

# Autoimmune liver disease

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Liver disease often presents with very nonspecific symptoms. These include fatigue, pain, fever, flu-like symptoms, jaundice, altered mental status, itching, abdominal distention, and weight gain. Early screening for liver function includes levels of liver enzymes, bilirubin, and certain liver proteins.

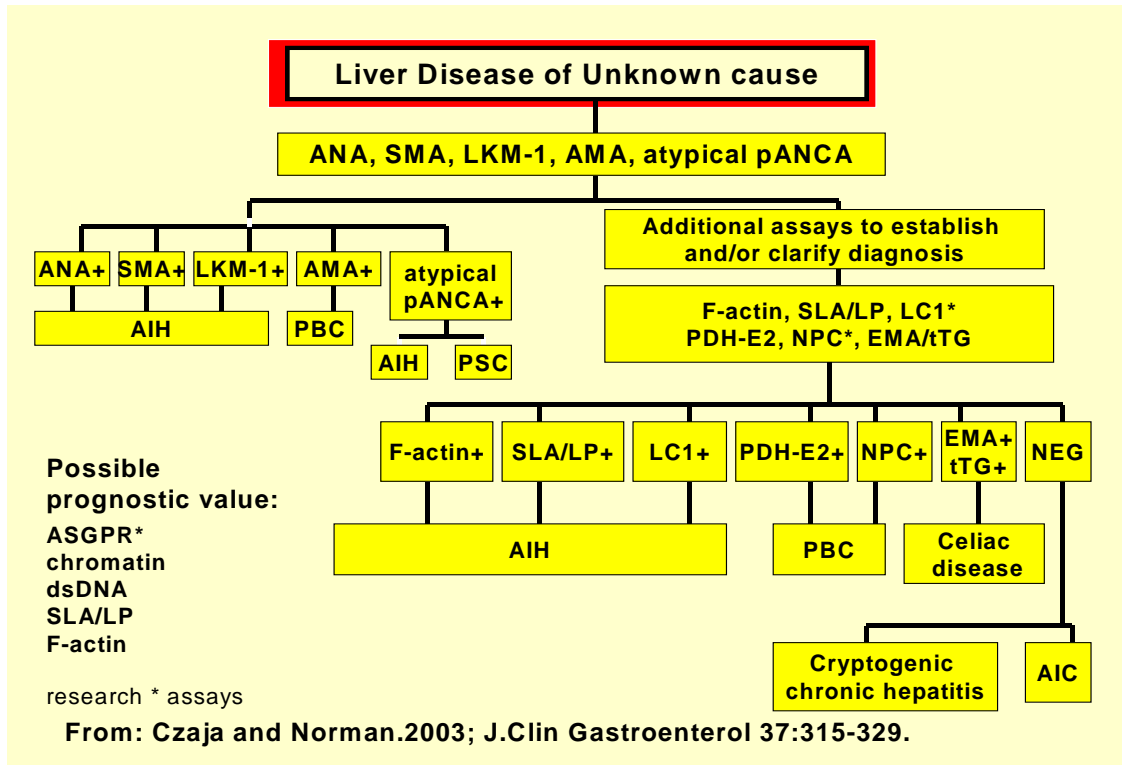
There are four separate enzymes included. These include aspartate aminotransferase (AST or SGOT), alanine aminotransferase (ALT or SGPT), alkaline phosphatase (AP) and gamma-glutamyl transferase (GGTP). AST and ALT are known as transaminases and AP and GGTP are known as cholestatic enzymes. Differential elevation of the enzymes is indicative of the type of liver damage. Elevation of the transaminases, especially the ALT is associated with inflammation and/or injury to liver cells. Common causes include autoimmune hepatitis (AIH), viral hepatitis, fatty liver, alcoholic liver disease, drug /medication-induced liver disease, herbal toxicity, genetic liver disease, liver tumors, heart failure and strenuous exercise. The elevation of AP and GGTP, on the other hand, are more frequently associated with possible blockage, injury or inflammation of the bile ducts. Common causes include primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), alcoholic or nonalcoholic fatty liver disease, liver tumors, drug-induced disease and gallstones.

Bilirubin elevations in liver disease are more frequently associated with worsening of liver disease or with bile duct blockage. It is often associated with increases in AP and GGTP.

The proteins that are monitored are albumin, prothrombin and certain immunoglobulins that are manufactured by the liver.

The major autoimmune diseases of the liver include autoimmune hepatitis (AIH), PBC and PSC. Elevation in the transaminases suggests AIH while elevation of AP and GGTP would suggest PBC or PSC.

The use of autoantibodies may aid in the differentiation of the specific disease. (See chart Liver disease of unknown cause)



## Autoimmune hepatitis

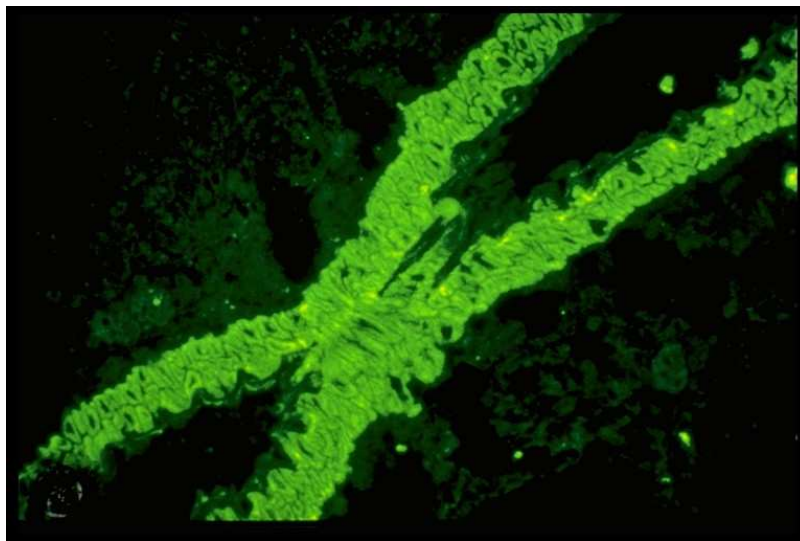
AIH is a chronic disease that results from immune mediated destruction of hepatic tissues in the absence of viral hepatitis. It is marked by progressive inflammatory destruction of hepatocytes, followed by development of cirrhosis and liver failure. It is detected predominantly in females. AIH is characterized by the presence of hypergammaglobulinemia, extrahepatic autoimmune syndromes, an increased prevalence of HLA-DR3 and/or HLA-DR4, and the presence of characteristic antibodies. Patients usually respond well to immunosuppressive treatment.

AIH is divided into three subtypes, AIH-1, AIH-2, and AIH-3. AIH-1, the most common type, is characterized primarily by the presence of antibodies to nuclear antigens (ANAs), smooth muscle (ASMA) and F-actin. Antibodies to atypical-ANCA may also occur. AIH-2 is characterized by antibodies to liver-kidney microsomal antibodies (cytochrome P450 2D6 (CYP2D6).) and LC-1. AIH-3 (sometimes considered a subset of AIH-1) is characterized by antibodies to soluble liver antigen /liver-pancreas (SLA/LP). It is important to note that autoantibodies are detected in many different liver diseases and alone are not diagnostic of autoimmune hepatitis.

## DIAGNOSTIC TESTS:

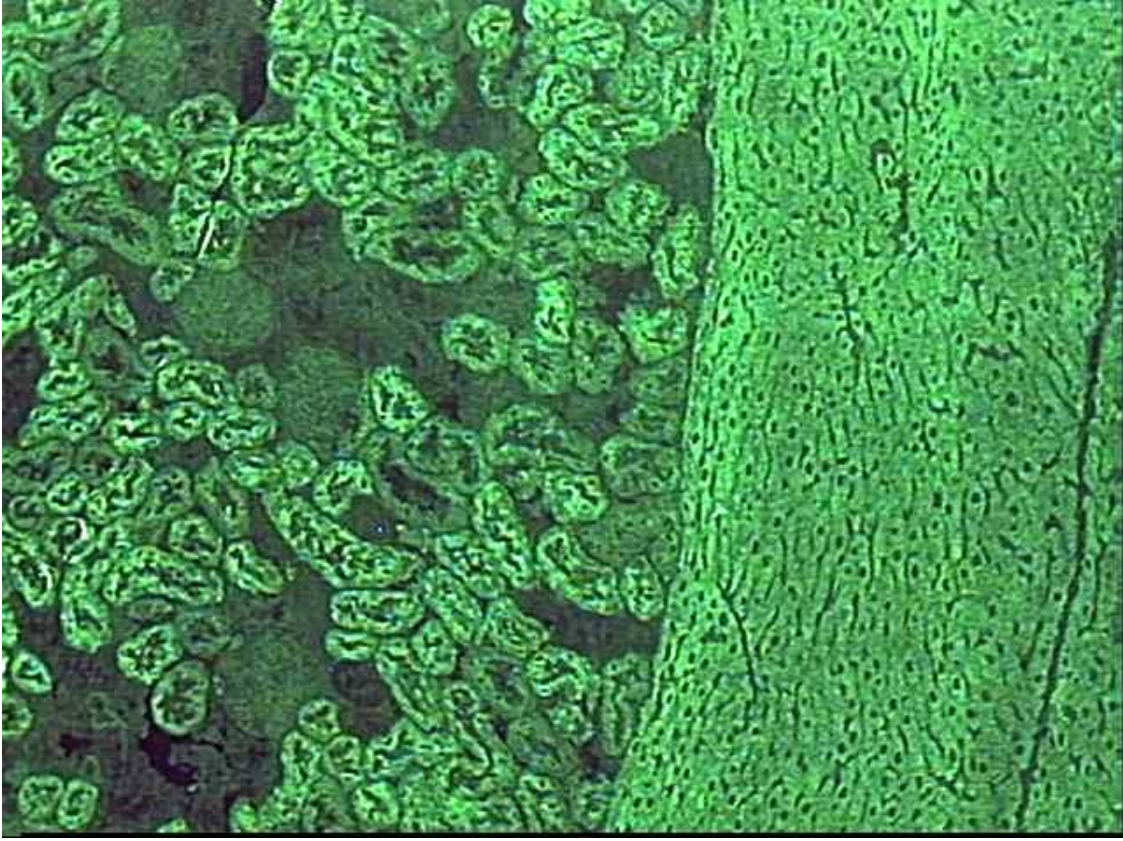
**Anti-nuclear antibodies:** The common substrate used for the detection of antibodies in liver disease is a composite of liver, kidney and stomach from rodents. This combination allows for the detection of antibodies to nuclear antigens, smooth muscle, LKM, mitochondria (AMA) and parietal cells at the same time. The ANA pattern is variable. The presence of antibodies to chromatin appears to be of prognostic value. These antibodies give a characteristically homogeneous pattern. Czaja *et.al.* (2003) demonstrated that antibodies to chromatin were commonly detected in AIH and were associated with disease activity. They characterized individuals who commonly relapsed after drug withdrawal.

□ **Smooth muscle antibodies:** The antibodies reacting to smooth muscle by IFA are a heterogeneous group of antibodies. They may be to actin, intermediate filaments, and tubulin or other minor components. They may stain the muscularis mucosae of the stomach and artery walls, the glomeruli and around the tubules. Titers > 1:160 are most associated with AIH. Titers ≤ 1:80 may be seen in other conditions.



**F-actin antibodies:** Antibodies to F-actin react with the polymerized form of actin. ELISA using antigen preserved in native conformation detects them. The test should be used in conjunction with not instead of ASMA by IFA.

**LKM antibodies:** There is a group of antibodies designated LKM. They comprise antibodies directed to cytochrome P450s and the uridine diphosphate (UDP)-glucuronosyltransferases (UGT). Antibodies to LKM-1, associated with AIH-2, react, with CYP2D6. The antibodies detected by IFA react with the hepatocellular cytoplasm and the distal third of the proximal tubules in the kidney.



Antibodies to LKM-2 from cases of drug-induced hepatitis caused by tienilic acid react with CYP2C9. Anti- LKM-3 is directed against family 1 UDP-glucuronosyltransferases (UGT1A), a superfamily of drug-metabolizing proteins located in the endoplasmic reticulum. Other antibodies have been identified that react with yet-unidentified proteins. LKM-3 antibodies may be detected in about 10% of AIH-2 patients and also in 6-10% of patients with HDV infection.

LKM-1 autoantibodies recognize a major linear epitope of CYP2D6 between amino acids 263 and 270. An ELISA using the cloned antigen is available.

**SLA/LP antibodies:** SLA/LP antibodies react with a 50 KD cytosolic protein associated with a UGA suppressor serine tRNA complex. ELISA using a recombinant protein detects the antibodies. 74% of patients with these antibodies will have other serological markers including ASMA and AMA. Patients with this antibody are usually female (90%) between 20-40 years of age with a more severe course of AIH.

**LC-1 antibodies:** LC-1 antibodies may be detected in about 50% of LKM positive AIH-2 patients. They appear to correlate with disease activity. The target antigen is formiminotransferase cyclodeaminase. By IFA, the antibodies stain the cytoplasm of the liver but not the kidneys.

Further reading:

1. Krawitt, EL. Autoimmune Hepatitis. *NEJM* 354: 54-66. 2006.
2. Strassburg CP, MP Manns. Autoantibodies and Autoantigens in Autoimmune Hepatitis. *Semin Liver Dis* 22: 339-351. 2002
3. Zachou, E Rigopoulou, GN Dalekos *J Autoimm Dis* 1:2. 2004
4. Czaja, AJ, Z Shums, WL Binder, SJ Lewis, VJ Nelson, GL Norman. Frequency and Significance of Antibodies to Chromatin in Autoimmune Hepatitis. *Digestive Diseases and Sciences* 48: 1658-1664. 2003
5. Czaja AJ, Z Shums, GL Norman. Frequency and Significance of Antibodies to Soluble Liver Antigen/Liver Pancreas in Variant Autoimmune Hepatitis. *Autoimmunity* 35: 475-483. 2002

# Primary Biliary Cirrhosis

Primary biliary cirrhosis (PBC) occurs primarily in adult females. It has not been observed in children. The predominant features include jaundice, pruritis, fatigue, persistent ill-defined abdominal discomfort, and hepatomegaly. Bilirubin generally increases over time. While PBC is seen in various ethnic populations, it is most frequently observed in Northern European populations. It has a lower frequency in Japan and other Asian areas.

PBC is a cholestatic liver disease. Cholestatic liver diseases are characterized by failure of the bile to flow. They are characterized by elevated levels of AP, GGTP, and bilirubin. AMA are detected in 90-95% of patients. PBC is classified into 4 stages on the basis of a liver biopsy. The stages range from damaged bile ducts in Stage 1 to cirrhosis in Stage 4.

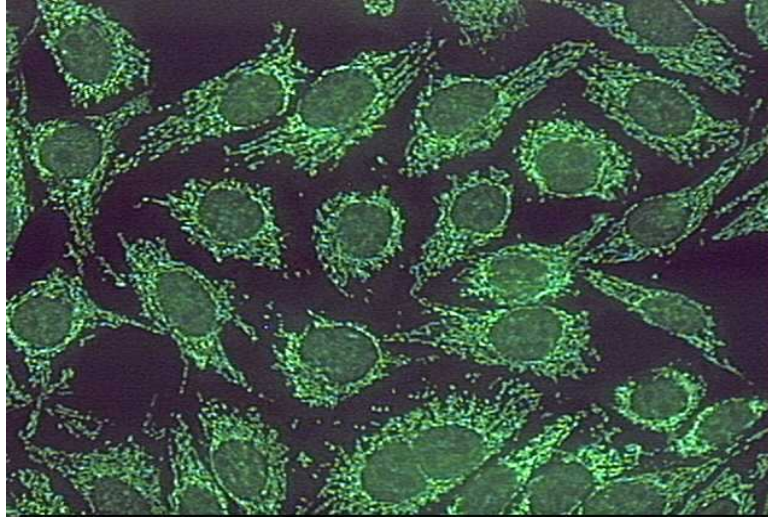
Researchers found that >90% of AMA identified react with subunits of the three 2-oxo-acid dehydrogenase complexes localized in the inner mitochondrial membrane or eukaryotic cells. They are involved in energy metabolism in the cell. These AMA associated with PBC are designated M2. The antibodies give a characteristic cytoplasmic pattern on HEp-2 cells. On the composite rodent kidney, stomach, liver slides, the staining is a strong granular cytoplasmic stain of the distal renal tubules, the parietal cells of the stomach and, the liver hepatocytes, with variable intense staining of the proximal renal tubules. Specific ELISA for M2 is available utilizing either purified M2 or a genetically engineered recombinant triple hybrid designated MIT-3. Some IFA negative PBC patients may be detected using ELISA.

Patients with PBC also have antibodies to several nuclear antigens. These include antigens to the nuclear envelope and nuclear pore complexes (gp210, p62 complex, Tpr, nuclear lamins) and to nuclear proteins sp100 and centromere.

## Diagnostic Tests:

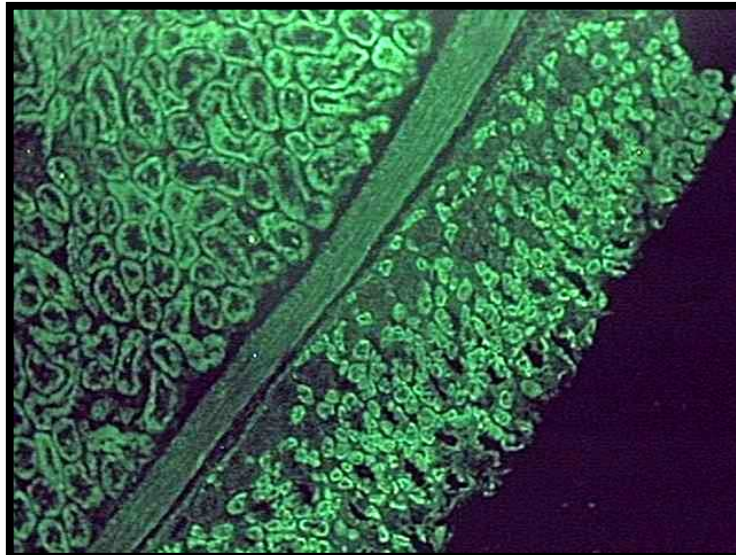
**Mitochondrial antibodies:** AMA are detected in > 95% of patients with PBC. They react with 2-oxy-acid dehydrogenases of the mitochondrial inner membrane. AMA give a coarse granular cytoplasmic stain on HEp-2.

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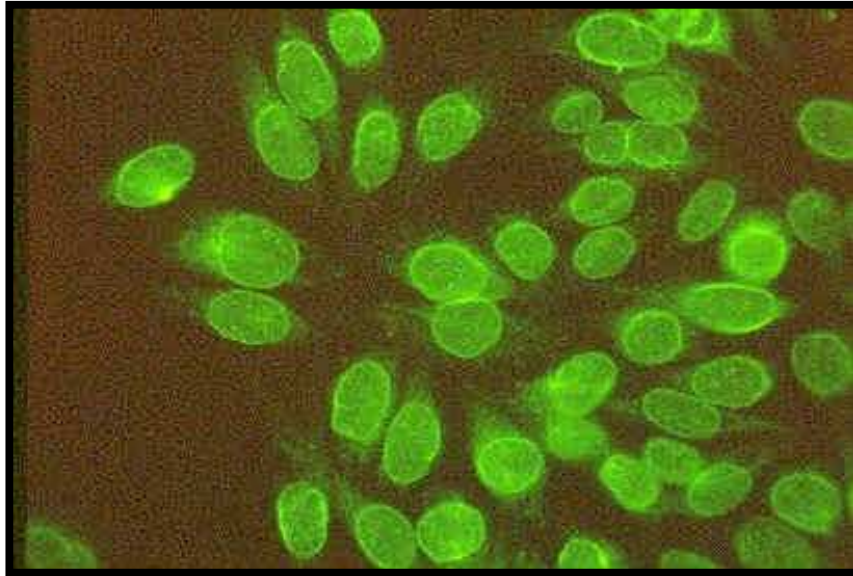
On the composite rodent kidney, stomach, liver slides, the staining is a strong granular cytoplasmic stain of the distal renal tubules, the parietal cells of the stomach and, the liver hepatocytes, with variable intense staining of the proximal renal tubules.

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ELISAs utilizing either purified M2 or a genetically engineered recombinant triple hybrid designated MIT-3 are available.

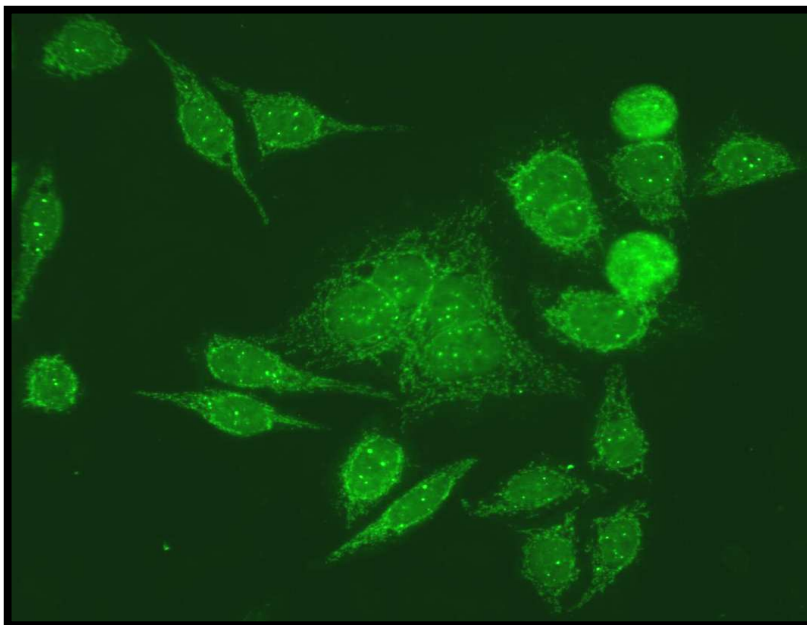
**Gp210 antibodies:** gp210 antibodies react with proteins of the central framework region of the nuclear pore complexes. They are detected in 10-41% of PBC sera. They are highly specific for PBC. They may be present in the absence of AMA. The presence of antibodies to gp210 is associated with hepatic failure and/or inflammatory arthropathy.



An ELISA utilizing a recombinant antigen is available.

**Sp100 nuclear dot antibodies:** sp100 antibodies react with proteins localized in 8-20 discrete nuclear dots in the nucleus. The 53 kD protein has been cloned. It migrates abberantly in a gel at 100 kD. The antibodies are detected in 27% of PBC patients and rarely in other conditions. They may be present in the absence of AMA.

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An ELISA utilizing a specific peptide is available.

**P62 complex antibodies:** p62 antibodies react with a complex of proteins located in the framework area of the nuclear pore complex. Antibodies are detected in 23-32% of PBC patients and occasionally in Sjögren's syndrome. They appear to be associated with progressive or advanced stage PBC. The IFA pattern is the same as gp210.

**Other antibodies:** Antibodies giving homogeneous, speckled, centromere and others may occur. They are not specific or diagnostic for PBC.

## Autoimmune Gastrointestinal Disease

### Celiac Disease

Celiac disease (CD) is diagnosed in patients having gluten-sensitive enteropathy (GSE). Patients are characterized by malabsorption that results from inflammatory injury to the mucosa of the small intestine after ingestion of wheat gluten, rye or barley proteins. There is a high prevalence of the disease in Western Europe and in the US and Australia. The prevalence is 1:120 – 1:300 in both Europe and North America. It is rare in African-Caribbean, Chinese, or Japanese. Over 95% of the patients express HLA DQ-2 heterodimer.

In infants, celiac presents between 4 and 24 months with impaired growth, diarrhea, and abdominal distension. It usually follows the introduction of cereal to the diet. If undiagnosed, children may present with short stature, iron or folate deficiency with anemia, and rickets due to malabsorption.

Adults with CD may present with episodic diarrhea, flatulence, weight loss and iron deficiency anemia that is the most frequent clinical presentation. Many patients may be asymptomatic. Patients that are diagnosed with dermatitis herpetiformis usually have asymptomatic celiac disease.

It is important that celiac disease be identified and treated, as there is a great chance of malignant transformation.

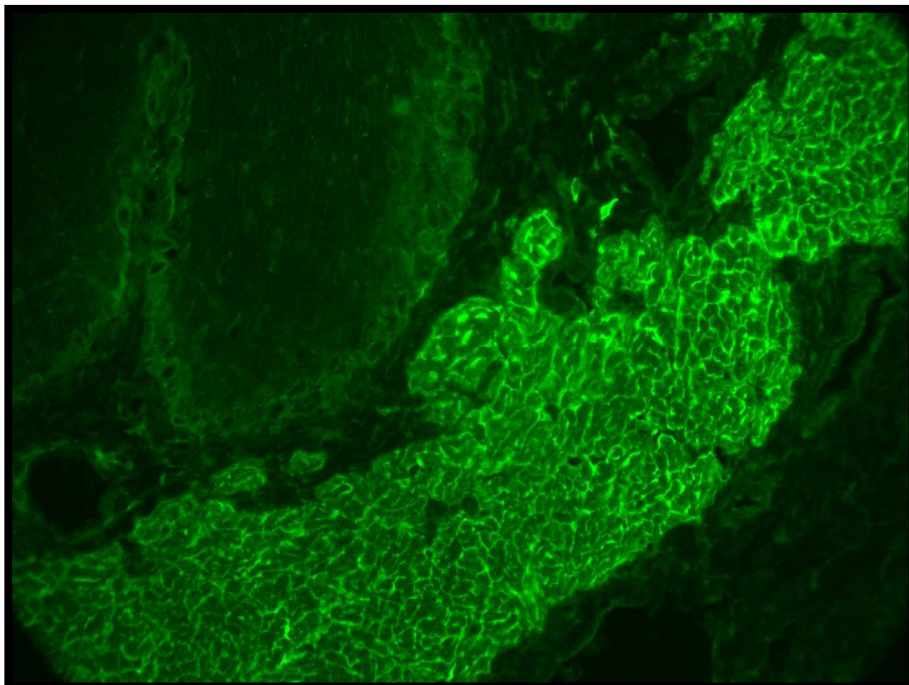
The antibody assays include the IgA endomysial (EMA), IgG and IgA tissue transglutaminase (tTG), and IgG and IgA gliadin. The IgA EMA and tTG are highly specific and sensitive for celiac. The definitive diagnosis is by biopsy. Treatment for CD is withdrawal of gluten from the patients diet. Anti-EMA and anti-tTG IgA tests are used to monitor compliance.

## Diagnostic Tests:

**Gliadin antibodies:** Gliadin is not a single protein but a heterogeneous group of proline- and glutamine-rich proteins in the alcohol-water-extractable portion of wheat gluten. Most patients with CD have antigliadin antibodies in the circulation. The level of these antibodies is broadly correlated with disease activity and decrease when patients are on a gluten-free diet. IgA antibodies to gliadin are quite specific for CD but not as sensitive as IgG antibodies. They are not as effective as IgA antibodies to endomysium or tTG.

It has been demonstrated that in the degradation of gliadin, a 33-mer peptide survives transit through the digestive enzymes to the small intestine. This 33-mer carries multiple copies of three epitopes immunogenic in CD. It has a high affinity for tTG. Once it is deamidated by tTG, the 33-mer elicits a response from T-cells from CD patients. A new ELISA using the major B-cell epitope antigenic peptide is available with high sensitivity and specificity.

**Endomysial antibodies:** IgA Endomysial antibodies are highly specific for CD. The IFA utilizes monkey esophagus or human umbilical cord tissue. On monkey esophagus, the samples are tested at 1:5 and 1:50 dilutions. The 1:5 increases the sensitivity but the 1:50 is necessary if ASMA are present. Most patients with active CD will still be EMA positive at 1:50. An IgA conjugate rather than IgG is used as a second antibody.



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