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Anti-glycoprotein 2 (anti-GP2) IgA and anti-neutrophil cytoplasmic antibodies to serine proteinase 3 (PR3-ANCA): antibodies to predict severe disease, poor survival and cholangiocarcinoma in primary sclerosing cholangitis

Ewa Wunsch[1] | Gary L. Norman[2] | Malgorzata Milkiewicz[1] | Marcin Krawczyk[3,4] | Chelsea Bentow[2] | Zakera Shums[2] | Michael Mahler[2] | Steffi Lopens[5] | Dirk Reinhold[6] | Andre Franke[7] | Christoph Schramm[8] | Dirk Roggenbuck[9,10] | Piotr Milkiewicz[1,4]

[1]Szczecin, Poland
[2]San Diego, CA, USA
[3]Homburg, Germany
[4]Warsaw, Poland
[5]Dahlewitz, Germany
[6]Magdeburg, Germany
[7]Kiel, Germany
[8]Hamburg, Germany
[9]Potsdam, Germany
[10]Senftenberg, Germany

Correspondence
Piotr Milkiewicz, Liver and Internal Medicine Unit, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland.
Email: p.milkiewicz@wp.pl

Summary

Background: Primary sclerosing cholangitis (PSC) is associated with progressive liver disease and cholangiocarcinoma. Although risk stratification is crucial for making clinical decisions, it is hindered by a scarcity of proven prognostic markers.

Aims: To assess the value of novel anti-glycoprotein 2 (anti-GP2) and anti-neutrophil cytoplasmic antibodies to serine proteinase 3 (PR3-ANCA) in combination with PSC-specific clinical and laboratory markers as predictors of quality of life, disease severity, and cholangiocarcinoma in two large, independent cohorts of PSC patients

Methods: Discovery (338 Polish patients) and validation (178 German patients) cohorts with PSC were evaluated. Anti-GP2 (isoforms 1/4) was detected by ELISAs and PR3-ANCA by chemiluminescence immunoassay. Clinical and laboratory data were collected and analysed. The outcome was defined as liver transplantation-free survival and occurrence of cholangiocarcinoma during follow-up.

Results: In the discovery group, anti-$GP2_{1/4}$ IgA and PR3-ANCA were associated with liver dysfunction, anti-$GP2_{1/4}$ IgA with risk scores for PSC and anti-$GP2_4$ IgA with cirrhosis. All cholangiocarcinoma patients were positive for PR3-ANCA and/or anti-$GP2_4$ IgA. The association between anti-GP2 IgA and liver biochemistry, risk scores, cirrhosis, impaired survival, and cholangiocarcinoma was confirmed in the validation cohort. Cox proportional-hazards regression indicated anti-$GP2_1$ IgA as an independent variable of poor outcome in both study cohorts. Analysis of the combined data showed that anti-$GP2_4$ IgA and PR3-ANCA were independent predictors for cholangiocarcinoma, while anti-$GP2_1$ IgA and PR3-ANCA were indicators for poor survival.

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The authors' complete affiliation are listed in Appendix 1.

Conclusions: Anti-GP2 and PR3-ANCA are prognostic antibodies in PSC as they identify patients at risk of severe disease, poor survival and biliary cancer.

1 | INTRODUCTION

Primary sclerosing cholangitis (PSC) is a slowly progressive biliary disease, which affects predominantly young to middle-aged adults and leads to end-stage liver disease in a significant proportion of cases.[1,2] Disease-related complications, such as pruritus and recurrent bacterial cholangitis may deeply impair patients' quality of life.[3] However, even in asymptomatic patients the disease is associated with the risk of progressive changes in the biliary tree and the occurrence of cholangiocarcinoma, a malignancy with a disastrous prognosis. The lifetime prevalence of cholangiocarcinoma in PSC patients varies between 5% and 10%[4-6] and about half of cases are detected within 2 years of the initial PSC diagnosis.[7] Unfortunately no effective pharmacological therapy is currently available[8] and liver transplantation remains the only curative treatment. PSC recurrence after surgery, however, is not an uncommon phenomenon.

No proven indicators to predict an unfavourable disease progression are currently known, posing a significant challenge for disease management. Hence, simple biochemical indicators of cholestasis, for example alkaline phosphatase or the non-specific model of end-stage liver disease (MELD), are primarily used for risk stratification or as clinical trial endpoints.[9] Cancer diagnostics and monitoring is limited to radiological imaging and insensitive serum cancer markers such as Ca19.9.[10] For these reasons, progression of biliary injury can be unpredictable and cholangiocarcinoma is therefore often diagnosed at a late stage when the cancer is inoperable. Novel prediction markers are therefore urgently needed.

To date the typical autoantibodies seen in PSC have not yet shown prognostic value.[11] In contrast, autoantibodies often support the diagnosis and serve as markers of disease severity and progression in other autoimmune disorders.[12] Although PSC is not considered a typical autoimmune disease, since immunosuppression fails to be an efficient treatment option, it is believed that autoimmunity plays an important role in the disease pathogenesis. An autoimmune background in PSC is suggested by associations with human leukocyte antigens[13] as well as other autoimmune disorders.[14] Moreover, while various autoantibodies have been associated with PSC, such as antineutrophil cytoplasmic antibodies (ANCA), antinuclear antibodies and smooth muscle antibodies,[15] their pathogenic role and correlations with clinical phenotype are weak. In particular ANCA, directed against yet unidentified subcellular constituents of neutrophil or myeloid cells, have been reported in 65%-95% of PSC patients mainly with concomitant ulcerative colitis.[16,17]

Recently, IgG to the serine protease proteinase-3 (PR3), a cytoplasmic ANCA target, was detected in up to 44% of patients with PSC.[18] PR3-ANCA is typically detected in small vessel vasculitis including granulomatosis with polyangiitis[19] and recently in patients with inflammatory bowel disease, specifically those with ulcerative colitis.[20] Interestingly, in the setting of PSC, Stinton et al have shown that PR3-ANCA does not seem to be significantly related to a co-diagnosis of inflammatory bowel disease, but is rather associated with worse liver biochemistry.[18] This was the first report suggesting potential association between an autoantibody profile and disease severity in PSC. Other studies identified IgA against the pancreatic major zymogen granule glycoprotein 2 (GP2) as a marker of disease severity in PSC.[21-24] Anti-GP2 antibodies (anti-GP2) are members of the family of pancreatic autoantibodies directed against proteins predominantly expressed in the exocrine pancreas.[25] In total, four human GP2 isoforms ($GP2_{1-4}$) were identified and both IgA, as well as IgG autoantibodies were detected in humans.[26-28] Pancreatic autoantibodies were first described in IBD[29] and further studies have indicated their usefulness as diagnostic markers in Crohn's disease.[30,31] Subsequent reports have indicated a link between anti-GP2, a distinct clinical PSC phenotype, and poor prognosis, especially in relation to the development of cholangiocarcinoma.[21,23,24] In this context, detection of anti-GP2 and PR3-ANCA may present a novel option for the enhancing outcome prediction and risk stratification in patients with PSC.

We therefore evaluated this possibility by performing a comparative evaluation of anti-GP2 and PR3-ANCA as biomarkers of disease severity and as predictive factors for early death, liver transplantation, and cholangiocarcinoma in two large independent European cohorts of patients with PSC. For the first time, quantitative autoantibody data obtained by immunometric assays were used, which will enable their use in routine clinical practice.

2 | MATERIAL AND METHODS

2.1 | Patients

We prospectively enrolled 338 consecutive Caucasian patients with PSC (discovery cohort): 218 (65%) males, median age 32 years (range 17-73 years), treated at two university medical centres in Poland (Warsaw and Szczecin). The diagnosis of PSC (n = 275, 81%) or PSC with autoimmune hepatitis (AIH) features (PSC/AIH variant) (n = 63, 19%) was established according to EASL Guidelines.[32] Ninety-one patients had cirrhosis confirmed by either histology or imaging techniques (computed tomography or liver elastography). All patients were screened clinically and endoscopically for concomitant inflammatory bowel disease: 181 (53%) subjects were diagnosed with ulcerative colitis, 27 (8%) with undifferentiated colitis and 13 (4%) with Crohn's disease.

An independent validation cohort of 178 consecutive Caucasian patients with PSC treated at university medical centre in Hamburg, Germany was also evaluated. The group consisted of 99 (56%)

TABLE 1 Demographic, clinical and laboratory data in study groups

Feature	Discovery cohort (n = 338)	Validation cohort (n = 178)	*P* value
Demographic and clinical data			
Age (y)	32 (25-39)	44 (34-53)	<0.0001****
Age at diagnosis (y)	28 (21-36)	38 (27-49)	<0.0001****
Disease duration (y)	1 (0-5)	3 (1-8)	<0.0001****
Gender (male/female)	218 (64.5%)/120 (35.5%)	99 (55.6%)/79 (44.4%)	0.06
Inflammatory bowel disease (yes/no)	221 (65.4%)/117 (34.6%)	112 (63.3%)/65 (36.7%)[&]	0.70
Inflammatory bowel disease subtype			
Ulcerative colitis	181 (53.6%)	86 (48.6%)	
Crohn's disease	13 (3.8%)	25 (14.1%)	
Undifferentiated colitis	27 (8.0%)	1 (0.6%)	<0.0001****
AIH features (yes/no)	63 (18.6%)/275 (81.4%)	15 (8.4%)/163 (91.6%)	<0.01**
Cirrhosis (yes/no)	91 (27.2%)/244 (72.8%)[$]	35 (20.0%)/140 (80.0%)[$]	0.09
Laboratory data			
Haemoglobin (g/dL, normal: 12-16)	14 (12-15)	14 (13-15)	<0.01**
Platelets (tys/uL, normal: 150-450)	240 (160-310)	260 (210-337)	<0.01**
Bilirubin (mg/dL; normal:<1)	1.0 (0.5-2.2)	0.6 (0.4-1.0)	<0.0001****
ALP (IU/L; normal: <120)	233 (136-418)	131 (91-206)	<0.0001****
GGT (IU/L; normal: <42)	200 (92-359)	96 (40-236)	<0.0001****
ALT (IU; normal: <30)	78 (41-136)	39 (27-79)	<0.0001****
AST (IU/L; normal: <30)	62 (35-103)	31 (23-53)	<0.0001****
Albumin (g/dL; normal: 3.8-4.4)	4.1 (3.7-4.5)	4.0 (3.7-4.4)	0.11
INR (normal: 0.9-1.2)	1.0 (1.0-1.1)	1.0 (0.95-1.0)	<0.0001****
Na (mmol/L; normal: 135-145)	140 (139-142)	139 (138-141)	0.03*
MELD (points)	7 (6-11)	6 (6-8)	<0.0001****
MELD-Na (points)	8 (7-12)	8 (6-9)	0.02*
Risk scores			
Mayo Risk score for PSC (points)	−0.4 (−1.0 to 0.6)[@]	−0.4 (−0.9 to 0.0)[#]	0.66
PREsTo risk at year 1 (%)	0.5 (0.4-0.9)	0.5 (0.4-0.8)	0.21
PREsTo risk at year 2 (%)	1.0 (0.8-1.8)	1.0 (0.7-1.5)	0.24
PREsTo risk at year 3 (%)	1.5 (1.2-2.6)	1.4 (1.1-2.2)	0.15
PREsTo risk at year 4 (%)	2.4 (1.9-4.2)	2.3 (1.8-3.6)	0.20
PREsTo risk at year 5 (%)	3.6 (2.9-6.4)	3.5 (2.7-5.5)	0.19

Note: The PREsTo was calculated only for patients who fulfilled eligibility criteria: 230 and 107 patients from discovery and validation cohort respectively. No data in: [#]57 (32%) patients; [@]39 (11.5%) patients; [$]3 (1%) patients; [#]2 (1%) patients; [&]1 (1%) patient. Data presented as number (%) or median (interquartile range). Mann–Whitney or Yates corrected chi-squared test; $P < 0.05$ is taken as significant. *<0.05. **<0.01. ***<0.001. ****<0.0001.

Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; INR, international normalised ratio; MELD, model of end-stage liver disease; Na, natrium; PREsTo, primary sclerosing cholangitis risk estimate tool; PSC, primary sclerosing cholangitis.

males and 79 (44%) females with a median age of 44 years (range 17-75 years). All included patients had no other concomitant liver disease that could impact the study measures such as alcoholic and non-alcoholic liver disease or viral hepatitis. The study groups did not contain patients with recurrent PSC after liver transplantation. Demographic, clinical and laboratory data of analysed patients are summarised in Table 1.

2.2 | Study parameters

The patients in the discovery cohort were included between October 2009 and March 2018, and subjects from the validation cohort—between September 2007 and September 2018. Patients' data were collected prospectively in local PSC registry programs and consisted of both baseline characteristics (demographic and clinical data, liver

biochemistry, quality of life measures) as well as the data collected in the follow-up. The follow-up schedule was based on an annual appointment, unless the patient's condition required a strict control. Baseline blood samples for autoantibodies, liver function tests and clinical parameters were collected at the same appointment. All serum samples were aliquoted and stored in local biobanks (Pomeranian Medical University, Szczecin Poland and Biobank Popgen, Institute for Epidemiology, Christian-Albrecht University, Kiel Germany) according to local protocols, until serological assessment. Patients from the discovery group were followed for up to 98 months (median follow-up time: 14 months, the end of follow-up period between March 2010 and June 2018) and those from the validation cohort for up to 143 months (median follow-up time: 47 months, the end of follow-up period between October 2010 and October 2019). During the observation period, dates of certain censored events, that is, liver transplantation or liver disease-related death, as well as the occurrence of cholangiocarcinoma, were documented.

2.3 | Autoantibodies

Autoantibodies were determined retrospectively in serum samples stored at baseline. Anti-GP2 IgA and anti-GP2 IgG were detected by isoform 4 (anti-$GP2_4$) and isoform 1 (anti-$GP2_1$)-based ELISAs (GA Generic Assays GmbH). These isoforms differ by 3 amino acids (valine-proline-arginine)[30] and were previously described in PSC.[23] All assays were carried out according to the manufacturer's instructions. The following cut-off values for seropositivity recommended by the manufacturer were applied: anti-$GP2_1$ IgG ≥ 33 U/mL; anti-$GP2_1$ ≥ 7 U/mL; anti-$GP2_4$ IgG ≥ 23 U/mL; anti-$GP2_4$ IgA ≥ 9 U/mL.[27]

PR3-ANCA IgG positivity was determined by a sensitive chemiluminescence assay (CIA), namely QUANTA Flash® PR3 (Inova Diagnostics, Inc) on the BIO-FLASH® Instrument (Biokit s.a.) using native PR3 antigen coupled to paramagnetic beads. A detailed description of the methodology has been described.[18,33] Previous studies using this specific anti-PR3 CIA assay demonstrated that a value of 11.0 to 11.8 chemiluminescent units (CU) was an optimal cut-off value to distinguish ulcerative colitis from Crohn's disease, while a recent study on a Chinese inflammatory bowel disease cohort reported an optimal cut-off of 7.3 CU.[34] For our PSC cohort we have selected a similar cut-off of 10 CU.[20,35]

2.4 | Health-related quality of life

Quality of life measurement was performed in the discovery cohort only. The Medical Outcomes Study 36-Item Short Form Health Survey (SF-36) and PBC-40 were used to assess the relationship between analysed autoantibodies and health-related quality of life. The SF-36 is a well validated and broadly used generic questionnaire that can be used in various clinical conditions.[36] It contains 36 items divided into 8 domains of physical and mental health. Scores can be obtained for each scale or aggregated into 2 summary scores, a Mental Component Summary and a Physical Component Summary score. Scale scores range between 0 and 100, with the higher score indicating better quality of life. A license was obtained for the use of the SF-36 v.1 questionnaire in this study (Licence number QM044529). The PBC-40 was designed for evaluation of the health-related quality of life in patients with primary biliary cholangitis,[37] but its usefulness in PSC was recently established.[38] It contains 40 questions covering the following domains: Fatigue, Cognitive, Social-Emotional, Itch and Other Symptoms with higher scores indicating poorer quality of life.

2.5 | Risk of hepatic decompensation

In order to compare the predictive value of the antibodies with the existing risk scores, we calculated and analysed the Mayo Risk Score[39] as well as the Primary Sclerosing Cholangitis Risk Estimate Tool (PREsTo).[40] The latter was calculated only for patients who fulfilled eligibility criteria[40]: 230 and 107 patients from discovery and validation cohort respectively.

2.6 | Statistics

Descriptive statistical methods were used to analyse all variables. Continuous variables were presented as median values and the interquartile range (IQR). Categorical data are described using the number of observations and absolute frequencies. Follow-up time was time until death, liver transplantation, or the last contact with the patient. The Shapiro-Wilk normality test was used to examine the distribution of quantitative variables. The Mann-Whitney test and the unpaired t test were applied as appropriate to calculate the differences between subgroups. Correlation analysis was performed using the Spearman rank or Pearson's correlation method. Prevalence comparison between groups was performed by two-tailed Fisher's exact or Yates corrected Chi^2 test. Survival analysis was performed using the log-rank Mantel-Cox regression model. To identify independent variables for the risk prediction of censored event (ie liver transplantation or liver disease-related death) and the occurrence of cholangiocarcinoma, Cox proportional-hazards regression was performed including parameters with significant correlations ($P < 0.05$) in the univariate analysis as covariates with regard to clinical and laboratory parameters. Calculations and graphs were performed using STATISTICA (Tibco Software Inc 2017), GraphPad Prism for Windows (version 7.0), and MedCalc (MedCalc Statistical Software version 14.8.1, MedCalc Software bvba). A value of $P < 0.05$ was considered statistically significant.

2.7 | Ethics

Written informed consent was obtained from each patient included in the study. The study was performed following the principles of good clinical practice and in accordance to the ethical guidelines of

the 1975 Declaration of Helsinki (6th revision, 2008). The study protocol was approved by local ethics committees (Warsaw, Szczecin and Hamburg).

3 | RESULTS

3.1 | Discovery cohort

3.1.1 | The prevalence of anti-GP2 and PR3-ANCA autoantibodies

Anti-GP2$_1$ IgA and anti-GP2$_4$ IgA were positive in 23% and 17% patients respectively. Anti-GP2$_1$ IgG and anti-GP2$_4$ IgG were present in 4% and 9% patients respectively. The combined anti-GP2$_{1/4}$ positivity rate was 31% for IgA and 10% for IgG. PR3-ANCA were detected in 54% patients (Table S1).

3.2 | Anti-GP2 and PR3-ANCA positivity is related to PSC phenotypes

Anti-GP2$_1$ IgA was associated with older patients' age and longer disease duration, whereas PR3-ANCA was more common in younger patients both at survey and at the time of diagnosis. Anti-GP2$_4$ IgA positivity was linked with the presence of cirrhosis (odds ratio, OR = 2.7, 95% confidence interval, 95%CI = 1.5-5.0; P = 0.001). PR3-ANCA was significantly more common in PSC patients with concurrent inflammatory bowel disease (OR = 1.9, 95% CI = 1.2-2.9; P = 0.008), especially in those with concomitant ulcerative colitis (OR = 2.2, 95%CI = 1.4-3.6; P = 0.001) In contrast, there was no relationship between the presence of any anti-GP2 antibody analysed and inflammatory bowel disease (Table 2). As demonstrated in Table 3, anti-GP2$_{1/4}$ IgA and PR3-ANCA were significantly associated with poorer liver function tests and MELD scores. Moreover anti-GP2$_{1/4}$ IgA were associated with higher Mayo Risk scores, and anti-GP2$_1$ IgA—with higher risk of hepatic decompensation measured by PREsTo (Table 3). In contrast, anti-GP2$_{1/4}$ IgG reactivity did not show any association with analysed variables (Tables S2 and S3).

3.3 | Anti-GP2$_1$ IgA is associated with impaired quality of life

Patients with anti-GP2$_1$ IgA positivity reported a significantly impaired well-being in both applied questionnaires: two of five domains of PBC-40 (ie Other symptoms [15 vs 13 points, P = 0.04] and Itch [5 vs 3 points, P < 0.01] domains) and 4/8 domains of SF-36 (Physical functioning [85 vs 90 points, P < 0.01], Role limitation-physical [50 vs 75 points, P < 0.01], Role limitation-emotional [67 vs 100 points, P = 0.02] domains, and Physical Component Summary [58 vs 69 points, P = 0.03]). No similar significant correlations were found for any of the other autoantibodies analysed in this study.

3.4 | Anti-GP2$_1$ IgA and PR3-ANCA impact survival

During the median follow-up time of 14 months, a total of 84 (25%) patients reached a study defined end-point. Seventy-seven (23%) patients were transplanted and seven (2%) died (2 from

TABLE 2 Patients characteristics according to anti-GP2 IgA and PR3-ANCA status

	Anti-GP2$_1$ IgA			Anti-GP2$_4$ IgA			PR3-ANCA		
Feature	Negative (n = 259)	Positive (n = 79)	P value	Negative (n = 280)	Positive (n = 58)	P value	Negative (n = 152)	Positive (n = 182)	P value
Median (IQR), y									
Age at diagnosis	28 (21-35)	31 (20-40)	0.19	28 (20-35)	30.5 (22-41)	0.18	32 (22-40)	26 (20-34)	0.02*
Age at survey	31 (25-38)	34 (26-46)	0.04*	31 (25-38)	34 (26-46)	0.12	33 (26-42)	30 (24-37)	0.02*
PSC duration	1 (0-4)	3 (0-6)	0.01*	1 (0-5.5)	2 (0-4)	0.94	2 (0-4)	1 (0-6)	0.64
Number of patients									
Gender (male/female)	164 (63.3%)/ 95 (36.7%)	54 (68.4%)/ 25 (31.6%)	0.50	180 (64.3%)/ 100 (35.7%)	38 (65.5%)/ 20 (34.8%)	1.00	89 (58.6%)/ 63 (41.4%)	125 (68.7%)/ 57 (31.3%)	0.07
AIH features (yes/no)	54 (20.9%)/ 205 (79.1%)	9 (11.4%)/ 70 (88.6%)	0.07	53 (18.9%)/ 227 (81.1%)	10 (17.2%)/ 48 (82.8%)	0.85	28 (18.4%)/ 124 (81.6%)	35 (19.2%)/ 147 (80.8%)	0.89
Cirrhosis (yes/no)[b]	65 (25.2%)/ 193 (74.8%)	26 (33.8%)/ 51 (66.2%)	0.15	65 (23.4%)/ 213 (76.6%)	26 (45.6%)/ 31 (54.4%)	<0.01**	36 (23.7%)/ 116 (76.3%)	55 (30.7%)/ 124 (69.3%)	0.18
Inflammatory bowel disease (yes/no)	169 (65.3%)/ 90 (34.7%)	52 (65.8%)/ 27 (34.2%)	1.00	181 (64.6%)/ 99 (35.4%)	40 (69%)/ 18 (31.0%)	0.65	88 (57.9%)/ 64 (42.1%)	131 (72.0%)/ 51 (28.0%)	<0.01**

Abbreviations: AIH, autoimmune hepatitis; anti-GP2, anti-glycoprotein 2; PR3-ANCA, anti-neutrophil cytoplasmic antibodies to serine proteinase 3; PSC, primary sclerosing cholangitis. *<0.05, **<0.01. ***<0.001. ****<0.0001.

[b]No data in three patients. Mann–Whitney and Fisher's exact test; P < 0.5 is taken as significant. *<0.05.

TABLE 3 The association between anti-GP2 IgA, PR3-ANCA, liver biochemistry and risk scores

Feature	Anti-GP2$_1$ IgA			Anti-GP2$_4$ IgA			PR3-ANCA		
	Negative (n = 259)	Positive (n = 79)	*P* value	Negative (n = 280)	Positive (n = 58)	*P* value	Negative (n = 152)	Positive (n = 182)	*P* value
Haemoglobin (g/dL)	14 (12-15)	13 (11-14)	<0.01**	14 (12-15)	12 (11-14)	<0.01**	14 (12-15)	13 (12-15)	0.20
Platelets (tys/uL)	253 (176-311)	209 (128-289)	0.01*	246 (176-309)	206 (104-328)	0.07	226 (166-290)	253 (159-314)	0.19
Bilirubin (mg/dL)	0.9 (0.5-1.8)	1.6 (0.7-4.2)	<0.0001****	0.9 (0.5-1.9)	1.7 (0.6-4.0)	0.02*	0.9 (0.5-1.8)	1.1 (0.6-2.3)	0.02[a]
ALP (IU/L)	216 (126-378)	317 (183-499)	<0.001***	241 (136-426)	219 (134-318)	0.35	197 (110-339)	294 (165-473)	<0.0001[a]
GGT (IU/L)	200 (88-379)	200 (108-314)	0.88	204 (96-397)	146 (72-272)	0.03*	150 (73-312	214 (120-394)	<0.01[a]
ALT (IU/L)	76 (40-134)	81 (43-149)	0.65	79 (44-135)	69 (34-138)	0.19	65 (37-114)	87 (50-156)	<0.01[a]
AST (IU/L)	59 (33-99)	76 (43-117)	0.05	63 (36-102)	62 (32-119)	0.73	56 (29-94)	70 (39-113)	<0.01
Albumin (g/dL)	4.2 (3.8-4.5)	3.9 (3.4-4.4)	<0.0001****	4.2 (3.8-4.5)	4.0 (3.3-4.3)	<0.01**	4.2 (3.9-4.5)	4.1 (3.5-4.5)	0.10
INR	1 (1-1.1)	1.1 (1-1.2)	<0.01**	1 (0.98-1.1)	1.1 (1.0-1.2)	<0.01**	1.0 (0.99-1.1)	1.1 (1.0-1.2)	<0.01[a]
Na (mmol/L)	140 (139-142)	140 (138-142)	0.76	140 (139-142)	140 (138-141)	0.41	140 (139-142)	140 (139-141)	0.02[a]
MELD (points)	7 (6-10)	10 (7-15)	<0.0001****	7 (6-10)	9 (7-15)	<0.01**	7 (6-10)	8 (7-11)	<0.01[a]
MELD-Na (points)	8 (7-10)	11 (8-15)	<0.0001****	8 (7-11)	10 (7-16)	<0.01**	8 (6-10)	9 (7-12)	<0.01[a]
Risk scores									
Mayo Risk score for PSC (points)	−0.6 (−1.1 to 0.4)	−0.1 (−0.5 to 1.2)	<0.0001****	−0.5 (−1.1 to 0.4)	0.0 (−0.6 to 1.5)	<0.01**	−0.6 (−1.2 to 0.6)	−0.4 (−0.9 to − 0.6)	0.25
PREsTo year 1 (%)	0.5 (0.4-0.8)	0.8 (0.4-1.8)	<0.01**	0.5 (0.4-0.9)	0.6 (0.4-0.8)	0.92	0.5 (0.4-0.8)	0.5 (0.4-0.9)	0.13
PREsTo year 2 (%)	0.9 (0.8-1.6)	1.5 (0.8-3.5)	<0.01**	1.0 (0.8-1.8)	1.1 (0.8-1.6)	0.83	0.9 (0.8-1.6)	1.0 (0.8-1.8)	0.29
PREsTo year 3 (%)	1.4 (1.2-2.4)	2.2 (1.2-5.1)	<0.01**	1.5 (1.2-2.6)	1.6 (1.1-2.4)	0.79	1.4 (1.2-2.4)	1.5 (1.2-2.6)	0.32
PREsTo year 4 (%)	2.2 (1.9-3.8)	(3.5-1.9-8.1)	<0.01**	2.3 (1.9-4.2)	2.6 (1.8-3.8)	0.85	2.2 (1.9-3.8)	2.5 (1.9-4.2)	0.21
PREsTo year 5 (%)	3.4 (2.9-5.9)	5.4 (3.0-12.4)	<0.01**	3.6 (2.9-6.5)	4.0 (2.9-5.9)	0.98	3.4 (2.9-5.8)	3.8 (3.0-6.5)	0.23

Note: Data presented as median (interquartile range). Mann–Whitney test; $P < 0.5$ is taken as significant. *<0.05. **<0.01. ***<0.001. ****<0.0001.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; anti-GP2, anti-glycoprotein 2; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase; INR, international normalised ratio; MELD, model of end-stage liver disease; Na, natrium; PR3-ANCA, anti-neutrophil cytoplasmic antibodies to serine proteinase 3; PREsTo, Primary Sclerosing Cholangitis Risk Estimate Tool; PSC, primary sclerosing cholangitis.

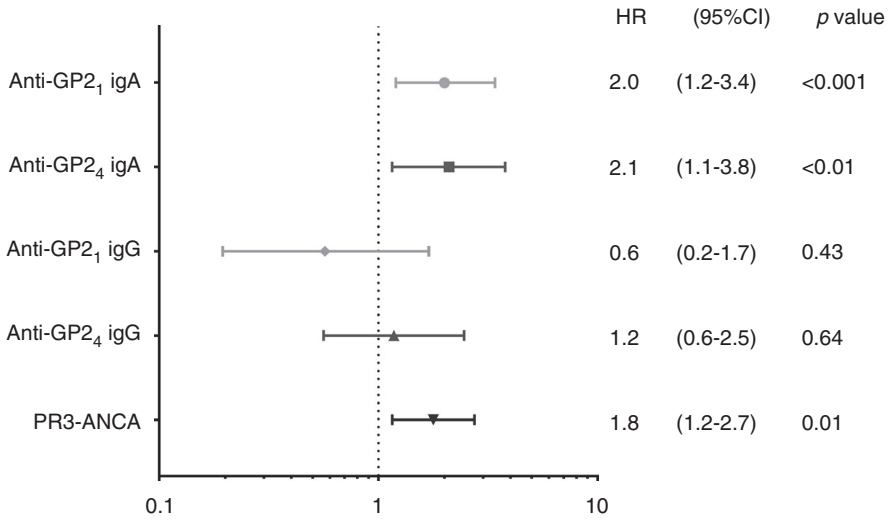

FIGURE 1 The hazard ratio forest plot of liver transplantation-free survival in the context of PR3-ANCA and anti-GP2 positivity. Abbreviations: anti-GP2, anti-glycoprotein 2; PR3-ANCA, anti-neutrophil cytoplasmic antibodies to serine proteinase 3

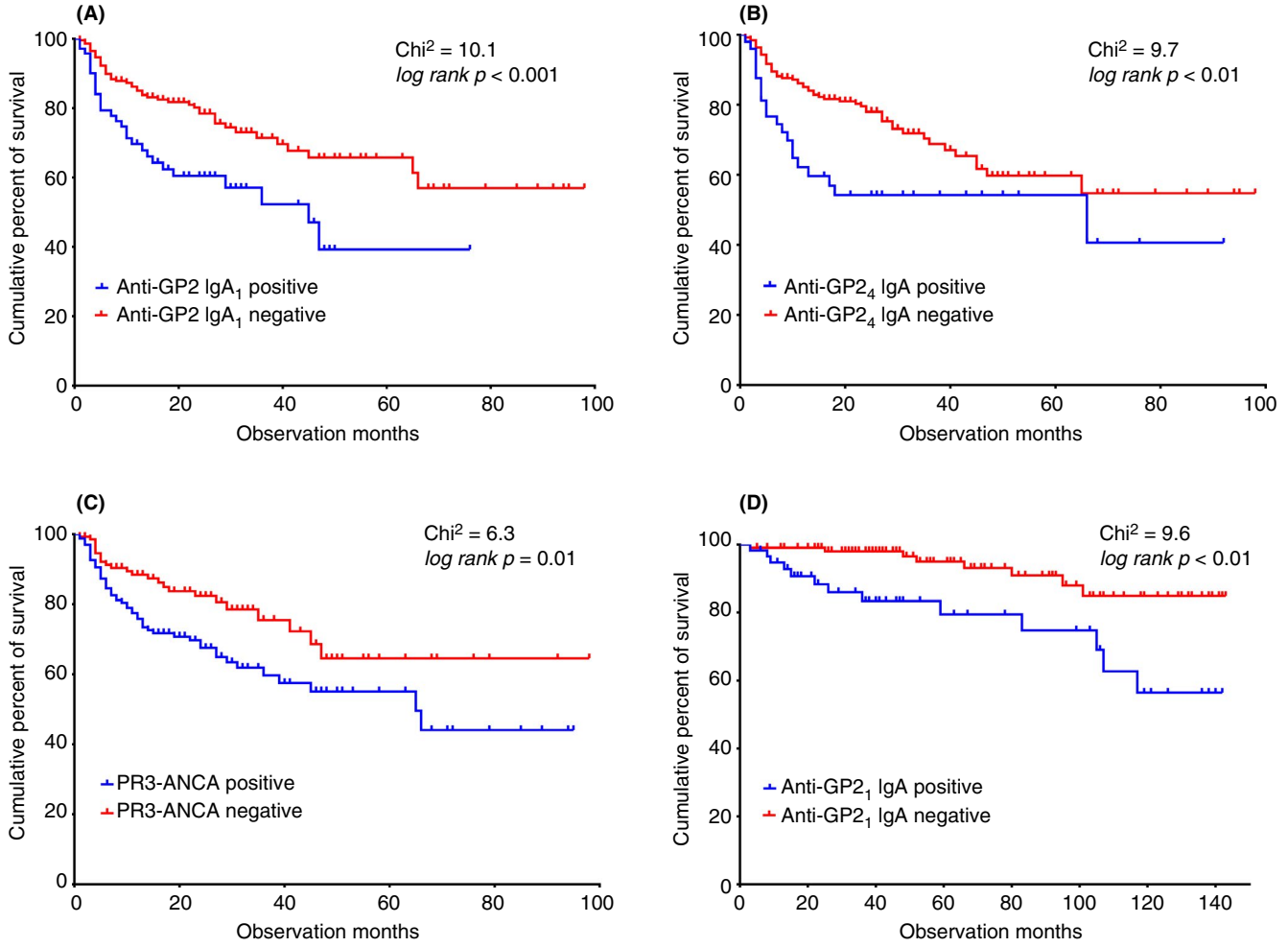

FIGURE 2 Kaplan-Meier survival curves for patients with primary sclerosing cholangitis from the discovery cohort (A-C) depending on anti-$GP2_1$ IgA, anti-$GP2_4$ IgA and PR3-ANCA status; and from the validation cohort (D), depending on the anti-$GP2_1$ IgA status. Survival curves significantly differed between anti-$GP2_1$ IgA-positive and negative patients in both cohorts, and between anti-$GP2_4$ IgA and PR3-ANCA in patients from discovery cohort, during observation periods. Abbreviations: anti-GP2, anti-glycoprotein 2; PR3-ANCA, anti-neutrophil cytoplasmic antibodies to serine proteinase 3 [Colour figure can be viewed at wileyonlinelibrary.com]

cholangiocarcinoma and five due to decompensated cirrhosis). The event-free survival rate for the whole study cohort was 75%.

In order to detect a relationship between the autoantibody status and the outcome, we calculated hazard ratios and performed the Log-rank (Mantel-Cox) test. The hazard ratios presented in Figure 1 indicated that PR3-ANCA and anti-$GP2_{1/4}$ IgA were associated with the occurrence of liver transplantation or death. Kaplan-Meier curves clearly demonstrated that positive anti-$GP2_{1/4}$ IgA and PR3-ANCA were associated with a shorter, transplantation-free survival (Figure 2A-C). In contrast, anti-$GP2_{1/4}$ IgG did not demonstrate a significant impact on survival in the analysed patients (data not shown). To identify independent variables for the risk prediction of a censored event, we performed a multivariate Cox hazards regression analysis. In the first step, we indicated significant variables in the univariate part of regression analysis (Table S4). In the second step, we incorporated significant clinical and laboratory parameters into the multivariate analysis. This analysis indicated that PSC cases with anti-$GP2_1$ IgA positivity were at risk of a poor survival (Table 4).

3.5 | Association between analysed autoantibodies and the risk of biliary cancer

During the follow-up, cholangiocarcinoma was diagnosed in 13 (4%) cases. Among these patients 12 (92%) were PR3-ANCA positive, 5 (39%)—anti-$GP2_4$ IgA positive and 3 (23%)—anti-$GP2_1$ IgA positive. Noteworthy, all patients (100%), who developed cholangiocarcinoma, were positive for either PR3-ANCA or anti-$GP2_1$ IgA. PR3-ANCA and anti-$GP2_4$ IgA were associated with an hazard ratio of 4.5 (95% CI = 1.5-13.6) and 4.4 (95% CI = 1.0-18.5), respectively, for developing biliary cancer. The Kaplan-Meier curves for the association between the antibodies and occurrence of cholangiocarcinoma is presented in Figure S1.

3.5.1 | Validation cohort

The autoantibody prevalences are shown in Table S1. The results from the validation group confirmed the association between

TABLE 4 Cox proportional-hazards regression of independent variables for the risk prediction of poor survival (liver transplantation or death) and the occurrence of cholangiocarcinoma in analysed groups of patients with primary sclerosing cholangitis

Dependent variable	Independent variable	Exp (*b*)	95% CI Exp (*b*)	*P* value
Discovery cohort				
Survival	Anti-GP2$_1$ IgA	1.806	1.122-2.908	0.01
	Albumin	0.173	0.118-0.254	<0.0001
Validation cohort				
Survival	Anti-GP2$_1$ IgA	30.037	2.745-328.640	<0.01
	Platelets	0.980	0.967-0.994	<0.01
Combined cohorts				
Survival	Anti-GP2$_1$ IgA	1.987	1.153-7.838	<0.01
	PR3-ANCA	1.983	1.227-3.213	<0.01
	Albumin	0.301	0.198-0.458	<0.0001
	ALT	0.995	0.991-0.998	<0.01
	AST	1.009	1.005-1.013	<0.001
	Platelets	0.997	0.995-0.998	<0.001
Cholangiocarcinoma	Anti-GP2$_4$ IgA	3.007	1.153-7.838	0.02
	PR3-ANCA	4.108	1.388-12.154	0.01
	Age	1.038	1.002-1.077	0.04
	Female gender	0.210	0.048-0.920	0.04

Note: Multivariate Cox proportional-hazard analysis; $P < 0.05$ is taken as significant.

Abbreviations: ALT, alanine aminotransferase; anti-GP2, anti-glycoprotein 2; AST, aspartate aminotransferase; CI, confidence interval; Exp(b), relative risk of event; PR3-ANCA, anti-neutrophil cytoplasmic antibodies to serine proteinase 3.

anti-GP2$_1$ IgA and longer disease duration and between PR3-ANCA and younger patients' age. The relationship with the occurrence of cirrhosis was also confirmed for anti-GP2$_1$ IgA. Similarly, as in the study group, anti-GP2$_{1/4}$ IgA was associated with more severe liver biochemistry and risk scores. With respect to PR3-ANCA, a correlation with lower albumin levels was found (Table S5).

During the median follow-up period of 47 months, 22 (12%) patients reached an end-point and 6 (2%) developed cholangiocarcinoma. The Log-rank (Mantel-Cox) test confirmed the association between anti-GP2$_1$ IgA and poor outcome (Figure 2D). The multivariate Cox regression analysis confirmed anti-GP2$_1$ IgA as an independent variable for the prediction of survival (Table 4).

3.5.2 | Combined outcome analysis

In order to enhance the power of observed associations in a larger group of cases we performed the multivariate Cox regression analysis for poor survival and occurrence of CCA taking into account combined data set. It was especially necessary to ensure the minimum number of cases for a more reliable statistical model for the occurrence of cholangiocarcinoma.[41] The analysis confirmed anti-GP2$_4$ IgA and PR3-ANCA as independent predictors for biliary cancer and indicated anti-GP2$_1$ IgA and PR3-ANCA as independent determinants of death or liver transplantation (Table 4).

4 | DISCUSSION

The putative pathogenic role of PSC-specific autoantibodies remains to be clarified by appropriate studies.[11] As the correlation of autoantibody levels with disease activity in PSC has yielded inconsistent results, we performed a complex analysis of potential associations between anti-GP2 and PR3-ANCA autoantibodies, clinical phenotype, and outcome. The major strength of the present study is the inclusion of two large, independent and well-characterised PSC cohorts to validate significant associations between autoantibodies status and distinct PSC phenotypes. To the best of our knowledge, this is the largest study with a replication cohort on the risk prediction potential of autoantibodies and their associations with the quality of life in PSC. These cohorts were not matched and differed significantly in terms of the demographics of included patients with their common denominator being the primary diagnosis of PSC. This, in our opinion, strengthens our conclusions by showing that the described phenomenon can indeed be associated with the disease itself and not with demographic features or concomitant IBD. We had a unique opportunity to perform direct comparative analyses of two different antibodies, anti-GP2 and PR3-ANCA, in the same patients using sensitive assays that are commercially available. For the first time, we detected anti-GP2 antibody in patients with PSC using a novel quantitative ELISA, thus enabling the quantitative measurement of autoantibody levels and the processing of large numbers of specimens. Our comprehensive observations encompassed

demographic, clinical and laboratory parameters and were further enhanced by measures of patients' disease-related symptoms. Finally, the character of the study allowed us to establish the ability and significance of the analysed antibodies to predict poor survival and biliary cancer. The latter was a key goal of our study, as to date no model for risk stratification of PSC patients exists which incorporates serological markers of the disease.

Both PR3-ANCA and anti-GP2 have been considered as potential biomarkers for the differential diagnosis and assessment of disease severity in IBD.[20,22,30,42-44] Recent studies indicate that the autoantibodies also may have an impact on the clinical picture of PSC. Jendrek et al demonstrated an association of anti-GP2 IgA with more advanced liver disease as measured by the Mayo Risk score.[21] Moreover, in a report by Tornai et al anti-GP2 IgA positive PSC patients had more severe liver biochemistry and more often cirrhosis when compared to anti-GP2 negative cases.[24] Furthermore, it was confirmed in a recent report that anti-$GP2_{1/4}$ IgA detected by immunofluorescence assay was an independent predictor of cirrhosis in PSC.[23] Our study confirmed these previous observations. In both patient cohorts analysed, we found several significant associations between anti-$GP2_{1/4}$ IgA and poorer liver function tests as well as MELD and Mayo Risk score. Finally, anti-$GP2_4$ IgA in the study group and anti-$GP2_1$ IgA in the validation cohort were significantly more common in patients with cirrhosis. Notably, in both analysed cohorts we also found significant associations between positive PR3-ANCA and exacerbated liver biochemistry, in line with the observations of Stinton et al[18]

Our study gave us a unique insight on the potential association between antibodies, disease-related symptoms and patients' quality of life measures. In both applied (generic and disease-specific) questionnaires, we found a negative impact of anti-GP2 IgA on patients' daily life, particularly in respect to the physical aspects of well-being. Although previous studies searched for factors determining quality of life among patients with PSC,[3,38,45,46] our current data are the first observations linking the autoantibody profile with patient's functioning. In our opinion, these results have significant practical clinical importance. Health-related quality of life emphasises the impact of a disease on the everyday life and from the patients' point of view, it is one of the most valid aspects of disease severity. Therefore, the identification of factors determining quality of life allows the targeting of patient populations needing special attention.

We believe that the fundamental value of our findings lies in the discovered impact of autoantibody status on the disease outcome. Our data showed that the presence of PR3-ANCA and anti-GP2 IgA are predictors of the disease-related death or liver transplantation. The indicated association between PR3-ANCA and poor survival is a novel and original finding. Our results regarding anti-GP2 IgA are in line with those reported by Jendrek et al, in which anti-GP2 IgA positivity was also a predictor for poor survival rates.[21] In contrast to our study, however, this effect was primarily based on PSC-associated biliary tract cancer, while the main reason of censored events in our group was liver transplantation due to non-cancer complications. The cause of this diversity can be explained by a lower occurrence of cholangiocarcinoma in our patients. Notably, our data emphasise the role of anti-GP2 IgA as an indicator of more severe PSC phenotype with the need of liver transplantation, irrespectively to cancer development. These conclusions are supported in another report, where the presence of anti-GP2 IgA, but not classical autoantibodies was associated with poor transplantation-free survival not related to biliary cancer.[24] Strikingly, however, among 13 patients who developed cholangiocarcinoma in the discovery cohort, all patients were either PR3-ANCA (12/13) or anti-$GP2_{1/4}$ IgA (6/13) positive. Multivariate Cox regression analysis of combined cohorts indicated that PR3-ANCA and anti-$GP2_4$ IgA were independently associated with biliary cancer. These results deserve particular attention, as the association of PR3-ANCA positivity alone and PR3-ANCA/anti-GP2 IgA combined positivity with tumorigenesis is an original finding.

Novel prediction models for the risk stratification in PSC are urgently needed and being actively pursued. Recently, two new scoring systems, representing a combination of simple biochemical and clinical variables, the PREsTo and the Amsterdam-Oxford Model were proposed as novel non-invasive tools for outcome prediction in patients with PSC.[40,47] However, those models were designed to predict hepatic decompensation or poor transplantation-free survival rather than the occurrence of cholangiocarcinoma. Notably, they did not incorporate autoimmune markers of PSC, because the typical autoantibodies seen in PSC have not yet shown prognostic value. Intriguingly, however, in our analysis the calculated risk indexes of PREsTo correlated significantly with the presence of anti-$GP2_1$ IgA in both analysed cohorts.

In our opinion, these findings demonstrate strong support for the value of novel antibodies as a risk markers for unfavourable disease course and suggest that new or modified prognostic models incorporating antibody status could result in novel predictive scores for severe disease course, poor survival and finally development of cholangiocarcinoma.

Although our study is a clinical observation, it allows us to draw some conclusions on the pathogenesis of PSC. Previous studies revealed a significant positivity for the IgA subclass of anti-GP2 (31%-52%),[21,24] in contrast to much lower prevalence of the corresponding IgG reactivities (less than 1%). Our current study confirmed this phenomenon in two independent cohorts. This is an intriguing finding as it supports a role for a mucosal loss of tolerance against GP2 in the pathophysiology of PSC. Tornai et al showed that the majority of the detected anti-GP2 IgA in PSC is contained the secretory component[24] as a result of elevated retro-transport of secretory IgA from mucosal surfaces to the circulation. However, this effect seems to be an inflammatory bowel disease-independent entity. In line with previous reports,[21,23,24] our data confirmed that the occurrence of anti-GP2 in PSC was not related to the underlying bowel disease. In light of recent reports on the presence of a biliary microbiota, a dysregulated immune response to it, as discussed for the pathophysiology of inflammatory bowel disease in relation to the intestinal microbiota, could play a role in the pathophysiology of PSC.[11,48-50]

We acknowledge some limitations of our study. First, it is possible that several differences between the cohorts could lead to some

discrepancies in observed frequencies and associations. Particularly the PR3-ANCA prevalence in the validation cohort was surprisingly very low. This finding differs significantly from the results seen in the study group, as well as previous observations, and suggest the need for further evaluations. Moreover, patients from both study groups belonged to referral-based cohorts from different European countries with different allocation models for liver transplantation. As this fact can impact the clinical profile and outcome, further population-based studies are needed to confirm our results. Finally, the relatively low occurrence of biliary malignancy and some unavailable data on our PSC patients hindered the risk stratification analyses. For this reason, especially for the occurrence of cholangiocarcinoma, the valid Cox regression analysis required the compilation of both cohorts in order to attain the minimum number of cases for a reliable statistical model.

Despite some limitations, our study produced several important conclusions. In summary, our findings confirmed the high prevalence of anti-GP2 IgA in patients with PSC using a novel ELISA. Moreover, anti-GP2 IgA and PR3-ANCA identified patient populations with a distinct, more severe disease phenotype and a shorter, non-cancer-related survival. The low prevalence and lack of clinical significance of anti-GP2 IgG reactivity adds further evidence to the hypothesis that anti-GP2 IgA could play pathogenic role in PSC. Finally, in our cohort all cholangiocarcinoma cases were associated with PR3-ANCA/anti-GP2 IgA combined positivity, and both antibodies were predictors of biliary malignancy. We conclude that the current study opens new avenues to construct and evaluate models which take into account antibody status to derive novel predictive scores for severe disease course, poor survival and finally development of biliary tract cancer.

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AUTHORSHIP

Guarantor of article: Piotr Milkiewicz.

Author contributions: EW, GLN, DR, MM and PM performed the research; EW, GLN, ZS, CB, CS, MM, MM1, SL, AF and DR1 collected and analysed the data; EW, and PM designed the research study and wrote the paper; and GLN, DR, MK contributed to the design of the study.

ORCID

Ewa Wunsch https://orcid.org/0000-0001-8740-5243
Marcin Krawczyk https://orcid.org/0000-0002-0113-0777
Piotr Milkiewicz https://orcid.org/0000-0002-1817-0724

REFERENCES

1. Dyson JK, Beuers U, Jones DEJ, Lohse AW, Hudson M. Primary sclerosing cholangitis. *Lancet*. 2018;391:2547-2559.
2. Karlsen TH, Folseraas T, Thorburn D, Vesterhus M. Primary sclerosing cholangitis – a comprehensive review. *J Hepatol*. 2017;67:1298-1323.
3. Cheung AC, Patel H, Meza-Cardona J, Cino M, Sockalingam S, Hirschfield GM. Factors that influence health-related quality of life in patients with primary sclerosing cholangitis. *Dig Dis Sci*. 2016;61:1692-1699.
4. Bergquist A, Ekbom A, Olsson R, et al. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol*. 2002;36:321-327.
5. Chapman MH, Webster GJ, Bannoo S, Johnson GJ, Wittmann J, Pereira SP. Cholangiocarcinoma and dominant strictures in patients with primary sclerosing cholangitis: a 25-year single-centre experience. *Eur J Gastroenterol Hepatol*. 2012;24:1051-1058.
6. Rizvi S, Eaton JE, Gores GJ. Primary sclerosing cholangitis as a premalignant biliary tract disease: surveillance and management. *Clin Gastroenterol Hepatol*. 2015;13:2152-2165.
7. Boberg KM, Bergquist A, Mitchell S, et al. Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical presentation. *Scand J Gastroenterol*. 2002;37:1205-1211.
8. Milkiewicz P, Wunsch E, Elias E. Liver transplantation in chronic cholestatic conditions. *Front Biosci*. 2012;17:959-969.
9. Trivedi PJ, Corpechot C, Pares A, Hirschfield GM. Risk stratification in autoimmune cholestatic liver diseases: opportunities for clinicians and trialists. *Hepatology*. 2016;63:644-659.
10. Razumilava N, Gores GJ, Lindor KD. Cancer surveillance in patients with primary sclerosing cholangitis. *Hepatology*. 2011;54:1842-1852.
11. Lopens S, Krawczyk M, Papp M, et al. The search for the Holy Grail: autoantigenic targets in primary sclerosing cholangitis associated with disease phenotype and neoplasia. *Auto Immun Highlights*. 2020;11:6.
12. Leslie D, Lipsky P, Notkins AL. Autoantibodies as predictors of disease. *J Clin Invest*. 2001;108:1417-1422.
13. Karlsen TH, Franke A, Melum E, et al. Genome-wide association analysis in primary sclerosing cholangitis. *Gastroenterology*. 2010;138:1102-1111.
14. Lunder AK, Hov JR, Borthne A, et al. Prevalence of sclerosing cholangitis detected by magnetic resonance cholangiography in patients with long-term inflammatory bowel disease. *Gastroenterology*. 2016;151:660-669.e664.
15. Angulo P, Peter JB, Gershwin ME, et al. Serum autoantibodies in patients with primary sclerosing cholangitis. *J Hepatol*. 2000;32:182-187.
16. Roozendaal C, de Jong MA, van den Berg AP, van Wijk RT, Limburg PC, Kallenberg CG. Clinical significance of anti-neutrophil cytoplasmic antibodies (ANCA) in autoimmune liver diseases. *J Hepatol*. 2000;32:734-741.
17. Duerr RH, Targan SR, Landers CJ, et al. Neutrophil cytoplasmic antibodies: a link between primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology*. 1991;100:1385-1391.
18. Stinton LM, Bentow C, Mahler M, et al. PR3-ANCA: a promising biomarker in primary sclerosing cholangitis (PSC). *PLoS One*. 2014;9:e112877.
19. Savige J, Gillis D, Benson E, et al. International consensus statement on testing and reporting of antineutrophil cytoplasmic antibodies (ANCA). *Am J Clin Pathol*. 1999;111:507-513.

20. Mahler M, Bogdanos DP, Pavlidis P, et al. PR3-ANCA: a promising biomarker for ulcerative colitis with extensive disease. *Clin Chim Acta*. 2013;424:267-273.
21. Jendrek ST, Gotthardt D, Nitzsche T, et al. Anti-GP2 IgA autoantibodies are associated with poor survival and cholangiocarcinoma in primary sclerosing cholangitis. *Gut*. 2017;66:137-144.
22. Papp M, Sipeki N, Tornai T, et al. Rediscovery of the anti-pancreatic antibodies and evaluation of their prognostic value in a prospective clinical cohort of Crohn's patients: The importance of specific target antigens. [GP2 and CUZD1] *J Crohns Colitis*. 2015;9:659-668.
23. Sowa M, Kolenda R, Baumgart DC, et al. Mucosal autoimmunity to cell-bound GP2 isoforms is a sensitive marker in PSC and associated with the clinical phenotype. *Front Immunol*. 2018;9:1959.
24. Tornai T, Tornai D, Sipeki N, et al. Loss of tolerance to gut immunity protein, glycoprotein 2 (GP2) is associated with progressive disease course in primary sclerosing cholangitis. *Sci Rep*. 2018;8:399.
25. Roggenbuck D, Bogdanos D, Conrad K. Loss of tolerance to one or two major targets in Crohn's disease or just cross-reactivity? *J Crohns Colitis*. 2013;7:e273-274.
26. Fukuoka S. Molecular cloning and sequences of cDNAs encoding alpha (large) and beta (small) isoforms of human pancreatic zymogen granule membrane-associated protein GP2. *Biochim Biophys Acta*. 2000;1491:376-380.
27. Röber N, Noß L, Goihl A, et al. Autoantibodies against glycoprotein 2 isoforms in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2017;23:1624-1636.
28. Roggenbuck D, Rober N, Bogdanos DP, et al. Autoreactivity to isoforms of glycoprotein 2 in inflammatory bowel disease. *Clin Chim Acta*. 2015;442:82-83.
29. Stöcker W, Otte M, Ulrich S, et al. Autoimmunity to pancreatic juice in Crohn's disease. Results of an autoantibody screening in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol Suppl*. 1987;139:41-52.
30. Roggenbuck D, Reinhold D, Baumgart DC, Schierack P, Conrad K, Laass MW. Autoimmunity in Crohn's disease-a putative stratification factor of the clinical phenotype. *Adv Clin Chem*. 2016;77:77-101.
31. Roggenbuck D, Reinhold D, Wex T, et al. Autoantibodies to GP2, the major zymogen granule membrane glycoprotein, are new markers in Crohn's disease. *Clin Chim Acta*. 2011;412:718-724.
32. European Association for the Study of the L. EASL clinical practice guidelines: management of cholestatic liver diseases. *J Hepatol* 2009;51:237-267.
33. Mahler M, Radice A, Yang W, et al. Development and performance evaluation of novel chemiluminescence assays for detection of anti-PR3 and anti-MPO antibodies. *Clin Chim Acta*. 2012;413:719-726.
34. Xu Y, Xu F, Li W, et al. The diagnostic role and clinical association of serum proteinase 3 anti-neutrophil cytoplasmic antibodies in Chinese patients with inflammatory bowel disease. *Scand J Gastroenterol*. 2020;55:806-813.
35. Arias-Loste MT, Bonilla G, Moraleja I, et al. Presence of anti-proteinase 3 antineutrophil cytoplasmic antibodies (anti-PR3 ANCA) as serologic markers in inflammatory bowel disease. *Clin Rev Allergy Immunol*. 2013;45:109-116.
36. Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care*. 1992;30:473-483.
37. Jacoby A, Rannard A, Buck D, et al. Development, validation, and evaluation of the PBC-40, a disease specific health related quality of life measure for primary biliary cirrhosis. *Gut*. 2005;54:1622-1629.
38. Raszeja-Wyszomirska J, Wunsch E, Krawczyk M, Rigopoulou EI, Bogdanos D, Milkiewicz P. Prospective evaluation of PBC-specific health-related quality of life questionnaires in patients with primary sclerosing cholangitis. *Liver Int*. 2015;35:1764-1771.
39. Kim WR, Therneau TM, Wiesner RH, et al. A revised natural history model for primary sclerosing cholangitis. *Mayo Clin Proc*. 2000;75:688-694.
40. Eaton JE, Vesterhus M, McCauley BM, et al. Primary sclerosing cholangitis risk estimate tool (PREsTo) predicts outcomes of the disease: a derivation and validation study using machine learning. *Hepatology*. 2020;71:214-224.
41. Peduzzi P, Concato J, Feinstein AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis. II. Accuracy and precision of regression estimates. *J Clin Epidemiol*. 1995;48:1503-1510.
42. Degenhardt F, Dirmeier A, Lopez R, et al. Serologic anti-GP2 antibodies are associated with genetic polymorphisms, fibrostenosis, and need for surgical resection in Crohn's disease. *Inflamm Bowel Dis*. 2016;22:2648-2657.
43. Horn MP, Peter AM, Righini Grunder F, et al. PR3-ANCA and panel diagnostics in pediatric inflammatory bowel disease to distinguish ulcerative colitis from Crohn's disease. *PLoS One*. 2018;13:e0208974.
44. Michaels MA, Jendrek ST, Korf T, et al. Pancreatic autoantibodies Against CUZD1 and GP2 are associated with distinct clinical phenotypes of Crohn's disease. *Inflamm Bowel Dis*. 2015;21:2864-2872.
45. Haapamaki J, Tenca A, Sintonen H, Barner-Rasmussen N, Farkkila MA. Health-related quality of life among patients with primary sclerosing cholangitis. *Liver Int*. 2015;35:2194-2201.
46. Kempinska-Podhorodecka A, Milkiewicz M, Jablonski D, Milkiewicz P, Wunsch E. ApaI polymorphism of vitamin D receptor affects health-related quality of life in patients with primary sclerosing cholangitis. *PLoS One*. 2017;12:e0176264.
47. de Vries EM, Wang J, Williamson KD, et al. A novel prognostic model for transplant-free survival in primary sclerosing cholangitis. *Gut*. 2018;67:1864-1869.
48. Liwinski T, Zenouzi R, John C, et al. Alterations of the bile microbiome in primary sclerosing cholangitis. *Gut*. 2020;69:665-672.
49. Roggenbuck D, Reinhold D, Schierack P, Bogdanos DP, Conrad K, Laass MW. Crohn's disease specific pancreatic antibodies: clinical and pathophysiological challenges. *Clin Chem Lab Med*. 2014;52:483-494.
50. Verdier J, Luedde T, Sellge G. Biliary mucosal barrier and microbiome. *Viszeralmedizin*. 2015;31:156-161.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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APPENDIX 1

The authors' complete affiliation details.

Ewa Wunsch: Translational Medicine Group, Pomeranian Medical University in Szczecin, Szczecin, Poland. Gary L. Norman, Chelsea Bentow, Zakera Shums, Michael Mahler: Research and Development, Inova Diagnostics, San Diego, CA, USA. Malgorzata Milkiewicz: Department of Medical Biology, Pomeranian Medical University in Szczecin, Szczecin, Poland. Marcin Krawczyk: Department of Medicine II, Saarland University Medical Center, Homburg, Germany; European Reference Network for Rare Liver Diseases; and Laboratory of Metabolic Liver Diseases, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland. Steffi Lopens: Medipan GmbH, Dahlewitz, Germany. Dirk Reinhold: Institute of Molecular and Clinical Immunology, Otto-von-Guericke University Magdeburg, Magdeburg, Germany. Andre Franke: Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany. Christoph Schramm: I. Department of Medicine and Martin Zeitz Centre for Rare Diseases, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; European Reference Network for Rare Liver Diseases; and Hamburg Center for Translational Immunology (HCTI), Hamburg, Germany. Dirk Roggenbuck: Faculty of Health Sciences, Joint Faculty of the Brandenburg University of Technology Cottbus – Senftenberg, the Brandenburg Medical School Theodor Fontane and the University of Potsdam, Germany; and Institute of Biotechnology, Faculty Environment and Natural Sciences, Brandenburg University of Technology Cottbus-Senftenberg, Senftenberg, Germany. Piotr Milkiewicz: Translational Medicine Group, Pomeranian Medical University in Szczecin, Szczecin, Poland; European Reference Network for Rare Liver Diseases; and Liver and Internal Medicine Unit, Medical University of Warsaw, Warsaw, Poland.