Laboratory tests in Wilson disease

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Copper Homeostasis and Physiology

- Copper is an essential trace mineral in human physiology.
- It acts as a cofactor of cupro-enzymes.
- In the blood, copper is bound to **ceruloplasmin,** the major copper-binding protein which can carry six copper atoms per protein (holoceruloplasmin).
- Copper loading of apoceruloplasmin (apoCp) is ATP7B protein dependent.
- Copper is absorbed in the intestine by the enterocytes and transported protein-bound by albumin or transcupreine to the sinusoidal plasma membrane in liver.

- Copper chaperones such as copper chaperones for superoxide dismutase (CCS) and antioxidant protein 1 (Atox1; donor for ATP7B) shuttle copper to specific intracellular targets.
- These targets are e.g. SOD1, ATP7A and ATP7B, respectively.
- In the normal hepatocyte under steady state conditions, ATP7B is predominantly located in the TGN, into which copper is transported for incorporation into apoCp to assemble enzymatically-active holoCp.
- The metal-binding domain in the nucleotide-binding domain of the Cp protein has six copper-binding sites, from which copper is accepted from Atox1 via protein-protein interaction.
- If levels of intracellular copper are high, ATP7B translocates its cellular compartment to biliary canalicular-associated structures and facilitates the process of copper excretion into the biliary tract.
- *ATP7B* is primarily expressed in the liver and its mutations cause

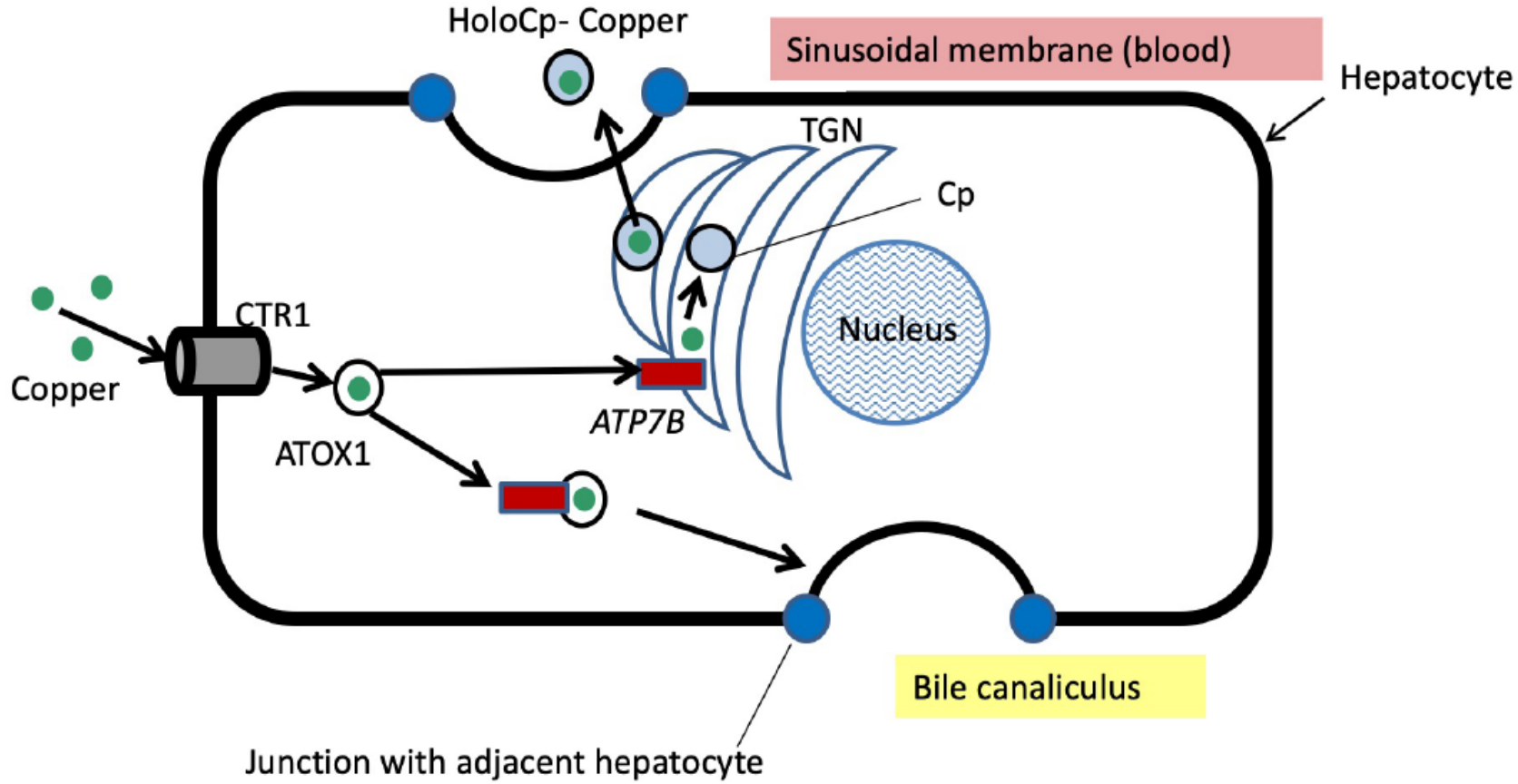


Figure 3. Copper homeostasis in the hepatocyte. Role of Wilson ATPase (ATP7B) in the hepatocellular disposition of copper: a hepatocyte is shown, with one side connected with the bile canaliculus, the other connected with the sinusoidal membrane. Starting at the left side of the diagram, copper (small green dots) is taken up by CTR1 (grey square), picked up and carried by ATOX1 to the Wilson ATPase (ATP7B; red square) in the trans-Golgi network (TGN). The Wilson ATPase either directs copper to production of caeruloplasmin (Cp; blue round shaped) or to excretion into bile. When intracellular copper concentrations are low or normal, the Wilson ATPase participates in the production of holocaeruloplasmin (HoloCp-Copper) in the Golgi apparatus; holocaeruloplasmin is then secreted into the blood. When intracellular copper concentrations are elevated, the Wilson ATPase expedites biliary excretion of copper.

Laboratory tests

- Liver enzymes
- CBC
- Ceruloplasmin
- Urinary Excretion of Copper
- Liver Biopsy with Quantitative Copper Determination
- Serum Copper and Non-Caeruloplasmin Bound Copper
- Exchangeable Copper and Relative Exchangeable Copper
- Genetic testing

Diagnostic testing for Wilson disease

Diagnostic test	Findings in Wilson disease	Comments
Slit lamp examination (or optical tomography)	▪ Kayser-Fleischer rings.	▪ Kayser-Fleischer rings are golden brownish rings that result from fine pigmented granular deposits of copper in Descemet's membrane in the cornea close to the endothelial surface.
24-hour urinary copper excretion	▪ A level >40 mcg/24 hours (>0.64 micromol/24 hours) is suggestive of Wilson disease and warrants further testing.	▪ To assess accuracy of the collection, we measure urinary creatinine excretion. ▪ 24-hour urine creatinine excretion should be between 15 and 20 mg/kg body weight. Values substantially above or below this estimate suggest over- and under-collection, respectively.
Serum ceruloplasmin	▪ Low level (<20 mg/dL [200 mg/L]).	▪ Ceruloplasmin is a 132-kd protein synthesized by hepatocytes and secreted into the circulation. ▪ Ceruloplasmin with bound copper is referred to as holoceruloplasmin (representing most of circulating ceruloplasmin), whereas ceruloplasmin that does not contain bound copper is referred to as apoceruloplasmin. ▪ Ceruloplasmin is an acute phase reactant and may be increased above basal levels with inflammatory states.
Serum copper concentration	▪ Typically low in proportion to the reduction in ceruloplasmin.	▪ In acute liver failure due to Wilson disease, copper may be markedly elevated (>200 mcg/dL [31.4 micromol/L]).
Liver biopsy	▪ Hepatic copper concentration is usually >250 mcg/g dry weight. ▪ Histologic findings may include fatty infiltration within hepatocytes, glycogen inclusions within nuclei, and portal fibrosis.	
Genetic testing	▪ Biallelic, pathogenic (disease-causing) variants affecting both ATP7B alleles are required for the diagnosis.	▪ Wilson disease is an autosomal recessive disorder and is the result of mutation in *ATP7B*, a gene encoding a copper transport protein, ATP7B.
Brain imaging	▪ MRI findings include abnormal T2 signals in the basal ganglia, brainstem, and white matter.	▪ Brain imaging may be normal in patients with Wilson disease who do not have neuropsychiatric involvement.

This table summarizes diagnostic testing for evaluating patients with suspected Wilson disease. This table is intended for use in conjunction with additional UpToDate content. For more details, please refer to UpToDate topics on clinical features and diagnosis of Wilson disease.

Table 1. Diagnostic scoring system developed at the 8th International Meeting on Wilson disease, Leipzig, 2001 (ref. 155).

Typical clinical symptoms and signs		**Other tests**	
Kayser-Fleischer rings		**Liver copper** (in the absence of cholestasis)	
Present	2	>250 µg (>4 µmol)/g dry weight	2
Absent	0	50–249 µg (0.8–4 µmol)/g	1
Neurologic symptoms*		Normal: <50 µg (<0.8 µmol)/g	-1
Severe	2	Rhodanine-pos. granules†	1
Mild	1	**Urinary copper** (in the absence of acute hepatitis)	
Absent	0	Normal	0
Serum caeruloplasmin		1–2 x ULN	1
Normal (>0.2 g/L)	0	>2 x ULN	2
0.1–0.2 g/L	1	Normal but >5 x ULN after D-penicillamine	2
<0.1 g/L	2	**Mutation analysis**	
Coombs-negative haemolytic anaemia		On both chromosomes detected	4
Present	1	On 1 chromosome detected	1
Absent	0	No mutations detected	0

TOTAL SCORE	**Evaluation**
4 or more	Diagnosis established
3	Diagnosis possible, more tests needed
2 or less	Diagnosis very unlikely

*Or typical abnormalities at brain magnetic resonance imaging; †If no quantitative liver copper available; ULN, upper limit of normal.

Serum aminotransferases

- In patients with Wilson disease, the serum aminotransferases are usually mildly to moderately elevated, and the aspartate aminotransferase (AST) concentration is usually higher than the alanine aminotransferase (ALT) concentration.

- AST to ALT ratio (often >1 in patients with Wilson disease)

Serum ceruloplasmin concentration

- Approximately 85 to 90 percent of patients with Wilson disease have low serum ceruloplasmin levels (<20 mg/dL or 200 mg/L)

- a serum ceruloplasmin concentration less than 20 mg/dL (200 mg/L) in a patient who also has Kayser-Fleischer rings is considered to be diagnostic of Wilson disease

- A very low serum ceruloplasmin concentration (<5 mg/dL or <50 mg/L) provides strong evidence for the diagnosis of Wilson disease.

- However, low ceruloplasmin levels can be seen in patients without Wilson disease, and normal or elevated ceruloplasmin levels may be seen in patients with Wilson disease

- Normal values for serum ceruloplasmin vary by age.
- They are very low during early infancy through approximately six months
- peak in early childhood (approximately 30 to 50 mg/dL or 300 to 500 mg/L)
- then decline to the adult range (20 to 35 mg/dL or 200 to 350 mg/L)

Limitations of serum ceruloplasmin

- a low ceruloplasmin level is not sufficient to make a diagnosis of Wilson disease, and a normal level does not rule out Wilson disease

- serum ceruloplasmin **alone has a low positive predictive value** in patients undergoing evaluation for liver disease

- Approximately 10 to 20 percent of **asymptomatic heterozygous carriers** have serum ceruloplasmin concentrations less than 20 mg/dL (200 mg/L).

- Other causes of **low serum ceruloplasmin** concentrations include:
  - Disorders that cause marked renal or enteric protein loss, such as nephrotic syndrome or protein-losing gastroenteropathy.
  - End-stage liver disease of any cause.
  - Rare diseases such as Menkes disease, aceruloplasminemia, and copper deficiency in patients receiving inadequate copper with parenteral nutrition.

Box 2
Reasons for a low level of serum ceruloplasmin

- Wilson disease
- Carrier (heterozygote) for Wilson disease
- Copper deficiency
- Nephropathy with loss of large proteins in the urine
- Enteropathy with loss of proteins in the gut
- Excess zinc ingestion
- Menkes disease or other *ATP7A* mutations
- Aceruloplasminemia or carrier state for this disorder

- On the other hand, serum ceruloplasmin concentrations may be normal or elevated in patients with Wilson disease.

- One cause of normal serum ceruloplasmin in patients with Wilson disease is the presence of **acute hepatitis**, which can increase serum ceruloplasmin values to the normal range.
- Other causes include **pregnancy**, **estrogen supplementation**, **and use of oral contraceptives**.
- In addition, ceruloplasmin is **an acute phase reactant**, so levels may be elevated in the

- In addition, **the method** used for measuring ceruloplasmin may influence the results.
- Serum ceruloplasmin can be measured enzymatically, by antibody-dependent assays, by radial immunodiffusion, and by nephelometry.
- The results are generally similar, except for the antibody-dependent and the immunodiffusion assays, which may **overestimate** the ceruloplasmin levels.
- The overestimation may occur because the two testing methods **do not discriminate between apoceruloplasmin and holoceruloplasmin**

Urinary copper excretion

- Urinary copper excretion is useful for **the diagnosis** of Wilson disease and for **monitoring therapy.**

- Wilson disease is typically associated with 24-hour urinary copper excretion of **>100 mcg** (>1.6 micromol), although lower values have been described in **up to 25 percent** of asymptomatic patients with confirmed disease.

- Values >40 mcg/24-hours (0.64 micromol/24-hours) are **suggestive** of Wilson disease.

- Elevated urinary copper excretion may also be seen in patients with **other forms of chronic active liver disease** and **in heterozygotes for Wilson disease.**

- The 24-hour collection is begun at the usual time the patient awakens.
- At that time, the first void is discarded and the exact time noted.
- Subsequently, all urine voids are collected, with the last void timed to finish the collection at exactly the same time the next morning.
- The time of the final urine specimen should

- In order to assess the completeness of the collection, **the urinary creatinine excretion** should be measured.
- The 24-hour urine creatinine excretion should be **between 15 and 20 mg/kg** body weight.
- Values substantially above or below this estimate **suggest over- and under-collection**, respectively, and should call into question the accuracy of the 24-hour urinary copper excretion result.
- The test should **not be used in patients with renal failure**.
- **Spot urine collections are highly variable** and are not reliable for making a diagnosis of Wilson disease.

- Normal values for urinary copper excretion vary among laboratories but are in the range of ≤30 to 40 mcg/day (0.48 to 0.64 micromol/day).

- A value >40 mcg/day (0.64 micromol/day) warrants further investigation.

- Care should be taken to avoid copper contamination of the urine collection containers. The container should be rinsed with **distilled (not tap) water**, or a new, disposable container should be used.

- Prior to analysis, a small amount of hydrochloric acid (30 mL of 6N solution) is usually added to the urine **to prevent precipitation of copper hydroxide**, which occurs when the urine is alkaline and may result in falsely-low urinary copper concentrations.

Penicillamine challenge

- Because urinary copper excretion can be increased in a variety of liver diseases, penicillamine challenge has been proposed as a means to **increase sensitivity and specificity**.

- Penicillamine greatly increases urinary copper excretion in patients with Wilson disease, and to a lesser extent, in patients with other forms of liver disease.

- has not been well-standardized in adults

- However, the penicillamine challenge has been **standardized in children**, where it can be used as an adjunctive test.

- In children, the test could be helpful in cases:
  - where urinary copper excretion is near the upper limit of normal but the suspicion for Wilson disease is high,
  - or conversely, in cases where the urinary copper excretion is mildly elevated but an alternative diagnosis is likely.

- The penicillamine challenge is performed by giving a 500 mg dose of penicillamine (regardless of the patient's weight) at the beginning of the 24-hour urine collection and then again at 12 hours.

- Urinary copper excretion **greater than 1600 mcg** per 24-hours (>25 micromol) is much more likely in Wilson disease compared with other types of liver disease.

Liver biopsy

- A liver biopsy permits:
  - the quantification of the hepatic copper concentration
  - the examination of liver histology, including staining for copper.

- Liver biopsies in patients suspected of having Wilson disease should be at least 1 cm in length and should be placed in a dry, copper-free container.

Hepatic copper concentration

- Quantitative hepatic copper determination in patients with Wilson disease usually reveals **more than 250 mcg** (4 micromol) of copper per gram of dry weight (normal <50 mcg [0.8 micromol] per gram of dry weight).

- This is generally considered to be **the gold standard for diagnosis.**

- Normal hepatic copper concentrations have been described in patients with Wilson disease.

- There are several possible explanations for this:
  - Reports describing such patients may have been limited by use of older laboratory equipment and use of insufficiently sized specimens.
  - There can be **variability in copper distribution within the liver,** contributing to sampling error (particularly among patients with cirrhosis).
  - In patients with **acute liver failure,** massive release of copper from necrotic hepatocytes can lead

- Because of potential errors in evaluation of hepatic copper concentration, the hepatic copper concentration should always be evaluated **in the context of other diagnostic criteria.**

- Wilson disease is **not** excluded based solely on a hepatic copper concentration less than 250 mcg/g (4 micromol/g) since **approximately 15 percent of Wilson disease patients fall below this cutoff.**

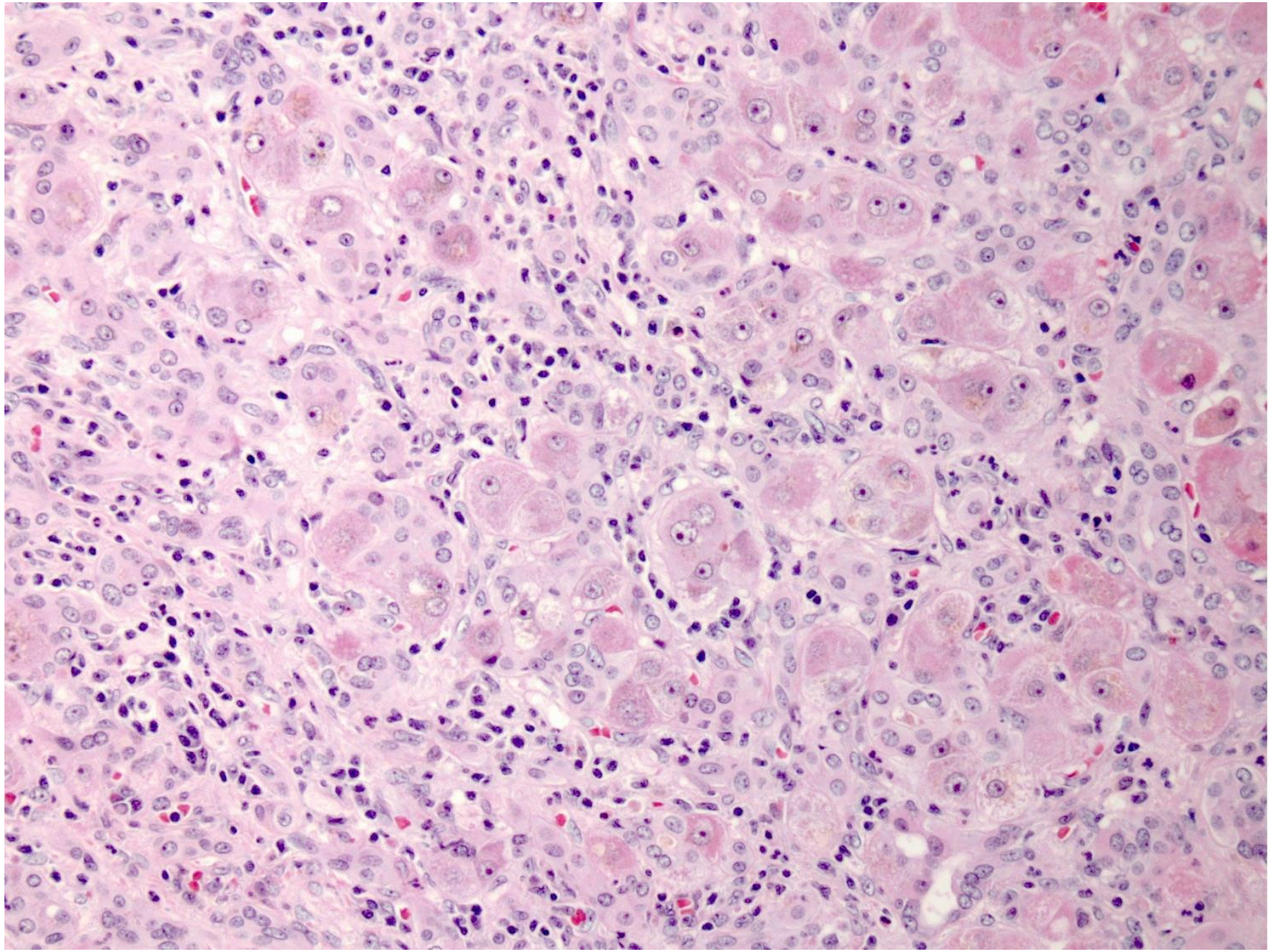
- On the other hand, a copper concentration greater than 250 mcg/g is virtually diagnostic **unless the patient has chronic cholestatic liver disease,**

Liver histology

- The earliest lesions in Wilson disease are seen **in the liver**, the site of initial copper accumulation.
- Histologic findings in patients with Wilson disease are similar to those of **autoimmune hepatitis and nonalcoholic steatohepatitis (NASH).**
- Early findings include **fatty infiltration** within hepatocytes, **glycogen inclusions within nuclei**, and **portal fibrosis.**
- At the time of diagnosis, cirrhosis is seen in 35 to 45 percent of patients.
- A special stain for copper deposition (Rhodanine and orcein) can be suggestive of Wilson disease.
- However, **copper stains have limited sensitivity**, and the absence of copper staining does **not exclude** the diagnosis.

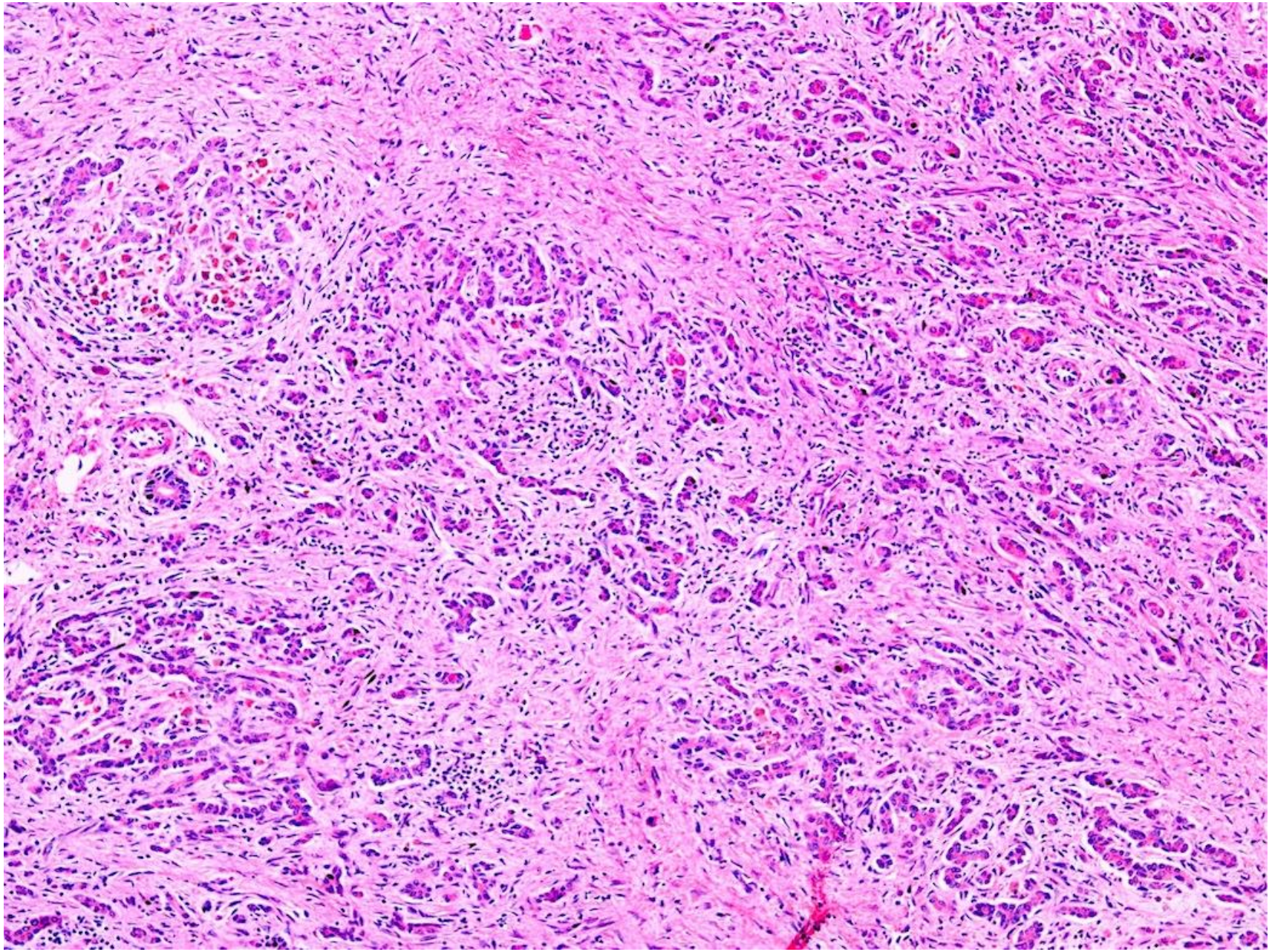
- Histologic features vary based on clinical presentation and stage.

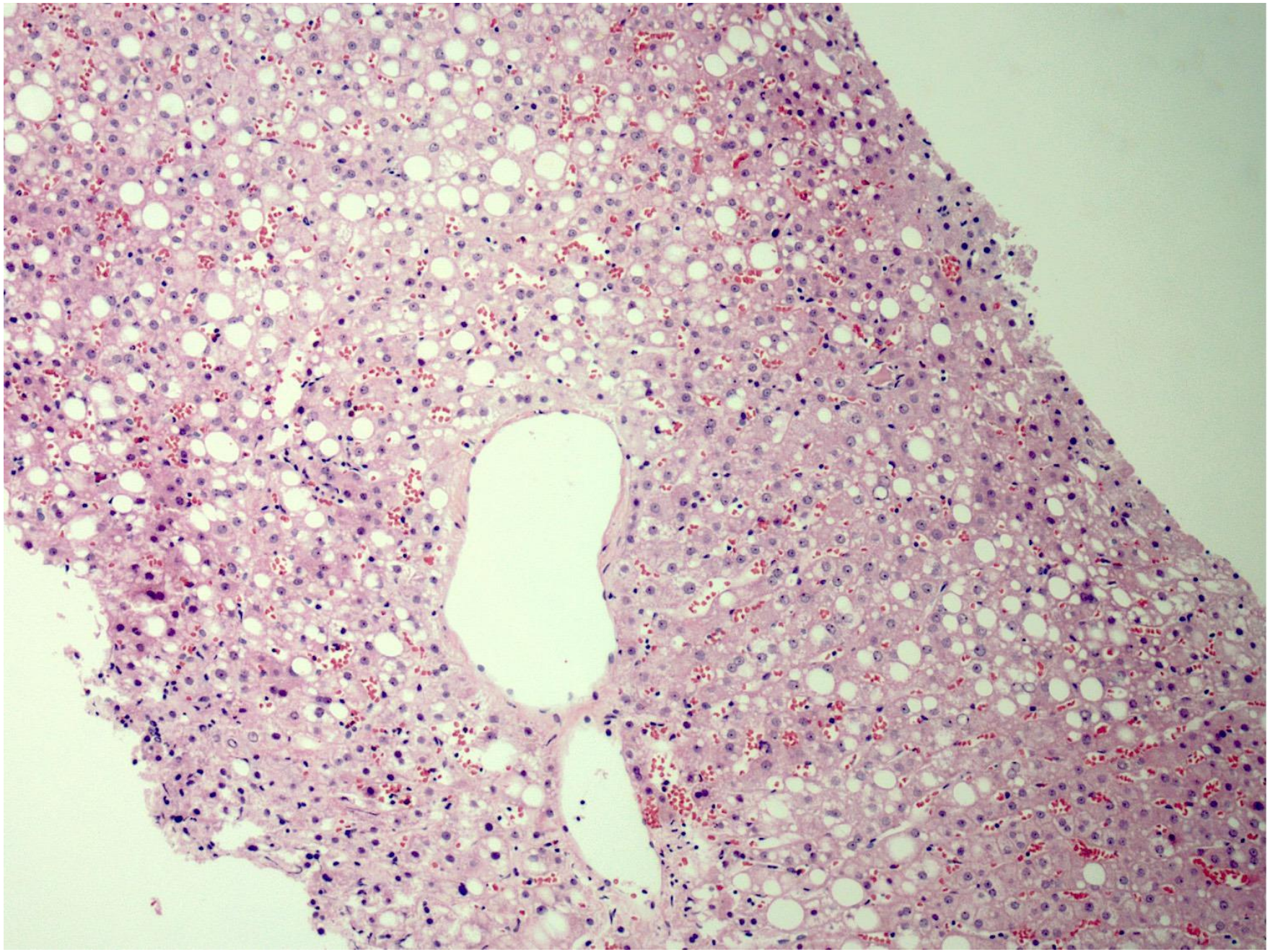
- Acute hepatitis:
  - Lobular and portal inflammation with lymphocytes and plasma cells
  - Can mimic autoimmune hepatitis
  - Hepatocyte ballooning mostly in periportal hepatocytes
  - Acidophil bodies
  - Acute cholestasis

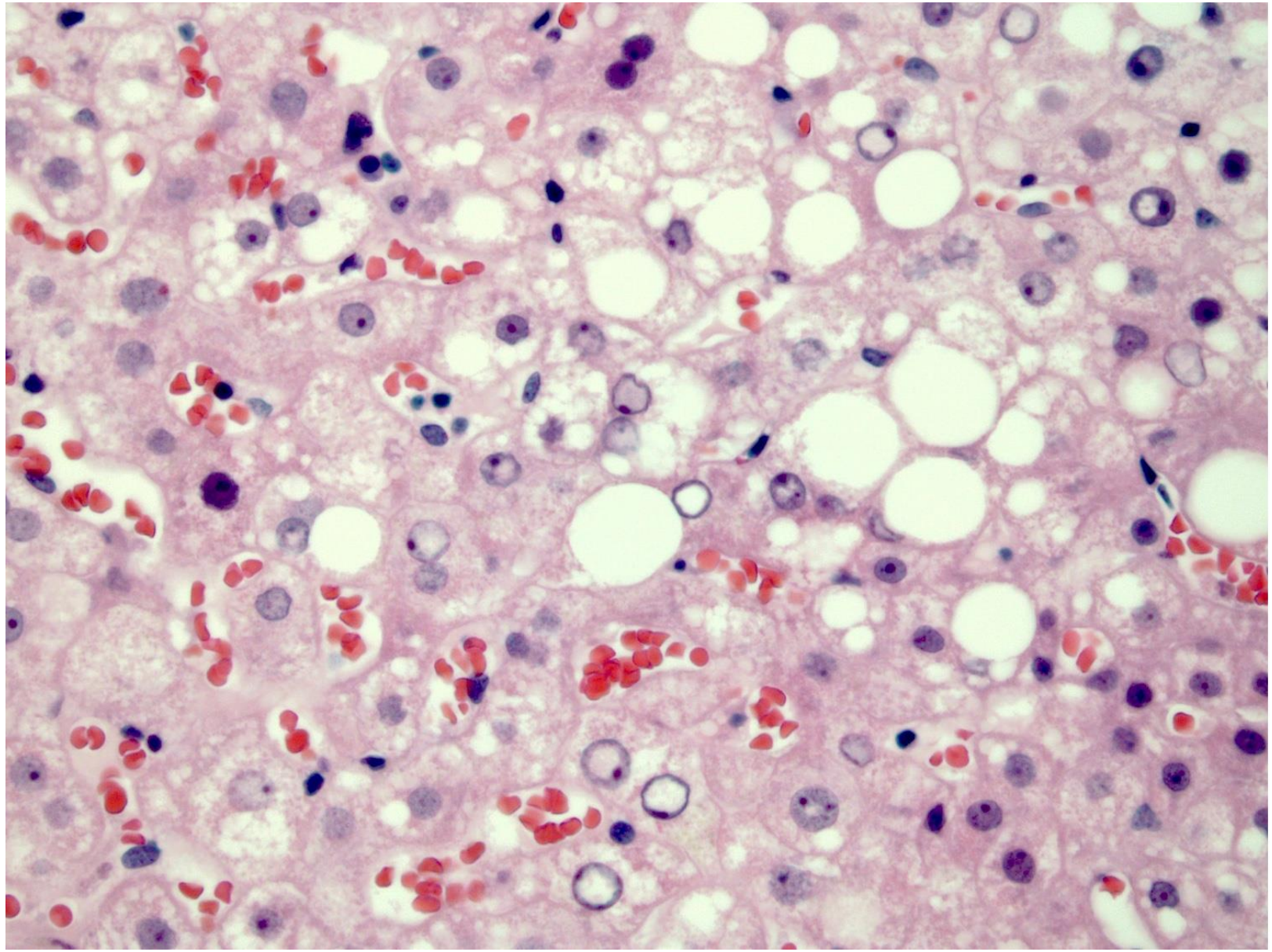


- Fulminant hepatitis：
  - Massive hepatocyte necrosis with parenchymal collapse
  - Hard to distinguish from other causes of fulminant hepatitis
  - Copper can be present in Kupffer cells or portal macrophages

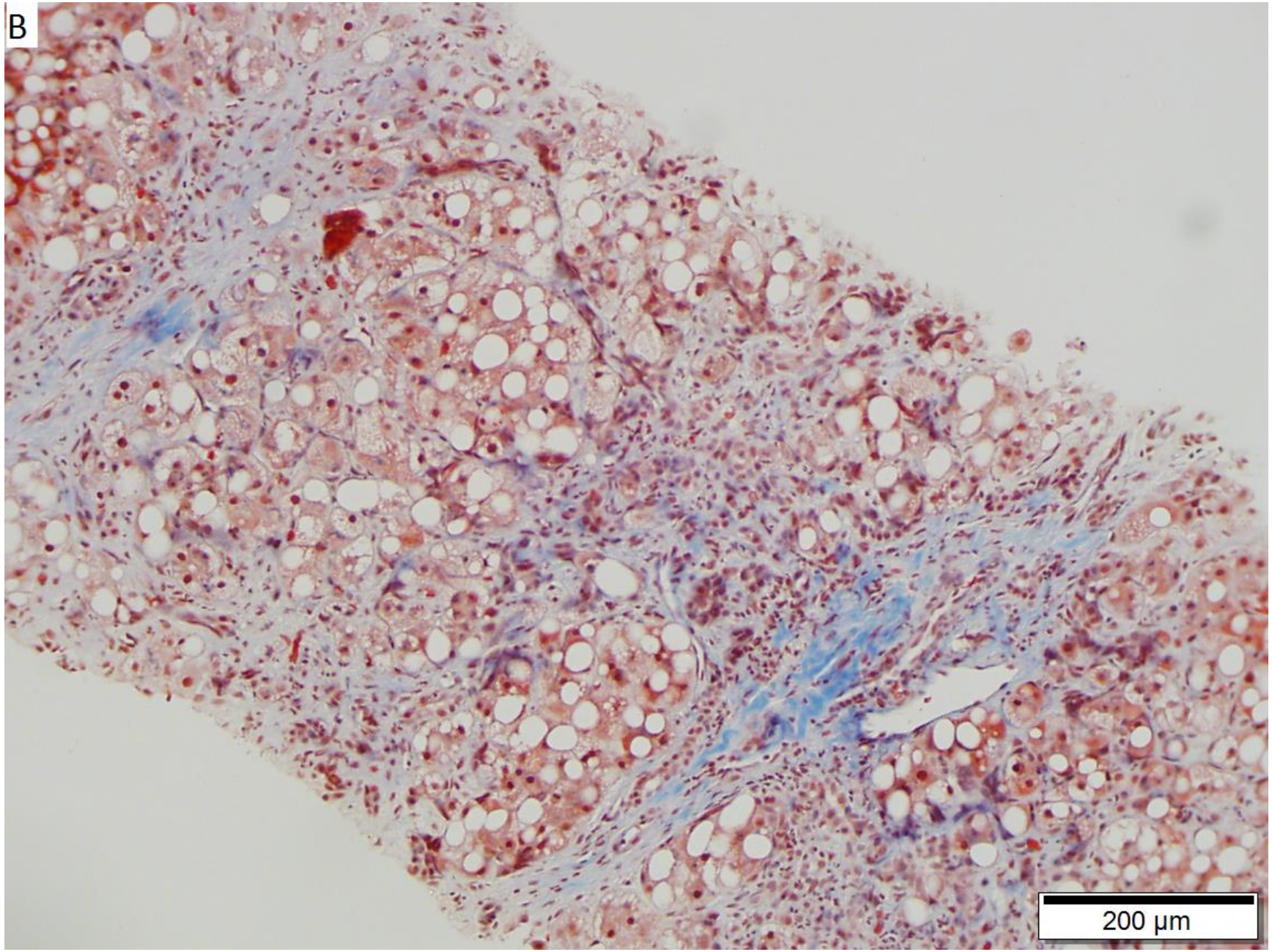
- Steatosis and steatohepatitis：
  - Large and small droplet macrovesicular steatosis
  - Glycogenated nuclei
  - Ballooning degeneration







- Chronic hepatitis:
  - Nonzonal, patchy steatosis
  - Mild chronic portal inflammation (predominantly lymphocytic with some plasma cells) with interface activity
  - Acidophil bodies
  - Periportal glycogenated nuclei
  - Periportal Mallory-Denk bodies (MDB)
  - Copper deposition within periportal hepatocytes
  - Hemosiderin pigment deposition, including Kupffer cells in a subset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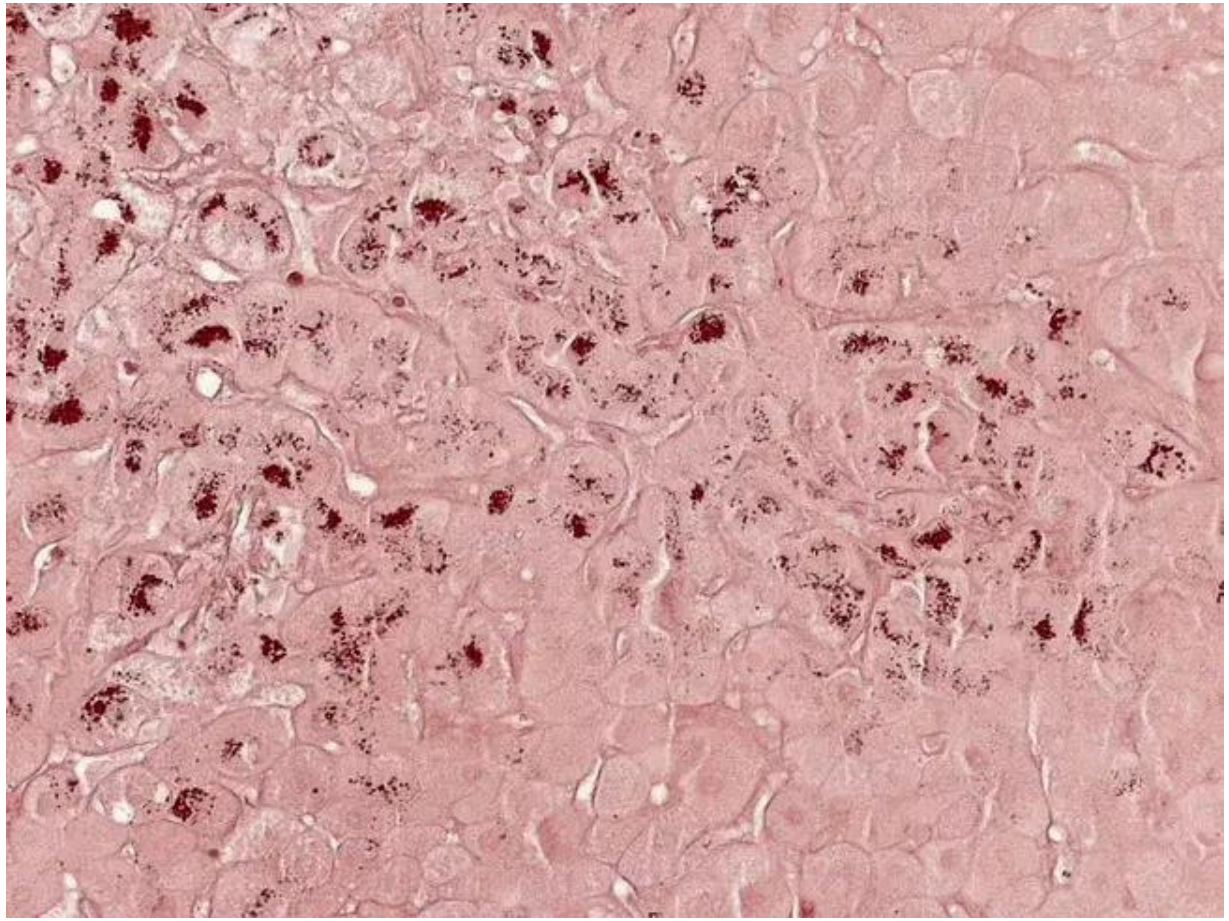
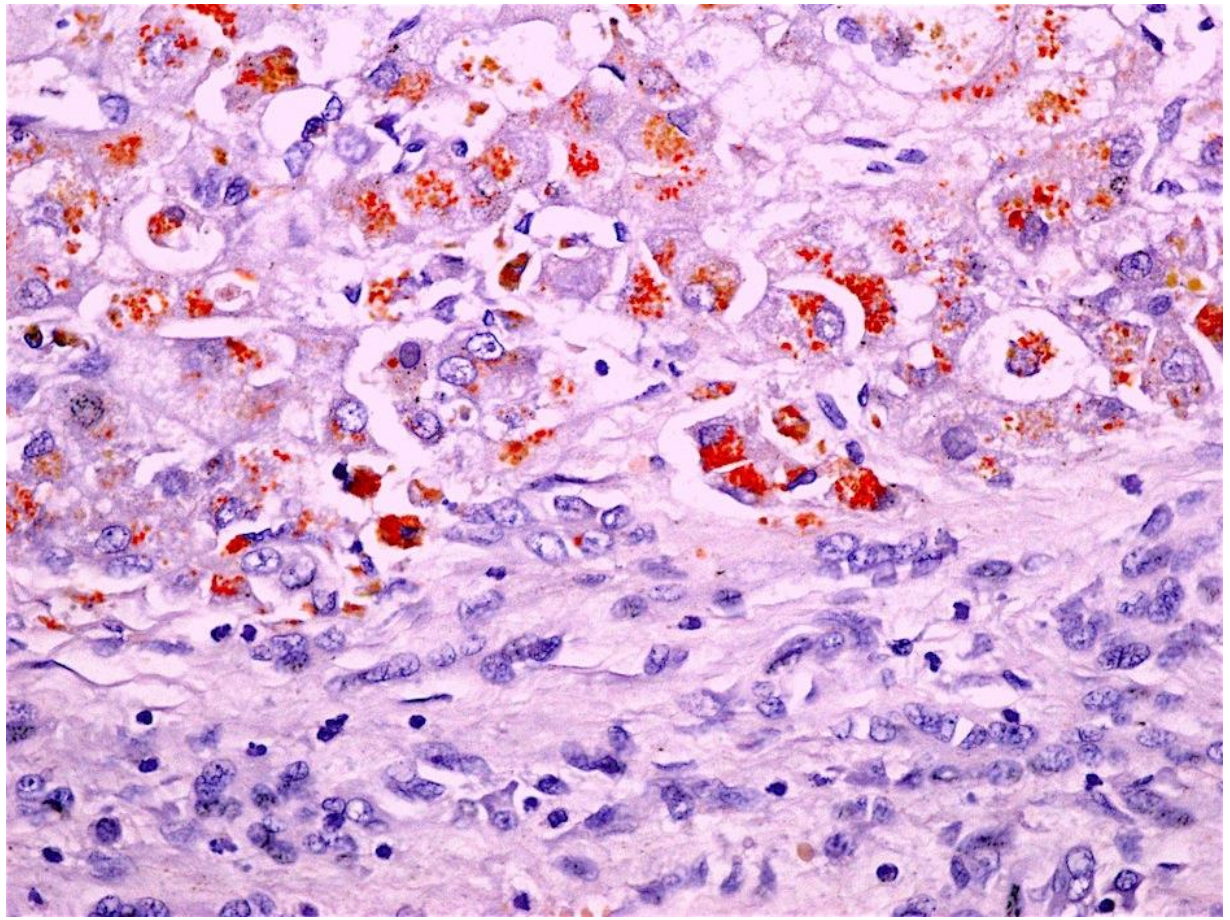
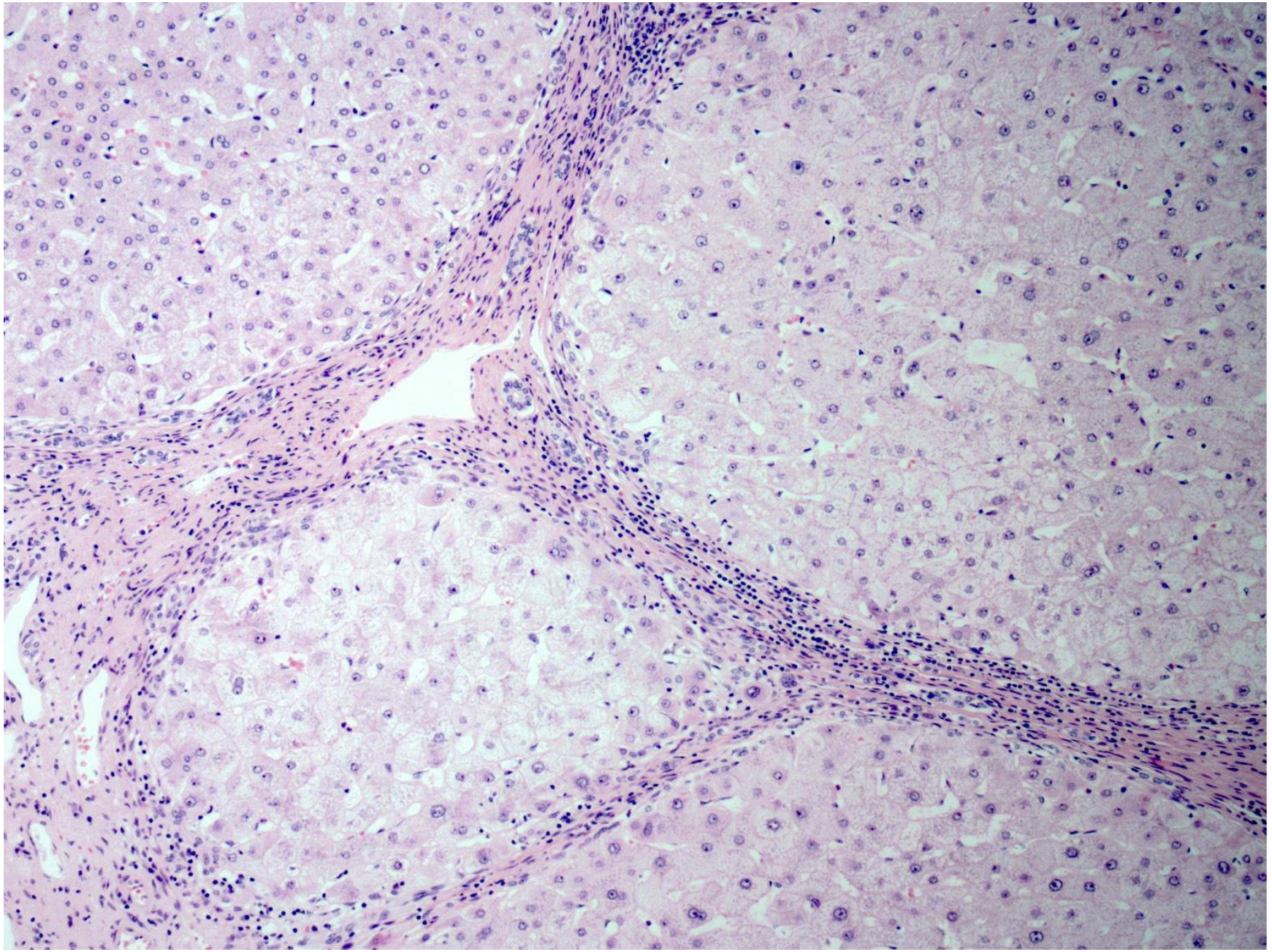




- Cirrhosis:
  - Nodular liver (micro or macronodular)
  - Steatosis
  - Hepatocyte anisonucleosis
  - Acidophil bodies
  - Hepatocyte ballooning and Mallory-Denk bodies
  - Satellitosis in some cases
  - Central vein fibrosis
  - Copper deposition is variable and patchy



Genetic testing

- Genetic testing for Wilson disease is used when:
  - the diagnosis remains unclear despite liver biopsy or
  - to aid with the screening of family members when the mutation in the proband is known.

- However, **the abundance of disease-specific mutations** complicates genetic testing.

- ATP7B can be affected by mutations at many different sites. Over 300 different mutations in the *ATP7B* gene have been identified in patients with Wilson disease. The H1069Q mutation is one of the most common mutations, with an allelic frequency of 10 to 40 percent.

- In populations with a higher frequency of a predominant mutation, diagnosis of Wilson disease using **allele-specific probes for specific mutations** may be an option.

- Since most patients are compound heterozygotes, the identification of one mutation supports the diagnosis, and the identification of two mutations confirms the diagnosis.

- Mutation analysis by **whole-gene sequencing** is another option to identify mutations in ATP7B and is available at some laboratories.

- **First-degree relatives** of patients diagnosed with Wilson disease should be screened for the disease.

- Among **siblings**, genotyping studies based upon the proband should be performed if possible.

- For **the child of a patient** with Wilson disease and **in cases where genetic testing is not possible**, the evaluation is the same as that for a patient with clinical features suggestive of Wilson disease.

Serum copper concentration

- In Wilson disease, the serum copper concentration is **decreased** in proportion to the reduction in serum ceruloplasmin, despite the presence of copper overload.

- Measurement of serum copper levels includes **both ceruloplasmin-bound and non-ceruloplasmin-bound copper**.

- Determination of **the serum non-ceruloplasmin-bound copper concentration** has been proposed as a diagnostic test for Wilson disease.

- However, the test is dependent on the adequacy of the methods for measuring both serum copper and

- The sensitivity and specificity of the non-ceruloplasmin-bound copper concentration for diagnosing Wilson disease **have not been well-established,** and it is generally not part of the diagnostic evaluation for Wilson disease.

- However, it may be used **to monitor therapy.**

- The non-ceruloplasmin-bound copper concentration is usually determined **indirectly.**

- The concentration is estimated from the serum total copper and serum ceruloplasmin concentrations.

- There are approximately 3.15 mcg of copper per mg of ceruloplasmin.

- Thus, non-ceruloplasmin-bound copper can be approximated using the following equation:
  - Non-ceruloplasmin-bound copper (mcg/dL) = serum copper (mcg/dL) – (3.15 x serum ceruloplasmin [mg/dL])
- For Système International (SI) units:
  - Non-ceruloplasmin-bound copper (mcg/L) = serum copper (mcg/L) – (3.15 mcg/g x serum ceruloplasmin [mg/L])

- The non-ceruloplasmin-bound copper can also be measured directly with **atomic absorption.**

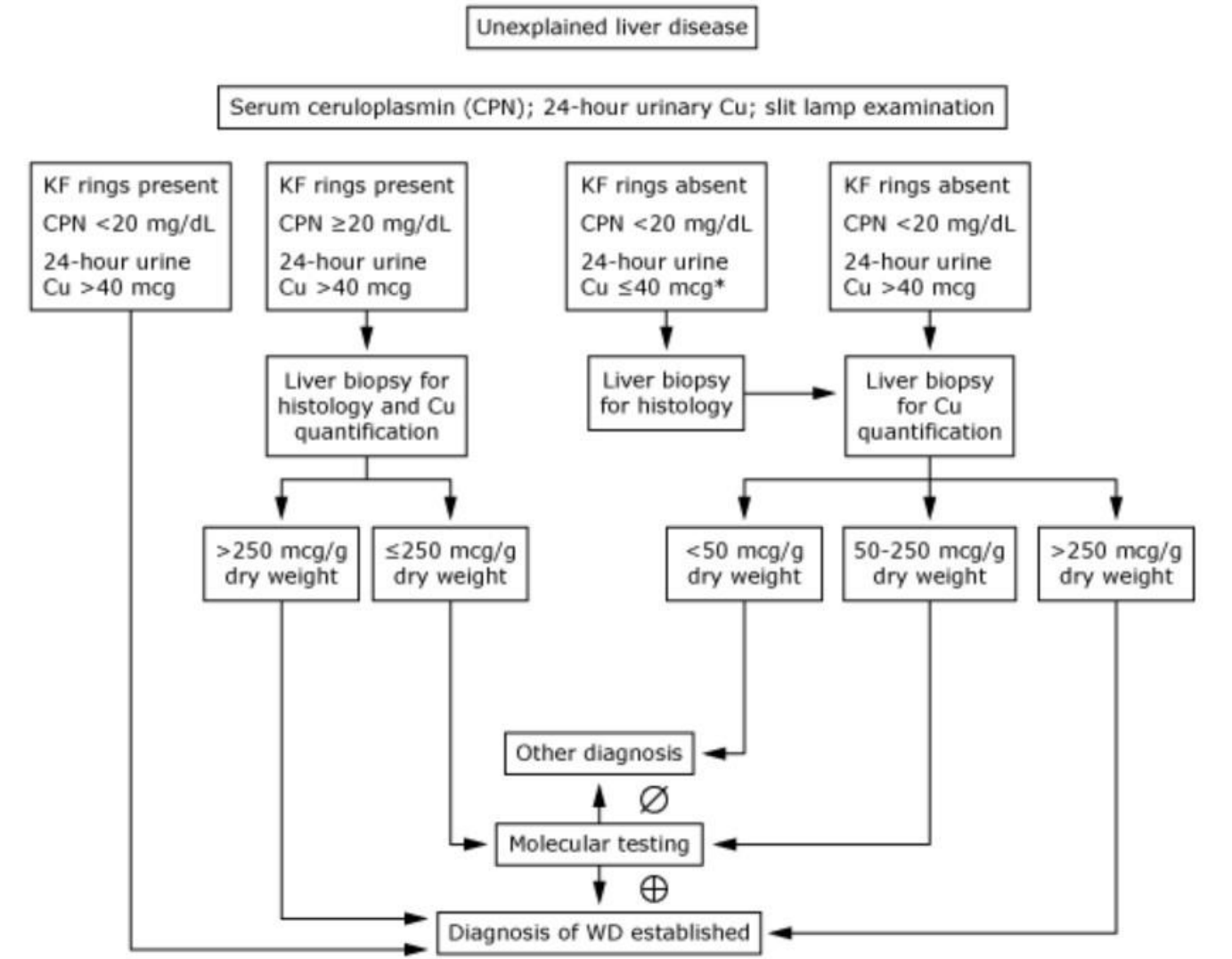
- **Elevated or normal levels of serum copper** in the setting of a decreased serum ceruloplasmin indicate that the concentration of non-ceruloplasmin-bound copper is **increased.**

- A normal non-ceruloplasmin-bound copper level is <15 mcg/dL (150 mcg/L).

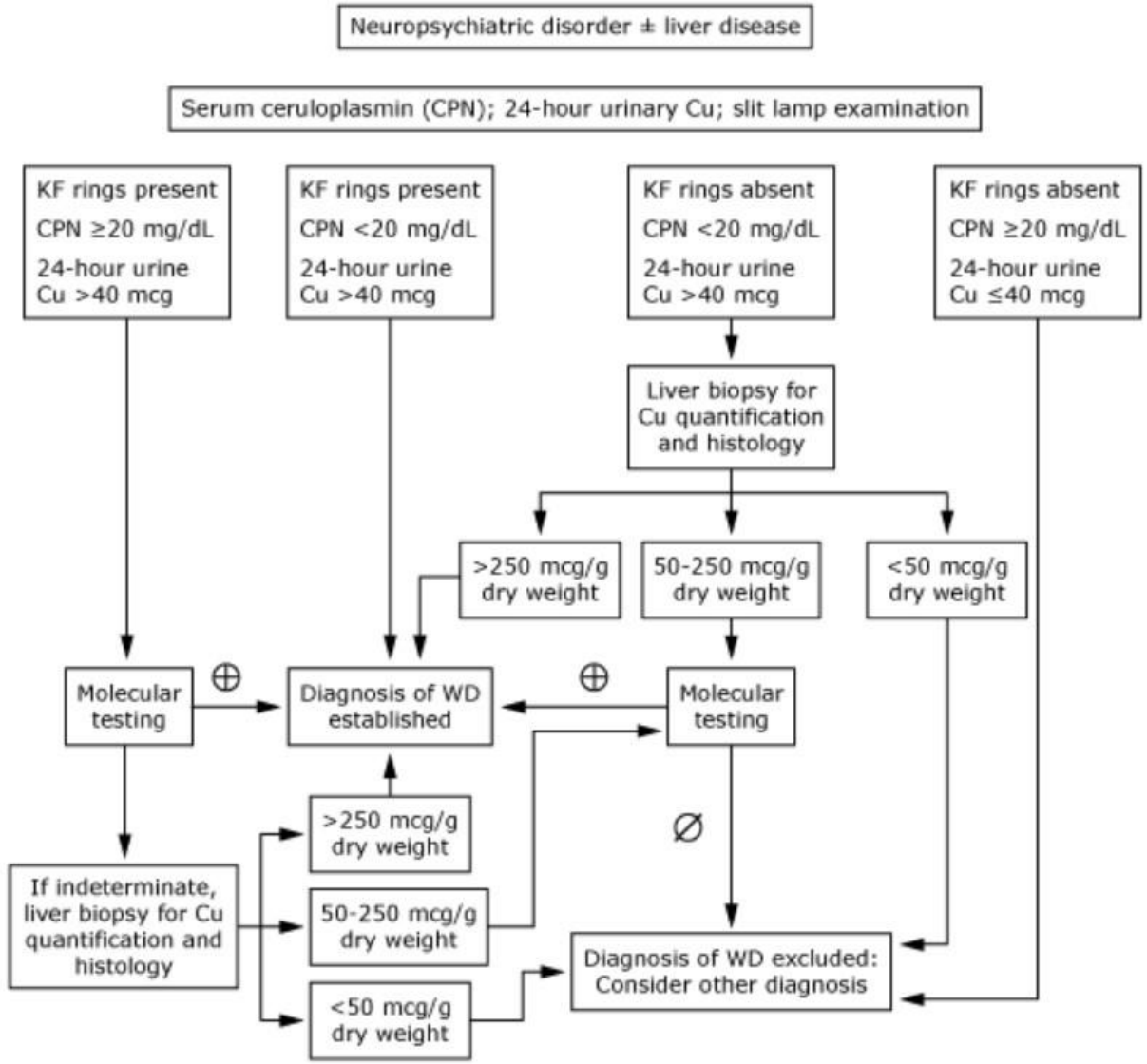
- In patients with untreated Wilson disease, the serum non-ceruloplasmin-bound copper concentration is typically **above 20 to 25 mcg/dL** (200 to 250 mcg/L), though it may be elevated:
  - in cases of acute liver failure due to any etiology,
  - in chronic cholestasis, and
  - in cases of copper intoxication.

- In patients receiving treatment for Wilson disease, a non-ceruloplasmin-bound copper concentration of <5 mcg/dL (50 mcg/L) suggests **possible systemic copper depletion.**

Approach to diagnosis of Wilson disease (WD) in a patient with unexplained liver disease



Approach to diagnosis of Wilson disease (WD) in a patient with a neurological disorder or psychiatric disease with or without liver disease



Screening for Wilson disease (WD) in sibling or child of a patient with secure diagnosis of WD