fe testing
      – This approach **will miss** minimal or mild patchy iron in hepatocytes or Kupffer cells
      – But there is **little/no clinical consequence** to very small amounts of iron in the liver
    • More details in hand out
Up-Front Stains

• H&E: Helpful in **all** biopsies
• Trichrome: Helpful in **almost all** biopsies
• Iron stain: Helpful in **most** biopsies
• PASD stain: Helpful in **many** biopsies
Up-Front Stains

• H&E: Helpful in **all** biopsies
• Trichrome: Helpful in **almost all** biopsies
• Iron stain: Helpful in **most** biopsies
• PASD stain: Helpful in **many** biopsies
  – A1AT globules can be hard to see on H&E
  – PASD doesn't add much to follow-up biopsies in individuals with chronic liver disease
Up-Front Stains

• H&E: Helpful in all biopsies
• Trichrome: Helpful in almost all biopsies
• Iron stain: Helpful in many biopsies
• PASD stain: Helpful in many biopsies
• Reticulin Stain: Helpful in some cases
Up-Front Stains

- H&E: Helpful in **all** biopsies
- Trichrome: Helpful in **almost all** biopsies
- Iron stain: Helpful in **many** biopsies
- PASD stain: Helpful in **many** biopsies
- Reticulin Stain: Helpful in **some** cases
  - Main role in non tumor pathology is nodular regenerative hyperplasia
  - Stain could be ordered based on H&E findings and clinical findings
Up-Front Stains

• H&E: Helpful in all biopsies
• Trichrome: Helpful in almost all biopsies
• Iron stain: Helpful in many biopsies
• PASD stain: Helpful in many biopsies
• Reticulin stain: Helpful in some cases
• PAS stain: Helpful in rare cases
Up-Front Stains

- H&E: Helpful in all biopsies
- Trichrome: Helpful in almost all biopsies
- Iron stain: Helpful in many biopsies
- PASD stain: Helpful in many biopsies
- Reticulin stain: Helpful in some cases
- PAS stain: Helpful in rare cases
  - Primarily in working up intra-cytoplasmic globules
  - Limited value as an up front stain
Up-Front Stains

- H&E
- Trichrome
- Iron
- PASD
- Reticulin
- PAS
Up-Front Stains

H&E
Trichrome
Iron
PASD
Reticulin
PAS

All cases
Up-Front Stains

H&E
Trichrome
Iron
PASD
Reticulin
PAS

All biopsies
Baseline biopsies
Others, PRN
Up-Front Stains

<table>
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<tr>
<th>Stain</th>
<th>Application</th>
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<tr>
<td>H&amp;E</td>
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<td>Baseline biopsies</td>
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<td><strong>Reticulin</strong></td>
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Special Stains for the Medical Liver Biopsy

• In your handout:
  – A comprehensive list of *almost* all of the stains I have ever used, with some annotation
  – I cannot cover them all today
  – We’ll touch on just a few
    • Focused on the most commonly used stains
Trichrome
Trichrome stain

Fibrosis Stage

Many different staging systems
Staging schemas are similar

- Essentially all are based on the following conceptual stages:
  - No fibrosis
  - Portal fibrosis (AKA periportal fibrosis)
  - Bridging fibrosis
  - Cirrhosis
Staging schemas are similar

Staging systems vary only in how they subdivide these conceptual stages
General Comparison of the Most Commonly Used Staging Systems

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<th>HAI</th>
<th>METAVIR</th>
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Trichrome stain

• Giving a “F” number is **not necessary** for clinical care
  – But if you or your clinical colleagues like them, they are perfectly fine to use
• All work pretty well for clinical care
• See handout of more details
Adequacy

• Adequacy:
  – General rule of thumb for clinical care:
    • **Bigger** is better
    • At least 1 cm and or 10 portal tracts
    • Smaller sized biopsies tend to understage
Staging pitfalls

Pitfalls:
A few make up most (~80%)
Staging Pitfalls: Portal Fibrosis

Marked portal inflammation or ductular reaction
Staging pitfalls: bridging fibrosis

- Bridging necrosis
Staging pitfalls: Cirrhosis

• Intact cores are better than fragmented cores
  – Fragmented biopsy does not equal cirrhosis
  – *Very fragmented* specimens are associated with cirrhosis
    • Specimens with >12 fragments in one study
  – Stage *what you see*, indicate limitations
    • Don’t “upstage” what you cant see because of fragmentation
Iron Stain
Hepatic Iron

- Perls iron stain
- Interpretation is typically straightforward
- See handout for more details
  - Quantitative iron analysis
  - Hepatic iron index
Hepatic Iron

“Guidelines” on when to suggest genetic testing

\(H(a)\)emochromatosis

Based on

iron stain results
Hepatic Iron

“Guidelines” on when to suggest genetic hemochromatosis testing

5 rules
Hepatic Iron

1. Work up for neonatal hemochromatosis
   – Neonate or stillbirth with
     • Marked liver injury/necrosis
     • Hepatic iron
     • *extrahepatic* iron deposits

(no known genetic tests, but can prevent future pregnancy problems with IgG)
Hepatic Iron

2. Mild iron
   - Age less than 40
   - No fibrosis
   - No other history/chronic liver disease to explain the iron
3. Moderate or marked iron
   - Any age
   - Non-cirrhotic liver
   - Can have chronic liver disease, eg HCV, NASH
   - No history of transfusion dependent anemia
Hepatic Iron

4. Marked iron in any liver
   – regardless of fibrosis stage
   – Regardless of age
   – Can have chronic liver disease, eg HCV, NASH
     • With no history of transfusion dependent anemia
Hepatic Iron

Summary of rules 2-4
(assumes no history of transfusion dependent anemias)

Iron levels
Marked:  All
Moderate:  All non-cirrhotic livers
Mild: Only if young, non-cirrhotic, and no chronic liver disease on biopsy (eg hepatitis, NAFLD, etc)
Hepatic Iron

5. **Kupffer cell** predominant iron with history of **elevated ferritin** and **low saturation levels**
   - suggest evaluation for ferroportin disease
Using other stains: ? Cholestatic liver disease

- Evidence for chronic cholestatic disease
  - CK7 (looking for intermediate hepatocytes, which is seen as staining of periportal hepatocytes)
  - Copper (periportal copper deposition)
  - These stains are useful only on non-cirrhotic livers
Immunohistochemical stains for liver tumors
Basik Approach to Diagnosis

Hepatocellular Carcinoma
Basic Approach to HCC

Let's say you have a biopsy of the liver

You examine the biopsy carefully

You identify a possible mass
Basic Approach to Diagnosis

You’re ready to take it to the next level
Basic Approach to Diagnosis

Immunohistochemistry!
Basic Approach to Diagnosis

OK! But answer this question **FIRST:**

Based on H&E findings, which of the following is true:

A. The tissue is all liver, but I’m not sure if some of it is cancer.
B. The tissue has definite cancer, but I’m not sure if its HCC or metastatic.
Basik Approach

Get this First question right!

(Getting it wrong leads to stains that aren't needed—often limiting the quality of the final diagnosis)
Basic Approach

Answer A. The biopsy is all liver, but I’m not sure if there is cancer

• *Reticulin stain*
• Ki-67
• Glypican 3
• CD34

• Consider others depending on the H&E differential; eg Glutamine synthetase for FNH

This list is in your handout
B. The tumor is definitely cancer, but is it HCC?

- HepPar1
- Glypican 3
- Arginase 1
- Albumin in situ hybridization
- CD10 or pCEA
- AFP

This list is in your handout
Basic Approach

Special stains; sensitivity and specificity

HepPar1 vs Glypican 3 vs Arginase 1

• These “big 3” are the most widely used
• Their absolute performance in any study depends on
  • Tumor grade
  • Underlying liver disease
  • Hepatocellular carcinoma morphologies included in study

All perform best with moderately differentiated tumors
  – For poorly differentiated tumors, I prefer a panel
Laws of IHC for HCC

Immutable laws of IHC
Laws of IHC for HCC

No. 1. The first paper is the best.

The sensitivity and specificity of a stain invariably falls as more papers are published.
Laws of IHC for HCC

No. 2. Immunostains are most powerful when used with H&E
Laws of IHC for HCC

No. 3. If H&E fits poorly with IHC, resolve with additional studies
Example

• 74 year old female
• 4.3 cm single liver mass
• Needle bx
HepPar
Outside hepPar control
(on same slide as tissue)
Work-up

Because the submitted HepPar was negative, I worked up for metastatic clear cell carcinoma.

– The only stain (from a long list) that was positive was cam5.2.
Repeat HepPar
Arginase
Diagnostic pitfall: entrapped hepatocytes
No. 4. If you want to use a diagnostic stain, use it enough to be good at using it.
Laws of IHC for HCC

A difficult case is the wrong time for a stain you’re not familiar with.
Laws of IHC for HCC

1. Sensitivity/specificity fall as data increases.
3. If morphology and IHC disagree, resolve with additional studies.
4. If you want to use a diagnostic stain, use it often enough to be good at using it.
Immunostains for Prognosis

Immunohistochemical markers

CK19, worse
P53, worse
AFP, worse
Beta catenin, better

None of these have entered routine clinical care at most USA and Canada centers (yet).

– CK19 is more widely used in Europe
Immunostains for Carcinoma subtype

• Fibrolamellar carcinoma
  – High level of over diagnosis
    • Up to 20%, even in articles published in top level specialty journals
  – Special stains can resolve almost all of these cases, and maybe all of them
    • All fibrolamellar carcinomas are CD68 and CK7 positive
    • If a tumor is negative for either, the odds are extraordinarily low that your diagnosis of fibrolamellar carcinoma is correct
Molecular Tests

• Molecular tests for diagnosis
  – Fibrolamellar carcinoma.
    • Highly sensitive and specific intra-chromosomal deletion leading to a DNAJB1-PRKACA fusion
    • If the tumor does not have this translocation, the odds are extraordinarily low that your diagnosis of fibrolamellar carcinoma is correct
    • Can detect by RT-PCR or FISH
Molecular Tests

• Molecular tests for therapy
  – Preliminary studies reporting VEGFA amplification is a useful marker for Sorafenib therapy.
    • Not been well validated

• FGFR2 translocations in cholangiocarcinoma
  – 15-45% of cases positive
  – Testing by RT-PCR or FISH
  – Predicts response to FGFR2 inhibitors