

## Anti-Gp210 and Anti-Sp100 Antibody Status and Ursodeoxycholic Acid Response in Primary Biliary Cholangitis

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### ABSTRACT

**AIMS:** Primary biliary cholangitis is a chronic biliary liver disease, for which the prognosis is poorer in patients who do not respond satisfactorily to the mainstay of treatment, ursodeoxycholic acid. Evidence suggests the presence of primary biliary cholangitis specific antinuclear antibodies such as anti-gp210 or anti-sp100 are associated

with poorer clinical outcomes, and possibly with non-response to ursodeoxycholic acid. We aimed to analyze the association between these primary biliary cholangitis-specific antinuclear antibodies, anti-gp210 and anti-sp100, and ursodeoxycholic acid response in primary biliary cholangitis.

**MATERIAL AND METHODS:** A retrospective audit was performed on 92 patients with primary biliary cholangitis for whom specific antinuclear antibody status and ursodeoxycholic acid response data was available. The response to ursodeoxycholic acid, assessed using Barcelona and Paris II criteria, was analyzed according to anti-gp210 and/or anti-sp100 positivity.

**RESULTS:** There was a non-significantly lower ursodeoxycholic acid response rate among anti-gp210 positive patients (12/18, 66.7%) compared with anti-gp210 negative patients (57/74, 77.0%,  $p = 0.5439$ ); and in anti-sp100 positive patients (15/21, 71.4%) compared with anti-sp100 negative patients (54/71, 76.1%,  $p = 0.886$ ). On univariate analysis and multivariate analysis, there was no significant change in odds of ursodeoxycholic acid response with either anti-gp210 or anti-sp100 positivity.

**CONCLUSION:** This study found that there was no association between anti-gp210 or anti-sp100 antibody status and response to ursodeoxycholic acid in a European primary biliary cholangitis cohort. This is in contrast to previous literature. However there was a trend towards an association between primary biliary cholangitis-specific antinuclear antibody positivity and lower ursodeoxycholic acid response rates.

**Key words:** Liver cirrhosis- biliary; Primary biliary cholangitis; Ursodeoxycholic acid; Anti-sp100; Anti-gp210.

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## INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic, progressive liver disease, which can carry a poor prognosis. In spite of the advent of beneficial therapy with ursodeoxycholic acid (UDCA), approximately 40% of patients will develop cirrhosis within 10 years of diagnosis<sup>[1]</sup>. Non-response to UDCA, which can occur in up to 40% of patients, is associated with a worse prognosis and decreased survival<sup>[2]</sup>. In the age of new second-line therapies for PBC, both licensed and in development, it is therefore imperative to identify non-responders early so as to alter their care to ensure the best management<sup>[3,4,5]</sup>.

Women under 50 years old and men of any age are less likely to respond to UDCA<sup>[6]</sup>, as are patients with ductopenia (>50% loss of bile ducts) on baseline liver biopsy<sup>[7]</sup>. The presence of the antinuclear antibody (ANA), anti-glycoprotein-210 (anti-gp210), has also been shown in a Japanese PBC population to be associated with UDCA non-response<sup>[8]</sup>, though this finding has not been validated, nor has it been evaluated in European populations. Anti-gp210 is highly specific for PBC, is positive in 16-44% patients, and can be particularly helpful for diagnosis in patients negative for anti-mitochondrial antibody (AMA)<sup>[9,10]</sup>. Another PBC-specific ANA is anti-sp100, which is present in 21% of patients with PBC<sup>[11]</sup>, but data relating to an association with UDCA response is lacking (See Figure 1).

There is an association of positivity to anti-gp210 or anti-sp100 with worse clinical outcome<sup>[11,12]</sup>, and likewise there is also a strong link of UDCA non-response to disease progression<sup>[7]</sup> and poorer prognosis<sup>[2,13]</sup>. However, it is not clear whether the presence of these antibodies is associated with response to UDCA. The aim of this study was to analyze the association of PBC-specific ANA with UDCA response in a European PBC cohort.

## METHODS

### Study population

A retrospective audit was performed on a PBC cohort at the John Radcliffe Hospital, Oxford, UK. The Oxford clinical PBC database was interrogated. To be included on this database, patients must meet standard clinical diagnostic criteria for PBC<sup>[14,15]</sup>. This database is updated prospectively with information from regular clinics and includes data on patient demographics, medication, treatment response, and blood test results (including liver biochemistry, platelets, hemoglobin and autoantibody status). Alkaline phosphatase (ALP) measurement was classified as the amount times the upper limit of normal (xULN) as the reference range varied according to the assays used over the years at the institution. Patients with unknown UDCA response, concurrent liver pathology including overlap syndrome with autoimmune hepatitis, use of fibrates, and/or unknown PBC-specific ANA status were excluded from analysis.

### Determination of UDCA response

Patients were defined as an UDCA-responder if they fulfilled one or both of the Barcelona criteria or Paris II criteria at one year post UDCA initiation (See Box 1)<sup>[2,13]</sup>. Accordingly, UDCA-non-response was if both criteria were not met.

### Immunoassays

PBC-specific ANA status (anti-gp210 and anti-sp100) was determined using the Euroimmune liver immunoblot protocol [Euroline 3 immunoblot kit (Euroimmun, Leipzig, Germany)]. This was chosen over indirect immunofluorescence as the diagnostic test of choice due to the reduced operator dependence of immunoblot. Anti-M2 and

the antibody against enhanced multi-peptide branch recombinant M2 antigen (anti-3E-BPO) were also detected using this protocol, both of which are specific to PBC. Briefly, 15 µl of patient sample was added to each pre-wet immunoblot strip and incubated for 30 minutes at room temperature. Strips were washed with supplied buffer before being incubated with enzyme conjugate for 30 minutes at room temperature. Strips were washed again as before and incubated for 10 minutes at room temperature with supplied substrate. Reactions were stopped with distilled water. Strips were air-dried and analyzed using the Euroline scan (Euroimmun, Leipzig, Germany). Results >20IU were reported as positive (see Figure 1C).

Anti-mitochondrial antibodies (AMA) were measured using indirect immunofluorescence. Patient serum was tested using NOVA Lite<sup>®</sup> mouse liver-kidney-stomach tissue slides (Inova Diagnostics, San Diego, California, USA). Staining was performed using a QUANTA-Lyser<sup>®</sup> 240 robotic workstation (INOVA, San Diego, California, USA), with samples screened at a 1:20 dilution. Slides were dual read and analyzed using an Olympus BX41 ultraviolet microscope (Olympus, Tokyo, Japan).

### Statistical analysis

The primary endpoint was the proportion of UDCA-responders among patients who were anti-gp210 positive versus anti-gp210 negative patients. Secondary endpoints included proportion of responders according to anti-sp100 status, as well as response according to the individual response criteria of both Barcelona and Paris II criteria. Response according to Barcelona alone and Paris II criteria alone was also analyzed.

Proportions were compared using Chi squared test. Univariate and multivariate logistic regression analyses for UDCA response were carried out against several variables including gender, age at diagnosis of PBC, alkaline phosphatase (ALP) at baseline (last result before initiating UDCA therapy), baseline alanine transaminase (ALT) or aspartate transaminase (AST), baseline bilirubin, baseline albumin, anti-gp210 status and anti-sp100 status. Missing values were imputed using the mean for normally distributed variables and the median for skewed variables. As the routine transaminase analyzed at the John Radcliffe Hospital changed from AST to ALT during the study period, ALT was used for analysis where available, and AST substituted if ALT was not available. This has been done in previous large PBC cohorts before in risk stratification and is therefore an accepted practice<sup>[16,17]</sup>.

The statistical software used was R 3.5.0 Core Team (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2018. URL <https://www.R-project.org/>).

## RESULTS

### Demographics

Of 204 patients existing on the Oxford PBC database (median follow up 10.8 years, IQR 5.3-15.5), 140 patients had PBC-specific ANA status known, and 92 also had UDCA-response documented, therefore 92 patients were included in the final analysis. Of the 48

#### Box 1 Criteria for assessing response to UDCA.

Paris II[13]	ALP and AST levels 1.5 × ULN and normal bilirubin, after 1 year of UDCA treatment.
Barcelona[2]	Decrease in ALP greater than 40% of pre-treatment ALP, or sation of ALP, after 1 year of treatment with UDCA.

ALP: alkaline phosphatase; AST: aspartate transaminase; ULN: upper limit of normal; UDCA: ursodeoxycholic acid.

patients for whom UDCA response was unknown, the reasons were: they had not been given or were intolerant (and so stopped) UDCA ( $n = 18$ ), they had not been on UDCA for 1 year at the time of the study ( $n = 3$ ), biochemical values at the start or after 1 year of treatment were unknown (for example they had moved from a different hospital and their previous medical records were not available) ( $n = 20$ ), they had another liver condition (such as overlap syndrome or bile duct pathology) that prevented the response of their PBC to UDCA being recorded accurately ( $n = 4$ ), they had overlap syndrome as well as missing biochemical data ( $n = 2$ ), or they were on UDCA and prednisolone and therefore their response to UDCA alone could not be ascertained ( $n = 1$ ).

Baseline demographics are shown in Table 1. The median age at diagnosis was 53.9 years (IQR 48.9-60.3, range 22.4-75.9) and 76.1% were female. 68/92 (73.9%) of the overall cohort were responders by at least one of Paris II or Barcelona criteria, (see Table 1).

**Antibody status**

73 patients (79.3%) were AMA positive, 18 (19.6%) were anti-gp210

positive, and 21 (22.8%) were anti-sp100 positive (see Figure 2). Of the AMA negative patients ( $n = 19$ ), 4 (21.1%) were positive for either anti-gp210 or sp100 and a further 4 (21.1%) were positive for either anti-M2 or anti-3E(BPO). (see Figure 3). Overall 81 patients (88.0%) had an antibody(s) supportive of a diagnosis of PBC, and of the 11 who did not, 8 had diagnostic liver biopsies. The remaining 3 patients had liver biopsies with non-specific changes but clinical phenotypes supporting a diagnosis of PBC.

**UDCA response according to PBC-specific ANA status**

There was a lower UDCA response rate among anti-gp210 positive patients (12/18, 66.7%), as compared with anti-gp210 negative patients (57/74, 77.0%), but this result was not significant ( $p = 0.544$ ). Of patients who were anti-sp100 positive, 15/21 (71.4%) were UDCA responders compared with 54/71 (76.1%) of anti-sp100 negative patients, but again this was not significant ( $p = 0.886$ ). (See Table 2 and Table 3).

When analyzing association of PBC-specific ANA status and UDCA response using the individual criteria of Barcelona and Paris

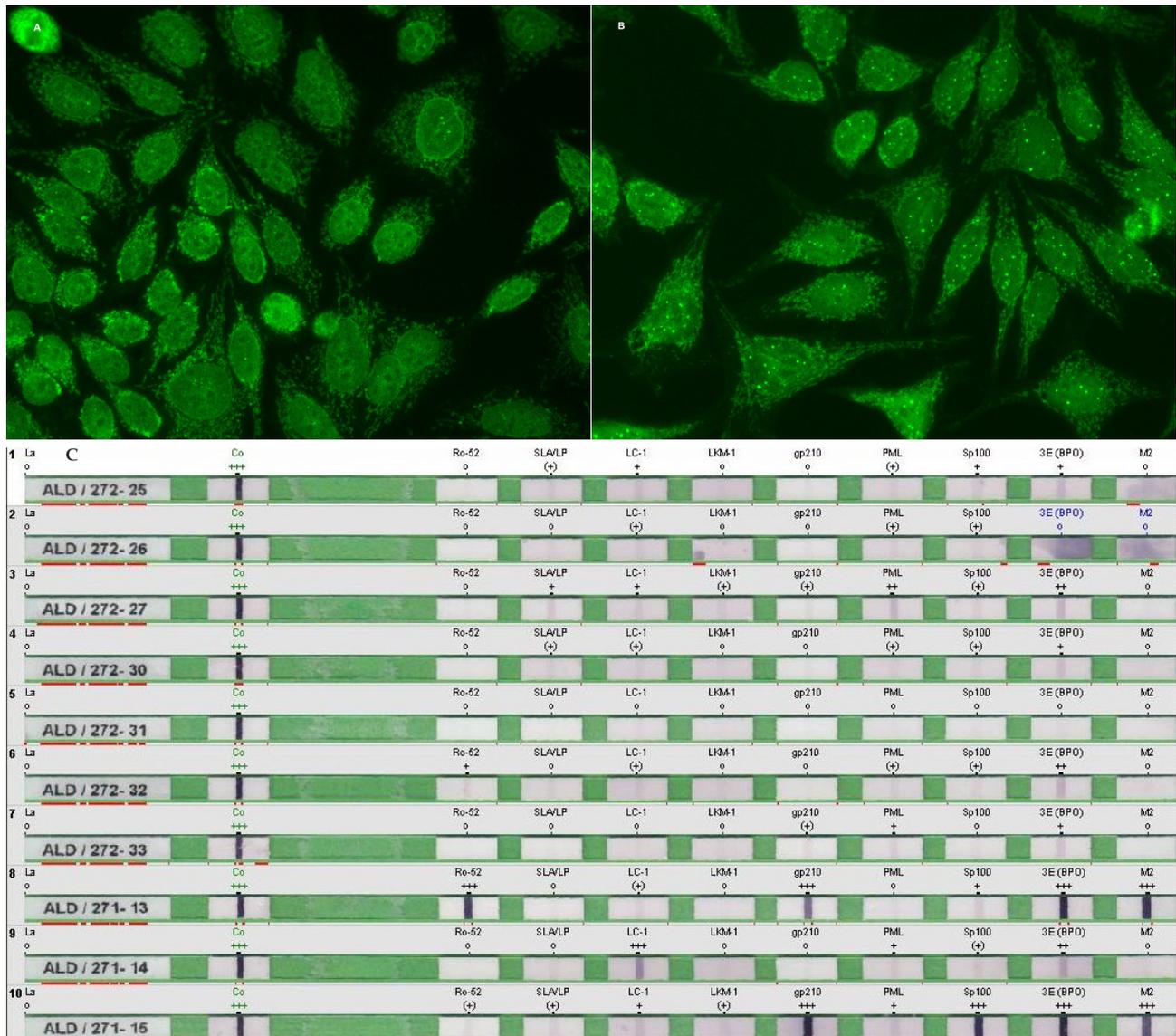
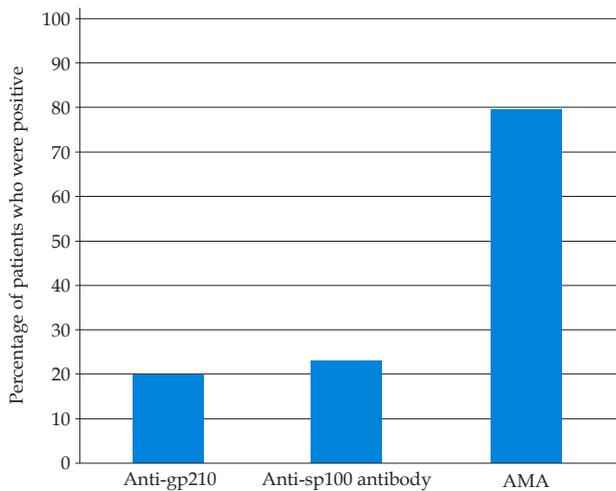


Figure 1 PBC specific antinuclear antibodies. Indirect immunofluorescence on HEp-2000 cells demonstrating. (A) nuclear rim staining typical of anti-gp210 positivity, (B) multinuclear dot staining seen in anti-sp100 positivity; (C) Euroimmune liver immunoblot image showing positivity for anti-gp210 (patients 8 and 10) and anti-sp100 (patient 10).

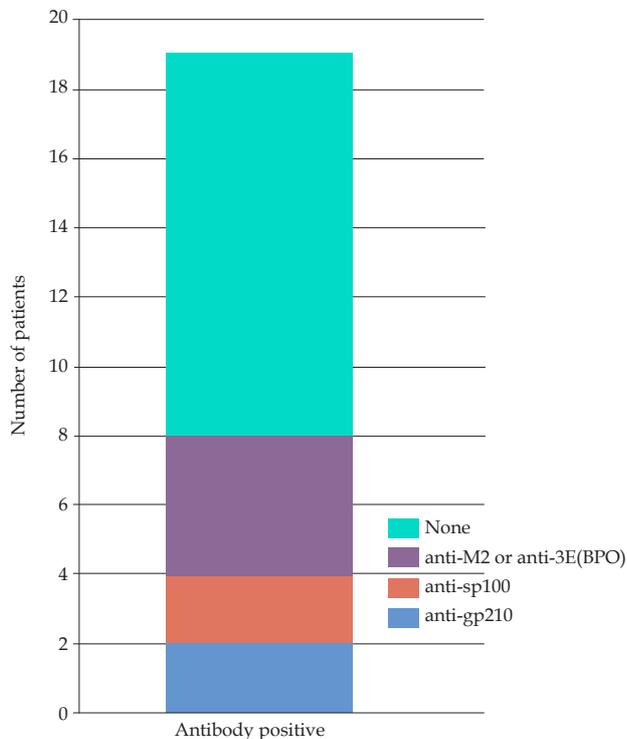
II criteria, similar trends were seen with lower responses among those who were antibody positive, but did not reach statistical significance. (See Table 2 and Table 3).

**Univariate and multivariate analysis of impact of various variables on UDCA response**

On univariate analysis there was no significant change in odds of UDCA response with either anti-gp210 or anti-sp100 positivity – OR 0.596 ( $p = 0.366$ ) for anti-gp210 positive patients and OR 0.787 ( $p = 0.667$ ) for anti-sp100 positive patients. There were similar non-significant findings by univariate analysis when assessing UDCA



**Figure 2** PBC-specific ANA status of the patient cohort. Of the 92 patients in the cohort, 19.6% were positive for anti-gp210, 22.8% were positive for anti-sp100, and 79.3% were positive for AMA.



**Figure 3** Anti-gp210 and anti-sp100 positivity status of AMA-negative patients. 2 of the 19 AMA negative patients (10.5%) were positive for anti-gp210, 2 (10.5%) were positive for anti-sp100. And 4 were positive for anti-M2 and/or anti-3E(BPO). No AMA negative patients were positive for both anti-gp210 and anti-sp100.

response by the individual Paris II or Barcelona criteria against positivity for anti-gp210 or anti-sp100 (see Table 4).

**Table 1** Baseline demographics of the patients included in the analysis.

Variable	Patients (n = 92)
Median age (IQR, range)	53.9 (48.9-60.3, 22.4-75.9)
Female sex, n (%)	70 (76.1)
ALP, xULN (IQR)	2.1 (1.4-2.8)
ALT/AST, IU/L (IQR)	55.5 (34.0-88.3)
Bilirubin, mmol/L (IQR)	9.0 (6.0-12.0)
Albumin, g/L (IQR)	44.0 (43.0-46.0)
Platelets, $\times 10^9/L$ (IQR)	268.5 (226.8-314.8)
UK-PBC 5 year risk score (IQR)	0.8* (0.5-1.6)
Responder to UDCA by at least of Paris II or Barcelona criteria, n (%)	68 (73.9)

\*this number represents the probability of liver transplant or liver related death; e.g. 0.8 represents an 80% chance. ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; IQR, interquartile range; UDCA, ursodeoxycholic acid; ULN, upper limit of normal.

**Table 2** Ursodeoxycholic acid response and anti-gp210 status.

	Anti-gp210 +ve (n = 18)	Anti-gp210 -ve (n = 74)	p-value
UDCA response †	12 (66.7%)	57 (77.0%)	0.544
UDCA response by Barcelona criteria	9 (50.0%)	46 (62.1%)	0.499
UDCA response by Paris II criteria	8 (44.4%)	48 (64.9%)	0.186

† UDCA response by fulfilling criteria of at least one of Barcelona or Paris II criteria. UDCA- ursodeoxycholic acid.

**Table 3** UDCA response and anti-sp100 status.

	Anti-sp100 +ve (n = 21)	Anti-sp100 -ve (n = 71)	p-value
UDCA response †	15 (71.4%)	54 (76.1%)	0.886
UDCA response by Barcelona criteria	11 (52.3%)	44 (62.0%)	0.593
UDCA response by Paris II criteria	13 (62.0%)	43 (60.1%)	1

† UDCA response by fulfilling criteria of at least one of Barcelona or Paris II criteria. UDCA: ursodeoxycholic acid.

**Table 4** Univariate analysis of anti-gp210 and anti-sp100 antibody status on the odds ratio of UDCA response

Antibody positivity	OR of UDCA response (p-value)		
	Barcelona criteria alone	Paris II criteria alone	Either Barcelona or Paris II criteria
Anti-gp210	0.609 ( $p = 0.348$ )	0.433 ( $p = 0.117$ )	0.596 ( $p = 0.366$ )
Anti-sp100	0.675 ( $p = 0.432$ )	1.058 ( $p = 0.912$ )	0.787 ( $p = 0.667$ )

UDCA: ursodeoxycholic acid.

**Table 5** Multivariate analysis of anti-gp210 positivity, anti-sp100 positivity, patient demographics and baseline biochemistry on the odds ratio of UDCA response.

Variable	OR of UDCA response (p-value)		
	Barcelona criteria alone	Paris II criteria alone	Barcelona or Paris II criteria
Anti-gp210	0.611 (0.400)	0.840 (0.799)	0.789 (0.724)
Anti-sp100	0.694 (0.516)	2.942 (0.179)	0.983 (0.980)
Female sex	1.718 (0.337)	0.946 (0.933)	1.416 (0.603)
Age (per year)	1.021 (0.398)	1.089 (0.009)*	1.058 (0.068)
ALP xULN (per unit increase)	0.985 (0.937)	0.439 (0.009)*	0.750 (0.179)
Platelets (per unit increase)	0.997 (0.322)	1.006 (0.168)	1.001 (0.773)
Bilirubin (per unit increase)	1.107 (0.029)*	1.029 (0.489)	1.225 (0.010)*
ALT/AST (per unit increase)	0.997 (0.523)	0.984 (0.095)	0.993 (0.145)
Albumin (per unit increase)	1.133 (0.122)	0.966 (0.733)	1.039 (0.684)

\*significant result ( $p < 0.05$ ), UDCA, ursodeoxycholic acid.

On multivariate analysis, including other factors such as female sex, age, and baseline biochemistry, there was no change in odds of UDCA response with either anti-gp210 or anti-sp100 positivity, when assessing response with Barcelona, Paris II or either criteria (see Table 5).

When assessing response using Paris II criteria alone, increased age was associated with increased odds of UDCA response (OR 1.089,  $p = 0.009$ ) and an increased ALP was associated with decreased odds of response (OR: 0.439,  $p = 0.009$ ).

Increased bilirubin at baseline was associated with a higher odds of UDCA response when assessed by Barcelona criteria alone (OR 1.107,  $p = 0.029$ ) or using response with either criteria (OR 1.225,  $p = 0.010$ ). When assessing UDCA response, when defined as responding by at least one criterion, no other variable, including age and gender, was significant.

## DISCUSSION

This study found that there was no association between anti-gp210 or anti-sp100 antibody status and response to UDCA in a European PBC cohort. Our finding is in contrast to the findings of Nakamura *et al* who found that anti-gp210 positivity was associated with a worse biochemical response to UDCA<sup>[8]</sup>. A possible explanation for the difference in findings is that our study only included 92 PBC patients in whom antibody status and UDCA response was known compared with 164 patients in the Japanese study, which may have led to inadequate power to find a statistically significant difference.

Additionally, we used Paris II and Barcelona criteria after 1 year of treatment to assess patient response to UDCA, as these are the criteria used clinically in the John Radcliffe Hospital. Nakamura *et al.* used a different measure of treatment response, comparing ALP, ALT and IgM at 2 years post treatment initiation. Results at 2 years that were  $\leq$  ULN were classed as good response,  $\leq 1.5 \times$  ULN classed as fair response and  $> 1.5 \times$  ULN classed as a poor response. Furthermore, a proportion of patients were also on bezafibrate and/or prednisolone in addition to UDCA, making the treatment being assessed different from our population (who were on UDCA alone). Finally, the differing demographics could have influenced the findings. The median age in Nakamura's study was 49.5 years (compared to 53.9y) and 89.6% were female (compared to 76.1%)<sup>[8]</sup>. All of these differences in study design may offer potential explanation for the differences in our results.

Although our study did not find an association of anti-gp210 or anti-sp100 positivity with UDCA response (or lack thereof), it is well known that ANA's are of utility in clinical practice, especially for diagnostic purposes. The primary aim of this paper was to determine if there was a role for these antibodies in predicting UDCA response, however they are also useful diagnostically, being recommended by the European Association for the Study of the Liver (EASL) as part of the serological screening of patients presenting with symptoms suggestive of cholestatic liver disease. EASL states that ANA testing can be especially useful in patients who are AMA negative to allow a PBC diagnosis to be made<sup>[14]</sup>. Indeed, in our study, 21.1% of AMA-negative patients were positive for either anti-sp100 or anti-gp210, which in clinical practice, not only helps with diagnosis but also obviates the need for a liver biopsy to make the diagnosis, thereby saving risk to the patient.

We did not investigate the clinical utility of the antibodies in this paper. For example we could have investigated whether patients with positive anti-gp210 or anti-sp100 status were more likely to experience clinical end points such as liver transplantation and death.

The number of patients on our database with documented clinical outcomes was small - of the 204 patients on the database, 8 (3.9%) had received liver transplants and 18 (8.8%) had died (of which 8 were documented as not being due to PBC), therefore only 18/204 (8.8%) of patients had experienced PBC related clinical outcomes. In our study cohort of 92 patients specifically, there were four patients (4.3%) who experienced liver decompensation, of whom one required a transplant and one died. We felt that investigating the relationship of ANA status and clinical outcomes would be too underpowered for such an analysis. A possible explanation for the low proportion of clinical outcomes in our cohort of 204 is the relatively short median follow up (10.8 years) with relation to the time it usually takes to develop a clinical outcome in PBC. As PBC is a slow, progressive disease, this may not have been sufficient follow up time for clinical outcomes to occur in our patients.

The finding of raised bilirubin at baseline associated with a higher odds of UDCA response is intriguing and not in keeping with the known relationship of raised bilirubin with poor clinical outcome<sup>[18]</sup>. This association of increased bilirubin at baseline with favorable UDCA response was also seen in our study when analyzing UDCA response according to Barcelona criteria alone (increased OR of 1.107 per unit measure). Yet, in the original Barcelona criteria paper, UDCA responders were more likely to have a lower bilirubin at baseline as compared with non-responders. This finding should therefore be interpreted with caution.

However, increased ALP at baseline was found to be associated with a decreased odds of UDCA response according to Paris II criteria (though not Barcelona or combined criteria), which is in keeping with the literature of higher ALP associated with poorer outcome<sup>[18]</sup>. Additionally, younger age is associated with a poorer clinical outcome and lower likelihood of UDCA response (Paris I criteria) in a large UK PBC cohort<sup>[5]</sup>, and accordingly we found an association of higher age with greater odds of UDCA response (by Paris II criteria).

The determination of probability of a good response in PBC patients is becoming increasingly important in the advent of effective second line therapies. A trial investigating the efficacy of obeticholic acid with or without UDCA found biochemical response rates of 46-47% in patients who did not respond, or where intolerant to UDCA alone<sup>[4]</sup>. Similarly, a trial investigating the use of bezafibrates in combination with UDCA, in patients who did not respond to UDCA alone, found a biochemical response rate of 31%<sup>[5]</sup>. Therefore risk stratification should be a priority to allow patients to be started on second line therapies promptly, if UDCA is unlikely to be efficacious.

It is essential to acknowledge the recent efforts of the UK-PBC Consortium and the Italian PBC Study Group in designing a prediction model for UDCA response. They had a derivation cohort of 2703 patients with PBC, and validated the model in a cohort of 460 patients<sup>[17]</sup>. They too found that raised ALP at diagnosis and younger age were associated with a lower probability of UDCA response. However, they did not evaluate the effect of PBC-specific ANAs on the odds of having a response to UDCA, which leaves room for further study.

It is important to note some of the limitations of this study. Firstly, there were 48 patients in whom the UDCA response was unknown and these patients were excluded, which reduced the size of the cohort and so the power of the study.

Additionally, the demographics of our study cohort are somewhat atypical for a Northern European PBC population in that more patients than expected were male (23.9%) compared to the previously reported ratio of roughly 10:1 female:male. The reason for this is

uncertain, however, increasing prevalence in males is being reported in the literature, perhaps with increased awareness, such as a ratio of 4.2:1 (F:M) in a recent Danish cohort<sup>[19]</sup>. It is also possible that as the disease tends to more advanced at presentation in males<sup>[6]</sup>, these patients were more likely to be started on UDCA in order to treat the disease more aggressively and therefore more male patients were included in our analysis. Lastly, of course, given the small sample size, it may be our over-representation of males was purely by chance. Indeed if one looked at the whole cohort of 204 patients on their PBC database before we excluded ineligible patients for our study, the proportion of males is lower at 16.2%.

Furthermore, 73.9% of the cohort responded to UDCA by at least one criteria, which is higher than the response rate of 61% quoted by Parés *et al.*<sup>[2]</sup> however it is still in keeping with more recent response rates reported in the literature such as 70-76%<sup>[17,20]</sup>.

Additionally, only 79.3% of our cohort was AMA positive, with reports in the literature citing that typically more than 95% of PBC patients are AMA positive<sup>[21]</sup>. It is again unclear as to why this should be the case, though the utilization of PBC-specific ANAs such as anti-gp210 and anti-sp100 is a relatively recent practice, which may have led to an increase in diagnosis of AMA-negative cases. We showed that a further 4 cases of AMA-negative patients (by indirect immunofluorescence) were positive for an AMA-specific antigen [anti-M2 or anti-3E(BPO)], which is a more specific test, therefore bringing our proportion of true AMA-negative patients to 15/92 (16.3%). We endeavoured to check that those who were truly negative for any PBC antibodies (AMA, anti-sp100, anti-GP210 or anti-M2) indeed had a liver biopsy (and/or a clinical phenotype) supporting a diagnosis of PBC, which was the case.

In conclusion, this study did not show an association between anti-gp210 or anti-sp100 and response to UDCA. However a trend was seen of positivity to these antibodies, particularly anti-gp210, and UDCA non-response, and the study may have been underpowered to find a possibly true association. These PBC-specific ANAs are important clinically for diagnosis, particularly in AMA-negative patients, as well as potentially for predicting clinical prognosis. Future studies could include PBC-specific ANAs as, in the advent of new second-line therapies in PBC, it is of increasing importance to predict UDCA response.

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