Chapter 4
The Pathology of Autoimmune Hepatitis

W. Carl Jacobs and William A. Ahrens

Introduction

Autoimmune hepatitis (AIH) was first described by Jan Waldenström in 1950 as a severe form of chronic active hepatitis involving women [21]. It is characterized as an unresolving form of inflammatory liver injury in the setting of autoantibodies and hypergammaglobulinemia. The disease affects women disproportionately at roughly a 4:1 ratio in comparison to men [5]. For the decades that followed Waldenström's initial characterization of the disease, there remained significant ambiguity for diagnostic criteria of the disorder known then as "autoimmune chronic active hepatitis" [12]. In the early 1990s, the International Autoimmune Hepatitis Group (IAHG) was ultimately formed in the wake of this uncertainty, in the attempt to provide expert consensus and clarity for the disease that still remains elusive in many ways today [1].

As with other autoimmune diseases, autoimmune hepatitis may follow a relapsing and remitting disease course and as such may present acutely with significant lymphoplasmacytic inflammatory activity and no signs of chronicity. Alternatively, AIH may present as an acute flare superimposed on chronic injury and hepatic fibrosis, or as cirrhosis with non-specific inflammatory activity. There is significant overlap between autoimmune hepatitis and other pathologic entities involving the liver, and as a result the diagnosis by definition requires clinical and pathologic correlation to rule out other processes.

W. C. Jacobs (✉) · W. A. Ahrens
Carolinas Pathology Group, Atrium Health, Charlotte, NC, USA
e-mail: Carl.Jacobs@atriumhealth.org

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Autoimmune Hepatitis Types

Autoimmune hepatitis occurs in all age groups, but most patients are young or middle aged. Approximately 20% of adults with AIH present after the age of 60 [6, 7, 16].

Type 1 autoimmune hepatitis is by far the most common form of AIH, especially in the adult population. It classically involves the autoantibodies anti-nuclear antibody (ANA) and anti-smooth muscle antibody (SMA). Perinuclear anti neutrophil cytoplasmic antibodies (p-ANCA) are also found in 50–96% of patients with type 1 AIH [3]. Of note, ANA and SMA are often mildly elevated in immune-mediated drug injury as well, therefore low level positivity is considered non-specific and only mildly supportive of a diagnosis of autoimmune hepatitis. Similarly, IgG may be elevated in immune-mediated drug injury as well as in the setting of active infection. Specificity for autoimmune hepatitis increases with higher titers.

Autoimmune Hepatitis Scoring Systems

A number of scoring systems have been proposed to aid in the diagnosis of autoimmune hepatitis. A unifying theme of all systems is that histology is a cornerstone for diagnosis and the inclusion of histologic and clinical features typical of both autoimmune hepatitis and other entities that may mimic or overlap with the disease process are present. The diagnosis, in short, must be made by demonstrating clinical and histologic features typical of autoimmune hepatitis, as well as excluding clinical and histologic features typical of other entities.

The Revised International Autoimmune Hepatitis Group modified scoring system, established in 1999, is a sensitive and specific scoring system to assess the likelihood of autoimmune hepatitis using both histologic and clinical parameters. In 2009, the IAHG introduced a simplified scoring system for the diagnosis of autoimmune hepatitis [11]. This simplified scoring system applies 1–2 points for autoantibodies at certain levels of titers, 1–2 points for elevated IgG level, 0–2 points for histology atypical for, compatible with, or typical for autoimmune hepatitis, respectively, and finally 2 points for the absence of viral hepatitis for a maximum score of 8. A score of 6 is defined as probable autoimmune hepatitis, and a score greater than or equal to 7 is defined as definite autoimmune hepatitis [11].

To be considered typical for autoimmune hepatitis under the 2009 IAHG simplified scoring system, a biopsy must demonstrate three features:

1. Interface hepatitis (lymphoplasmacytic infiltrates extending into the lobule)
2. Emperipolesis (active penetration of a hepatocyte by a lymphocyte or plasma cell)
3. Hepatic Rosette formation (not specifically defined by the IAHG).

Figures 4.1, 4.2, 4.3 and 4.4 show histologic features compatible with AIH. A biopsy is considered “compatible with” AIH under this system if it shows some but

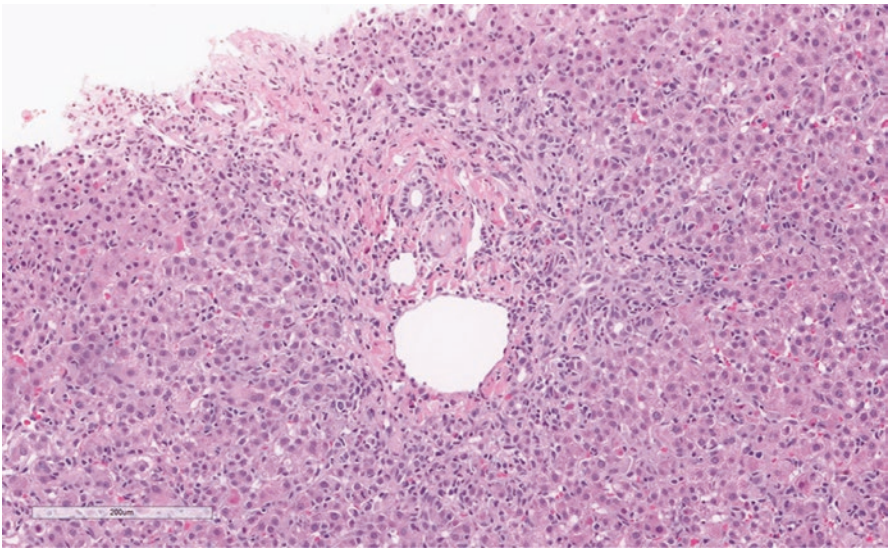

Fig. 4.1 Medium power image showing autoimmune hepatitis with portal, periportal and lobular activity

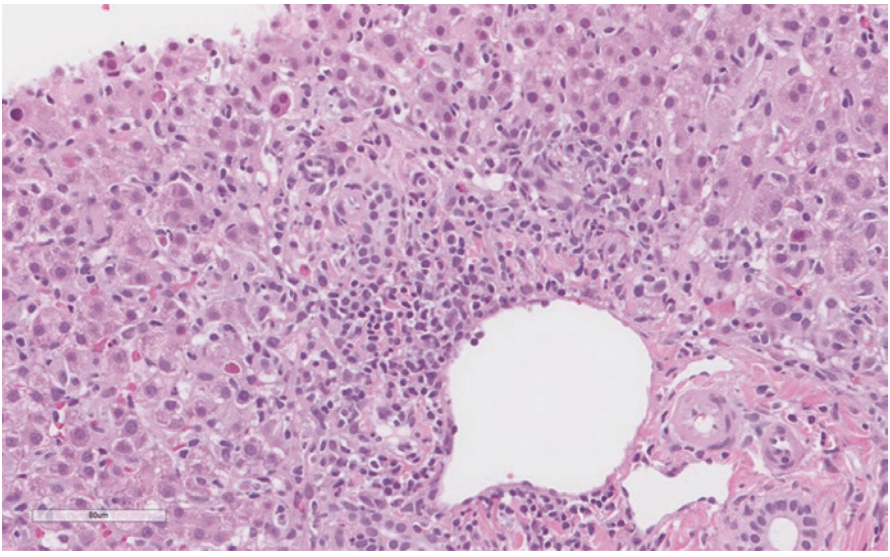

Fig. 4.2 Higher power image showing interface activity – periportal lymphoplasmacytic inflammation with apoptotic hepatocytes or "piecemeal necrosis"

not all three features. Histologic patterns "atypical for" autoimmune hepatitis was defined as biopsies showing signs of another discrete diagnosis such as steatohepatitis. Effectively, a definitive diagnosis of autoimmune hepatitis by this simplified scoring system requires some level of autoantibodies, elevated IgG, histologic support, and the exclusion of viral hepatitis.

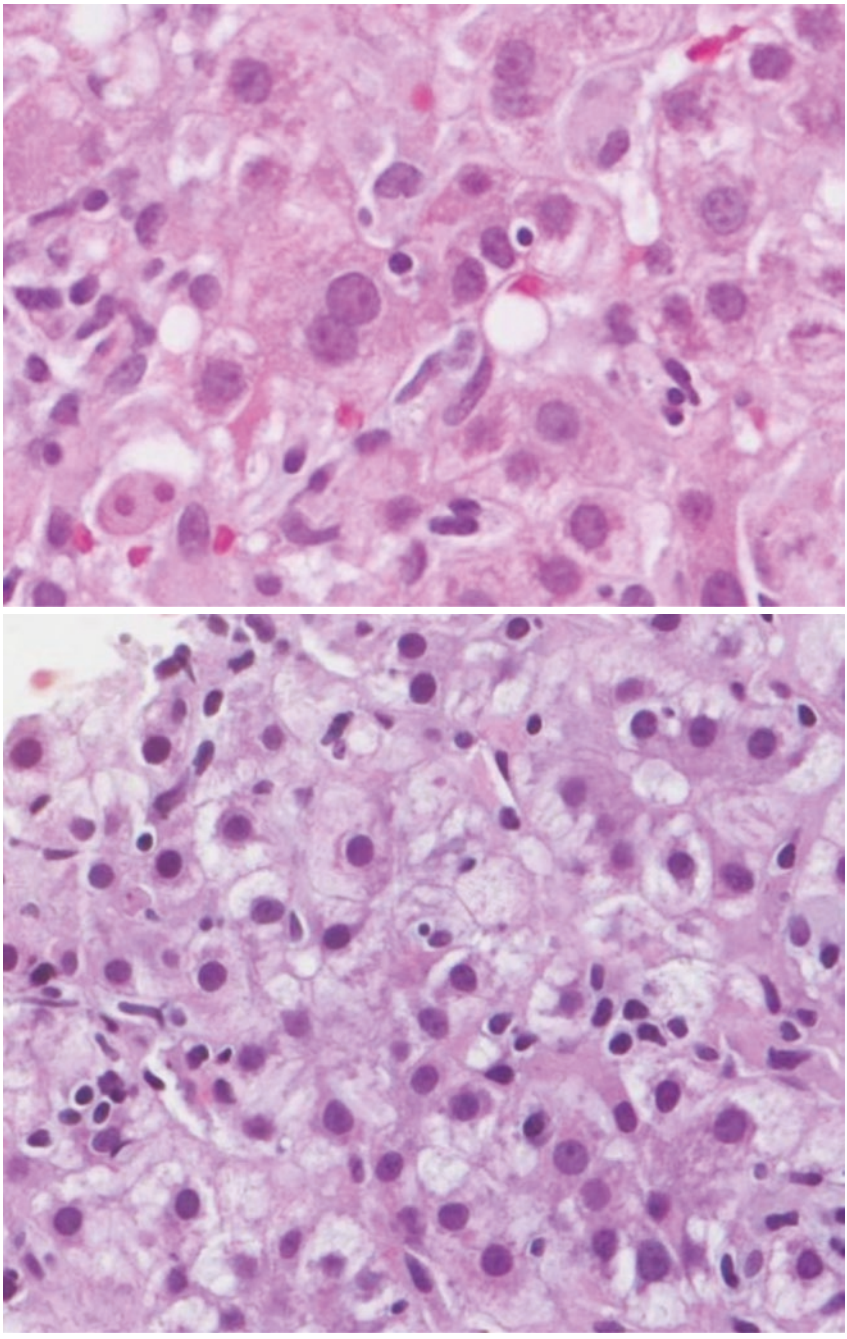

Fig. 4.3 Emperipolesis (active penetration of a hepatocyte by a lymphocyte or plasma cell) –In these photos lymphocytes with pericellular clearing are seen entirely within the confines of a hepatocyte

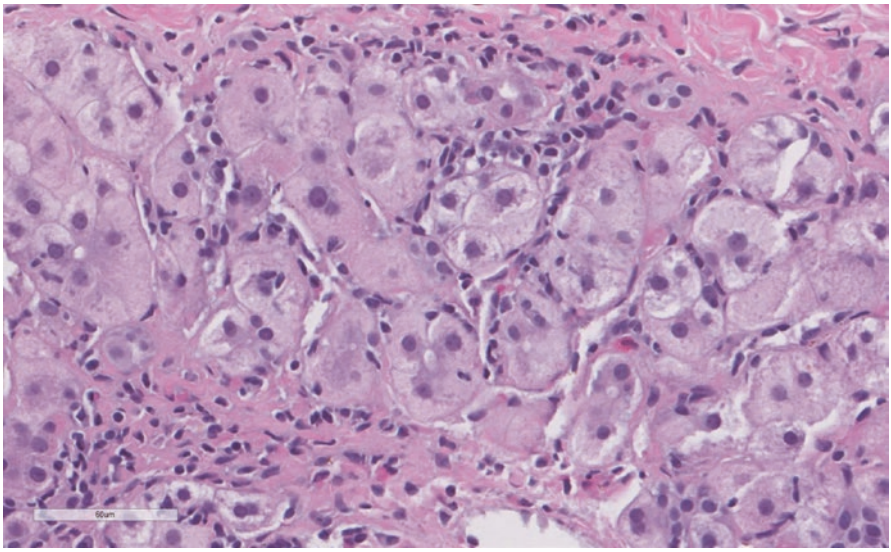

Fig. 4.4 Hepatocyte rosette formation in a case of active autoimmune hepatitis: clusters of hepatocytes centered around a central space

Newly Discovered Problems with Classic Autoimmune Hepatitis Histology

There are problems with the histologic criteria proposed by the IAHG. On the one hand, there seems to be significant interobserver variability in the recognition and interpretation of what constitutes emperipolesis and hepatic rosette formation. Like the IAHG, many studies have not clearly defined criteria for identifying rosettes (most strictly defined in a couple studies as an arrangement of hepatocytes around a central luminal space). Similarly, emperipolesis suffers from a lack of specific defined diagnostic criteria in many studies and therefore the incidence of these features varies drastically from study to study [2]. In addition, other entities such as drug injury and infection have been shown to demonstrate emperipolesis and hepatic rosette formation, therefore both the sensitivity and specificity of these features have been called into question.

Studies have shown wide ranging numbers with respect to classic histologic features of autoimmune hepatitis. Rosettes have been reported in 29–75% of autoimmune hepatitis, 11% of primary biliary cholangitis, 2–41% of drug induced liver injury, 23% of chronic viral hepatitis, and 4% of Wilson's disease [2].

In one study, emperipolesis was identified in 65% of acute autoimmune hepatitis cases and in 77% of non-autoimmune acute hepatitis cases. Rosettes were identified in 33% of acute autoimmune hepatitis cases and in 38% of non-autoimmune acute hepatitis cases. Both emperipolesis and rosettes were identified in only 26% of autoimmune hepatitis, yet found in 31% of non-autoimmune hepatitis cases [2].

Interface activity and necrosis were also found to be non-specific histologic features. Interface activity was present in 80% of autoimmune hepatitis cases and 77% of non-autoimmune hepatitis cases. Confluent necrosis was noted in 40% of autoimmune hepatitis cases, and in 69% of non-autoimmune hepatitis cases. Significantly, numerous plasma cells were much more commonly found in autoimmune hepatitis cases (75%) in comparison to non-autoimmune hepatitis cases (8%) [2].

Acknowledging the limitations with specificity of "classic" histologic features of autoimmune hepatitis, interface hepatitis, emperipolesis and rosette formation may be indicative of severity of hepatitis rather than specific to a given etiology. Many older studies that found these features specific to autoimmune hepatitis failed to control for stage of fibrosis or grade of inflammation [10].

Several recent studies have mentioned the presence of Kupffer cells with "hyaline droplets" [20] or "hyaline globules" [10] which appear to be more common in autoimmune hepatitis than other entities although they have been seen in other processes as well, including viral hepatitis C (Fig. 4.5) [4]. This feature is thus being considered in newer scoring systems. In addition, some authors have proposed scoring systems that include periportal positivity for copper and CK7 stains as markers of chronic biliary disease, which are not typically found in cases of isolated autoimmune hepatitis [2].

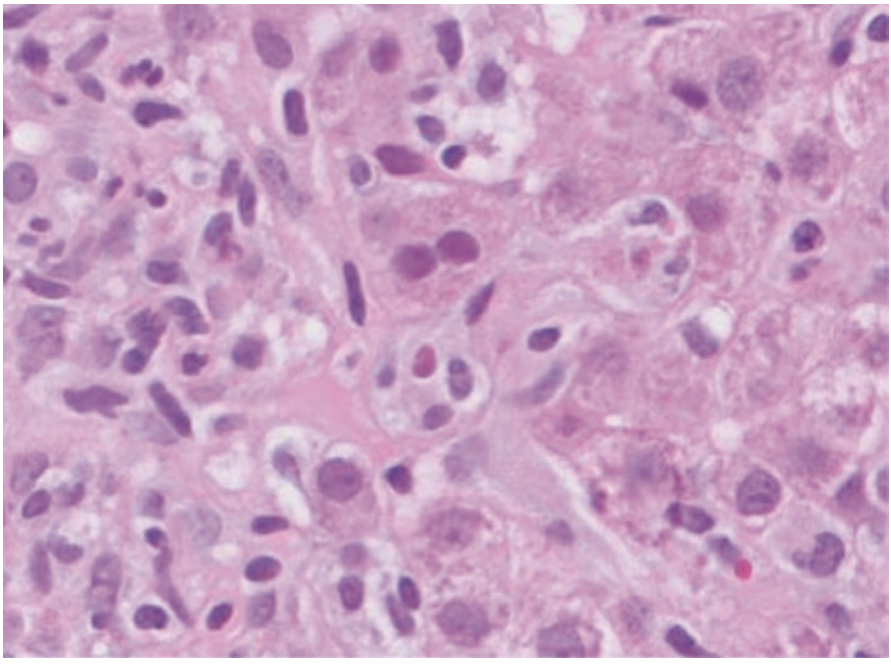

Fig. 4.5 Kupffer cell with hyaline globule – these are readily seen on PAS and PAS-D stains, although this one is also easily seen on routine H&E stain. They are often seen in autoimmune hepatitis and may be related to the immune regulatory functions of Kupffer cells [4]

Acute, Acute on Chronic, and Chronic Presentation of Autoimmune Hepatitits

Autoimmune hepatitis can present at any age and any population, but it is more likely to present acutely in children and young adults [9, 17] and often presents with chronic changes/fibrosis in an older patient population.

In the acute setting, autoimmune hepatitis may present classically with a striking portal, periportal, and lobular lymphoplasmacytic infiltrate, with interface activity including apoptotic hepatocytes at the portal-periportal interface or limiting plate (historically termed 'piecemeal necrosis'). Other common features include hepatocyte rosette formation, emperipolesis, lobular eosinophils (in contrast to portal eosinophils which some studies have shown may be more common in drug injury [19]), and zone 3 damage with pericentral necrosis and plasma cell central venulitis. Portal and lobular ceroid-laden macrophages are often seen and will be highlighted by PAS stains with and without diastase. They signify 'resolving/ongoing' liver injury, representing the liver's attempt to clean up and remove damaged cells. In more severe acute presentations, parenchymal drop out is common (Figs. 4.6 and 4.7). This will sometimes present as bridging parenchymal collapse that can result in a nodular appearance that can be confused for cirrhosis both radiologically and histologically (Figs. 4.8, 4.9 and 4.10). Extreme cases may present as fulminant hepatitis with extensive panlobular necrosis, necessitating emergent liver transplant.

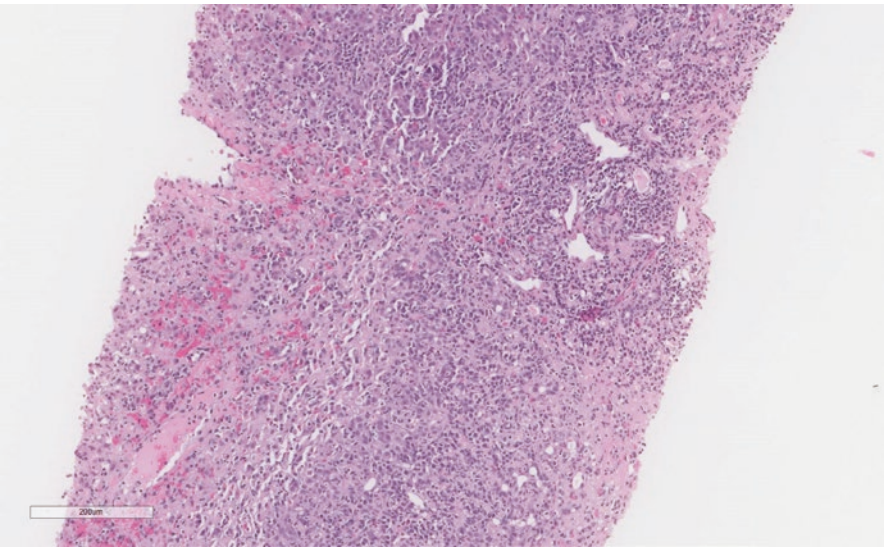

Fig. 4.6 Acute autoimmune hepatitis with bridging parenchymal collapse, numerous plasma cells, and central venulitis

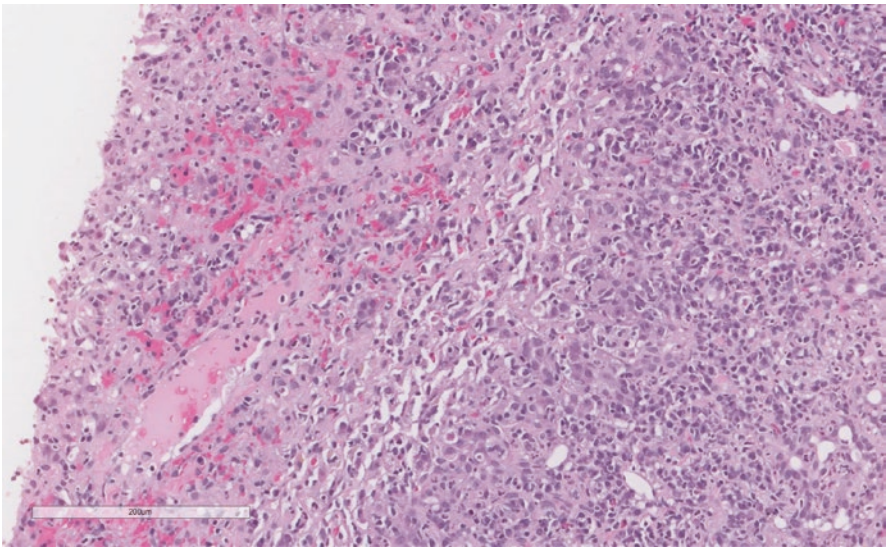

Fig. 4.7 Higher power image showing central venulitis with pericentral necrosis and bridging parenchymal collapse. Numerous plasma cells are seen admixed with lymphocytes, eosinophils and neutrophils in this case

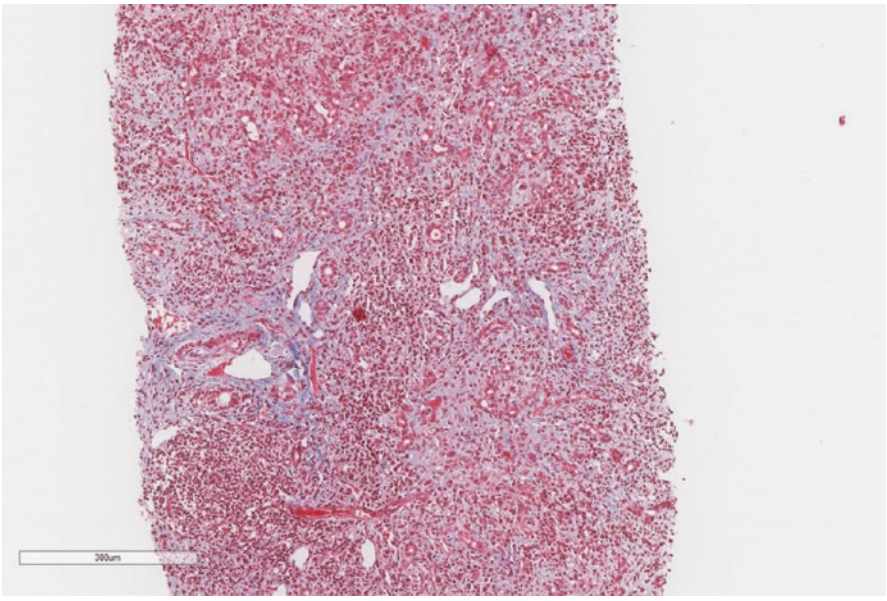

Fig. 4.8 Trichrome stain showing a portal tracts (dark blue) as well as panlobular parenchymal collapse (pale blue) associated with diffuses bile ductular proliferation. A bile ductular reaction is typical to all cases of significant acute hepatitis with parenchymal loss. Bridging parenchymal collapse can be mistaken for cirrhosis both radiologically and histologically

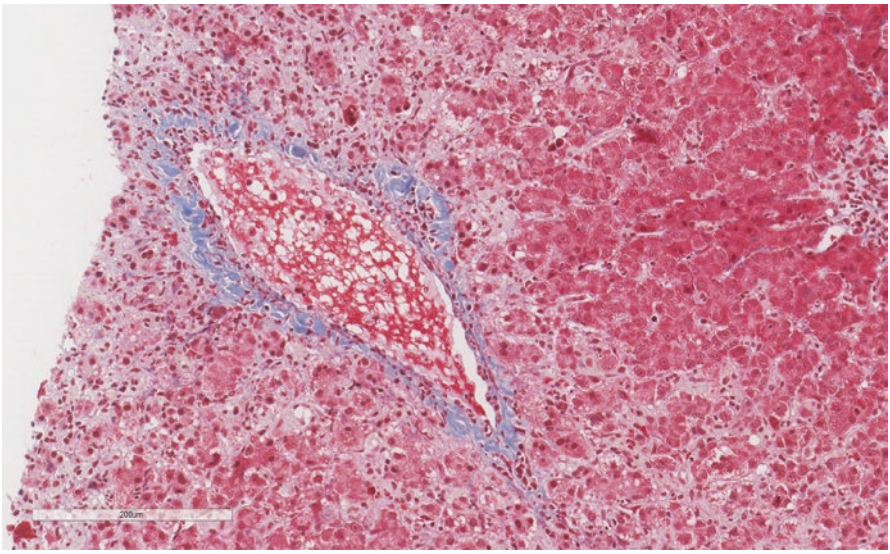

Fig. 4.9 High power image of a central vein of a largely intact central vein (dark blue), with pericentral hepatocyte loss/drop out (pale blue) associated with lymphoplasmacytic inflammation and admixed ceroid laden macrophages (Trichrome stain)

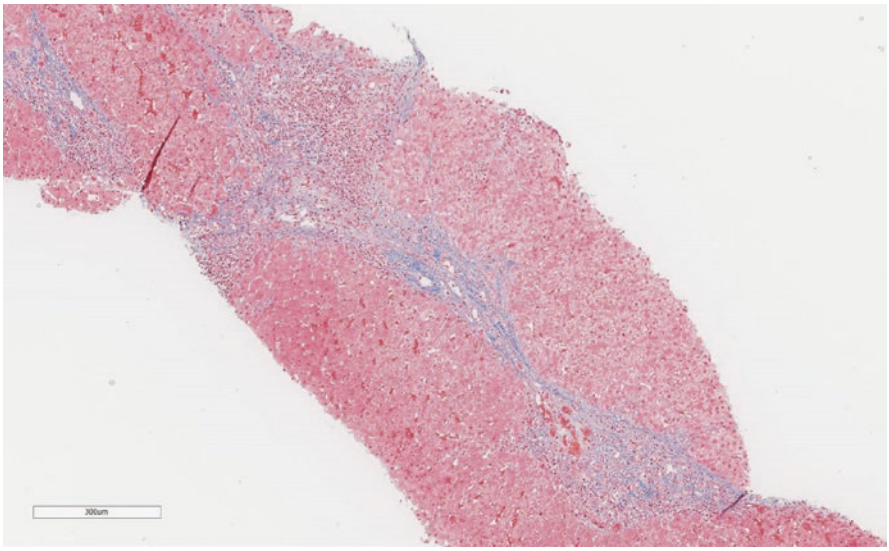

Fig. 4.10 At least bridging fibrosis is shown here in a case of acute on chronic hepatitis. Staging of fibrosis can be difficult in the setting of acute on chronic liver injury and often must be deferred if there is significant parenchymal collapse, but in this case the trichrome stain is definitive. Reticulin stain may be a helpful adjunct stain to distinguish collapse from fibrosis

Reticulin stain and trichrome stain are both helpful in distinguishing true fibrosis from parenchymal collapse in the setting of significant acute hepatitis. Mature collagen/fibrosis will stain darkly with trichrome stain whereas parenchymal collapse will look paler (in comparison to the native portal tracts which act as a positive control for fibrous tissue in the setting of acute hepatitis where there is no true fibrosis). Reticulin stain is typically pale gray in portal tracts and areas of fibrosis but will stain the reticulin fibers of the hepatic plate darkly. In the setting of parenchymal collapse, reticulin will highlight areas of lost hepatocytes/collapsed plates as the dark black reticulin fibers are typically retained.

If a liver biopsy is obtained after steroid therapy has been initiated, the diagnosis may be difficult or impossible to histologically confirm. Immunosuppression can lead to rapid resolution of the lymphoplasmacytic inflammatory infiltrate, leaving empty appearing, previously expanded portal tracts, reduced lobular activity, and non-specific portal and lobular ceroid-laden macrophages cleaning up the previous damage.

Patients presenting with an acute flare superimposed on chronic autoimmune hepatitis will show some degree of hepatic fibrosis and varying degrees of lymphoplasmacytic inflammation (Figs. 4.11 and 4.12). Patients with advanced fibrosis or cirrhosis may demonstrate very limited inflammatory activity and therefore show a non-specific histologic picture of end stage liver disease.

In the setting of cirrhosis and limited inflammation, sometimes the best a pathologist can do is to exclude features typical for other specific entities such as primary biliary cholangitis and primary sclerosing cholangitis which will both show an irregular/biliary pattern of fibrosis, and may show ductopenia which is not typical

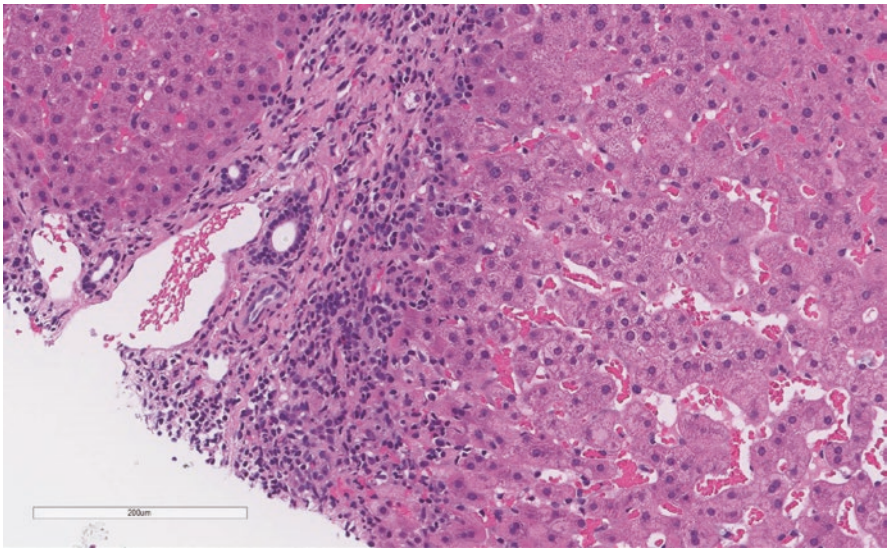

Fig. 4.11 Significant ongoing portal/periportal lymphoplasmacytic activity is seen in this case of acute on chronic autoimmune hepatitis. The trichrome stain shows at least bridging fibrosis (see previous photo)

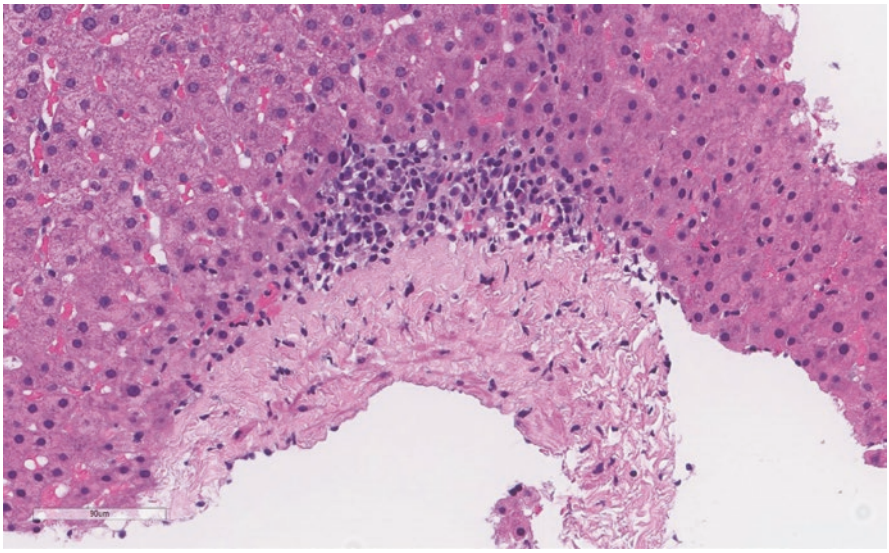

Fig. 4.12 A striking plasma cell dominant population is seen adjacent to a larger central vein in this case of acute on chronic autoimmune hepatitis

for autoimmune hepatitis. The biopsy may also show the residual nodular scars highlighting the areas of lost interlobular native bile ducts pathognomonic for primary sclerosing cholangitis. Remote toxic/metabolic liver injury may still show residual intracytoplasmic hyaline and sinusoidal fibrosis, even in the absence of steatosis.

Differentiating Autoimmune Hepatitis from Infection and Drug Induced Liver Injury

The differential diagnosis of acute autoimmune hepatitis includes infection as well as immune-mediated drug injury. Acute viral hepatitis, especially acute hepatitis B or acute hepatitis C infection, will often show prominent plasma cells. Acute viral hepatitis is less likely than autoimmune hepatitis to demonstrate interface activity. Since interface activity is seen in at least some cases of acute viral hepatitis as well, distinguishing between these entities ultimately requires clinical and serologic correlation (Fig. 4.13). Epstein Barr virus (EBV) infection may also show some overlapping features with autoimmune hepatitis including the presence of plasma cells, though typically EBV hepatitis will show a more prominent sinusoidal lymphocytic infiltrate. In situ hybridization for EBV (EBER) can confirm or exclude this entity.

Women are more likely than men to present with autoimmune hepatitis and are also more likely to present with drug induced liver injury that shows an autoimmune-like histology including frequent plasma cell infiltration, hepatocyte rosette formation and lobular disarray. Men, in contrast, are more likely to show cholestasis in

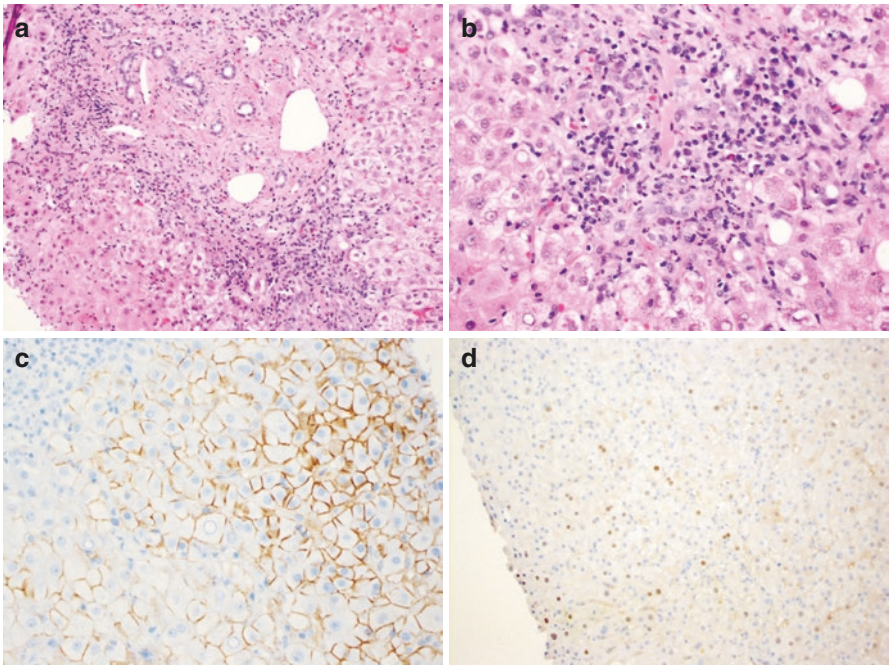

Fig. 4.13 Forty-nine year old female with 2 week history of jaundice. History of doxycycline therapy 1 week ago. Remote history of cholecystectomy with current biliary stones. Autoimmune and viral serologies still pending at the time of biopsy. (**a**) Histologic sections show a portal and lobular hepatitis with interface activity and patchy prominent plasma cells. There is also a bile ductular proliferation. (**b**) Mixed lobular inflammation with conspicuous plasma cells. (**c**) Immunostains for HBsAg (**c**) and HBcAg (**d**) were both positive, confirming the diagnosis. The overall findings are compatible with acute viral hepatitis B, with the biliary proliferation representing either a component of the acute hepatitis or biliary obstruction from cholelithiasis

drug induced liver injury [15]. Autoimmune serologies were later confirmed to be negative. Drug induced liver injury may present with a mixed histologic pattern of injury and inflammation, whereas autoimmune hepatitis will often, but not always, have a striking plasma cell predominant infiltrate. Other features classically considered specific to autoimmune hepatitis including hepatocyte rosette formation, intra-acinar eosinophils, plasma cell rich central venulitis with pericentral hemorrhagic necrosis, and emperipolesis may be less helpful than previously believed in making a diagnosis of autoimmune hepatitis. may be a helpful feature to favor autoimmune hepatitis over immune-mediated drug injury, as drug injury will not typically present with fibrosis [8], except in cases of chronic injury from drug or supplement use. Features that may favor drug induced liver injury include canalicular and hepatocellular cholestasis, prominent neutrophils, and intra-acinar lymphocytes [19].

Ultimately, plasma cell rich liver biopsies in patients who have been exposed to potential offending drugs or supplements will often require clinical correlation over a period of months to arrive at a definitive diagnosis. Immune mediated drug injury

may take months to resolve after the offending agent has been removed. These patients respond to steroid therapy as well, and the only way to definitively confirm immune mediated drug injury may be to taper the immunosuppression therapy and follow up liver enzymes over time to assess for prolonged resolution. Autoimmune hepatitis (idiopathic or drug induced AIH) are more likely to relapse after cessation of immunosuppression, whereas immune mediated drug injury will not, provided that the offending agent has been successfully identified.

Autoimmune Hepatitis with PBC and PSC Overlap

Patients presenting with autoimmune hepatitis not uncommonly present with an overlap syndrome. In the setting of advanced fibrosis, periportal copper and CK7 positive cholangiocytes may be seen in isolated autoimmune hepatitis and are non-specific (Figs. 4.14 and 4.15). However, these findings should not be seen in less advanced cases of autoimmune hepatitis and thus, if present, they should prompt consideration for a chronic biliary process such as primary biliary cholangitis or primary sclerosing cholangitis.

Autoimmune hepatitis/Primary biliary cholangitis (PBC) overlap syndrome can often be diagnosed with certainty based on histologic and clinical findings. For any

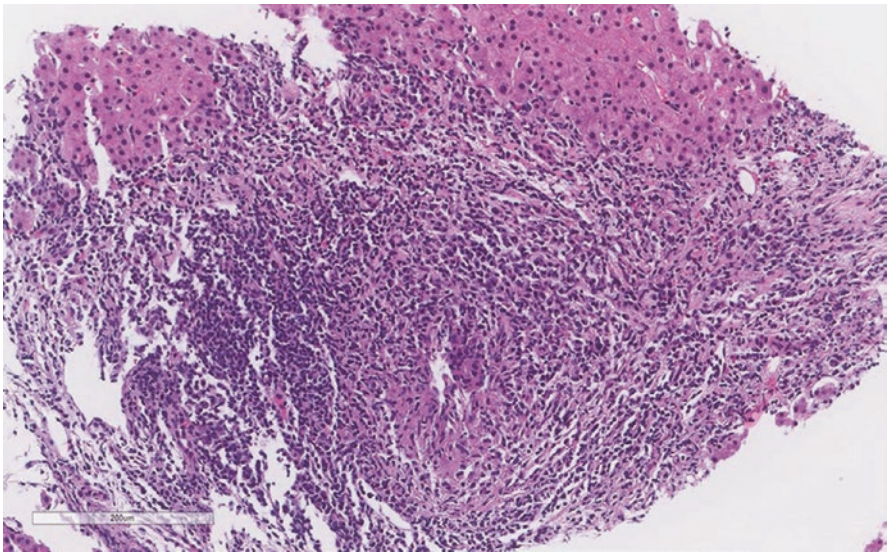

Fig. 4.14 A single portal granuloma associated with localized duct destruction is seen in this case of primary biliary cholangitis-autoimmune hepatitis overlap. A striking plasma cell population is seen in this portal tract, which may be seen in PBC alone, however the case also showed prominent interface and lobular lymphoplasmacytic activity which is not typically seen in PBC. Serologic workup revealed elevated ANA and anti-smooth muscle antibody, as well as elevated IgG and IgM, supporting the diagnosis

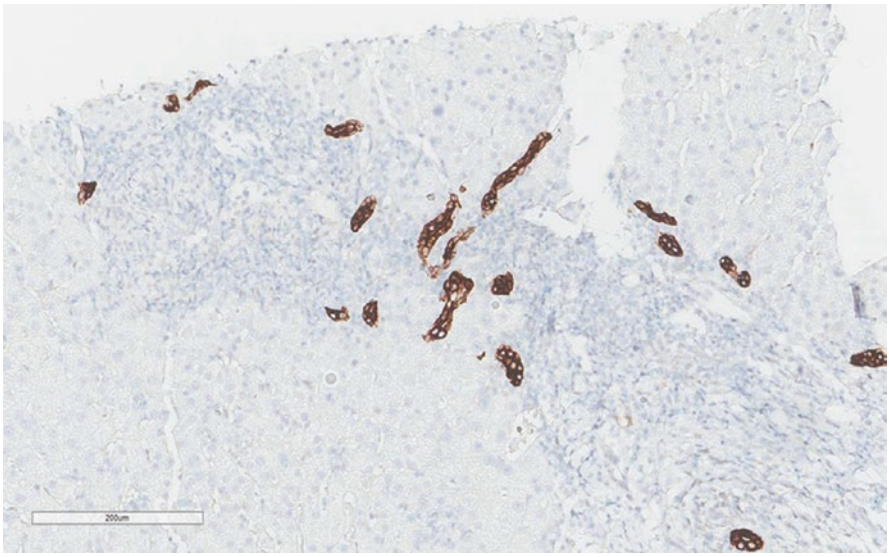

Fig. 4.15 Immunostain for CK7 in this case of PBC-AIH overlap highlights a biliary proliferation (which is not typically seen in cases of AIH, except in the setting of parenchymal collapse and hepatic regeneration). Notably, the CK7 stain also shows marked reduction in canals of Hering. The typical portal tract will demonstrate 3–16 canals of Hering [14], which appear on immunostains for CK7 or CK19 as isolated cholangiocytes or short strings of cells arrayed around the portal tract at the limiting plate. Only very rare canals were identified in this case (1–2 canals of Hering are seen in this image). Canals of Hering are an early marker for PBC, although the finding is not entirely specific and loss has been documented in drug injury (specifically methotrexate) as well [18]. In this particular case, the finding is supportive of the diagnosis of PBC-AIH overlap

patient with positive AMA, a component of PBC should be considered. PBC will often have prominent plasma cells in portal tracts, so even a striking portal plasma cell infiltrate does not necessarily denote a component of autoimmune hepatitis. However, lobular activity should not be prominent in PBC alone, therefore classic features of PBC combined with interface and lobular activity with scattered apoptotic hepatocytes (ideally including prominent lobular plasma cells) should prompt consideration for a component of AIH. Autoimmune hepatitis may show some subtle bile duct damage, but will not typically show the classic florid duct lesions or granulomatous duct destruction typical in PBC. In addition, evaluation of canals of Hering using CK7 or CK19 might be helpful, as these are often reduced or lost in PBC. Loss of canals of Hering and CK7 positive cholangiocytes may be the central histologic findings of so-called “minimal change PBC”, a diagnosis which must be made in conjunction with clinical/serologic findings [13].

Autoimmune hepatitis/primary sclerosing cholangitis overlap requires similar diagnostic rationale. Significant lobular activity is not typical in PSC. Autoimmune hepatitis will not typically demonstrate peribiliary concentric fibrosis (Figs. 4.16, 4.17 and 4.18). Native ductopenia, another clue pointing towards a chronic biliary process, is not typically seen in autoimmune hepatitis. Portal nodular scarring in areas of lost bile ducts is virtually diagnostic for a component of PSC.

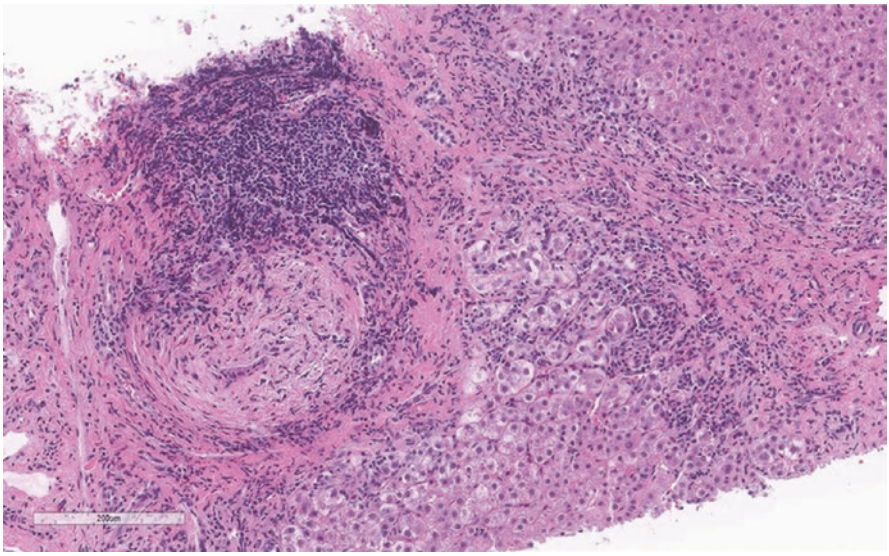

Fig. 4.16 Autoimmune hepatitis-primary sclerosing cholangitis overlap. On the left peribiliary concentric fibrosis is seen surrounding what is left of an interlobular bile duct (this is nearly a complete nodular scar). On the right periportal plasma cell rich inflammation is seen with hepatocyte rosette formation. This biopsy is from a pediatric patient with ulcerative colitis and elevated ANA and anti-smooth muscle antibody

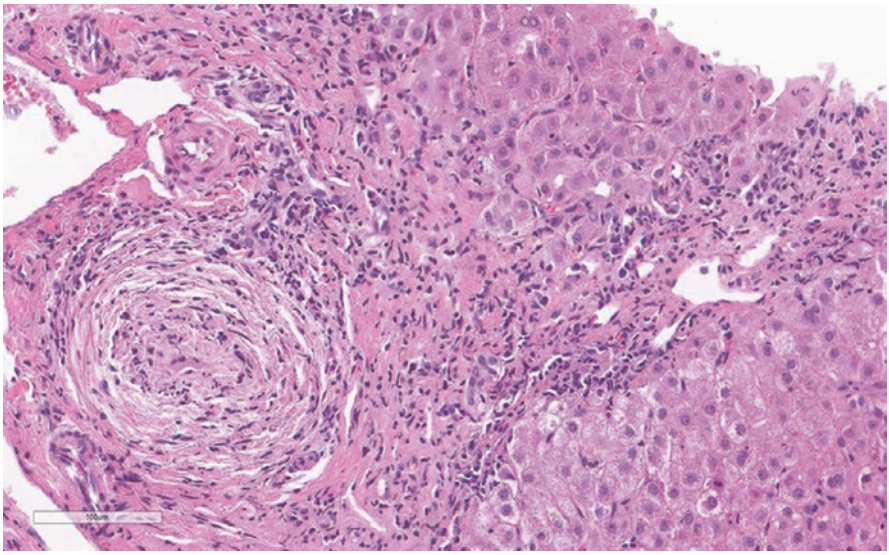

Fig. 4.17 Autoimmune hepatitis-primary sclerosing cholangitis overlap. On the left peribiliary concentric fibrosis is seen surrounding what is left of an interlobular bile duct (this is nearly a complete nodular scar). On the right periportal plasma cell rich inflammation is seen with hepatocyte rosette formation

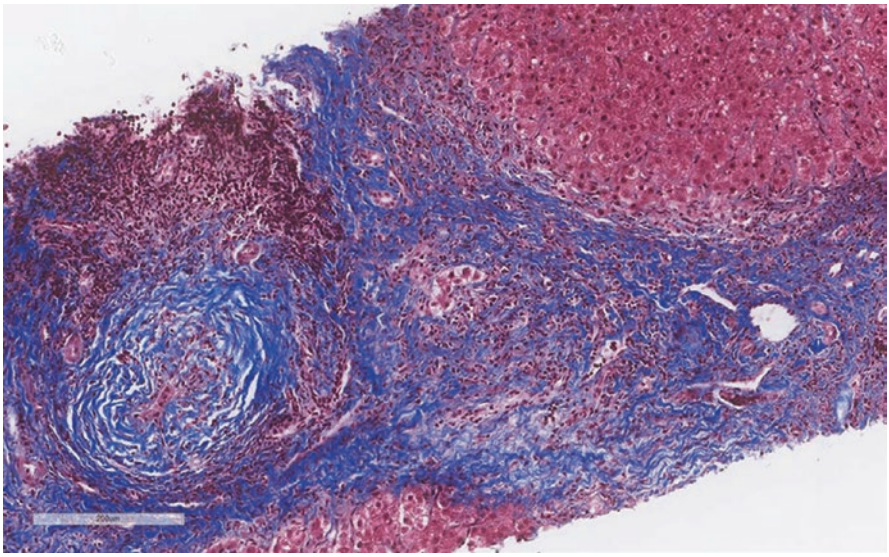

Fig. 4.18 Trichrome stain highlights the concentric periductal fibrosis and advanced fibrosis in this case of autoimmune hepatitis-primary sclerosing cholangitis overlap

IgG4 mediated disease is another consideration for patients with prominent plasma cells and bile duct damage. Neither autoimmune hepatitis, nor primary sclerosing cholangitis should present with many IgG4 positive plasma cells therefore immunostain for IgG and IgG4 can lead to the correct diagnosis in these cases.

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