



Primary sclerosing cholangitis: a narrative review of diagnostic and prognostic biomarkers

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Background and Objective: Primary sclerosing cholangitis (PSC) is an autoimmune biliary fibrosing disease characterized by inflammation and injury of the intra- and/or extrahepatic bile ducts. The pathogenesis of PSC is poorly understood but is believed to be multifactorial, involving genetic predisposition, immunological dysregulation, and environmental influences. These may include disturbances in the gut-liver axis such as immune dysfunction in the colon and liver, alterations in the fecal and biliary microbiome, conjugation of bile acids into toxic species, and compromised intestinal epithelial integrity due to colitis, resulting in translocation of bacterial byproducts to the liver. There is a critical need for diagnostic and prognostic biomarkers that would enhance management and outcomes for patients with PSC. Additionally, validation of such biomarkers could serve as measurable endpoints when conducting future clinical trials. This aim of this paper is to review the available literature on candidate diagnostic and prognostic biomarkers in the adult and pediatric PSC populations.

Methods: Original studies investigating biomarkers in serum, bile, and tissue published until November 2024 were systematically searched on PubMed, with a specific focus on newer studies published in the past 10 years and pediatric studies. Small studies with fewer than 10 patients in each study group, animal model studies, and studies with a focus on biomarkers for cholangiocarcinoma were excluded.

Key Content and Findings: Diagnostic and prognostic biomarkers summarized in this review include autoantibodies, markers of innate and adaptive immune responses, extracellular vesicles, epigenetic modifications, microbiome, proteins involved in lipid metabolism and bile acid homeostasis, and markers of fibrogenesis. Novel concepts for future biomarker discovery and implementation, including the potential for insights to be gained from the pediatric PSC population, are explored.

Conclusions: There is a critical need for further biomarker discovery for PSC as it will provide clues to disease pathogenesis and uncover candidate targets for therapeutic intervention.

Keywords: Autoantibody; epigenetics; extracellular vesicles; innate immunity; microbiome

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Introduction

Primary sclerosing cholangitis (PSC) is a progressive fibrosing cholangiopathy characterized by inflammation and injury of the intra- and/or extrahepatic bile ducts leading to biliary fibrosis. It is a rare diagnosis with an estimated incidence of up to 1.3 per 100,000 adults (1-4) and 0.2 per 100,000 children (5). Disease progression can be highly variable but ultimately results in biliary cirrhosis and end-stage liver disease. PSC is associated with significant morbidity, including portal hypertensive complications, biliary infections, and cholangiocarcinoma (CCA) (5-8). The rate of liver transplant has been reported to be approximately 40% at a median of 10 years after PSC diagnosis in adults (9) and 14% at a median of 4 years after diagnosis in children (7). Pediatric PSC has many unique characteristics compared to adults, including a 5 to 10-fold higher rate of PSC overlap with autoimmune hepatitis (AIH) and a higher incidence of isolated small duct PSC (7). Furthermore, pediatric PSC liver histology is more inflammatory and less fibrotic compared to adults, with lower rates of cirrhosis at presentation and higher rates of asymptomatic patients. This robust inflammatory environment may provide a window to early events in disease pathogenesis, prior to the onset of significant fibrosis and cirrhosis. However, this population has yet to be well studied.

PSC is associated with inflammatory bowel disease (IBD) and particularly ulcerative colitis (UC) in ~80% of cases, while only 5–7% of patients with UC will develop PSC (10,11). It remains unclear why certain patients with IBD develop PSC and it has been proposed that PSC-IBD should be considered as a unique disease entity (12). There are no prognostic biomarkers to predict which IBD patients will go on to develop PSC. Patients with PSC-UC have a unique IBD phenotype characterized by pancolitis, rectal sparing, and backwash ileitis. These patients have a relatively mild IBD clinical course including lower rates of colectomy, but conversely have higher rates of colorectal carcinoma (11,13,14).

As demonstrated in this review, the link between PSC and UC has facilitated biomarker discovery and provided clues to disease pathogenesis. As such, several defects in the ‘gut-liver axis’ are implicated in the pathogenesis of PSC, including immunologic disturbances in the colon and liver, a distorted fecal and biliary microbiome, conjugation of bile acids into toxic species, and disruption of the intestinal epithelial barrier due to colitis that results in bacterial

or bacterial byproduct translocation to the liver (15,16). Immunologic mechanisms that have been proposed include homing of gut-imprinted T lymphocytes to the liver following clonal expansion due to shared integrin molecules in the gut and liver endothelia (17,18). Furthermore, the “leaky gut” theory proposes a role for the innate immune system in the pathogenesis of PSC. It is suggested that in the setting of colonic inflammation, the intestinal microbiota is altered and the disrupted epithelial barrier allows bacterial byproducts to enter the portal circulation, triggering local innate immune activation through toll-like receptor signaling (15,16,18-21). The immune system in the liver and in the intestines has receptors that recognize microbial products, resulting in recruitment of pro-inflammatory cells and subsequent bile duct injury (20). Additionally, B-cell mediated immune responses appear to play a role given that autoantibodies such as perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and immunoglobulin A (IgA) targeting glycoprotein 2 (anti-GP2) have been observed in patients with PSC. These findings will be discussed at greater length in this review.

With regard to a genetic association with disease, more than 20 susceptibility genes have been identified through genome wide association studies, however the combined impact of genetic susceptibility factors on overall PSC risk is estimated to be less than 10% (16). The strongest association that has been identified is the human leukocyte antigen (HLA) complex on chromosome 6. Genetic variants on the HLA complex are known to be associated with autoimmune conditions, supporting an autoimmune etiology for PSC and involvement of the adaptive immune system (22).

There is a critical need for both diagnostic and prognostic biomarkers that would alter screening, management and counseling for patients with PSC, as well as provide clues to disease pathogenesis and end-points for clinical trials. A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (23). Given the lack of a single prognostic marker for PSC, non-invasive prognostic models that utilize clinical and biochemical variables have been developed. The revised Mayo risk score (rMRS) estimates adult PSC patient survival and the Amsterdam-Oxford and United Kingdom-PSC scores predict transplant-free survival (24-26). The enhanced liver fibrosis (ELF) score entails serological measurements of three surrogates of fibrogenesis

(hyaluronic acid, tissue inhibitor of metalloproteinase 1, and type III procollagen amino-terminal propeptide); this score also predicts transplant-free survival (27). In pediatrics, the Sclerosing Cholangitis Outcomes in Pediatrics (SCOPE) index, which uses biochemical variables and cholangiography, was developed to assess risk for disease progression and complications in children under 18 years of age (28). Biomarkers highlighted in this review and future biomarker discovery may complement these models in the prognosis of disease severity. Validation of such biomarkers would provide measurable endpoints that could be used as outcome measures when conducting clinical trials. Furthermore, biomarker discovery will likely provide clues to disease pathogenesis and has the potential to uncover candidate targets for therapeutic intervention.

This paper will review the available literature on candidate diagnostic and prognostic biomarkers in the adult and pediatric populations (*Table 1*). These biomarkers include autoantibodies, markers of innate and adaptive immune responses, extracellular vesicles, epigenetic modifications, the microbiome, proteins involved in lipid metabolism and bile acid homeostasis, and markers of fibrogenesis (*Figure 1*). We present this article in accordance with the Narrative Review reporting checklist (available at <https://tgh.amegroups.com/article/view/10.21037/tgh-25-13/rc>).

Methods

For the purpose of this narrative review, original studies investigating biomarkers in serum, bile, and tissue published until November 2024 were systematically searched on PubMed, with a specific focus on newer studies published in the past 10 years. Small studies with fewer than 10 patients in each study group, animal model studies, and studies with a focus on biomarkers for CCA were excluded (*Table 2*). Although biomarkers to identify CCA are an important component of disease management in the adult population, this will not be highlighted and the reader is referred to a review on the topic of CCA biomarkers by Catanzaro *et al.* (71). Additionally, transient elastography and other radiologic biomarkers are beyond the scope of this review and have recently been summarized by Tow *et al.* (72). We conclude with discussing the unmet needs and future directions of PSC biomarker research and how biomarker discovery, particularly in the pediatric population, will enable further understanding of disease pathogenesis and the advancement of therapeutic interventions.

Potential diagnostic and prognostic biomarkers

Activated immune responses

Autoantibodies

Autoantibodies play a pivotal role in autoimmune diseases and their presence in PSC has been a subject of in-depth investigation. A number of autoantibodies have been observed in serum of patients with PSC, but none are disease specific. Their association with PSC is not clear and may simply reflect an immunologic epiphenomena (73). PSC is associated with other autoimmune diseases and genome-wide association studies have shown a strong association with HLA haplotypes; therefore, immune mediated factors may be involved in its pathogenesis (74).

The most widely studied autoantibody in PSC is the anti-neutrophil cytoplasmic antibody (ANCA), present in 65–95% (75,76) of patients with PSC, yet its immunologic basis and clinical significance is not well understood. ANCAs were first discovered in 1989 (77) and are a group of autoantibodies that target proteins in the cytoplasm of neutrophils. ANCAs are divided into two classes based on their pattern of immunostaining: perinuclear ANCA (pANCA) entails staining of the perinuclear cytoplasm, and cytoplasmic ANCA (cANCA) includes diffuse granular staining of the cytoplasm. pANCA has been associated with antibodies against a variety of antigens including lactoferrin, elastase and myeloperoxidase (78). In PSC and AIH, pANCA autoantibodies do not often react with these classical pANCA antigens (79); rather, they react with the nuclear envelope antigens in neutrophils, changing their designation to atypical pANCAs (aANCA).

cANCA is often associated with autoimmune vasculitis, such as granulomatosis with polyangiitis and microscopic polyangiitis. cANCA is attributed to the presence of autoantibodies specifically targeting serine proteases such as proteinase 3 (PR3-ANCA). PR3-ANCA has been associated with IBD and, in particular, UC (80). PR3 has been shown to activate many inflammatory factors and proteolytic enzymes [interleukin (IL)-1b, IL-8, tumor necrosis factor- α , etc.] (81) and by doing so may contribute to biliary inflammation. Furthermore, autoantibodies against PR3 may lead to PR3 inactivation, leading to interference of PR3-dependent caspase 3 cleavage, which modulates neutrophil death and inflammation (82).

With this background, there have been a number of studies investigating the prevalence of ANCA subtypes in patients with PSC, with or without UC, and whether or not its presence is correlated with disease severity and

Table 1 Summary of candidate diagnostic and prognostic biomarkers

Biomarker	Biospecimen Source	Dx or Px	Predict transplant-free survival?	Sample size (n): PSC/IBD/healthy control/liver disease control	Ref.	Year
Autoantibodies						
ANCA	Serum	Neither	No	241/274/368/–	(29)	2017
	Bile	Px	No	39/–/–/33	(30)	2013
PR3-ANCA	Serum	Dx	Not assessed	244/–/–/254	(31)	2014
	Serum	Px	Yes	522/–/–/–	(32)	2021
	Serum	Dx	Not assessed	22/117/164/–	(33) [†]	2022
Anti-GP2	Serum	Dx and Px	Yes	138/62/52/–	(34)	2017
	Serum	Px	Yes	65/–/100/488	(35)	2018
Anti-GP2 isoforms	Serum	Dx	Not assessed	212/–/95/50	(36)	2018
	Serum	Px	Yes	522/–/–/–	(32)	2021
Innate and adaptive immune responses						
Calprotectin	Bile	Dx	Not assessed	56/–/–/38	(37)	2014
	Bile	Dx and Px	Yes	106/–/–/20	(38)	2016
Calprotectin (S100A8/9)	Bile	Dx and Px	Not assessed	89/–/–/30	(39)	2017
IL-8	Serum	Px	Yes	305/96/100/–	(39)	2017
Osteopontin	Serum	Dx and Px	Yes	68/32/29/–	(40)	2024
Soluble CD163	Serum	Px	Yes	297/–/–/–	(41)	2021
Mannose receptor	Serum	Px	Yes	297/–/–/–	(41)	2021
Soluble CD14	Serum	Px	Yes	166/–/100/–	(42)	2019
Eotaxin-1 and -3	Serum	Dx	Not assessed	58/–/50/144	(43)	2014
CCL24 (eotaxin-2)	Serum	Neither	Not assessed	45/–/30/–	(44)	2024
VAP-1	Serum	Dx and Px	Yes	134/50/54/145	(45)	2018
Soluble CD40	Serum	Dx	Not assessed	22/28/19/–	(46)	2019
Extracellular vesicles						
Aminopeptidase N	Serum	Dx	Not assessed	30/–/32/–	(47)	2017
Ficolin-1	Serum	Dx	Not assessed	30/–/32/–	(47)	2017
Neprilysin	Serum	Dx	Not assessed	30/–/32/–	(47)	2017
IL13-Ra1	Serum	Dx and Px	Not assessed	25/–/25/–	(48)	2022
Epigenetic modifications						
<i>NINJ2</i> CpG hypermeth.	Serum	Dx	Not assessed	27/27/22/–	(49)	2022
<i>NOX5</i> CpG hypermeth.	Serum	Dx	Not assessed	78/–/40/–	(50)	2020
<i>IL17F</i> hypometh.	Serum	Dx	Not assessed	78/–/40/–	(50)	2020
miR-200c	Serum	Dx	Not assessed	70/–/70/20	(51)	2016
miR-122	Serum	Px	Yes	134/–/–/–	(52)	2018

Table 1 (continued)

Table 1 (continued)

Biomarker	Biospecimen Source	Dx or Px	Predict transplant-free survival?	Sample size (n): PSC/IBD/healthy control/liver disease control	Ref.	Year
Microbiome						
<i>Enterococcus</i>	Bile	Px	Yes	189/–/–/–	(53)	2023
	Bile	Dx	Not assessed	43/–/–/22	(54)	2020
	Stool	Dx	Not assessed	64/42/1120/–	(55)	2019
	Stool	Dx	Not assessed	52/43/52/–	(56)	2016
	Stool	Dx	Not assessed	43/32/31/–	(57)	2017
	Stool	Dx	Not assessed	13/15/23/–	(58) [†]	2017
<i>Veillonella</i>	Stool	Dx	Not assessed	85/36/263/–	(59)	2017
	Stool	Dx	Not assessed	30/–/23/–	(60) [†]	2020
<i>Megasphaera</i>	Stool	Dx	Not assessed	30/–/23/–	(60) [†]	2020
<i>Firmicutes</i>	Stool	Dx	Not assessed	30/–/23/–	(60) [†]	2020
Vitamin B6	Serum & Stool	Dx and Px	Yes	136/93/158/–	(61)	2021
	Serum	Dx and Px	Yes	373/–/100/–	(62)	2023
BCAA	Serum & Stool	Dx and Px	Yes	136/93/158/–	(61)	2021
AAA-IgA	Serum	Dx and Px	Yes	67/172/153/–	(63)	2017
Lipid metabolism & bile acid homeostasis						
LXR/RXR and FXR/RXR	Serum	Dx	Not assessed	80/–/–/18	(64)	2022
AGPAT1	Colonic tissue	Dx	Not assessed	25/28/7/–	(65)	2022
Autotaxin	Serum	Px	Yes	252/–/–/–	(66)	2019
C4	Serum	Px	Yes	330/–/100/–	(67)	2022
Fibrogenesis						
Pro-C3	Serum	Px	Not assessed	234/–/–/–	(68)	2024
	Serum	Px	Yes	138/52/–/–	(69)	2018
MMP7	Serum	Dx	Not assessed	12/–/–/42	(70) [†]	2020

[†], denotes pediatric study. AGPAT1, 1-acetylgllycerol-3-phosphate O-acyltransferase 1; ANCA, anti-neutrophil cytoplasmic antibody; AAA, anti-F-actin antibodies; BCAA, branched-chain amino acid; C4, 7 α -hydroxy-4-cholesten-3-one; CCL24, eotaxin-2; CpG, cytosine-phosphate-guanine dinucleotide; Dx, diagnostic; FXR, farnesoid X receptor; GP2, glycoprotein 2; hypermeth., hypermethylation; IBD, inflammatory bowel disease; IgA, immunoglobulin A; IL, interleukin; LXR, liver X receptor; MMP7, matrix metalloproteinase 7; miR, microRNAs; PR3, proteinase 3; Pro-C3, propeptide of type III collagen; PSC, primary sclerosing cholangitis; Px, prognostic; RXR, retinoid X receptor; S100A8/9, subunits of calprotectin; VAP-1, vascular adhesion protein-1.

risk of liver transplant. Hov *et al.* characterized the clinical and genetic associations of ANCA in PSC (29). ANCA was analyzed in the serum of patients with large duct PSC, healthy controls, and UC without PSC. ANCA was present in 80% of patients with PSC, the majority being pANCA (70%), and ANCA-positive patients were diagnosed at an earlier age (33 *vs.* 41 years, $P < 0.001$). HLA-B 08 and

DRB1-03 were more prevalent in the ANCA-positive cohort. Transplant free survival was similar in the PSC ANCA-positive and ANCA-negative groups.

Lenzen *et al.* demonstrated that immunoglobulin G (IgG)-ANCA in the bile correlated with risk of PSC and dominant strictures but not with risk of death or liver transplantation (30). There was a higher frequency of IgG-

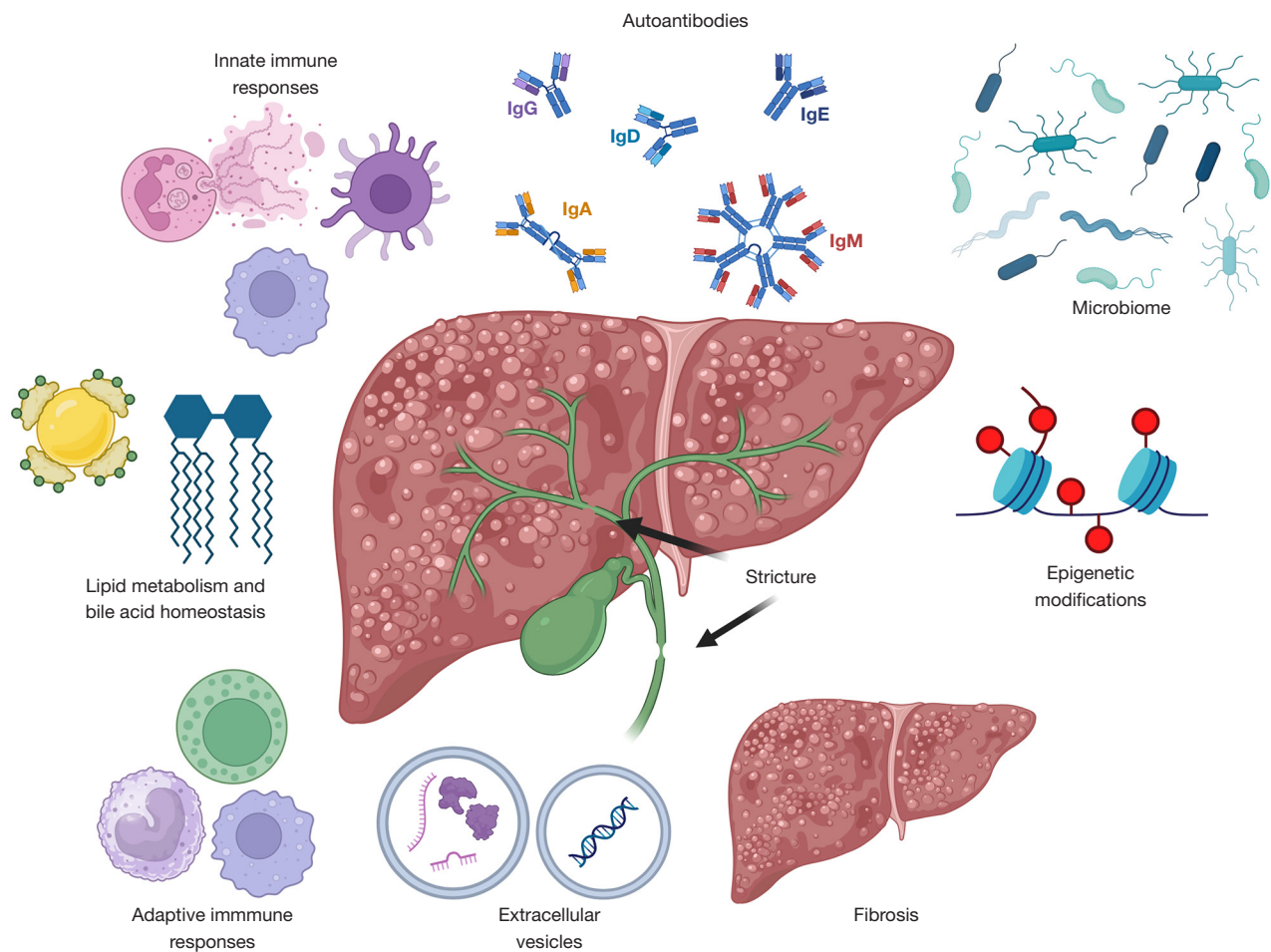


Figure 1 Overview of key areas of biomarker discovery in primary sclerosing cholangitis. Ig, immunoglobulin.

Table 2 Summary of search strategy	
Items	Specification
Date of search	October 1, 2023 to December 1, 2024
Database	PubMed
Search terms	Primary sclerosing cholangitis AND “biomarker” OR “biologic marker” OR “prognostic” OR “diagnostic”
Timeframe	Up to November 2024 with specific focus on newer studies published since 2014
Inclusion and exclusion criteria	Inclusion: original studies, studies investigating serum, bile or tissue diagnostic or prognostic biomarkers Exclusion: studies with n<10 PSC patients, animal model studies, studies with a focus on cholangiocarcinoma, studies with a focus on radiologic or transient elastography markers, articles not published in the English language
Selection process	The search and selection of articles were conducted by P.S.R. These were the reviewed and the most relevant papers selected by all authors including senior authors E.D.Z., L.F. and C.L.M.

PSC, primary sclerosing cholangitis.

ANCA in the bile of PSC than those without PSC (38% *vs.* 6%, $P=0.001$). In addition, IgG-ANCA in bile was associated with a 10-fold higher risk of PSC ($P=0.005$) and, using logistic regression, IgG-ANCA was an independent predictor of PSC with odds ratio (OR) of 10.8 [95% confidence interval (CI): 2.1–55.8, $P=0.005$]. They also demonstrated that IgG-ANCA was significantly associated with the presence of dominant strictures, severity of cholangiographic features using Amsterdam score, number of endoscopic retrograde cholangiopancreatographies (ERCPs) performed and number of interventions performed. They did not find an association between serum ANCA and disease activity and progression and postulated that bile ANCA is more representative of ongoing injury and a strong local immune response.

With regard to PR3-ANCA, Stinton *et al.* detected PR3-ANCA in 39% of patients with PSC (11% of controls; $P<0.0001$) and aANCA in 41% of patients with PSC (20% of controls; $P<0.0001$) (31). PR3-ANCA-positive status was significantly associated with younger age, higher ALT and alkaline phosphatase and lower platelet levels. Wunsch *et al.* (32) identified PR3-ANCA in 55% of patients with PSC and highest frequencies occurred in those patients with PSC and IBD (OR 1.9; $P=0.008$). The presence of PR3-ANCA was associated with shorter transplant-free survival [hazard ratio (HR) 4.5, 95% CI: 1.5–13.6]. With regard to pediatric PSC, Laass *et al.* (33) found that the prevalence of PR3-ANCA was 41% in those with UC compared to 11% in Crohn's disease ($P<0.00001$) and PR3-ANCA was more prevalent in UC with PSC than those without PSC (68% *vs.* 31%; $P=0.0014$). Median PR3-ANCA values were significantly higher in those with PSC than without PSC and PR3-ANCA positivity was associated with statistically higher liver enzyme levels.

Another intriguing autoantibody in PSC is anti-glycoprotein 2 (anti-GP2). Anti-GP2 is a member of a family of pancreatic autoantibodies directed against proteins that are predominantly expressed in the exocrine pancreas (83). GP2 is an immune modulator (84) and microbe sensor (85), interacting with pathogenic bacteria and modulating both innate and adaptive immune responses to intestinal microbiota. A pathogenic role for biliary GP2 has recently been suggested as Lopens *et al.* found GP2 to be expressed in the peribiliary glands and was identified in the bile of patients with PSC (86). Anti-GP2 antibodies are known to target the pancreas; however, they have also been detected in the bile of patients with PSC (86,87). It is plausible that there are interactions between anti-GP2

antibodies and biliary epithelial cells which trigger immune responses resulting in biliary injury and fibrosis. Jendrek *et al.* identified anti-GP2 IgA in 52% of patients with PSC compared to 2% of controls ($P<0.001$) (34) and the association of anti-GP2 with PSC was independent of the presence of IBD. Anti-GP2 was significantly associated with disease severity based on rMRS, bilirubin and transplant-free survival. Tornai *et al.* (35) detected anti-GP2 antibodies in 46% of patients with PSC, 5% of other liver diseases and 0% in healthy controls ($P<0.001$). Anti-GP2 antibodies were associated with a more severe PSC phenotype (higher liver enzymes, lower albumin, increased rMRS, cirrhosis) and were significantly associated with shorter transplant-free survival ($P<0.01$), which was an independent predictor after adjusting for rMRS (HR 4.69, 95% CI: 1.05–21.04; $P=0.043$). Interestingly, flow cytometry demonstrated that 68% of the anti-GP2 IgA antibodies were bound with a secretory component and the authors postulated that this binding was due to active retro-transportation of anti-GP2 from the gut lumen to the mucosa (and hence, potentially to the portal circulation).

Antibody production to specific isoforms of GP2 (GP2₁₋₄) has also been investigated in PSC. Detection of anti-GP2₁ and/or anti-GP2₄ IgA had a 66% sensitivity and 98% specificity for the diagnosis of PSC; the presence of these antibodies was associated with cirrhosis (36). Similar to the findings of Jendrek *et al.*, there was no association of anti-GP2 and PSC with or without IBD (34). Wunsch *et al.* identified anti-GP2₁ and/or anti-GP2₄ in 31% of patients with PSC (32). Anti-GP2₄ IgA was associated with cirrhosis (OR 2, $P=0.001$) and anti-GP2_{1/4} IgA was significantly associated with disease severity based on rMRS, Model for End-stage Liver Disease (MELD) score, and liver function tests. Quality of life was lower in those with anti-GP2₁ IgA-positive. Both anti-GP2₁ and anti-GP2₄ were associated with decreased transplant free survival ($P<0.01$). The authors postulated that PSC pathophysiology may involve loss of mucosal tolerance against GP2.

These studies demonstrate that autoantibodies detected in serum and/or bile may function as diagnostic and prognostic biomarkers. PR3-ANCA performs better than aANCA for the diagnosis of PSC and may help differentiate PSC from other immune disorders. Anti-GP2 antibodies may help identify a subgroup of patients with a more severe phenotype. Whether or not these autoantibodies actually contribute to the pathophysiology of PSC or can be used to stratify disease severity is speculative at this point. As research progresses, a deeper understanding of

these autoimmune markers will pave the way for targeted therapeutic strategies and improved clinical management of PSC.

Innate and adaptive immunity

The immune system includes both innate (immediate) and adaptive (delayed) responses that entail pro- or anti-inflammatory pathways. Constituents of innate immunity include neutrophils, macrophages, natural killer cells and eosinophils (88). Neutrophil activation is associated with the generation of calprotectin, a “danger signal” that promotes inflammatory and autoimmune responses. Voigtländer *et al.* found that calprotectin was significantly increased in the bile of patients with PSC compared to other liver disease controls. The bile calprotectin levels were higher in patients with high rMRS and positively correlated with alkaline phosphatase levels, suggesting that bile calprotectin was a biomarker of disease severity (37). Similarly, Gauss *et al.* identified high levels of bile calprotectin in PSC compared to controls (38). High bile calprotectin concentrations were associated with the presence of microbes, dominant strictures and a decreased transplant-free survival. Unfortunately, ERCP-guided collection of bile is invasive and thus limits bile calprotectin as a feasible biomarker.

An unbiased proteomics approach was utilized to identify a panel of proteins that predicted disease severity in PSC. PSC-related proteins were first characterized from bile specimens and then measured in the serum as potential candidate biomarkers (39). Bile proteins associated with PSC diagnosis and grade of cholangiographic changes included IL-8, matrix metalloproteinase 9, and S100A8/9 (subunits of calprotectin), among others. S100A8 was the single marker that best distinguished mild from advanced PSC. IL-8 is a chemokine that attracts neutrophils to sites of inflammation and is involved in neutrophil survival and activation (89). Patients with PSC were stratified according to tertiles of serum IL-8, resulting in excellent discrimination for transplant-free survival (39).

With regard to macrophage activation as a potential biomarker in PSC, a recent study utilizing a mouse model of PSC identified an infiltrative monocyte-derived macrophage population in the liver that produced osteopontin (40). Inhibition of osteopontin protected the mice from developing PSC. This study also analyzed serum from patients with PSC and found that osteopontin levels were significantly increased in PSC compared to controls and serum osteopontin levels correlated with liver fibrosis

grade and was predictive of transplant-free survival.

Other markers of macrophage activation, soluble cluster of differentiation (CD) 163 (sCD163) and mannose receptor (sMR), are increased in a variety of autoimmune liver diseases, including PSC (41). sCD163 and sMR serum levels increased with PSC disease severity based on rMRS and ELF test and sCD163 predicted transplant-free survival (area under the receiver operating characteristic curve: 0.79) (41). In addition, macrophage soluble CD14 was significantly increased in PSC and predicted increased risk for liver transplant or death (42).

Eosinophil activation as a biomarker in PSC was analyzed based on serum expression of two eosinophil chemokines, eotaxin-1 (E1) and eotaxin-3 (E3) (43). E1 was significantly elevated in PSC compared to other autoimmune liver diseases and E3 was higher in all autoimmune liver diseases compared to healthy controls. In a study that utilized an unbiased serum proteomics approach, a unique protein signature was identified to predict PSC disease and was associated with high ELF scores (44). This study examined the role of CCL24 (eotaxin-2), a chemokine with fibroinflammatory activity, including recruitment of neutrophils and monocytes (90). High levels of CCL24 were associated with the presence of cirrhosis in PSC. Collectively, these studies suggest that innate immune activation contributes to biliary injury and outcomes in PSC and further investigations of innate immune proteins as biomarkers is warranted.

Adaptive immunity involves cellular (T cell) and humoral (B cell) responses. Humoral responses include antibody production which is detailed above. Cellular adaptive immunity includes the activation of T cells and associated pro-inflammatory cytokine production (88). Trivedi *et al.* analyzed vascular adhesion protein-1 (VAP-1) in patients with PSC, a constituent involved in intestinal mucosal T cell migration into the liver (45). VAP-1 was significantly elevated in the liver of patients with PSC compared to other immune-mediated diseases. *In-vitro* inhibition of VAP-1 resulted in decreased adhesion of gut tropic α4β7-positive T cells, a key T cell that contributes to liver injury in PSC. In regard to VAP-1 as a biomarker, high serum soluble VAP-1 levels predicted worse transplant-free survival. Lampinen *et al.* analyzed the immunophenotype of patients with UC-only *vs.* PSC-UC and identified CD40 as a strong candidate diagnostic biomarker for PSC (46). Antigen-presenting cells express CD40, which binds to CD40 ligand on T cells, promoting T cell activation.

Extracellular vesicles (EVs)

EVs are membrane-bound vesicles released by cells that have a role in cell-to-cell communication and are increasingly recognized to have a wide range of effects on target cells. They can contain lipids, proteins, and nucleic acids. There is a growing body of evidence to suggest that EVs play an important role in liver pathophysiology (91). Specifically in PSC pathogenesis, it has been found that cholangiocytes undergo cellular senescence and release EVs (92). These EVs have been implicated in inducing proliferation and malignant progression of human cholangiocytes and immune cell activation and migration. In a comparative analysis of the proteomic content of serum EVs, 161 proteins were differentially expressed in patients with PSC versus healthy controls (47). Specifically, aminopeptidase N, ficolin-1, and neprilysin demonstrated the best diagnostic capacity for PSC. Another proteomic study of EVs found elevated levels of circulating EVs in patients with PSC and further identified 282 proteins that were differentially regulated in patients with PSC compared with healthy controls (48). Among these proteins, IL13-Ra1 was the most unique marker for PSC EVs compared with healthy control EVs and higher levels were associated with increased severity of liver fibrosis. IL-13Ra1 is a receptor for IL-13, which has been implicated for its role in inflammatory and autoimmune conditions, as well as fibrotic conditions. In addition to studying the proteomic profile of EVs, the transcriptomic profile of serum and urine EVs has been explored. When compared with a group of healthy controls and patients with UC, patients with PSC were found to have unique transcriptomic signatures in both serum and urine EVs (93). Although the investigation of EVs in patients with PSC is still in its early stages, EVs have demonstrated potential to serve as non-invasive biomarkers for diagnosing PSC.

Epigenetic modifications

Epigenetic modifications occur through heritable mechanisms that involve transcriptional regulation without changes to the DNA sequence, such as by DNA methylation, histone modification, and non-coding RNA sequences. While genome-wide association studies have identified some risk loci for PSC, it is estimated that genetic variants contribute less than 10–20% to disease pathogenesis thereby implicating epigenetic modifications in the heritability of this disease (16,94). Recent studies

have found clues to the role of epigenetic regulation in the development of PSC. de Krijger *et al.* investigated the peripheral blood DNA methylome, yielding a model that could discriminate between patients with PSC-UC from UC-only using a predictive model with 18 cytosine-phosphate-guanine dinucleotide (CpG) sites. Four of these CpG loci were annotated to the *NIN72* gene with hypermethylation found in PSC-UC patients compared to UC-only patients (49). *NIN72*, which encodes nerve injury induced protein 2, is highly expressed in myeloid cells and peripheral leukocytes, suggesting a role in immune-mediated disease. Similarly, Moore *et al.* analyzed the DNA peripheral blood methylome and identified a hypermethylated intronic CpG in *NOX5* [encodes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase] in patients with PSC with and without IBD and hypomethylation of an exon of *IL17F* (pro-inflammatory cytokine) in patients with PSC (50). Importantly, this study demonstrated that some of the top differentially methylated CpGs (DMCs) in peripheral blood were also observed in liver tissue, highlighting the potential utility of DMCs as non-invasive biomarkers.

MicroRNAs (miRNAs) are non-coding RNA that are involved in post-transcriptional gene regulation and have been implicated in normal physiology as well as the control of disease states. Bernuzzi *et al.* assessed specific serum miRNAs as diagnostic biomarkers for PSC and found miR-200c to be downregulated in patients with PSC compared with controls (51). miR-200c has been shown to directly target neural cell adhesion molecule 1 (NCAM1), a known hepatic stem/progenitor cell marker; and inactivation of miR-200c is known to induce the epithelial-mesenchymal transition (95). In a cohort of patients with PSC, Friedrich *et al.* studied the prognostic value of miR-122, which is primarily expressed in the liver and has been associated with other liver-disease processes (52). Patients with low levels of miR-122 were found to have impaired actuarial transplant-free survival, while miR-122 was found to be an independent predictor of survival in patients with PSC (52).

Microbiome

The high occurrence of IBD with PSC implies that the gut-liver axis is playing a significant role in disease pathogenesis. The gut-liver axis involves bidirectional communication between the gut and the liver; intestinal blood enters the liver via the portal vein and bile ducts directly

communicate with the intestines, delivering bile (15). Gut microbiota or byproducts that enter the portal venous system may promote inflammatory and autoimmune responses within the liver. It is known that PSC-IBD patients have biliary (53,54) and ileocolonic (59) microbiota that are distinct from those with IBD only. Bile cultures obtained at the time of ERCP in patients with or without PSC revealed unique microbial dysbiosis in patients with PSC, with decreased biodiversity and increased abundance of *Enterococcus* (54). Furthermore, *Enterococcus* in the biliary fluid strongly correlated with the concentration of the noxious secondary bile acid tauroolithocholic acid, suggesting a potential mechanism of biliary injury. This group also showed that biliary colonization with *Enterococcus faecalis* (or *faecium*) strongly correlated with increased incidence of decompensated cirrhosis, death or need for liver transplantation (53).

The gut microbial profile in adult PSC-IBD patients is likewise distinct from IBD only and healthy individuals (59). Analysis of stool samples revealed that patients with PSC have a markedly reduced bacterial diversity, with a predominance of *Veillonella* genus. *Veillonella* abundance, in combination with other PSC-associated genera, accurately discriminated PSC from healthy controls. Importantly, *Veillonella* was most prevalent in advanced PSC, with a positive correlation of *Veillonella* and the PSC rMRS (59). When controlling for the moisture content in stool, *Enterococcus* was identified as a significant pathogen in PSC, based on a strong positive correlation with serum alkaline phosphatase (55). The overrepresentation of *Enterococcus* in stool samples from patients with PSC has been supported in a number of studies, including one pediatric study (56-58). Similarly, analysis of the stool microbiome in children with PSC showed a predominance of *Veillonella*, as well as *Megasphaera* genera, in those >10 years of age (60). The microbiome profile that characterized children with PSC <10 years of age was predominantly *Veillonella* and *Firmicutes* genera. There was a positive correlation between the abundance of *Veillonella* and bilirubin in the PSC-IBD cohort. Intriguing recent data suggests that the salivary microbiome in PSC-IBD is unique compared to IBD only and may function as a diagnostic biomarker in PSC (96,97).

The unique compositional microbiome changes in PSC are associated with functional changes in microbial metabolism. Kummel *et al.* analyzed fecal gene signatures of patients with PSC and discovered decreased expression of genes related to vitamin B6 (Vit B6) and branched-chain amino acid (BCAA) metabolism compared to healthy

controls (61). Furthermore, targeted plasma metabolomics revealed decreased concentrations of Vit B6 and BCAA in PSC and these biomarkers were associated with reduced liver transplant-free survival. A follow-up study confirmed these findings and found deficiencies in the active form of Vit B6 [pyridoxal 5'-phosphate (PLP)] in up to 38% of patients with PSC overall and in 52% of patients with PSC at the time of liver transplant (62). PLP independently predicted liver transplant-free survival, including transplant recipients with recurrent disease. This work suggests that altered microbial metabolism of essential nutrients results in deficiencies of Vit B6 (PLP), Vit B6-associated metabolic pathways, and BCAA, contributing to PSC disease pathogenesis.

An additional contribution of intestinal dysbiosis to PSC pathogenesis may be related to a disruption in the gut barrier function, whereby bacterial byproducts enter the portal circulation and directly or indirectly (via pro-inflammatory pathways) damage biliary epithelia. Analysis of a panel of serologic markers that reflect gut barrier dysfunction revealed that anti-F-actin antibodies (AAA), a marker of structural intestinal mucosal damage, were most frequent in PSC-IBD, compared to IBD only and healthy controls (63). AAA-IgA was an independent predictor of cirrhosis and high rMRS. Interestingly, AAA-IgA positivity was associated with higher frequencies of anti-microbial antibodies and the enterocyte damage marker, intestinal fatty acid binding protein. This suggests that AAA-IgA could be used as a prognostic biomarker of disease severity in PSC. In summary, these studies suggest that microbial dysbiosis in PSC is associated with intestinal barrier dysfunction and translocation of bacterial byproducts. One can speculate that either the microbiome and/or serum markers of intestinal barrier dysfunction are plausible prognostic biomarkers for disease severity in PSC.

Lipid metabolism and bile acid homeostasis

Lipids play numerous roles in the body, including energy storage and metabolism, signal transduction, and immunoregulation. Investigations of changes in lipid metabolism in PSC have shown increased serum levels of bile acids (especially taurine and glycine conjugates of primary bile acids), phosphatidylcholine and -ethanolamines, lysophosphatidylinositols and decreased concentration of some fatty acids, sphingomyelin and triglyceride species (98). A serum proteomics study of patients with PSC compared to healthy controls revealed

112 proteins that were significantly different between groups that could form the basis for a future diagnostic biomarker panel (64). Liver X receptor/retinoid X receptor (LXR/RXR) and farnesoid X receptor/retinoid X receptor (FXR/RXR) activation pathways were among the top pathways enriched for in the serum protein database. FXR regulates bile acid synthesis and LXR regulates metabolism of cholesterol and bile acids. In the setting of UC with or without PSC, a promising diagnostic biomarker involved in lipid metabolism is 1-acetylgllycerol-3-phosphate O-acyltransferase 1 (AGPAT1) (65). Based on a proteomics discovery platform, significantly higher amounts of AGPAT1 were present in colonic tissue biopsies in PSC-UC patients compared to UC only. AGPAT1 is an enzyme responsible for the conversion of phospholipid lysophosphatidic acid into phosphatic acid. Another prognostic biomarker in PSC that is involved in dysregulated lipid metabolism is autotaxin. Autotaxin hydrolyzes lysophospholipids to lysophosphatidic acid, a mediator of cholestasis-associated pruritus. High serum autotaxin levels predicted decreased transplant-free survival and strongly correlated with AST to Platelet Ratio Index, rMRS and MELD scores (66). The field of dysregulated lipid metabolism in the setting of chronic liver disease is growing and will likely yield multiple diagnostic and prognostic biomarkers for PSC.

PSC is associated with the retention of bile acids within the liver and serum, which likely contributes to progressive biliary injury and fibrosis (99,100). Bile acid levels and composition have been shown to be dysregulated in PSC and the identification of bile acid receptors has led to the discovery of signaling pathways that are involved in regulating cholangiocyte function (100). Mousa *et al.* investigated the utility of bile acids as a clinical biomarker and found that patients with PSC had increased bile acid levels, conjugated fraction, and primary-to-secondary bile acid ratios relative to controls. Moreover, a serum bile acid profile created using a machine learning model in patients with PSC accurately predicted hepatic decompensation (C-statistic 0.95) (101). Similarly, a unique bile acid profile has been identified in bile samples from patients with PSC compared to controls with and without cholestasis (102).

There has been recent interest in the suppression of bile acid synthesis as a potential therapy in cholestatic liver diseases. As such, a recent study found that advanced PSC was associated with suppression of bile acid synthesis, and that serum 7 α -hydroxy-4-cholesten-3-one (C4), which is a circulating bile acid precursor was found to be negatively associated with total bile acids (67). Furthermore, a

50% reduction in C4 corresponded to increased risk for liver transplantation or death after accounting for other factors (67). Together these studies suggest a role for bile acids in the pathogenesis of PSC and offer a potential biomarker for both clinical use and clinical trials.

Fibrogenesis

PSC is characterized by inflammation and fibrosis of intra- and or extrahepatic bile ducts and the degree of fibrosis is strongly associated with disease progression and outcomes. A final common pathway of immune cellular crosstalk leads to activation of stellate cells and portal myofibroblasts with fibrosis, resulting in collagen deposition, fibrosis and bile duct strictures. Proposed serum biomarkers of fibrogenesis in PSC include IL-37 expression (103) and propeptide of type III collagen (pro-C3) (68,69). In a pediatric cohort, matrix metalloproteinase 7 (MMP7), which has previously been implicated as a diagnostic biomarker in biliary atresia, was investigated as a biomarker of biliary injury and fibrosis in patients with autoimmune liver disease (70). MMP7 is an enzyme involved in extracellular matrix remodeling in response to cellular injury. In this study, serum MMP7 levels were found to be significantly higher in patients with either PSC or AIH/PSC overlap compared to those with AIH without cholangiopathy. These findings were not confounded by IBD severity. Furthermore, there was a strong correlation between serum concentrations of MMP7 and liver tissue *MMP7* mRNA expression.

Beyond serum biomarkers, liver stiffness evaluation is another non-invasive assessment of fibrosis that should be considered as stiffness may be an excellent biomarker of disease activity and progression (104). In adults with PSC there is evidence of a correlation of vibration-controlled transient elastography (VCTE) to histologic fibrosis severity (105,106). In children with PSC, Dillman *et al.* demonstrated that magnetic resonance imaging (MRI) elastography liver stiffness correlated with histologic severity of fibrosis, suggesting the use of MRI elastography as a non-invasive method to assess PSC progression (107).

Conclusions

In the past decade there have been significant inroads into the discovery of diagnostic and prognostic biomarkers for PSC. Many of the biomarkers highlighted in this review directly impact the immune environment within the liver and biliary system, resulting in chronic inflammation and

fibrogenesis. A subset of the biomarkers associated with immune activation may be potential targets for future therapeutic interventions. Given that the majority of biomarker studies were performed in small cohorts, multi-centered validation studies are essential to determine the accuracy and applicability of these biomarkers. Herein lies the largest unmet need, as validation studies require a very large group of patients and ideally would include participants worldwide. A strength of this review includes the identification of unique biomarkers discovered in children with PSC. The robust inflammatory environment within the livers of children with PSC may offer insights into early events in disease pathogenesis before the development of significant fibrosis and cirrhosis. Upon validation, diagnostic biomarkers will enhance the ability to identify PSC at earlier stages, facilitating timely intervention and screening, thus improving patient outcomes. Prognostic biomarkers could assist in the planning of liver transplantation and be utilized as surrogate endpoints in clinical trials.

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Footnote

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