EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | MONOTHEMATIC CONFERENCE

PRIMARY BILIARY CIRRHOSIS (PBC)

MILAN, ITALY

MAY **23 - 24 / 2014**

PROGRAMME AND ABSTRACTS

The EASL Building
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WELCOME **MESSAGE**

Dear Colleagues,

It is our great pleasure to welcome you to the European Association for the Study of the Liver (EASL) Monothematic Conference on Primary Biliary Cirrhosis (PBC), which will take place in Milan, Italy on May 23-24, 2014. PBC is still an enigmatic liver disease but recent acquisitions on its genetic architecture and on a number of key open questions such as the reasons for its female preponderance and why only small-size bile ducts are affected have been reported. In addition, a number of animal models of PBC have been described.

We think it is timing to have a dedicated meeting for critically review these recent developments:

- the real role of genetics and epigenetics in the development of the disease
- why there are large geographical variations in disease frequency across Europe
- why PBC occurs predominantly in women
- what causes disabling symptoms such as fatigue
- why the autoimmune attack is focused on the biliary epithelium

New theories on potential environmental triggers, such as chemical xenobiotics, will be explored together with the processes within the unique immunological milieu of the liver which lead to the breaking of self-tolerance. We hope you will find this conference of interest. A large number of true experts will highlight recent progress in understanding the pathogenesis of this enigmatic disease and areas for future research efforts.

We look forward to welcoming you in Milan for this important scientific European event.

SCIENTIFIC ORGANISING COMMITTEE

Ulrich Beuers | Pietro Invernizzi | Albert Parès

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Istituto Clinico Humanitas
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20089 Rozzano, Milano
Italy
www.humanitasedu.it/sedi/milano

INFORMATION ABOUT MILAN
City Web Site: Tourism Office Milan:
www.turismo.milano.it

LANGUAGE
The official language of the conference is English.

CLIMATE
The month of May is characterized by rising daily high temperatures, with daily highs increasing from 21°C to 26°C over the course of the month, exceeding 31°C or dropping below 16°C only one day in ten.

NAME BADGES
All participants are kindly requested to wear their name badges throughout the EASL Monothematic Conference on PBC in order to be admitted to the lecture halls and other scheduled activities.

REGISTRATION & ACCOMMODATION
All participants should register online. Hotel accommodation for the EASL Monothematic Conference on PBC will be offered to participants. Detailed information, as well as access to the online registration and accommodation page, is available on the website.

REGISTRATION DESK
The onsite registration desk at the conference venue, will be opened:

Thursday, May 22	16.00 pm – 19.30 pm
Friday, May 23	07.30 am – 18.30 pm
Saturday, May 24	8.00 am – 15.00 pm

Group Registration: Dedicated timing will be allocated to group registration pick-ups. These timing will be communicated separately to group leaders.

VISA REQUIREMENTS
Visa regulations depend on your nationality and country of origin. Please contact your local Embassy / Consulate for full and official instructions on the specific visa regulations and application procedures that apply to you. It is the responsibility of the participant to obtain a visa if required.

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Official letters of invitation designed to help overcome administrative difficulties in certain countries may be requested. It must be understood that such letters do not represent a commitment on the part of the Organising Committee or conference to provide any financial assistance.

CME ACCREDITATION
The 'EASL Monothematic Conference: Primary Biliary Cirrhosis (PBC)' is accredited by the European Accreditation Council for Continuing Medical Education (EACCME) to provide the following CME activity for medical specialists. The EACCME is an institution of the European Union of Medical Specialists (UEMS), www.uems.net.

The EASL Monothematic Conference: Primary Biliary Cirrhosis (PBC)' is designated for a maximum of (or 'for up to') 11 hours of European external CME credits. Each medical specialist should claim only those hours of credit that he/she actually spent in the educational activity.

Through an agreement between the European Union of Medical Specialists and the American Medical Association, physicians may convert EACCME credits to an equivalent number of AMA PRA Category 1 Credits™. Information on the process to convert EACCME credit to AMA credit can be found at www.ama-assn.org/go/internationalcme.

Live educational activities, occurring outside of Canada, recognized by the UEMS-EACCME for ECMEC credits are deemed to be Accredited Group Learning Activities (Section 1) as defined by the Maintenance of Certification Program of The Royal College of Physicians and Surgeons of Canada.

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Any pharmaceutical companies producing drugs participating as Sponsors or Exhibitors to events held in Italy must be registered at the Italian Ministry of Health. Application to participate MUST be presented at the Italian Ministry of Health latest 70 days prior the beginning of the event. Applications after this deadline date will be rejected.

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Session Evaluation Forms - The session evaluation forms will be available online. A link will be sent to you by e-mail after the conference. You are requested to kindly complete the forms for each session that you attend.

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GENERAL **INFORMATION**

TRANSPORT TO AND FROM VENUE
From Malpensa Airport
Take the A8 motorway for Milan. Enter the tangenziale ovest (west ring road) and exit at Ticinese/Rozzano. Follow the route given in the paragraph from the motorways.

From Linate Airport
Take the tangenziale est (east ring road) for Genoa, enter the tangenziale ovest (west ring road) and exit at Ticinese/Rozzano. Follow the route given in the paragraph from the motorways.

From the Central Station
Take the internal city ring road (dei Bastioni) to Porta Ticinese, follow the route given in the paragraph from the city centre.

From the City Centre
Go straight on from Porta Ticinese, Corso San Gottardo, via Meda, via Montegani, via dei Missaglia, then follow the indications Istituto Clinico Humanitas, or Basiglio Milano 3.

From the motorways
From all of the motorway exits follow indications for tangenziale ovest (west ring road) and, once on it, exit at Ticinese/Rozzano. After the stop from the ring road onto deiGiovi road (ss35), turn right. Turn right again at the traffic light, in via M. Amiata which becomes via Isonzo, then right again following the indications for Istituto Clinico Humanitas.

LIST OF PARTICIPANTS
To be displayed on the notice board located in the registration area.

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Informal for all occasions.

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This will be a non-smoking event.

BANKING
The official currency in Italy is the Euro (€).

CURRENCY EXCHANGE
Foreign currency can be exchanged at banks, bureau de change and automatic currency exchange machines.

SAFETY AND SECURITY
Please do not leave bags or suitcases unattended at any time, whether inside or outside the session halls. Hotels strongly recommend that you use their safety deposit boxes for your valuables.

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To the extent permitted by mandatory law, the Organiser is not able to take any responsibility whatsoever for injury or damage involving persons and property during the conference.

Participants are advised to take out their own personal travel, health and property insurance for their trip. Equally, participants are encouraged to take out insurance to cover costs such as flight, accommodation, registration fee and others in the case the event is cancelled or postponed for any reasons mentioned within these Guidelines.

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If using a Powerpoint (or any other computer) presentation, please note you need to bring it on a CD, a DVD or on a «disk on key» memory stick (using the USB port in the computer) and load it on to one of the conference computers in the Speakers' Ready Room located near the plenary hall (follow "Speakers Ready Room" signs) at least 1 hour before the start of the session. You may supply your own laptop computer as a back-up.

If combining video films with PowerPoint, please make sure to check the content in the session hall where your lecture is taking place during a coffee or lunch break prior to your session, at least 30 minutes before the start of the session - even after checking it in the Speakers' Ready Room.

Please note that the conference computers in the session halls are being supplied with Windows 7 and Office 2010.

Important note for Macintosh users:
In order to use MAC presentations on a PC compatible computer please note that you need to prepare them according to the instructions below, before bringing presentations to the Speakers' Ready Room:

1.	Use a common font, such as Arial, Times New Roman, Verdana etc. (Special fonts might convert to a default font when using a PowerPoint based PC).
2.	Insert pictures as JPG files (not TIF, PNG or PICT - these images will not be visible on a PowerPoint based PC).
3.	Use a common movie format, such as AVI, MPG and WMV (MOV files from QuickTime will not be visible on a PowerPoint based PC).

Please note that VHS Video projection, 35 mm' slide projection and overhead projection (projection of transparencies) will not be available.

IMPORTANT NOTE:
It is mandatory that all oral presenters prepare a disclosure slide as the first slide in their presentation. If you have nothing to disclose, this slide must be included and indicate "nothing to disclose"

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | SCHOOL OF HEPATOLOGY

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LIVER CIRRHOSIS: A SYSTEMIC DISEASE

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SCIENTIFIC **PROGRAMME**

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THURSDAY, MAY 22, 2014	
16:00 - 20:00	**REGISTRATION**

FRIDAY, MAY 23, 2014	
SESSION 1	**EPIDEMIOLOGY, NATURAL HISTORY AND PATHOPHYSIOLOGY I: GENETICS**
8:30 - 9:00	EPIDEMIOLOGY AND NATURAL HISTORY OF PRIMARY BILIARY CIRRHOSIS **Mauro Podda**, *Italy*
9:00 - 9:30	PRIMARY BILIARY CIRRHOSIS IN FAR EAST **Ma Xiong**, *China*
9:30 - 10:00	GENOME-WIDE ASSOCIATION STUDIES **Gideon Hirschfield**, *UK*
10:00 - 10:30	EPIGENETICS OF PRIMARY BILIARY CIRRHOSIS **Pietro Invernizzi**, *Italy*
10:30 - 11:00	**COFFEE BREAK**
SESSION 2	**PATHOPHYSIOLOGY II: BILIARY PATHOPHYSIOLOGY**
11:00 - 11:30	MODULATION OF CHOLANGIOCYTE SECRETION **Mario Strazzabosco**, *Italy*
11:30 - 12:00	BILIARY PATHOPHYSIOLOGY: ROLE AND MODULATION OF AE2 **Juan F. Medina**, *Spain*
12:00 - 12:30	ROLE OF HCO3-: THE BILIARY HCO3- UMBRELLA **Ulrich Beuers**, *The Netherlands*
12:30 - 13:00	MECHANISMS OF CHOLANGIOCYTE CELL DEATH IN PRIMARY BILIARY CIRRHOSIS **Domenico Alvaro**, *Italy*
13:00 - 13:45	**LUNCH & POSTER VIEWING**
13:45 – 14:00	**DISCUSSION** **Robert Mitchell-Thain & Ingo van Thiel**

SESSION 3	PATHOPHYSIOLOGY III: AUTOIMMUNITY AND ENVIRONMENTAL FACTORS
14:00 – 14:30	TOLERANCE AND IMMUNE REGULATION **David Adams**, *UK*
14:30 – 15:00	THE ENVIRONMENT: INNATE IMMUNITY AND PRIMARY BILIARY CIRRHOSIS **M. Eric Gershwin**, *USA*
15:00 – 15:30	ROLE OF ANIMAL MODELS **Peter Fickert**, *Austria*
15:30 – 16:00	B CELLS AND AUTOANTIBODIES **Dimitrios Bogdanos**, *UK*
16:00 – 16:30	**COFFEE BREAK**
SESSION 4	**PATHOPHYSIOLOGY OF EXTRAHEPATIC MANIFESTATIONS**
16:30 – 17:00	AUTOTAXIN IN CHOLESTATIC ITCH **Ronald Oude Elferink**, *The Netherlands*
17:00 – 17:30	PATHOPHYSIOLOGY OF EXTRAHEPATIC MANIFESTATIONS **David Jones**, *UK*
17:30 – 18:00	OSTEOPOROSIS **Nuria Guañabens**, *Spain*

SCIENTIFIC **PROGRAMME**

SATURDAY, MAY 24, 2014	
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9:30 - 10:00	SURROGATE MARKERS **Henk R. van Buuren**, *The Netherlands*
10:00 - 10:30	THE MODEL DRUG: UDCA **Raoul Poupon**, *France*
10:30 - 11:00	**COFFEE BREAK & POSTER VIEWING**
SESSION 6	**THERAPEUTIC APPROACHES BEYOND UDCA**
11:00 - 11:30	FXR VS PPAR AGONISTS: COMPETITORS OR FELLOW-COMBATANTS? **Michael Trauner**, *Austria*
11:30 - 12:00	CORTICOSTEROIDS, ANTIVIRALS, OTHER AGENTS **Andrew L. Mason**, *Canada*
12:00 - 12:30	PRIMARY BILIARY CIRRHOSIS: AUTOIMMUNE HEPATITIS OVERLAP SYNDROME **Olivier Chazouillères**, *France*
12:30 - 13:30	**LUNCH & POSTER VIEWING**
SESSION 7	**THERAPEUTIC APPROACHES: LIVER TRANSPLANTATION AND EXTRAHEPATIC MANIFESTATIONS**
13:30 - 14:00	ADVANCES IN TREATMENT OF PRURITUS AND OSTEOPOROSIS **Albert Parès**, Spain
14:00 - 14:30	LIVER TRANSPLANTATION AND DISEASE RECURRENCE **James Neuberger**, *UK*
14:30 - 15:00	HEPATOCELLULAR CARCINOMA AND EXTRAHEPATIC MALIGNANCIES **Annarosa Floreani**, *Italy*
15:00 - 15:30	**GENERAL DISCUSSION AND CONCLUSIONS**

POSTER BOARDS

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P03	YI	YOUNGER PATIENTS PRESENTING WITH PBC ARE MORE LIKELY TO HAVE COGNITIVE IMPAIRMENT	Laura Griffiths
P04		FREQUENCY OF CHOLELITHIASIS IN DIFFUSE LIVER DISEASES WITH CHOLESTASIS	Sayfullo Avezov
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EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | MASTERCLASS

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DECEMBER **11 - 13 / 2014**

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INVITED **SPEAKERS' ABSTRACTS**

EPIDEMIOLOGY AND NATURAL HISTORY OF PRIMARY BILIARY CIRRHOSIS

Mauro Podda*

Corresponding author's e-mail: mauro.podda@humanitas.it

The epidemiology and natural history of primary biliary cirrhosis remain largely elusive and may present important clues to disease pathogenesis. A systematic review of population based studies indicated a wide range in the yearly incidence (0.33-5.8/100.000) and point prevalence (1.91-40.2/ 100.000) rates. Though different ethnic representations may also contribute it is likely that methodological issues, based on the retrospective survey of diagnosed cases, and time trend play a major role, also in view of the prolonged asymptomatic period of the disease. In a complementary fashion, the search for serum AMA in unselected population sera may identify the largest possible number of patients who have or will develop the disease. Finally, the median female to male ratio for PBC is classically accepted as 9-10:1 but is significantly lower for AMA prevalence (2.5:1). On the other hand, the natural history of untreated PBC is traditionally described as a gradual progression through four phases: preclinical, asymptomatic, symptomatic, and liver failure. Though in most patients the phases are subsequent, their duration shows wide variations individually and sometimes one or two symptomatic phases may be skipped. There is also wide variability among different reports, mainly due to the pattern of patient referral and recruitment in single centers, similarly to what described above for epidemiologic studies. Furthermore most of recent studies combine UDCA-treated and untreated patients. We are convinced that the true "natural history" of PBC will be never accurately known.

PRIMARY BILIARY CIRRHOSIS IN FAR EAST

Xiong Ma*

Corresponding author's e-mail: maxiongmd@hotmail.com

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by high-titer serum antimitochondrial autoantibodies (AMA) and autoimmune-mediated destruction of small and medium-sized intrahepatic bile ducts. According to Ludwig's classification, liver histology identifies four PBC stages. Recently, a Japanese group proposed a new staging and grading system for PBC that takes into account necroinflammatory activity and histological heterogeneity. Our study demonstrated that the immunostaining of dendritic cell marker CD11c is a sensitive tool to identify liver granulomas in PBC. PBC is closely associated with a greater risk of overall cancer and hepatocellular carcinoma (HCC), furthermore, males are at risk of developing HCC at any histological stage of PBC. Ursodeoxycholic acid (UDCA) is the only licensed therapy for PBC so far, while the biochemical response to UDCA is a strong predictor of long-term outcome. A Chinese study showed that biochemical responses at the sixth month can be used in place of those evaluated after 1 year of UDCA therapy. Bezafibrate is a dual PPARs/PXR agonist with potent anti-cholestatic efficacy in early-stage PBC patients with an incomplete biochemical response to UDCA therapy. Combination of UDCA and immunosuppressors appears to be the best therapeutic option for PBC-autoimmune hepatitis (AIH) overlap syndrome. Our group found that plasma IgG ³1.3xULN had a sensitivity of 60% and a specificity of 97% in identifying cases of corticosteroid-responsive PBC-AIH overlap syndrome in China, while the use of a higher threshold of 2.0xULN (one of Paris criteria) reduced the sensitivity to 10%. In summary, PBC is emerging as an important disease among the etiology of chronic liver diseases in our clinics. The trend toward an earlier diagnosis of the disease and more effective medical treatment, may improve the outcomes of PBC patients especially in Far East.

GENOME WIDE ASSOCIATION STUDIES

Gideon Hirschfield*

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Primary biliary cirrhosis is the archetypal autoimmune disease. With the strong epidemiology of disease, and associated autoimmune conditions, in patients and their families, an important genetic component to disease risk has always been recognised. Genome wide association studies have within the last few years made significant advances into the definition of genetic risk factors. With the ability to harness well phenotyped cohorts, and utilise a variety of different gene array platforms, new insights into PBC pathophysiology have been made, particularly with a focus on the IL-12 and JAK-STAT signalling cascades. These insights remain in parallel to the strong HLA association identified from prior studies, as well as the new genome wide studies. With this data we now have an array of strongly immune focused risk loci associated with disease initiation. These are feeding our understanding of the events associated with disease risk, and underpinning concepts for new immunomodulatory studies. Further studies focused more on disease phenotype, such as itch or treatment response, are also important as they will provide different mechanistic insights.

EPIGENETICS OF PRIMARY BILIARY CIRRHOSI

Pietro Invernizzi [1*]
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Primary biliary cirrhosis (PBC) has been considered for long time a multifactorial autoimmune disease presumably arising from a combination of environmental and genetic factors, with genetic inheritance mostly suggested by familial occurrence and high concordance rate among monozygotic twins. In the last decade, genome-wide association studies (GWAS), new data on sex chromosomes defects and instabilities, and initial evidence on the role of epigenetic abnormalities have strengthened the crucial importance of genetic and epigenetic factors in determining the susceptibility PBC. Although high-throughput genetic studies are revolutionizing the search for genetic influences on PBC and have the potential to be translated in clinical and therapeutic application, more biological knowledge on candidate genes is now needed. In addition to genetics, accumulating evidence has demonstrated that epigenetics is involved in the pathogenesis of PBC. Epigenetic modifications, particularly DNA methylation, are known regulatory mechanism of gene expression and appear as ideal candidates to explain the environmental influence on individual susceptibility to PBC. However, although abnormal DNA demethylation has been shown in CD4+ T cells in women with other autoimmune diseases, the actual involvement of epigenetic mechanisms exemplified by abnormal DNA methylation in PBC has not been extensively studied. Several epigenetic aberrancies of the X chromosome have been reported that would be most likely involved in the female predominance of PBC. In addition, it has been demonstrated a significantly lower levels of DNA methylation of a number of gene promoters in PBC. For example, low methylation of the CD40L promoter was found in CD4+ T cells from PBC patients as compared with controls. Interestingly, this decreased methylation was inversely correlated with levels of serum IgM in PBC patients. The findings of an absence of genetic modifications of the CD40L gene in concert with decreased DNA methylation of the CD40L promoter in PBC patients suggests that environmental factors rather than genetics must play a major role in the pathogenesis of elevated serum IgM in PBC. In the future it will be very important to further study and understand the real role of epigenetic abnormalities in PBC.

MODULATION OF CHOLANGIOCYTE SECRETION

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Physiology of cholangiocyte secretion

The biliary epithelium forms a branching system of conduits within the liver where bile flows from the hepatocytes to the gallbladder and intestine and is organized in a complex tridimensional network that starts at the canals of Hering and forms tubules (interlobular, septal, major ducts, and hepatic ducts) embedded into the portal space. The biliary tree is lined by cholangiocytes, epithelial cells with absorptive and secretory properties that actively contribute to bile formation, regulating its volume, pH and composition according to physiological needs.

The morphology and function of cholangiocytes varies along the biliary tree: cholangiocytes lining the large interlobular and major ducts are mostly involved in secretory functions, whereas cholangiocytes in the smaller bile duct branches, cholangioles and ducts of Hering have the ability to proliferate in response to liver damage, participate in the liver reparative response, and undergo limited phenotypic changes. This functional specificity is consistent with the fact that most cholangiopathies show a site restricted bile duct injury. For instance, primary biliary cirrhosis (PBC) targets specifically the interlobular bile ducts, whereas primary sclerosing cholangitis (PSC) affects the larger intrahepatic and extrahepatic ducts. Interestingly, the "small duct" variant of PSC, where damage is restricted to the finest branches of the biliary tree, has distinct clinical manifestations.

Bile formation starts at the hepatocyte canalicular membrane, with the secretion of bile acids, other organic and inorganic solutes, electrolytes and water. As the primary bile flows through the bile ducts on its route toward the duodenum, its composition is regulated by the intrahepatic bile duct epithelium that reabsorbs fluids, amino acids, glucose and bile acids, while secreting water and electrolytes. Twenty years of investigation have partly unveiled the complexity of the transport function of cholangiocytes and of its regulation, a topic that has been reviewed also recently and which pathophysiological consequences (impairment of the "bicarbonate umbrella") will be discussed later by Dr Beuers.

Ultimately, secretion of fluids and their alkalinization in the bile ducts is mainly associated with a net flux of chloride (Cl-) and bicarbonate (HCO3-) into the lumen which induces the secretion of water and regulates bile pH. In contrast with hepatocytes, where the major driving force for bile production is the active secretion of bile acids by adenosine triphosphate (ATP)-driven transporters, cholangiocytes secrete fluid and electrolytes in response to paracrine or endocrine stimuli acting on the basolateral or apical side of the cell. A number of ion channels and transporters have been identified and shown to have a polarized subcellular distribution. As in all mammalian cells, the driving force for facilitated membrane transport in cholangiocytes is provided by the Na+/K+ ATPase, which actively extrudes sodium (Na+) from the cell and, together with potassium (K+) channels, maintains the transmembrane potential. At the basolateral side, the Na+ gradient regulates the Na+/H+ exchanger isoform 1 (NHE1 or SLC9A1) and the Na+:HCO3- symporter (SLC4A4) which mediate the reabsorption of HCO3- necessary for acid extrusion, while the Na+/K+/2Cl- cotransporter (NKCC1), a major determinant of fluid secretion, mediates the chloride uptake into the cell. On the apical side of the cell, Cl- efflux is mainly mediated by a cyclic-adenosine monophosphate (cAMP) activated, slow conductance, Cl- channel encoded by the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The opening of chloride channels (CFTR) in the apical membrane leads to an efflux of Cl- and the generation of a lumen-negative potential which induces the release of water into the lumen through aquaporines (AQP-1 and AQP-4). The Cl- gradient regulates the Na+ independent Cl-/HCO3- exchanger (AE2 or SLC4A2) which extrudes bicarbonate into the bile providing biliary alkalinization, in accordance with intracellular pH. Other carriers such as the Na+ dependent glucose transporter (SGLT1 or SLCA21), the glutamate transporter and the ileal bile acid transporter (iBAT or SLC10A2) expressed on the apical membrane of cholangiocytes, mediate the re-absorption of biliary constituents, such as glucose and glutathione break down products and conjugated bile acids. This is particularly important because bile acids can stimulate proliferation of biliary epithelial cells. Bile acids are then secreted in the peribiliary plexus via t-ASBT, a truncated isoform of the apical sodium-dependent bile acid transporter (ASBT or SLC10A1). This cholehepatic circulation of bile acids is also important in the overall regulation of bile secretion. This most simplified secretory model has the advantage of being of more immediate use to understand its implications in cholangiopathies.

Modulation of cholangiocyte secretion

The secretory function of the bile ducts is finely regulated by rapid hormone-mediated signaling; here will quote only the major ones. The net amount of fluid and secreted HCO3- is determined by the integration of different pro-secretory (secretin, glucagon, VIP, acetylcholine, bombesin) and anti-secretory (somatostatin, endothelin1) stimuli, just to name a few. Most of the hormones regulating cholangiocyte secretion in interlobular/ septal ducts ultimately act on the adenyl cyclases (ACs), the transmembrane enzymes that regulate the intracellular level of the second messenger cAMP, converting ATP to cAMP. Secretin, the main choleretic hormone, increases cAMP/PKA. This activates CFTR, and consequently stimulates Cl- and HCO3- efflux and inhibits the Na+/H+ exchanger-3 (NHE3 or SLC9A3) -dependent Na+ absorption. Cholinergic agonists, _-adrenergic agonists and HCO3- mediated signals also regulate bile secretion through the cAMP and PKA pathway. Adenyl Cyclases (ACs) may thus represent an important means of integration of multiple secretory signals. So far nine different isoforms of AC have been identified (AC1-9), each displaying tissue specific expression and regulation.

The secretory functions of the biliary epithelium are also regulated by molecules (such as bile salts, glutathione and purinergic nucleotides) secreted by hepatocytes into the canalicular bile, and delivered to receptors and transporters located in the apical membrane of cholangiocytes. For instance, ATP, which is released into the bile by hepatocytes or by cholangiocytes themselves, can bind to apical P2Y2 purinergic receptors and stimulate apical Ca2+-activated Cl- channels and basolateral Na+/H+ exchanger (NHE1 or SLC0A1), thus promoting Cl- efflux into the bile and basolateral HCO3- influx. Certain bile acids may also stimulate cholangiocyte secretion of HCO3- by inducing ATP secretion by CFTR and purinergic activation of apical Ca++-activated or volume-activated Cl- channels.

Role of cAMP and Ca2+ signaling

Several recent studies suggest that cAMP and the cross-talk between CA2+ signaling and cAMP is crucial for important secretory functions, such as ion secretion, cell proliferation and secretion of angiogenic factors.

The amount of cAMP produced by a given cell results from the activity of several adenylyl cyclase (AC) isoforms, which respond to specific stimuli and second messengers. At least 7 of them are expressed in cholangiocytes. Among them, AC8 is regulated by Ca2+/calmodulin, whereas AC5 and AC6 are inhibited at physiological Ca2+ concentrations (100–200 nmol/L), but can be activated at the Ca2+ concentrations measured in polycystin-defective cells. Thus, at the [Ca2+]i measured in cystic cholangiocytes, AC6 becomes more prone to activation than AC8. Of note, AC6 gene silencing abolishes shear stress-induced signaling in polarized cholangiocytes. In addition to bile secretion, increased epithelial levels of cAMP stimulate also the proliferative activity of cholangiocytes via the PKA/Src/Raf/MEK/ERK1/2 cascade.

Cholangiocyte secretion and inflammation

The biliary epithelium represents the primary target in several Inflammation conditions, from primary biliary cirrhosis to sclerosing cholangitis, graft rejection, and sepsis.

Inflammation negatively affects the secretory function of cholangiocytes. It is well know that ductal cholestasis often accompanies sepsis, an effect though to be related to the exposure of the biliary epithelium to circulating endotoxins. We have shown that LPS, INFɣ, and TNFα inhibit cAMP-dependent CFTR-mediated fluid secretion in isolated bile ducts units. Further studies demonstrated that this effect is due to upregulation of NOS2, with NO-mediated nytrosylation of adenyl cyclases and inhibition of cAMP production. In fact cholestasis caused by NO or pro-inflammatory cytokines was prevented by exposure to antioxidants and NO scavengers. These observations may explain why biochemical cholestasis is evident before the establishment of ductopenia or cirrhosis. Inhibition of the PKA-dependent Ras/ERK1/2 pathway may also contribute to reduced biliary proliferation and ductopenia. Furthermore, NO-dependent nytrosylation of DNA quality control mechanism may contribute to inflammation-related biliary carcinogenesis in PSC.

On the other hand, it is now clear that changes in key ion transporter protein impact on innate and adaptive immunity. As it will be shown later by Dr. Medina, AE2 deficient mice show several features consisted with PBC, including anti mitochondria antibodies; interestingly AE2-deficient CD8+T-cells show impaired intracellular pH regulation, enhanced production of IL-2 and membrane expression of its receptor IL-2Rα and increased cell proliferation.

Our group has recently demonstrated that cholangiocytes deficient in CFTR are more prone to respond to LPS with increased production of pro-inflammatory cytokines and increasede biliary damage. Lack of CFTR, in fact, negatively affects the endotoxin tolerance of the biliary epithelium, i.e. the ability of cholangiocyte to temper its inflammatory response to activation of Toll-like receptors (TLR). In CFTR- defective cells activating phosphorylation of TLR4 is increased, leading to an augmented NF-kB-mediated inflammatory response. This mechanism, rather that defective Cl-secretion appears to be responsible for CF-related liver damage in this model. These findings change our concept of the pathogenesis of CF-cholangiopathy, once considered a prototypic channellopathy leading to ductal cholestasis and suggest new therapeutic approaches.

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BILIARY PATHOPHYSIOLOGY: ROLE AND MODULATION OF AE2

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The canalicular bile is fluidized and alkalinized along the bile ducts through hydroionic fluxes at the biliary epithelium. The hormone secretin stimulates this process, which involves biliary secretion of bicarbonate via Cl^-/HCO_3^- anion exchange. There is strong evidence supporting the notion that this exchange is mediated by AE2, a membrane protein also known to be relevant for the regulation of the pH_i. Thus, intracellular alkalinization may greatly stimulate AE2 for its exchange function according to the transmembrane ion gradients, which results in intracellular acid loading because of HCO_3^- efflux and Cl^- influx. Previously, we had reported a diminished expression of AE2 mRNA in liver biopsies and peripheral blood mononuclear cells from patients with primary biliary cirrhosis (PBC). Also the expression of the AE2 protein is decreased in PBC livers. Interestingly, microfluorimetric analysis in cholangiocytes isolated from PBC patients and cultured for a few passages showed that the cAMP-stimulated AE activity is diminished in PBC as compared to both healthy and diseased controls. And positron emission tomography (PET) studies indicated that PBC patients, even at the early stages of the disease, failed to secrete bicarbonate to bile in response to secretin, a defect that could be partially reversed after several months of treatment with UDCA. More recently, we reported that miR-506 is upregulated in cholangiocytes of PBC patients and that the mRNA for AE2 may be a target of miR-506. Altogether, these findings sustain our hypothesis that dysfunctions related to AE2 have a role for the pathogenesis of PBC. According to this model, the reduced expression of AE2 in cholangiocytes may cause cholestasis and oxidative stress in these bile duct cells. On the other hand, inadequate AE2 function in lymphocytes can disturb pH_i regulation in these immune cells (mainly in the $CD8^+$ T-cell population) and alter their homeostasis leading to autoimmunity. Cholangiocyte alterations together with dysregulated homeostasis of immune cells could then favor the development of both nonsuppurative destructive cholangitis and serum antimitochondrial antibodies. The notion that AE2 abnormalities may be involved in the pathogenesis of PBC is further supported by our findings in $Ae2_{a,b}^{-/-}$ mice as these animals can indeed develop biochemical, histological, and immunologic alterations that resemble PBC (including development of serum AMA). Analysis of HCO_3^- transport systems and pH_i regulation in cultured cholangiocytes obtained from normal and $Ae2_{a,b}^{-/-}$ mice confirmed that AE2 is the transporter responsible for the Cl^-/HCO_3^- exchange in these cells. Finally, both $Ae2_{a,b}^{+/+}$ and $Ae2_{a,b}^{-/-}$ mouse cholangiocytes (but not human or rat cholangiocytes) exhibited a Cl^--independent bicarbonate transport system, essentially a Na^+-bicarbonate cotransport (NBC) system. This system is upregulated in $Ae2_{a,b}^{-/-}$ mouse cholangiocytes and could partially contribute to pH_i regulation in the absence of AE2.

ROLE OF HCO3-: THE BILIARY HCO3- UMBRELLA

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Hydrophobic bile salts induce cytotoxicity in many cell types, including hepatocytes, at micromolar concentrations. Notably, human biliary epithelial cells are exposed to high millimolar concentrations of hydrophobic bile salts under physiologic conditions without signs of cytotoxicity. This remarkable resistance against bile salt-induced toxicity is incompletely understood. Formation of mixed micelles of bile salts with phospholipids in bile may be regarded as a protective mechanism, but are unable to sufficiently reduce the concentration of free hydrophobic bile salt monomers to less than low millimolar levels in bile. Human biliary HCO3- secretion accounts for 25%-40% of total bile flow and by far exceeds that of rodents. The physiologic role of the enormous difference in biliary HCO3- secretion between humans and rodents has remained obscure. We recently hypothesized that biliary HCO3- secretion might constitute a biliary HCO3- umbrella on the apical cholangiocyte surface, a so far unrecognized protective mechanism of cholangiocytes against bile salt-induced toxicity1.

Intracellular accumulation of hydrophobic bile salts is a prerequisite for their cytotoxic effects. Uncontrolled, carrier-independent membrane traffic and cell invasion of bile salts is determined by their polarity and degree of protonation. Glycine conjugates account for the majority of bile salts in human bile, have a pKa of ~4 and at physiologic pH are protonated, apolar and thus cell permeable at micromolar amounts. Even small changes in local biliary pH close to the apical membrane of cholangiocytes thus have a dramatic effect on glycine-conjugated bile salt / bile acid ratio and, thereby, sensitivity of cholangiocytes towards glycine-conjugated bile acids. In rodents, which have a more hydrophilic, less toxic bile salt pool with mainly taurine conjugates (pKa of ~1-2) changes in biliary pH would have a minor, negligible effect on bile salt protonation and toxicity. By altering sensitivity towards bile salt toxicity and increasing frequency of apoptotic events in cholangiocytes, genetic and acquired defects disrupting the biliary HCO3--umbrella may be a common pathogenetic factor in various cholangiopathies.

Genetic variants of key modulators of the biliary HCO3- umbrella and the cholangiocyte apical glycocalix - which stabilizes the alkaline pH microclimate close to the apical membrane - have been associated with manifestation or progression of chronic cholestasis, as demonstrated for AE2 in PBC, TGR5 and FUT2 in PSC, and CFTR in cystic fibrosis-associated liver disease. A link between post-transplant nonanastomotic stricturing of denervated bile ducts and dysfunctional vagal muscarinic receptor 3-dependent stimulation of cholangiocyte HCO3- secretion could also be considered1. Experimental evidence in human cholangiocyte cell lines in vitro supports the concept that a biliary HCO3- umbrella protects human cholangiocytes against damage by bile acid monomers2. Thus, defects of the biliary HCO3- umbrella might lead to development of chronic cholangiopathies.

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MECHANISMS OF CHOLANGIOCYTE CELL DEATH IN PRIMARY BILIARY CIRRHOSIS

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Cholangiocytes lining interlobular bile ducts are the primary target of damage in the course of Primary Biliary Cirrhosis (PBC). A balance between cholangiocyte proliferation and death characterizes the progression of PBC where, in the advanced stages, predominance of cell death leads to ductopenia. For many years, based on morphologic criteria, three modes of cell death have been considered: apoptosis, autophagy and necrosis. However, similar phenotypes may result from different biochemical and functional events and, therefore, the Nomenclature Committee on Cell Death recently proposed a functional classification based on the main pathways involved, the caspase dependence and the inhibitory interventions. Extrinsic apoptosis by death receptors (Fas/FasL, TNF/TNF-R1, TRAIL/DR4,5) is certainly the mechanism more diffusely investigated in PBC. Indeed, different studies demonstrated overexpression of death receptors and related ligands and pathways in PBC cholangiocytes and surrounding mononuclear cells, as well as enhanced serum levels of soluble FasL and Trail. However, a relative resistance of normal cholangiocytes to a direct Fas-mediated killing has been reported. Rather, cholangiocytes are particularly sensitive to CD40 mediated apoptosis, where CD154 induction of FasL and autocrine/paracrine Fas activation takes place. In addition, different intracellular mechanisms may counteract apoptosis by cell death receptors. For example, intracellular B7-H4 appears to prevent Fas/FasL-mediated cholangiocyte apoptosis during the progression of PBC. Cholangiocytes are exposed to biliary components which could co-participate in inducing damage including, hydrophobic bile salts (BS) and oxysterols. Isolated cholangiocytes are particularly sensitive to apoptotic damage by hydrophobic BS but, in the intact liver, this is not the case, suggesting defensive mechanisms operating in vivo. Biliary phospholipids, biliary bicarbonate and the IGF1 system are among potential defensive mechanisms which could be defective in PBC thus facilitating BS-induced cholangiocyte apoptosis. Caspase-dependent intrinsic apoptosis with the involvement of the perforin/genzymeB pathway has been also described to play a role in damaging PBC cholangiocytes, though to a lesser extent than extrinsic apoptosis. Autophagy has been recently investigated in PBC where it was associated with senescence and autoimmunity. Markers of autophagy (LC3, LAMP-1) are expressed in damaged small bile ducts of PBC patients but, autophagic cell death can be only claimed when blocked by autophagy inhibitors.

Therefore, the real impact of autophagic cell death in determining bile duct loss is unknown. Markers of autophagy are co-expressed with markers of senescence including, p16INK4a and p21WAF1/Cip1. Cellular senescence can be triggered by a number of cellular stresses, including telomere dysfunction, oxidative stress and nontelomeric DNA damage. To this regard, telomere length was demonstrated to be reduced in PBC cholangiocytes (and blood mononuclear cells) and this could be involved in initiating a process of senescence that favour cholangiocyte loss by autophagy. However, the exact mechanism how cellular senescence may cause bile duct loss in PBC is only speculative. Senescence cells do not proliferate in response to injury; they could be removed by necrosis, apoptosis, or anoikis. Alternatively, senescence seen in cell components of ductular reaction (DR), could impair proliferation of stem/progenitor cells devoted to replace damaged cholangiocytes in interlobular bile ducts thus, resulting in bile duct loss. Senescence and autophagy have been also implicated in the breakdown of immunotollerance against PDC-E2 and, therefore, in the immmunopathogenesis of PBC. The impairment of IGF1 system and the loss of estrogen receptor expression characterizing cholangiocytes in advanced PBC stages may play a major role in favouring senescence. Finally, p53-dependent WAF1 upregulation has been shown in PBC cholangiocytes. WAF1 (p21WAF1/Cip1) is a potent and reversible inhibitor of cell-cycle progression at both the G1 and G2 checkpoints, and an important molecule of cell-cycle regulation and homeostasis. Upregulation of WAF1 and p53 in response to genotoxic damage such as oxidative stress associated with inflammation, followed by irreversible G1 arrest could also represent a major mechanism of cellular senescence of biliary epithelial cells in PBC. In conclusion, different mechanisms of cell death have been described in PBC, the relative impact being probably different during the progression of the disease. Although immunologic mediated extrinsic apoptosis by death receptors seems to be a major mechanism in the early stages, autophagy and senescence are certainly implicated and, probably predominated in the advanced ductopenic stages.

THE ENVIRONMENT, INNATE IMMUNITY AND PRIMARY BILIARY CIRRHOSIS

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Environmental stimulation is a major factor in the initiation and perpetuation of autoimmune diseases. We have addressed this issue and focused on primary biliary cirrhosis (PBC), an autoimmune disease of the liver. Immunologically, PBC is distinguished by immune mediated destruction of the intra hepatic bile ducts and the presence of high titer antimitochondrial autoantibodies (AMA) directed against a highly specific epitope within the lipoic acid binding domain of the pyruvate dehydrogenase E2 subunit (PDC-E2). We submit that the uniqueness of AMA epitope specificity and the conformational changes of the PDC-E2 lipoyl domain during physiological acyl transfer could be the lynchpin to the etiology of PBC and postulate that chemical xenobiotics modification of the lipoyl domain of PDC-E2 is sufficient to break self-tolerance, with subsequent production of AMA in patients with PBC. Indeed, using quantitative structure activity relationship (QSAR) analysis on a peptide-xenobiotic conjugate microarray platform, we have demonstrated that when the lipoyl domain of PDC-E2 was modified with specific synthetic small molecule lipoyl mimics, the ensuing structures displayed highly specific reactivity to PBC sera, at levels often higher than the native PDC-E2 molecule. Hereby, we discuss our recent QSAR analysis data on specific AMA reactivity against a focused panel of lipoic acid mimic in which the lipoyl di-sulfide bond are modified. Furthermore, data on the immunological characterization of antigen and Ig isotype specificities against one such lipoic acid mimic; 6,8-bis(acetylthio) octanoic acid (SAc), when compared with rPDC-E2, strongly support a xenobiotic etiology in PBC. This observation is of particular significance in that approximately one third of patients who have taken excessive acetaminophen (APAP) developed AMA with same specificity as patients with PBC, suggesting that the lipoic domain are a target of APAP electrophilic metabolites such as NAPQI. We submit that in genetically susceptible hosts, electrophilic modification of lipoic acid in PDC-E2 by acetaminophen or similar drugs can facilitate loss of tolerance and lead to the development of PBC.

Our work on environmental factors should be placed in the context of the biliary microenvironment of PBC. In particular, the interleukin (IL)-12/IL-23 mediated Th1/Th17 signaling pathway has been associated with the etiopathogenesis of primary biliary cirrhosis (PBC). To address the cytokine microenvironment specifically in the liver, we examined the localized expression of cytokine subunits and their corresponding receptors using previously optimized immunohistochemistry with an extensive panel of antibodies directed at IL-12p70, IL-12p35, IFN-γ, IL-12RB2, IL-23p40, IL-23p19, IL-17 and IL-23R using liver from PBC (n=51) and non-PBC (n=80) control liver disease patients. Multiple portal tracts in each patient were blindly evaluated and individually scored. We report herein that although IL-12/Th1 and IL-23/Th17 staining were detected in all of the liver sections, they were primarily localized around the damaged interlobular bile ducts in PBC. Most importantly, Th17 skewing was prominent in advanced PBC patients with intensive secretion of IL-23p19 by inflamed hepatocytes around IL-23R, IL-12RB2, and IFN-γ expressing degenerated cholangiocytes. Our novel finding on the direct association of Th17 skewing and disease severity illustrates the significance of the IL-23/Th17 pathway in the perpetuation of IL-12/Th1-mediated immunopathology in PBC. Furthermore, localized IL-23p19 production by hepatocytes may enhance pro-fibrotic Th17 signalling and pro-inflammatory IFN-γ production that contribute to PBC pathology. In conclusion, our data emphasize the pathogenic relevance of IL-12/Th1 and IL-23/Th17 in the evolution of PBC. Of significance, however, the shift from a Th1 to a Th17 imbalance at advanced stages of the disease suggests the necessity to consider modulation of the IL-23/Th17 pathway as a potential target for therapeutic intervention. PBC is a multi-hit disease and likely has several overlapping etiological factors. It is likely a disease which includes innate and adaptive responses and, in particular, a significant bystander effect, which leads to chronic inflammation. The presence of continued bystander inflammatory cells explains in part the recurrence of PBC in some patients following liver transplants. The bridge between innate and adaptive immunity should become a focal point to disrupt and down-regulate chronic autoimmune cholangitis.

ROLE OF ANIMAL MODELS

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Primary biliary cirrhosis (PBC) represents a prototypic autoimmune and immune mediated cholangiopathy with enigmatic etiology. This disease has distinct clinical, laboratory, immunological, and histomorphological characteristics. Well-characterized animal models for PBC are utterly needed to develop novel pathogenetic concepts and study new treatment strategies. The first aim of this presentation is therefore to outline a wish list including the characteristics of an ideal PBC animal model:

- Clear female predominance
- AMA positivity > 90%
- Loss of tolerance to mitochondrial autoantigens of the E2 subunit of the oxoacid-dehydrogenase pathway
- ANA positivity in 50-80% including special subtypes (e.g. speckled, anti-centromeres, anti-sp100, anti-gp 210)
- Chronic inflammation of small bile ducts with focal duct obliteration and epitheloid cell granuloma formation
- Slow disease progression with vanishing of bile ducts
- Biliary type of liver fibrosis
- PDC-E2 specific autoreactive CD4 T cells in liver and hilar lymphe nodes
- PDC-E2 specific autoreactive CD8 T cells in liver
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The second aim is to contrast this with a real life up-to-date overview of currently available mouse models and to provide a basis for discussion how we should characterize potential PBC models. The outlined clinical, immunological and histological characteristics of PBC represent the landmark for currently available mouse models. Consequently potential PBC animal models should be systematically investigated in regard to serum liver test abnormalities, immunological abnormalities, and longitudinal studies in regard to their liver phenotype. Although some of the currently available models show some individual characteristics of PBC, it is obvious that all of them have substantial limitations. Nevertheless some may be advantageous to study certain pathophysiological aspects of PBC. Due to the complex nature of PBC it seems to be very likely that no single ideal PBC model will ever be generated, but models will certainly help to clarify specific pathogenetic aspects and could turn out to be suitable to test potential drugs for treatment.

AUTOTAXIN IN CHOLESTATIC ITCH

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In patients with cholestasis, including intrahepatic cholestasis of pregnancy (ICP), serum autotaxin (ATX) activity correlates with itch intensity. Itch occurs irrespective of the nature of the cholestasis. Also in women with intrahepatic cholestasis of pregnancy (ICP) mild cholestasis is accompanied by itch and strongly elevated serum ATX activity. Autotaxin in the circulation is responsible for the formation of lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC). LPA is a potent bioactive lipid that signals through 6 LPA receptors. We hypothesize that ATX causes itch by formation of lysophosphatidate (LPA), activating sensory nerve endings. Several findings support this hypothesis; firstly, intradermal injection of LPA is mice causes scratch behavior that can be inhibited by simultaneous injection of a LPA receptor antagonist. Secondly, various treatments to reduce cholestasis also reduce itch and serum ATX levels. Thirdly, it has been recently reported by various groups that also in atopic dermatitis ATX in serum is elevated and correlates with the extent of itch. We have attempted to develop a mouse model for cholestatic itch. However, it appears that itch is not a symptom in cholestasis in rodents. In several models (bile duct ligation, ATP8B1 deficiency combined with bile salt feeding) severe cholestasis was not associated with any increase in serum ATX. Even combination of pregnancy and cholestasis (as a model for ICP) did not induce scratch behaviour. This lack of itch in cholestatic mice correlates with only minor increases in serum ATX as opposed to strong increases in humans. In order to be able to investigate the effect of high serum ATX levels we have developed a model in which mice are transduced with AAV harbouring the ATX gene. These animals are analysed for scratch behaviour and other parameters in the presence and absence of cholestasis. We have analysed the potential source of ATX in cholestatic patients. Staining of various tissues reveals that ATX is relatively strongly expressed in enteroendocrine cells of the small intestine. Conclusion: our data suggest that LPA produced by elevated ATX activity in the bloodstream may play a significant role in cholestatic pruritus.

PATHOPHYSIOLOGY OF EXTRAHEPATIC MANIFESTATIONS

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Fatigue in Primary Biliary Cirrhosis is a significant clinical problem and is the symptom with the greatest prevalence in PBC exceeding more classically associated symptoms such as pruritus. Fatigue is the factor with the greatest impact on patient quality of life and, in severe cases which account for approximately 20% of patients and can have a profound impact on patient's existence. The science of the pathogenesis of fatigue is in its infancy and has depended on the development of effective tools to quantify the problem. These are now in place which has led to an expansion of activity and also novel therapeutic studies. Fatigue in PBC appears to be unrelated to severity of the disease although in very advanced disease specific end stage factors can complicate and exacerbate fatigue. In terms of clinical associates there are associations between fatigue in PBC and both autonomic dysfunction and sleep disturbance. There can also be a significant component to depression although it is unusual for this to be a sole associated factor and it appears likely that depression in fatigue is mainly a manifestation of the consequences of profound fatigue and its effect on quality of life rather than a cause. Emerging data suggests that there are both central and peripheral factors in fatigue with both organic brain change manifestos neurophysiological abnormality and imaging abnormality being present in association with fatigue. It appears that this component of fatigue in PBC is irreversible with transplant raising implications for treatment. It is potentially the case that cholestasis itself drives this organic brain injury and this is an area of active research. There are important peripheral components to fatigue in PBC with metabolic abnormalities in muscle in patients, linked in terms of their expression with autonomic dysfunction. Paradigms around altering metabolic function in PBC, including through exercise therapy represent novel opportunities. It is likely that current research programmes exploring the pathogenesis of fatigue will lead to novel therapeutic interventions in the near future. This will be of significant benefit to patients.

OSTEOPOROSIS

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Osteoporosis is a frequent complication in patients with chronic cholestasis. In a series of 185 Spanish women with primary biliary cirrhosis (PBC), 37% had osteoporosis, and in a large US series, the prevalence was up to 20%. Advanced age, low body mass index and severity of the liver disease are the main risk factors for osteoporosis in patients with PBC. Mechanisms underlying osteoporosis in this liver disease are complex and poorly understood. In this setting, osteoporosis mainly results from low bone formation, related to the effects of retained substances of cholestasis, such as bilirubin and bile acids. Thus, in *in vitro studies*, unconjugated bilirubin and serum from jaundiced patients decrease osteoblast viability, differentiation and mineralization. Accordingly, bilirubin down-regulates RUNX2 (a master switch for osteoblast differentiation) gene expression. In addition, other mechanisms are involved in the low bone formation process. Indeed, high circulating sclerostin levels (an inhibitor of the Wnt pathway, which is involved in the regulation of osteoblastogenesis) have been found. Also, abnormal circulating levels of osteoprotegerin and the receptor-activator of NF-κB (RANKL), key osteoclastogenic cytokines, have been described in PBC and elevated circulating osteoprotegerin was associated with the severity of liver disease. In addition to the effects of retained substances of cholestasis on bone formation, lithocholic acid has a potential damaging effect on the vitamin D pathway. By contrast, ursodeoxycholic acid, which is the main treatment of cholestatic liver disease, neutralizes the damaging effects of bilirubin, lithocholic acid and sera from jaundiced patients on survival, differentiation and mineralization of osteoblastic cells. Furthermore, ursodeoxycholic acid counteracts the apoptotic consequences of bilirubin and lithocholic acid and therefore, it may have further beneficial effects on the decreased bone formation in cholestasis.

Increased bone resorption has also been described in cholestatic women with advanced disease and interestingly, jaundiced serum up-regulates RANKL/OPG gene expression ratio.

Other conditions in patients with chronic cholestasis, such as low vitamin D levels and poor nutrition in advanced stages, may be contributing factors to the full picture of bone disease.

RANDOMIZED TRIALS AND META-ANALYSES

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This talk focuses on the role of ursodeoxycholic acid (UDCA) for the treatment of primary biliary cirrhosis (PBC). The variety of randomized trials, the combined analysis of data sets and the several meta-analyses will be discussed. There have now been several randomized controlled trials of UDCA; the doses, the duration of treatment as well as the endpoints that were sought in the trials have all varied. This has led to some confusion into the role of UDCA therapy. Several of the initial randomized trials, particularly with extended evaluation, showed improvement in survival but because these were post-hoc analyses and not necessarily arrived at during the pre-specified duration of the study, they were questioned. A combination of data from three of the trials using a similar drug formulation with a dose of 13-15 mg/kg/day using clinical endpoints of death or liver transplantation was able to demonstrate that patients with PBC receiving UDCA at that dose had an improvement in life expectancy free of survival over the four years of the initial study intent. In fact, these data were used to gain U.S. Food and Drug Administration approval for legalized use of the drug for PBC in the United States. There were several subsequent meta-analyses and some were criticized for using studies of short duration (under two years) and several using inadequate doses. Subsequent studies have demonstrated that doses of 5-7 mg/kg/day were less effective in effecting biochemical improvement than higher doses. Doubling the doses from 13-15 to 25-28 mg/kg/day was not associated with improved biochemical effects. Furthermore the large datasets were able to help identify subsets of patients who could derive benefit from therapy. Initially, within four years, only patients with advanced disease had an improvement of life expectancy with UDCA therapy. Later analyses of longer term follow-up demonstrated that the proposal to only treat patients with advanced disease because of adherence of the findings from the combined analysis would have led to many unnecessary deaths if strategy of treating cirrhotic patients only was recommended. These data have eventually become much more widely accepted and serve as the basis for recommendations and practice guidelines from the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of the Liver (EASL) to use UDCA for the treatment of PBC in the dose of 13-15 mg/kg/day.

THE MODEL DRUG: UDCA

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The model drug: UDCA

Bile acids (BAs), or bile salts, are a family of steroids synthesized from cholesterol in the liver. Their primary functions traditionally include regulation of cholesterol homeostasis, its elimination in a soluble form, formation of canalicular and ductular bile, and solubilisation of dietary lipids and their intestinal absorption. BAs are now recognized as signalling molecules that regulate not only BAs synthesis, conjugation and transport but also lipid, glucose, energy and immune homeostasis through activation of FXR, TGR5, intracellular protein kinases, integrins and acid-sensing ion channels.

Therapy with natural BAs arose in the 1970s when it was discovered that oral administration of CDCA induced the dissolution of cholesterol gallstones. However, CDCA also induced biliary cirrhosis in some species, and was shown to be midly hepatoxic and to induce dose-dependent diarrhea in humans. Subsequently, UDCA was found to have similar efficacy in gallstone disease, but without side-effects. The markedly different behaviours of the two natural BAs was ascertained by numerous experimental studies *in vitro* and *in vivo*. Thus, UDCA was proposed as a potential therapeutic approach to chronic cholestatic disorders based on the following rationales: a) accumulation of toxic BAs might be at least partly responsible for liver injury in chronic cholestasis; and b) replacement of endogenous BAs by a non-toxic BA (UDCA) might protect the liver and retard the progression of these disorders. This hypothesis was first tested in primary biliary cirrhosis (PBC) in which UDCA was found to provide marked improvement in liver biochemistries. Later, placebo-controlled trials and long-term observational studies found that UDCA was associated with a slow-down progression of PBC towards liver failure. In primary sclerosing cholangitis, UDCA efficacy remained uncertain. A placebo-controlled trial using high doses of UDCA (30 mg/kg/day) showed that it was not only ineffective, but also harmful. Other clinical conditions in which UDCA therapy may be useful include cholestasis of pregnancy and *ABCB4/MDR3* deficiencies. An informal and subjective review of some aspects of UDCA's heritage will be discussed and highlighted with the hope to further speed up progress to tackle cholestatic liver diseases: BAs as toxicants, inflammagens, immunosupressors and mediators of the adaptative response to cholestasis; What is still unclear in UDCA biotransformation in health and disease; Lessons from past UDCA clinical trials in PBC;Not all PBC is the same; Traditional liver biochemistries turn to be surrogate markers of severity and outcome; Bicarbonate-rich choleresis and biliary innate immunity connection.

FXR VS PPAR AGONISTS: COMPETITORS OR FELLOW-COMBATANTS

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Our improved understanding of the molecular mechanisms of bile formation and cholestasis has opened new perspecitives for targeted therapies of cholangiopathies such as primary biliary cirrhosis (PBC). Nuclear receptors (NRs) are attractive targets for pharmacotherapy of cholestatic disorders, since their activation may orchestrate several key processes with beneficial effects counteracting or at least ameliorating cholestasis by reduction of hepatocellular retention of potentially toxic bile acids (BAs), protection of the bile duct epithelium from toxic bile, suppression of inflammation / bacterial translocation, reduction or even reversal of fibrosis and tumour prevention. The most relevant BA-activated NR for regulation of hepatobiliary homeostasis, bile secretion and thereby understanding and treating cholestasis, is the farnesoid X receptor (FXR, NR1H4). Furthermore other NRs such as fatty acid-activated peroxisome proliferator-activated receptors PPARs, in particular PPARα (NR1C1) and PPARγ (NR1C3) as regulators of inflammation, fibrosis and energy homeostasis, may also impact on biliary homeostasis and cholestatic liver injury. Due to their capability to broadly control hepatic BA metabolism, hepatic inflammation and fibrosis, NRs in general and BA-activated NRs in particular have emerged as promising therapeutic targets.

BA receptor/farnesoid X receptor (FXR) agonists (e.g., obeticholic acid (OCA), already successfully tested in PBC. Before entering clinical trails, the protective effects of FXR were demonstrated in several animal models. A non-BA synthetic FXR agonist GW4064 and a BA-derived 6α-ethyl derivative of CDCA derivative (OCA also known as 6-ECDCA or INT-747) had beneficial effects in mouse models of chemically-induced liver injury (ANIT and estradiol-induced) or in bile duct-ligation (BDL). A high affinity ligand for FXR (INT-767), but not INT-747/OCA, was able to cure bile duct injury and cholestatic liver injury in the Mdr2 (Abcb4)-/- cholangiopathy model. Interestingly, the therapeutic mechanisms not only involved suppression of BA synthesis, as well as anti-inflammatory and antifibrotic effects, but also induction of a bicarbonate-rich choleresis which may be a common denominator for successful treatment of cholangiopathies by reinforcing the bicarbonate umbrella. Combination therapy of UDCA with the OCA (INT-747) in phase II clinical trials in PBC patients not responding to UDCA showed substantial reduction of biochemical parameters of liver damage and cholestasis.

In line with the results obtained with combination therapy, OCA monotherapy in PBC patients also achieved a significant reduction of cholestasis. Dose dependent pruritus was the most common adverse event in patients receiving higher doses of OCA. A multicentre, placebo-controlled, randomized phase III clinical trial, testing lower doses of OCA in PBC patients who have not non-responded to standard UDCA has recently been completed and the results are eagerly awaited.

Fibrates have anti-cholestatic, anti-inflammatory, and anti-fibrotic effects in animal and in vitro studies. The mechanisms that underlie these effects are complementary, and largely mediated through activation of PPARα (and to a lesser degree of other PPAR isoforms). Fibrate treatment ameliorated liver biochemical tests in patients with suboptimal response to UDCA, either as mono-therapy or in combination with UDCA. These results, however, were obtained in smaller case series and pilot studies and the results of phase III studies are awaited. Recently available novel PPARα/δ ligands (so far only tested in diabetes and fatty liver) could also be of interest, but have not yet been tested in cholestatic conditions. In conclusion, we currently witness a revolution of expanding use of NR-targeting therapies in cholestatic liver diseases. The translation of expanding knowledge on NRs and novel insights into BA (patho)biology should result in optimization of the currently available therapies with careful selection of patients' subgroups benefiting from such novel targeted therapeutic approaches.

CORTICOSTEROIDS, ANTIVIRALS, OTHER AGENTS

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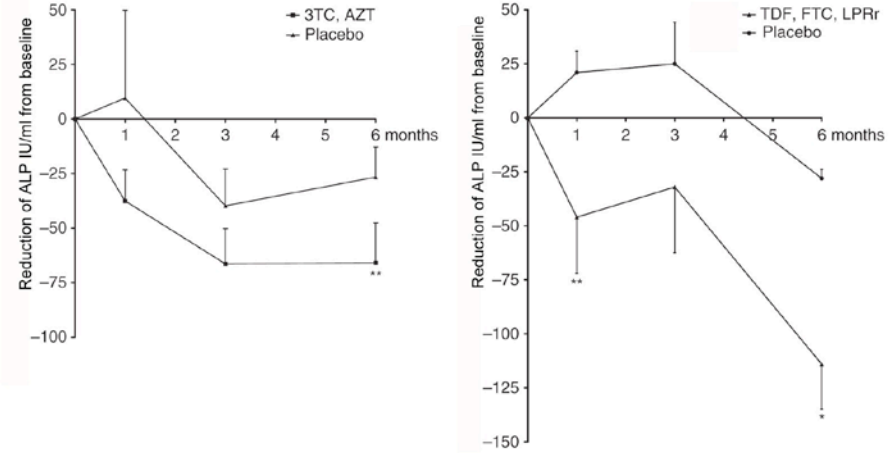
There is a need for further therapeutic intervention for PBC patients who have a suboptimal response to ursodeoxycholic acid, features of autoimmune hepatitis (AIH) and transplant recipients with recurrent PBC. The various adjunctive therapies under investigation include immunosuppressive regimens, anti-interleukin 12 therapy (Ustekinamab) to counterbalance the effects of genetic susceptibility, antiretroviral therapy to combat a putative betaretrovirus infection as well as FXR, PXR and PPAR agonists to augment choleresis and biliary phospholipid secretion. For example, randomized controlled trials using adjunct therapy with budesonide have demonstrated significant improvement in biochemical and histological features but its use in PBC patients has been limited because of concerns with risk versus benefit. Indeed, clinical improvement has been reported for many immunomodulatory agents including azathioprine, cyclosporine, colchicine, methotrexate and mycophenolate mofetil but none have been adopted due to toxicity or lack of efficacy. Given that PBC patients with overlap features of AIH experience progressive disease, there is consensus that this subgroup should be treated with adjuvant immunosuppressive therapy. It is paradoxical, however, that recurrent PBC following liver transplantation occurs earlier and is more severe for those maintained on tacrolimus, a more potent immunosuppressive agent than cyclosporine. Indeed, reports from multiple transplant centres have demonstrated the protective effect of cyclosporine against recurrent PBC and it is notable this calcineurin inhibitor also has demonstrable antiviral effects against many agents, including the human betaretrovirus, whereas tacrolimus does not. Our group continues to investigate the prevalence of a betaretrovirus related to mouse mammary tumour virus (MMTV) in patients with PBC as well as the use of anti-retroviral therapy. Recently, we reported the detection of proviral integration sites and retroviral RNA in the majority of PBC patients' biliary epithelium to justify this approach. Likewise, evidence of MMTV has been found in several spontaneous mouse models of PBC and linked with the development of the autoimmune phenotype. The NOD.c3c4 model has been especially useful for testing specific antiviral regimens because the mouse has cholangitis as result of MMTV. In this model, anti-retroviral therapy with tenofovir and emtricitabine (Truvada) and the HIV protease inhibitors lopinavir and ritonavir (Kaletra) resulted in lowering of betaretrovirus levels and abrogation of cholangitis.

Though some of the NOD.c3c4 mice developed viral resistance to less potent regimens of lamivudine and zidovudine (Combivir) in the absence of protease inhibitors. In pilot studies using antiretroviral therapy for PBC, we also noted biochemical rebound consistent with viral resistance to lamivudine and zidovudine. While only a few of the endpoints were observed, a significant reduction in hepatic biochemistry was seen with lamivudine and zidovudine treatment compared to control subjects (Figure 1). In a coded analysis of pre and post treatment biopsies, significant improvements were recorded in necroinflammatory activity, bile duct injury and the percentage of portal tracts containing bile ducts in liver biopsies. More recently, case studies have reported normalization of liver tests in PBC patients treated with the more potent combination anti-retroviral regimen of tenofovir and emtricitabine with lopinavir and ritonavir. Also, a preliminary analysis of a randomized controlled trial in PBC reported incremental biochemical improvement using this regimen as compared to lamivudine and zidovudine without a protease inhibitor (Figure 1). Further histological and virological studies will be required to determine whether this combined anti-retroviral regimen has any merit as an adjunct therapy for patients with PBC.

Figure 1:

Incremental improvement of hepatic biochemistry observed in PBC patients maintained on UDCA receiving combination antiretroviral therapy with a protease inhibitor. Patients treated with daily Lamivudine 150 mg (3TC) and Zidovudine 300 mg (AZT) developed a 66 IU/mL mean reduction in ALP (alkaline phosphatase) after 6 months (n=59), whereas those receiving daily Tenofovir/Emtricitabine 300/200 mg (TDF, FTC) and Lopinavir/ritonavir 800/200 mg (LPRr) for 6 months (n = 13) experienced a mean ALP reduction of 114 IU/mL [two-way ANOVA, * P < 0.001, ** P < 0.05].



PRIMARY BILIARY CIRRHOSIS – AUTOIMMUNE HEPATITIS OVERLAP SYNDROME

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PBC and AIH are classically viewed as distinct liver diseases. However, patients presenting with clinical, biochemical, serological, and/or histological features reminiscent of both diseases, either simultaneously or consecutively, have been repeatedly recognised. The term overlap syndrome is used to describe these settings. Unfortunately, lack of universal agreement on what precisely constitutes a PBC-AIH overlap syndrome (OS) has generated considerable confusion in the literature (1). The pathogenesis of PBC-AIH OS is debated and it remains unclear whether this syndrome forms a distinct entity or a variant of PBC or AIH. In this regard, the name overlap that strongly suggests the presence of 2 distinct diseases could be a misnomer but it has become very popular and has the advantage to draw physician's attention to particular features with clinical consequences.

Definition of PBC-AIH OS. The challenge remains that no autoimmune liver disease has an absolute diagnostic test, especially AIH. As a consequence, the clinical phenotypes of patients with the same OS designation exhibit considerable heterogeneity. The IAIHG criteria (either revised or simplified) are an attractive tool for making the diagnosis of OS in patients with an existing diagnosis of PBC. However, although widely used in this setting, the diagnosis performance of these criteria appears low and their use is not recommended for the diagnosis of OS (2). In 1998, we proposed criteria ("Paris criteria") for the diagnosis of PBC-AIH OS. In order to exclude simple "crossover" or "outlier" syndromes (one clear diagnosis while having one feature associated with another), presence of at least 2 of the 3 accepted key criteria was required for diagnosis of each disease (PBC: 1) alkaline phosphatase (AP) > 2 ULN or γ glutamyltranspeptidase (GGT) > 5 ULN, 2) presence of antimitochondrial antibodies (AMA), 3) a liver biopsy specimen showing florid bile duct lesions; AIH: 1) alanine aminotransferase (ALT) > 5 ULN, 2) serum immunoglobulin G (IgG) levels > 2 ULN or presence of smooth muscle antibodies (ASMA), 3) a liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis) (3). It is generally assumed that these criteria provide a diagnostic template that can be consistently applied and are currently the most commonly used tool for diagnosing PBC-AIH OS. The 1999 EASL guidelines endorsed these diagnostic criteria but specified that histologic evidence of moderate to severe lymphocytic piecemeal necrosis (interface hepatitis) was mandatory (4).

As a consequence, interface hepatitis is a fundamental component and histology is vital in evaluating patients with overlap presentation. Unfortunately, there are insufficiently reproducible ways to grade interface hepatitis, keeping in mind that mild interface hepatitis is likely a common mechanism of liver injury across autoimmune liver diseases.

Clinical presentation and prevalence. Whatever the criteria used, it appears that the simultaneous presence of features of both diseases is usual presentation although less commonly, the onset of AIH and PBC is temporally dissociated, usually with PBC presenting first. Interestingly, in most cases, it is possible to define one primary disorder ("dominant" disease), usually PBC (2). Indeed, in patients with a clinical presentation of AIH, biliary injury may be observed in 10-20% but biochemical cholestasis or presence of AMA is uncommon. In this regard, it has been proposed that OS represents an "hepatitic" form of PBC in genetically susceptible individuals (HLA-B8, DR3 or DR4 positive) (5). This would fit with the hypothesis that autoimmune disease can develop ("secondary" AIH) in any susceptible host if, for some reason, the local milieu becomes pro-inflammatory. However the jury is still out with regard to pathogenesis and a coincidence of two independent diseases or a representation of the middle a continuous spectrum between PBC and AIH have also been discussed. With no codified diagnostic approach, reported prevalence figures are variable but it is generally assumed that OS prevalence is approximately 8-10% in adult patients with PBC or AIH.

Natural history and treatment. Patients with OS have a more severe disease compared to conventional PBC as illustrated by a higher frequency of extensive fibrosis at presentation (despite a younger age in some reports) and most series support a worse outcome in terms of biochemical response to ursodeoxycholic acid (UDCA), progression of fibrosis and liver-related mortality (6, 7). Intensity of interface hepatitis is likely to play a key role in this faster progression. As a consequence, OS should always be considered once PBC has been diagnosed and in case of poor response to UDCA because of potential therapeutic implications (4). Despite the lack of controlled studies, common sense and small series (7, 8) strongly argue for adequate immunosuppression in these patients. EASL guidelines recommend adding steroids (eventually budesonide) either at the time of diagnosis of OS or in case of inadequate biochemical response after 3 months of UDCA (4). Again, OS should not be over-diagnosed in order not to expose unnecessarily PBC patients to the risk of steroid side effects. Interestingly, it has been suggested that doses of immunosuppressants could be lower and rate of successful withdrawal higher than in classical AIH (8). In UDCA treated PBC developing AIH ("sequential" OS), use of immunosuppressive treatment is mandatory.

The PBC-AIH OS is heterogeneous and ill-defined but it constitutes a clinical reality that must be accepted, refined, treated and studied (9). In this regard, international effort for collection of a large database and discovery of more specific molecular signatures with the ability to identify subgroups within the spectrum of autoimmune liver disease should be encouraged.

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NOTES

ADVANCES IN TREATMENT OF PRURITUS AND OSTEOPOROSIS

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Pruritus is one of the most frequent and unbearable manifestations in symptomatic PBC patients. Usually pruritus can be alleviated using cholestyramine or colestipol, two nonabsorbable basic polystyrenes which bind anions such as bile acids and other substances in the gut. Another potent bile acid sequestrant (colesevelam), which binds only bile acids, is not effective for cholestatic pruritus as reported in a placebo-controlled trial. The next step for the management of pruritus is rifampicin, a pregnane X receptor agonist and an enzyme-inducing antibiotic. This agent is widely used as second-line treatment and it has a dramatic effect resulting in a relief of pruritus in most cases. Moreover, it can be used safely for long-term periods. Other agents such as naltrexone and sertraline also reduce cholestatic itching, but the effects are less prominent and constant.

In patients with lack of response to the aforementioned treatments, albumin dialysis and plasmapheresis as well as biliary drainage can relieve pruritus. In a series of patients with resistant pruritus albumin dialysis resulted in a significant decrease of itching as assessed by a visual analogue scale. Compared with baseline, the visual analogue scale decreased by 72% immediately after treatment, and by 51% after 1 month. Pruritus diminished in all but one patient. Significant decreases in circulating bile acids, total bilirubin, cholesterol, and gamma-glutamyl-transferase were observed. Encouraging effects of fibrates on relieving pruritus have been reported as well. New agents addressed to block the ileal bile acid transport and, therefore the bile acid enterohepatic circulation, are also under investigation.

Osteoporosis resulting in a high risk of fracture is a common complication in patients with PBC. Its pathogenesis is poorly understood, but it mainly results from low bone formation. However, increased bone resorption has been described in women with advanced disease. For the prevention and treatment of osteoporosis good nutrition is recommended, as is the suppression of the risk factors for osteoporosis. Supplements of calcium and vitamin D, or the dose required to maintain normal levels should be provided. Although calcium and vitamin D supplement s are recommended, there are no data confirming the efficacy of these supplements in preventing bone loss in PBC.

There is no specific therapy for osteoporosis in PBC, but it has been demonstrated that different antiresorptive regimens of oral bisphosphonates are effective in increasing bone mass in these patients. In a recently published trial, monthly ibandronate and weekly alendronate increase bone mass and are well tolerated in postmenopausal women with osteoporosis or severe osteopenia, without impairment of liver function and cholestasis. The monthly regimen of ibandronate may be more convenient for patients who are taking multiple medications and certainly, adherence to therapy was higher for ibandronate than for alendronate. However, alendronate increased bone mineral density more consistently at the total hip. The two regimens induced a decrease in all markers of bone turnover, without significant differences in the magnitude of the change between treatment groups, although changes in the alendronate group were seen earlier than in the ibandronate group. An important issue with the use of oral bisphosphonates in patients with PBC is safety, since it is accepted that oral nitrogen-containing bisphosphonates may be associated with upper gastrointestinal side effects such as gastritis and esophagitis. This has precluded the use of oral bisphosphonates in patients who may have esophageal varices. Therefore, if the tolerability profile is poor, or a recent esophageal banding/sclerotherapy has been performed, other therapeutic options like parenteral bisphosphonates may be considered. Accordingly, parenteral bisphosphonates may stabilize bone mineral density without serious adverse events.

LIVER TRANSPLANTATION AND DISEASE RECURRENCE

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1. Primary biliary cirrhosis (PBC) is a complex disorder and recent genome-wide association studies have identified numerous risk loci for PBC which host genes involved in innate or acquired immune responses.
2. There is no curative treatment and some progress to end-stage liver disease requiring liver transplantation (LT).
3. Despite the apparent increase in the prevalence of PBC, both the proportion and numbers of patients undergoing transplantation for PBC is falling
4. Indications are as for other chronic liver disease and transplantation should be considered either when patients have a life expectancy of less than1 year (from end-stage disease or otherwise untreatable liver cell cancer) or intractable symptoms, such as intractable itching or encephalopathy.
5. Outcome after LT is excellent with current patient and graft survival exceeding 80% at 5 years.
6. While LT is effective in treating itching, lethargy improves but does not disappear so transplantation is not indicated for lethargy alone.
7. Recurrent disease is well described. rPBC can be detected by histology: AMA persist irrespective of recurrence.
8. rPBC occurs in about 45% at 5 years although reported rates vary. rPBC results in graft failure in fewer than 5% grafts.
9. Risk factors for rPBC include both host genetic factors and choice of calcineurin inhibitor.

Liver transplantation for PBC

Despite the rise in the reported prevalence of PBC, the absolute numbers and proportion of patients transplanted for PBC is falling. Reasons for this decline are not clear. Indications for LT in those with PBC are as for other chronic liver disease: that is end stage disease as characterised by:

- Progressive jaundice (especially when serum bilirubin exceeds 100umol/L)
- End stage disease such as shown by MELD >16, UKELD >49
- Complications as intractable ascites or progressive encephalopathy
- Liver cell cancer inappropriate for other therapeutic modalities and within local guidelines
- Symptomatic disease: as intractable pruritus, encephalopathy or ascites

Fatigue in PBC is often severe and disabling. Cross-sectional studies have shown no evidence of improved fatigue after LT. A prospective study showed fatigue improves after LT; however, but 44% had moderate to severe fatigue two years after LT suggesting fatigue alone is not an indication for LT Contraindications for LT are few and as for other indications. Outcomes: after LT for PBC are excellent with 10 year survival rates exceeding 80%.

Recurrence of PBC after Liver Transplantation: Despite (or because of?) PBC recurs in the majority of recipients and may be more aggressive than the original disease. The reported prevalence rates of rPBC range 0-35% and reported incidence rate is 21-37% at 10 years and 43% at 15 years and median reported time between 3 and 5.5 years. The reported recurrence frequency rate increases with time but varies in part because of different diagnostic criteria as well as variation in the use of protocol biopsies. Current data suggest UDCA does not influence patient and graft survival.
Graft loss due to recurrent disease is less common in PBC (graft lost from recurrent disease: 1% PBC; 6 AIH; and 8% PSC). .
The diagnosis of rPBC can be confidently made only on histological criteria since liver tests are non-specific and AMA persist irrespective of evidence of graft histology. Tacrolimus-based immunosuppression is associated with an increased risk and earlier recurrence of rPBC compared with ciclosporin. The HLA profile and HLA donor-recipient mismatch have controversial association in rPBC. There is an association between rPBC and a non-HLA locus (rs62270414) in position 3q25 which hosts the IL12A gene and there is an additive effect between this SNP and the choice of calcineurin-inhibitor at one year on the risk of rPBC (greatest with Tacrolimus at one year and rs62270414 genotype AG or GG, and least with Cyclosporin at 1-year and rs62270414 genotype AA.

Rejection after Liver Transplantation in Autoimmune Liver Disease
The reported incidence of ACR after LT shows significant variation between centres and over time: this is partially related to evolving immunosuppressive strategies, different policies on protocol biopsies and to a lesser extent, discrepancies in the diagnostic criteria for rejection but studies suggest patients transplanted for PBC have higher risk of ACR and LAR OR 2.1, compared to those with HCV. A pre-LT diagnosis of PBC and a young recipient age were the only independent predictors of LAR in the Cox logistic regression model.

De-Novo Autoimmune Hepatitis after Liver Transplantation
The clinical manifestations of dn-AIH are similar to those of recurrent AIH including a prominent plasma cell infiltrate with interface hepatitis, hypergammaglobulinaemia, increased serum IgG levels, and autoantibodies and has been described after LT for PBC

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NOTES

HEPATOCELLULAR CARCINOMA AND EXTRAHEPATIC MALIGNANCIES

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PBC is characterized by a long natural history influenced by a number of factors that modulate mortality risk, including: clinical signs/symptoms of liver disease, associated autoimmune conditions, comorbidity with associated non-autoimmune conditions (i.e. osteoporosis), and response to ursodeoxycholic acid (UDCA). Judging from a recent meta-analysis focusing on PBC and cancer risk that included approximately 16,300 PBC patients from several countries, PBC is closely associated with a greater overall risk of malignancy, and especially HCC, but not other extrahepatic cancers. Five studies in this meta-analysis reported a relationship between HCC and histological stage of PBC, and they all clearly indicated that HCC arises in advanced histological stages of PBC. This behaviour is commonly observed in Western countries for all types of liver disease with a viral and non-viral etiology, with only very few exceptions. The meta-analysis also confirmed our previous finding of a relative risk for HCC in female patients with stage IV PBC similar to that of female patients with cirrhosis resulting from other etiologies.

Few studies report statistical analyses on the risk factors associated with the onset of HCC. The most interesting finding concerns its association with male gender and with failure to respond to UDCA (although these data should be considered with caution). In our opinion, it is remarkable that UDCA may favourably influence the natural history of PBC in responders. A recent risk-factor analysis was conducted by the "Global PBC Study Group", involving 15 centres across North America and Europe, and spanning more than 40 years of follow-up for 3546 PBC patients, 131 of whom developed HCC. HCC risk stratification by 12-month response to UDCA (Rotterdam, Paris-I, or Toronto criteria) showed a significantly higher HCC incidence at 5 and 10 years in non-responders.

Screening for HCC with cross-sectional imaging, with or without alpha-fetoprotein at 6-month intervals, should be recommended for PBC patients with advanced disease (histological stage IV or Mayo prognostic score >4.1), or evidence of portal hypertension. The incidence/prevalence, risk factors and survival for extrahepatic malignancies (EM) was recently analysed for 753 PBC patients from two European centres (Padova in Italy and Barcelona in Spain).

The prevalence was similar in the two cities (9.7% in Padova and 9.4% in Barcelona). The overall cancer incidence was similar to the expected incidence in the general population of the same geographical areas. Logistic regression analysis showed that advanced histological stage and extrahepatic autoimmune diseases were significantly associated with the onset of EM. Survival was similar in those with and without EM, however, and actual survival was comparable with the one predicted by the Mayo model. Given the lack of any evidence-based association with other extra-hepatic liver malignancies, there is no reason to submit PBC patients to more intensive cancer screening than the general population.

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POSTER
ABSTRACTS

Poster P1 - YI

ENHANCED STRATIFICATION OF HEPATOCELLULAR CARCINOMA RISK IN PRIMARY BILIARY CIRRHOSIS: AN INTERNATIONAL COLLABORATIVE STUDY

Palak J. Trivedi [1, 2,*], Willem Lammers [3], Henk van Buuren [3], Harry Janssen [4, 3], Pietro Invernizzi [5], Pier M. Battezzati [6], Annarosa Floreani [7], Albert Pares [8], Cyriel Ponsioen [9], Christophe Corpechot [10], Raoul Poupon [10], Marlyn Mayo [11], Jayant Talwalkar [12], Andrew Burroughs [13], Frederik Nevens [14], Andrew Mason [15], Tony Bruns [16, 1], Ka-Kit Li [1, 2], Kris Kowdley [17], Marjolijn Leeman [3], Teru Kumagi [18, 4], Angela Cheung [4], Ana Lleo [5], Nora Cazzagon [7], Irene Franceschet [7], Llorenç Caballeria [8], Kirsten Boonstra [9], Liesbeth de Vries [9], Mohamad Imam [12], Giulia Pieri [13], Pushpjeet Kanwar [17], Keith Lindor [12, 19], Bettina Hansen [3], Gideon Hirschfield [1, 2] and Global PBC Study Group

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Introduction: Hepatocellular carcinoma (HCC) is an infrequent but poor prognostic development for patients with primary biliary cirrhosis (PBC). A better evaluation of risk factors is needed to help facilitate a more stratified approach to surveillance.

Aim: Identify candidate risk factors for HCC development across a multicentre international registry.

Methodology: Risk-factor analysis was performed across 15 centres from North America and Europe spanning >40-years follow-up using Cox proportional hazards assumptions, logistic regression and Kaplan-Meier estimates (SPSSv22).

Results: Of 4588 patients with PBC (median follow-up 7.1yrs; IQR 3.5-11.5), 146 developed HCC. Excluding those who developed HCC within 12 months of PBC diagnosis (n=23), median time to HCC was 7.3yrs (3.2–11.8). HCC was significantly more common in men (6% vs. 2%; $P=3.0x10^{-7}$), and on univariate analyses factors at diagnosis associated with future HCC development were male gender (HR:2.91; $P=1.0x10^{-6}$), serum AST (HR:1.24; $P=1.2x10^{-5}$), Rotterdam criteria for advanced biochemical disease (HR:9.00; $P=1.4x10^{-10}$), thrombocytopenia (HR:1.01; $P=7.0x10^{-12}$) and hepatic decompensation (HR:9.89; $P<4.0x10^{-18}$). Multivariate analysis (stepwise-backward model) restricted baseline association to male gender (HR:5.41; $P<0.01$), thrombocytopenia (HR:1.01; $P=0.01$) and serum albumin (HR:0.002; $P<0.001$). Use of ursodeoxycholic acid (UDCA) itself was not associated with future risk of HCC; however, at 12 months stratification by biochemical non-response was effective through Rotterdam (adj. HR:4.35; $P=1.6x10^{-4}$), Paris-I (HR:3.36; $P=0.001$) or Toronto criteria (HR:2.00; $P<0.05$). Five (4% vs. 0.2%) and 10-year (11% vs. 2%) HCC incidence was significantly increased for biochemical non-responders (Paris-I; $P=1.2x10^{-10}$), and by multivariate analysis non-response remained the most significant risk factor. Indeed, biochemical non-response predicted future risk of HCC development in patients with early stage disease (HR:3.60; $P<0.01$), moderate/advanced disease (HR:2.87; $P=0.02$), and when restricting the analysis to only male patients (HR:3.62; $P<0.05$).

Conclusions: Using a uniquely powered, internationally representative, cohort has allowed robust demonstration that 12-month biochemical non-response is associated with an increased risk of developing HCC in PBC. In the pursuit of stratified and personalised care, this can be used to inform surveillance strategies for patients.

Poster P2 - YI

SPECIFICALLY EXPRESSED MIRNA IN CD4+ T CELLS PARTICIPATES IN THE PATHOGENESIS OF PRIMARY BILIARY CIRRHOSIS

Ryo Nakagawa [1, 2,*], Hiroki Takahashi [1], Ryosuke Muroyama [2], Wenwen Li [2], Kaku Goto [2], Chisato Saeki [1], Yasuo Matsubara [2], Naoya Kato [2], Mikio Zeniya [1] and Department of Gastroenterology and Hepatology, The Jikei University School of Medicine

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Introduction: Primary biliary cirrhosis (PBC) is a chronic inflammatory autoimmune liver disease. Although detailed mechanisms of the pathogenesis of PBC remain unknown, $CD4^+$ T cells are suggested to play an important role. Recently microRNA (miRNA) was reported to be involved in the pathogenesis of PBC.

Aim: We analyzed the expression profile of miRNA and their target genes in $CD4^+$ T cells of PBC patients to reveal their participation in pathogenesis of PBC.

Methodology: Clinically and pathologically diagnosed 7 PBC patients and 7 healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. Total RNA, including miRNA, was extracted from $CD4^+$ T cells purified from peripheral blood. The comprehensive analysis of miRNA was undergone using microarray and quantitative real-time PCR (qRT-PCR). We predicted the target genes of miRNA, which was expressed specifically in PBC, using bioinformatics. The dynamics of predicted target genes were analyzed by microarray and qRT-PCR. Then, luciferase assay and miRNA mimic assay were performed to examine the binding of the specific miRNA to 3´untranslated region (3´UTR) of target genes. Finally, we tested the potential role of specifically expressed miRNA against target genes by overexpressing miRNA in cultured cells.

Results: Microarray miRNA study showed 2 increased and 13 decreased miRNAs in PBC ($p<0.05$). Among them, 5 miRNAs were validated to be down-regulated in PBC ($p<0.05$) by qRT-PCR. A total of 4,855 target genes were predicted from 5 miRNAs by bioinformatics. In the mRNA microarray analysis, the expression of 2,565 genes was significantly different between PBC and control.

Comparison of the target prediction and gene expression microarray study revealed 238 target genes were specifically expressed in PBC. Among 238 target genes, we analyzed 10 genes that were the target for more than 3 specifically expressed miRNAs to reveal the regulation of the target genes by miRNAs. The expression of 3 target genes, which were reported to be associated with T cell development and function, were validated by qRT-PCR. Luciferase assay and miRNA overexpression assay demonstrated that the specific miRNAs regulate these target genes by binding to their 3´UTR.

Conclusions: We have identified PBC specific expression of miRNA and their target genes. These miRNA may participate in the immunological pathogenesis of PBC through the regulation of the target genes in $CD4^+$T cells.

Poster P3 - YI

YOUNGER PATIENTS PRESENTING WITH PBC ARE MORE LIKELY TO HAVE COGNITIVE IMPAIRMENT

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Introduction: Data from the UK-PBC cohort have shown that patients presenting with PBC at a younger age have a greater symptom burden, particularly with regard to fatigue and autonomic dysfunction. Patients with PBC also report memory and concentration problems and previous studies have demonstrated that overt cognitive dysfunction is prevalent in this group yet unrelated to the severity of liver disease. The mechanism underpinning cognitive dysfunction in PBC is not fully understood. Studies have shown evidence of structural brain changes which may be secondary to cholestasis and there may also be associations with the autonomic dysfunction which is prevalent in PBC.

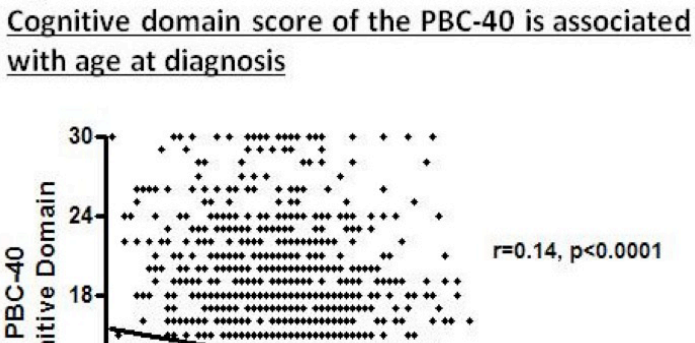
Aim: The aim of this study was to evaluate the prevalence of cognitive impairment in the UK-PBC patient cohort and identify relevant associations which may guide further research.

Methodology: We analysed data from the UK-PBC dataset which contains symptom information on over 2000 patients in the UK. The symptom data used in this observational study includes the cognitive domain of the PBC-40, a self –reported, disease-specific quality of life measure.

Results: Data on 2187 patients from the UK-PBC database were analysed. 27% of PBC patients had clinically significant cognitive dysfunction (defined using an evidence-based clinical cut-off for the cognitive domain of the PBC-40, derived from control populations). Patients without evidence of advanced liver disease (normal bilirubin and albumin) actually had a higher prevalence of clinically significant cognitive impairment (37%) than the group as a whole. Cognitive dysfunction was significantly associated with both a younger age at diagnosis and a younger age at entry into the study ($p<0.0001$). The <50 age group had significantly more cognitive dysfunction than the >60 age group ($p<0.0001$). Cognitive dysfunction was not associated with duration of disease or response to ursodeoxycholic acid therapy.

Conclusions: Cognitive dysfunction is surprisingly frequent in PBC and, contrary to expectation, it is alarming to see that it is significantly more common in patients presenting at a younger age. This argues strongly against the thought that the high prevalence of cognitive dysfunction seen in PBC may simply be a manifestation of advancing age or hepatic encephalopathy due to advanced liver disease. We believe that cognitive dysfunction is an under-recognised, unappreciated symptom in this patient group and the results highlight an important area for further research.

Figure:



Poster P4

FREQUENCY OF CHOLELITHIASIS IN DIFFUSE LIVER DISEASES WITH CHOLESTASIS

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Introduction: Cholelithiasis occurs more commonly in patients with chronic liver disease (CLD). We aimed to evaluate the prevalence of cholelithiasis in CLD patients and to define whether the presence of cholelithiasis is associated with cholestasis.

Aim: To study the incidence of cholelithiasis in diffuse liver diseases, according to cholestasis in Tajikistan.

Methodology: 1307 patients (544 men, 763 women, at the age of 17-70 years) with liver disease were examined. Chronic viral hepatitis (CH) was diagnosed in 332, liver cirrhosis (LC) in 540, primary biliary cirrhosis (PBC) in 23, nonalcoholic fatty liver disease (NAFLD) in 246, Gilbert's syndrome in 160, Dubin-Johnson syndrome in 6 patients. Cholelithiasis was diagnosed by ultrasound. The functional state of the gallbladder was evaluated according to sonography and fractional duodenal sounding of bile.

Results: Cholelithiasis is revealed in 312 cases of the total number of patients with liver disease, which is 5-8 times higher than in the general population. Cholelithiasis was often found in patients with primary biliary cirrhosis (in 47.8% of cases). In CH and LC without cholestasis gallstones in the gallbladder diagnosed in 11.8 and 23.4% of patients, in CH and LC with cholestasis in 21.4% and 37.5%, respectively. More than 60% of patients with LC and PBC revealed hypokinesia of the gallbladder. Cholelithiasis was diagnosed in 30.5% of patients with NAFLD, in 19.4% of patients with Gilbert's syndrome and in 16.7% of patients with Dubin-Johnson syndrome. Most patients with functional hyperbilirubinemia had large stones (diameters ≥15 mm), occurring often asymptomatically.

Conclusions: Gallstones are most common in patients with chronic diffuse liver disease with cholestasis. CH and LC are not only often associated with cholelithiasis, but can also be the cause.

Poster P5

DNA METHYLATION PROFILING OF THE X CHROMOSOME IN PRIMARY BILIARY CIRRHOSIS

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Introduction: Although the etiology of PBC remains enigmatic, there are several pieces of data supporting a strong genetic predisposition and environmental factors interact to produce a selective loss of tolerance. Moreover, there is a striking female predominance and recent data suggesting that this sex predisposition is secondary to epigenetic alterations on the X chromosome.

Aim: The goal of the present study was to rigorously define the X chromosome methylation profile of CD4, CD8, and CD14 cells from PBC patients and controls using a genome-wide approach, involving patients with PBC and healthy controls.

Methodology: Each subject provided peripheral blood mononuclear cells followed by purification of CD4, CD8, and CD14 subpopulations. Thence, genomic DNA was isolated, sonicated, and immunoprecipitated for analysis of methylation. All products were hybridized to a custom tiled 4-plex array containing 27,728 CpG Islands annotated by UCSC and 22,532 well-characterized RefSeq promoter regions. Furthermore, bisulfite sequencing was then used for validation on a subsequent group of independent samples from PBC patients and controls.

Results: We report herein a striking demethylation of CXCR3 in CD4 T cells from patients with PBC in addition to hypermethylation of UBE2A and FUNDC2 in CD8 T cells.

Conclusions: These data, including additional epigenetic results reported, reflects an intense genome-wide study of DNA methylation profiling of the X chromosome in lymphoid subpopulations. In conclusion, our data, particularly on the DNA demethylation of CXCR3, further emphasizes a potential role of CXCR3 in the pathogenesis of autoimmune cholangitis.

Poster P6 - YI

EVALUATION OF THE CONCEPT OF BIOCHEMICAL RESPONSE IN UDCA TREATED PATIENTS WITH PRIMARY BILIARY CIRRHOSIS. A DUTCH MULTICENTER STUDY

Wim J. Lammers [1,*], Marjolijn Leeman [1], Cyriel Y. Ponsioen [2], Kirsten Boonstra [2], Karel J. van Erpecum [3], Frank H. Wolfhagen [4], Johan P. Kuyvenhoven [5], Jan M. Vrolijk [6], Joost P. Drenth [7], Ellen M. Witteman [8], Carin M. van Nieuwkerk [9], Bas W. van der Spek [10], Ben J. Witteman [11], G. W. Erkelens [12], Mark A. Verhagen [13], S. A. van Tuyl [13], A. C. Poen [14], J. T. Brouwer [15], Frank ter Borg [16], Ger H. Koek [17], Theo J. van Ditzhuijsen [18], Bettina E. Hansen [1], Henk R. van Buuren [1] and Dutch Multicenter PBC Study Group

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Introduction: Biochemical response (BR) to ursodeoxycholic acid (UDCA) enables risk stratification in patients with primary biliary cirrhosis (PBC).

Aim: We aimed to evaluate to what extent BR influenced patient management and whether this changed over time.

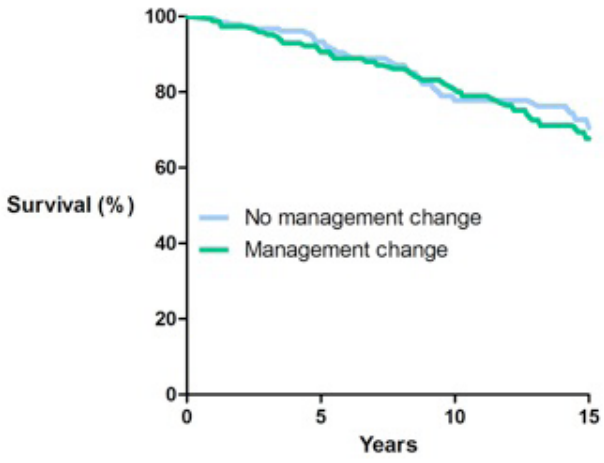
Methodology: Analysis of retro- and prospectively collected data comprising >40 general and university Dutch hospitals. Extensive efforts including site visits and detailed medical

chart review were undertaken to assess patient management in relation to BR. BR was defined using Barcelona, Paris 1&2, Rotterdam and Toronto criteria.

Results: Of 851 UDCA-treated patients (94% AMA+, 87% female, mean age: 54±12, median follow-up 8.9 (IQR 4.7-14.1) 466 (55%) were classified as non-responders according to ≥1 response criterion. 345/466 (74%) non-responders could be evaluated with respect to subsequent management and clinical course.
10-year transplant-free survival for responders versus non-responders was 89% vs 76% (p<0.0001). The UDCA dosage was increased and/or corticosteroid treatment was instituted in 81/345 (23%) non-responders. 58/345 (17%) were referred to specialized centers. Medical management was not altered in 206 (60%) of the non-responders. Survival for non-responders with or without management change was comparable (p=0.90, Figure1).
The proportion of non-responding patients in whom management was changed did not increase after the year 2006, when the concept of biochemical response was introduced (46% versus 34%, p=0.07).

Conclusions: BR to UDCA did affect medical management in a minority of cases. No evidence was found for growing impact of BR as a clinical tool in patient management in general practice in recent years. Awareness of the concept of biochemical response evaluation in PBC management should be increased.

Figure:



Poster P7

GENETIC INSIGHTS INTO PRIMARY BILIARY CIRRHOSIS – AN INTERNATIONAL COLLABORATIVE META-ANALYSIS AND REPLICATION STUDY

Heather J. Cordell [1], Younghun Han [2], Yafang Li [2], George F. Mells [3,*], Gideon M. Hirschfield [4], Gang Xie [5], Brian Juran [6], M. Eric Gershwin [7], Pietro Invernizzi [8], Michael F. Seldin [9], Kostantinos N. Lazaridis [6], Christopher I. Amos [2], Richard N. Sandford [3], Katherine A. Siminovitch [5] and the Canadian-US PBC Consortium, Italian PBC Consortium, and UK-PBC Consortium

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Introduction: Primary biliary cirrhosis (PBC) results from an interaction of genetic and environmental factors. To date, four genome-wide association studies (GWAS) and two Illumina® Immunoarray studies of PBC have helped delineate the genetic architecture of this disease. These studies have confirmed associations at the human leukocyte antigen (HLA)-region and identified 27 non-HLA susceptibility loci. Candidate genes are notably involved in the IL-12 signalling cascade.

Aim: To identify additional risk loci for PBC.

Methodology: We have undertaken genome-wide meta-analysis (GWMA) of discovery datasets from the North American, Italian and UK GWAS of PBC, respectively, with a combined, post-QC sample size of 2,745 cases and 9,802 controls. Genome-wide imputation of each discovery dataset was undertaken in MACH using HapMap3 as the reference panel; GWMA was undertaken using ProbABEL and PLINK. Following meta-analysis, the index single nucleotide polymorphisms (SNPs) at loci with $P_{GWMA}<1\times10^{-5}$ were genotyped in a validation cohort consisting of 3,716 cases and 4,261 controls.

To prioritise candidate variants and genes at confirmed risk loci, we used the ENCODE and the 1000Genomes datasets to identify SNPs within regulatory elements and non-synonymous (ns) SNPs in strong linkage disequilibrium (LD) with the index variant (r^2>0.8).

Results: We identified nine previously unknown risk loci for PBC. Functional annotation of these loci revealed one nsSNP in *CTSH* (15q24) and SNPs within regulatory elements that are predicted to affect expression of *DGKQ* (4p16), *PAM* (5q14), *CTSH* (15q24) and *IL21R* (16p12), that are strongly-correlated to the index variant. Other candidate genes include *IL12B* (5q31), which forms part of the IL-12 signalling cascade, and *CCL20* (2q36), which is involved in chemo-attraction of lymphocytes and dendritic cells towards epithelia and is expressed by T_H17 cells originating from $Foxp3^+$ T cells.

Conclusions: This uniquely powered international collaborative GWMA and replication study confirms additional immunologically relevant loci that are associated with the risk of developing PBC.

Table:

Table 1: Newly-identified risk loci for primary biliary cirrhosis

Chr	SNP (A1/A2)	Discovery P	Validation P	Joint P	OR (95% CI)	Candidate gene/s (number in region)
2q12	rs12712133 (A/G)	8.10×10^{-6}	7.90×10^{-5}	2.60×10^{-9}	1.14 (1.01-1.20)	IL1R1,IL18R1 (7)
2q36	rs4973341 (C/T)	2.30×10^{-7}	7.70×10^{-5}	8.50×10^{-11}	0.83 (0.79-0.88)	CCL20 (3)
4p16	rs11724804 (A/G)	1.30×10^{-7}	4.30×10^{-6}	3.30×10^{-12}	1.19 (1.14-1.26)	DGKQ (6)
5q14	rs526231 (T/C)	1.40×10^{-5}	9.40×10^{-5}	5.30×10^{-9}	0.87 (0.83-0.91)	PAM (4)
5q31	rs2546890 (G/A)	6.20×10^{-7}	1.90×10^{-5}	5.50×10^{-11}	0.86 (0.83-0.90)	IL12B (5)
6q23	rs6933404 (C/T)	4.10×10^{-7}	2.80×10^{-5}	5.50×10^{-11}	1.19 (1.13-1.26)	(2)
8q24	rs1529335 (A/G)	4.80×10^{-7}	5.60×10^{-3}	3.70×10^{-8}	0.84 (0.79-0.89)	PVT1,TMEM75 (3)
15q24	rs1369324 (T/C)	1.80×10^{-6}	2.60×10^{-3}	3.30×10^{-8}	0.87 (0.83-0.92)	CTSH (7)
16p12	rs1859308 (T/C)	4.40×10^{-5}	5.40×10^{-4}	9.50×10^{-8}	0.83 (0.78-0.89)	IL4R,IL21R (2)

Risk loci for primary biliary cirrhosis (PBC) identified by genome-wide meta-analysis (GWMA) of discovery and validation cohorts from the North American, Italian and UK genome-wide association studies of PBC, respectively. Chr, chromosome; SNP, single nucleotide polymorphism; A1, minor allele; A2, major allele; OR, odds ratio; CI, confidence intervals.

Poster P8

BASAL AND STIMULATED URINARY COPPER EXCRETION IN PBC PATIENTS DURING UDCA THERAPY

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Introduction: Urinary copper excretion (UCE) is increased in patients with cholestasis and cirrhosis. Hepatic copper concentrations in primary biliary cirrhosis (PBC) correlate with UCE and liver disease severity. D-penicillamine (D-PCA) can increase UCE and decrease aminotransferases, like in pts. with Wilson's disease. Ursodeoxycholic acid (UDCA) prolonged the time to liver transplantation or death. Data regarding basal and stimulated UCE in PBC during UDCA are limited

Aim: The aim of our study was to assess basal and D-PCA stimulated UCE in PBC patients on UDCA and to compare the same parameters in patients with alcoholic liver cirrhosis (ALC).

Methodology: 6 pts. with PBC who received UDCA 15 mg/kg/d and 10 subjects with ALC were studied. The median age of both groups was 60 (43-70) and 57 (31-73) years, respectively. All subjects were tested for 24-h UCE - basal and stimulated by single intake of 1g D-PCA.

Results: An increased basal UCE >0.64 mcmol/24h was found in 3/6 of PBC pts vs. 7/10 of subjects with ALC. Median basal UCD in both groups was 0.635 (0.32-2.1) mcmol/24h and 0.835 (0.46-2.4) mcmol/24h, respectively. Paired t-test indicated a significant increase of UCE after stimulation with D-PCA in both groups ($p<0.005$). Median stimulated UCE was higher in PBC patients than in ALC: 11.1 (5-16) mcmol/24h vs. 5.3 (0.81-14) mcmol/24h, respectively. One PBC pt. was tested prior and during UDCA. Both basal and stimulated UCE were higher before initiation of UDCA.

Conclusions: Basal UCE was abnormal in 62% of studied pts. After stimulation UCE increased both in PBC and ALC patients, but median value was two times higher in PBC patients suggesting persistence of copper retention even during standard UDCA therapy. This could enhance progression of liver fibrosis. One can speculate that some PBC patients may benefit from long-term D-penicilamine therapy added to UDCA. Further studies are needed to confirm or reject this hypothesis.

Poster P9 - YI

PRIMARY BILIARY CIRRHOSIS – AUTOIMMUNE HEPATITIS OVERLAP SYNDROME: CHARACTERISTICS AND THERAPEUTIC OPTIONS

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Introduction: Introduction: Primary biliary cirrhosis – autoimmune hepatitis overlap syndrome is poorly defined, without universal agreement on the diagnostic criteria. Weather this overlap syndrome requires immunosuppressive therapy additional to ursodeocycholic acid is a controversial issue.

Aim: Prevalence, characteristics of the overlap syndrome of primary biliary cirrhosis – autoimmune hepatitis and therapeutic options.

Methodology: The study was performed on 43 patients with primary biliary cirrhosis, the overlap syndrome being diagnosed using the criteria from the European Association for the Study of the Liver (EASL) guidelines for the management of cholestatic liver disease (2009).

Results: Distribution of the cases was: 69.76% primary biliary cirrhosis (30 patients), 23.25% overlap syndrome of primary biliary cirrhosis – autoimmune hepatitis (10 patients), 6.97% primary biliary cirrhosis and viral hepatitis, one of them also having Wilson disease (3 patients). Associated diseases were represented by: diabetes mellitus, asthma, autoimmune thyroiditis. The overlap syndrome of autoimmune hepatitis/primary biliary cirrhosis was treated with ursodeoxycholic acid in combination with immunosuppressant drugs (corticotherapy, azathioprine, mycophenolate mofetil) with very encouraging results.

Conclusions: The overlap syndrome of primary biliary cirrhosis – autoimmune hepatitis and the therapeutic options in this context are not well established worldwide. In this subgroup of patients the overlap of primary biliary cirrhosis – autoimmune hepatitis was predominant. Overlap syndromes respond favorable to immunosuppressive therapy in combination with ursodeoxycholic acid. Further prospective studies are necessary to establish a clear diagnostic and therapeutic consensus.

Poster P10 - YI

EXTRAHEPATIC AUTOIMMUNITY ASSOCIATED WITH PRIMARY BILIARY CIRRHOSIS (PBC).

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Introduction: There are limited information about extrahepatic autoimmunity (EAI) associated with PBC and on the impact of EAI on PBC survival.

Aim: To analyze the association between PBC and other autoimmune diseases and the impact of EAI on the natural history.

Methodology: 361 consecutive PBC patients between 1973 and 2013 were considered (22 males, 339 females; mean age 67.7±14.1 yrs, mean age at diagnosis 53.1±12.4 yrs); the presence of other EAI and extrahepatic malignancies (EM), histological stage at diagnosis, biochemical data, and physiological history were recorded. Survival analysis was performed using the Kaplan-Meyer model.

Results: The mean follow-up was 8±6.9 yrs; 140 patients (38.8%) had no EAI, 221 patients had at least one associated EAI; among these, 123 patients (34.1%) had one associated EAI, 70 patients (19.4%) 2, 23 patients (6.4%) 3, 4 patients (1.1%) 4, 1 patient (0.3%) 1. Hashimoto's thyroiditis was present in 45 patients (20.4%), Graves' thyroiditis in 7 (3.2%), Raynaud's syndrome in 65 (29.4%), Sjogren's syndrome in 124 (56.1%), systemic lupus erythematosus in 8 (3.6%), scleroderma in 22 (9.9%), rheumatoid arthritis in 22 (9.9%), cutaneus autoimmune diseases in 18 (8.1%), vasculitis in 8 (3.6%), celiac disease in 5 (1.4%), other EAI in 29 (13.1%). The proportion of patients with associated EAI for representative periods (1973-1980, 1981-1990, 1991-2000, 2001-2010, 2011-2013) remained stable (62.5%, 58.9%, 60.3%, 64.4%, 56.4%, respectively). No differences were observed between patients with or without EAI in terms of mean age at diagnosis (52.9±13.2 vs 53.2±11.8 yrs, p=n.s.), AMA positivity (83.6% vs 80.5%, p=n.s.), ANA positivity (43.6% vs 48.0%, p=n.s.), histological stage at diagnosis, smoking habits (23.6% vs 17.2%, p=n.s.), alcohol consumption (17.9% vs 19.0%, p=n.s.) and BMI>25 (29.3% vs 31.2%, p=n.s.).

There were significantly more females among PBC patients with associated EAI than without EAI (97.3% vs 88.6%, p=0.0017). The presence of at least one EAI significantly increased the risk of development of EM (OR 3.4, 95% CI: 1.2-9.4, p<0.02). The mean survival from diagnosis of PBC was similar in patients with or without EAI (281.4 vs 334.8 months respectively, p=n.s.).

Conclusions: EAI is often associated with PBC, especially in female patients. The presence of associated EAI increases significantly the risk of EM development, but does not reduce the survival.

Poster P11 - YI

SPECIFIC AUTOANTIBODIES FOR PRIMARY BILIARY CIRRHOSIS DO NOT PREDICT URSODEOXYCHOLIC ACID RESPONSE

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Introduction: 20-40% of patients with Primary Biliary Cirrhosis (PBC) will not respond to standard treatment with ursodeoxycholic acid (UDCA). This represents a management challenge to hepatologists. Antimitochondrial antibody (AMA) status does not appear to predict treatment response. Newer, more specific, but less sensitive, antibodies exist to aid in diagnosis of PBC and may affect long term clinical outcome.

Aim: The aim of this study was to determine if positivity for PBC specific autoantibodies predicts UDCA response.

Methodology: A database of all patients with PBC exists at John Radcliffe Hospital. Data collected includes patient demographics, treatment with UDCA (13-15mg/kg), liver enzymes, and antibody status for anti-sp100 (sp100), anti-glycoprotein210 (gp210), and anti-M2 (as determined by immunoblot assay [EuroLINE Profile for Autoimmune liver diseases] *See figure 1*). UDCA response according to both Barcelona and Paris II criteria were compared between groups positive (+ve) for each antibody with the negative group (-ve). Patients with concomitant liver conditions (including overlap), who did not receive UDCA, or for whom one year data was not available were excluded from analysis. Chi squared test was used for statistical comparisons.

Results: Of 148 patients with PBC on the database, sp100/gp210 status was available for 105 patients. 21/105 (20.0%) patients were gp210 +ve, 27/105 (25.7%) were sp100 +ve, and 18/105 (17.1%) were AMA -ve. Of those who were AMA -ve, 9/18 (50%) were positive for one of sp100, gp210, or anti-M2.

71 patients met inclusion and exclusion criteria for analysis with regards to UDCA response. The median age at diagnosis was 54y (29-73).

Of those who were gp210 +ve (19.7%), 35.7% had a primary response to UDCA according to Barcelona criteria, vs. 56.1% of those who were gp210 -ve, but this was not statistically significant (p=0.235). A similar proportion of responders was found according to Paris II criteria (42.9% of gp210 +ve, versus 61.4% of gp210 -ve, p=0.239).

25.3% were sp100 +ve, but there was no statistically significant difference in responders according to sp100 status either – Barcelona response: 38.9% (sp100 +ve) vs. 56.6% (sp100 -ve), p=0.257; Paris II response: 55.6% (sp100 +ve) vs. 58.5% (sp100 -ve), p=1.000.

Conclusions: Positivity for sp100, gp210, or anti-M2 was not associated with UDCA response at one year in patients with PBC. It may, however, act as an adjunctive diagnostic tool in AMA negative patients.

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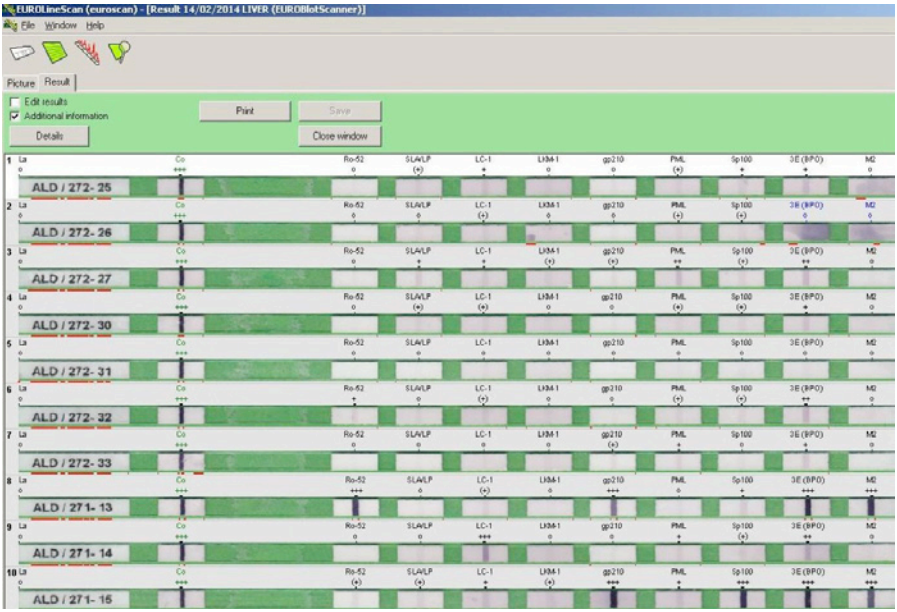
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NOTES

Poster P12 - YI

SERUM LEVELS OF CHOLESTEROL PRECURSOR AND PLANT STEROLS INDICATE DISTORTED CHOLESTEROL HOMEOSTASIS IN CIRRHOTIC PBC PATIENTS

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Introduction: In humans new cholesterol derives from *de novo* synthesis and intestinal absorption. Serum cholesterol precursor (e.g., lathosterol, desmosterol) and plant sterol levels (e.g., sitosterol, campesterol) represent valid surrogate marker for cholesterol biosynthesis and intestinal absorption, respectively.

Aim: Since chronic liver diseases modulate cholesterol homeostasis, we systematically investigated sterol serum levels in patients with primary biliary cirrhosis (PBC) with and without liver cirrhosis.

Methodology: Overall, we recruited 111 non-transplanted PBC patients (age 22 - 83 years, 101 females). In this cohort, a total of 30 individuals presented with liver cirrhosis at diagnosis. Serum levels of plant sterols, cholesterol and its precursors were measured by gas chromatography/mass spectrometry (GC/MS). Individuals with results suggesting familial hypercholesterolaemia or hyperphytosterolemia were excluded from subsequent analyses. Serum sterol levels were compared between cirrhotic and non-cirrhotic patients with non-parametric tests.

Results: PBC patients with liver cirrhosis demonstrated significantly higher sitosterol and campesterol levels than the non-cirrhotic individuals. (P = 0.0002 and P = 0.0067, respectively). Serum levels of lathosterol and desmosterol were lower in these patients (P = 0.0001 and P = 0.013, respectively), and they displayed a trend to lower serum cholesterol levels (P = 0.064). In cirrhotic patients we identified increased sitosterol:cholesterol and campesterol:cholesterol but decreased lathosterol:cholesterol ratios (all P < 0.0001). Overall, the ratios of phytosterols to cholesterol precursors were significantly (all P > 0.0001) increased in patients with liver cirrhosis as compared to non-cirrhotic individuals.

Conclusions: PBC patients with liver cirrhosis are characterized by decreased cholesterol synthesis and increased sterol absorption as compared to non-cirrhotic individuals. Determination of serum sterols may improve clinical assessment of patients with PBC. Further studies are however required to investigate the association between liver injury in PBC and cholesterol homeostasis.

Poster P13 - YI

PATIENT EXPERIENCE OF PRURITUS IN THE UK-PBC RESEARCH COHORT

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Introduction: Pruritus is a common problem in cholestatic liver diseases such as Primary Biliary Cirrhosis (PBC). Pruritus has negative impact on patient quality of life. There are limited studies on patient reported experience of cholestatic itch and its treatment.

Aim: To utilise the data from the UK-PBC Research Cohort: 1) to report the prevalence and severity of pruritus in patients with primary biliary cirrhosis (PBC), 2) to describe patient reported information on their experience of itch and anti-pruritic therapy they had received.

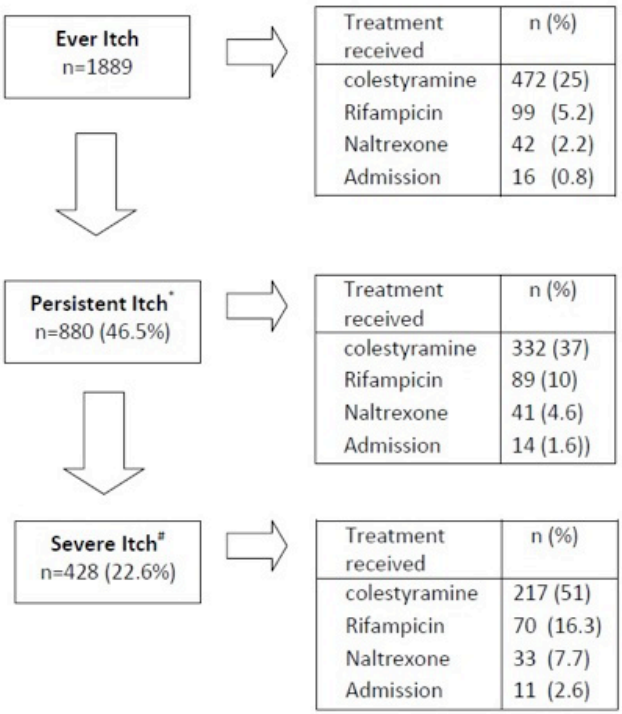
Methodology: This was an observational cross-sectional study of PBC patients recruited into the UK-PBC Research Cohort in which pruritus has been characterised as follows: 1) Frequency of itch (never, rarely, occasionally, frequently, all the time); 2) Experience of itch since diagnosis based on the PBC-40 itch domain; 3) Intensity of worst itch since diagnosis and in the last 7 days, measured using a visual analogue scale (VAS) and a 0-10 grading scale (GS); 4) Treatment for itch since diagnosis of PBC. We defined **persistent itch** as pruritus occurring 'frequently' or 'all the time', and **severe itch** as persistent itch combined with PBC-40 itch score ≥10.

Results: Data were available for 2705 PBC patients without a liver transplant. 1889 patients (69.8%) had experienced itch at some point in their illness. Of these, 880 (46.5%) had persistent itch. Figure 1 summarises main results. In those with persistent itch: intensity since diagnosis was ≥5 on VAS & GS in 766 (87%) patients; intensity in the last 7 days was ≥5 on VAS & GS in 478 (54.3%) patients and 428 (48.6%) patients had severe itch.These figures were closely correlated.

Patients with severe itch had received the following treatments: colestyramine (217, 51%), rifampicin (70, 16.3%) and naltrexone (33, 7.7%). 11 (2.6%) patients had been admitted to hospital for treatment of severe itching. Notably, 193 (45%) patients with severe pruritus reported no anti-pruritic treatment at all.

Conclusions: Our results highlight the prevalence of pruritus in PBC. In the UK-PBC cohort, approximately one-half of all PBC patients with itch had persistent itch and one-quarter had severe itch. However, it would seem that treatment of itch was unsatisfactory as many patients with severe pruritus did not receive anti-pruritic therapy. Our results also suggest need for improvement in the awareness and management of itch in PBC.

Figure 1: Results of patient experience and treatment of pruritus in UK-PBC Cohort.



Treatment received	n (%)
colestyramine	472 (25)
Rifampicin	99 (5.2)
Naltrexone	42 (2.2)
Admission	16 (0.8)

Treatment received	n (%)
colestyramine	332 (37)
Rifampicin	89 (10)
Naltrexone	41 (4.6)
Admission	14 (1.6))

Treatment received	n (%)
colestyramine	217 (51)
Rifampicin	70 (16.3)
Naltrexone	33 (7.7)
Admission	11 (2.6)

*Persistent itch: those who reported itch frequently or all the time since development of PBC
#Severe itch: persistent itch with a PBC-40 itch domain score of ≥10

Poster P14

LONG-TERM TREATMENT OF PRIMARY BILIARY CIRRHOSIS WITH THE FXR AGONIST OBETICHOLIC ACID SHOWS DURABLE EFFICACY

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Introduction: Obeticholic acid (OCA), 6-ethyl chenodeoxycholic acid (CDCA), is derived from CDCA and shows ~100x greater farnesoid X receptor (FXR) agonist activity than CDCA, the natural human ligand. OCA is being developed for the treatment of primary biliary cirrhosis (PBC). In a double blind (DB), placebo controlled study OCA 10mg and 50mg, given as monotherapy, showed highly significant reductions in alkaline phosphatase (ALP) and other biochemical analytes, compared with placebo. Pruritus was the main adverse event (AE) in the DB phase (placebo: 30%, OCA 10mg: 70%, 50mg: 94%; Kowdley, J. Hepatology 2011;54:S13). An open label, long-term study extension followed.

Aim: To evaluate the efficacy and safety associated with long-term OCA treatment

Methodology: During the ongoing extension, subjects initiated OCA at 10mg once daily and titrated to 50mg based on response. Ursodeoxycholic acid (UDCA) was added in 11 subjects.

Results: Subjects (N=28): mean age 58yrs; female: 78%; Caucasian: 96%. Baseline: ALP: 442±275U/L; bilirubin: 4.6±3.2mmol/L; GGT: 460±318U/L; ALT: 91±61U/L; AST: 72±39 U/L. Median(IQR) exposure was 3.2(2.6-3.6yrs); 9 subjects terminated early. Changes in liver chemistry are shown in the table. Pruritus was the most common AE (89%). Normalized exposure to pruritus (days/year of OCA) was comparable between the first year and overall but severity tended to decrease with continued treatment.

Conclusions: PBC is a rare, chronic cholestatic liver disease with persistent significant unmet need. In this study long-term OCA treatment maintained a durable improvement in ALP and other liver chemistry. Pruritus, while prevalent appeared to diminish in severity with continued therapy.

Table:

	ALP	GGT	ALT	AST	Bilirubin
% Δ at 2y					
All Subjects (n=23)	-31% (9)*	-48% (10)*	-38% (8)*	-22% (8)*	-17% (7)*
No UDCA use (n=15)	-29% (11)*	-39% (14)*	-26% (11)*	-11(9)*	-7% (9)
% Δ at 3y					
All Subjects (n=15)	-32% (12)*	-48% (11)*	-41% (8)*	-27% (8)*	-17% (10)
No UDCA use (n=10)	-33% (14)*	-46% (12)*	-41% (9)*	-22% (9)*	-8% (12)
Data are mean (SE). *p<0.05 change from baseline					

Poster P15

THE FXR AGONIST OBETICHOLIC ACID IMPROVES ALKALINE PHOSPATASE/BILIRUBIN RESPONSE CRITERION ASSOCIATED WITH TRANSPLANT-FREE SURVIVAL IN PRIMARY BILIARY CIRRHOSIS

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Introduction: Obeticholic acid (OCA), a potent farnesoid-X receptor agonist, is being developed for the treatment of Primary Biliary Cirrhosis (PBC). The low prevalence and slow progression of PBC makes clinical outcome (liver transplant or death) studies difficult. Two independent retrospective observational database studies (Global PBC and UK PBC Study Groups) confirmed PBC patients with ALP > 1.67x ULN or bilirubin > ULN have an increased risk of liver transplant or death [HR (95% CI): 3.53 (2.4-5.3); p < 1x10-10 and HR (95% CI): 4.77 (2.4-9.6); p = 1x10-5 for Global PBC and UK PBC Study Groups, respectively]. These biochemical response criteria (ALP <1.67x ULN, ≥15% reduction and bilirubin ≤ ULN) have been studied as the primary endpoint in the Phase 3 PBC OCA International Study of Efficacy (POISE).

Aim: The aim of this retrospective pooled analysis was to evaluate the POISE response criteria overall and across patient subgroups based on two controlled Phase 2 PBC OCA trials.

Methodology: Two 12-week, double-blind, placebo-controlled Phase 2 trials evaluated the safety and efficacy of OCA in PBC patients with persistently high ALP (≥1.5 -10x ULN) and bilirubin < 2x ULN, ±UDCA (n=224). All OCA doses showed highly significant decreases in ALP and bilirubin versus placebo. OCA was generally well-tolerated; dose-related pruritus, generally mild, was common. In the present analysis, subjects (overall and stratified by UDCA use, age at time of diagnosis, gender, and BMI) above the POISE entry criteria were retrospectively analyzed for response (n=189).

Results: Patient demographics were similar across treatment groups overall (mean age = 55 years; female [> 90%]; White [> 95%]). Significantly more OCA-treated patients met the POISE response criteria versus placebo, overall and in both the OCA monotherapy and UDCA-background therapy studies. Consistent trends were observed across the patient subgroups analyzed.

Conclusions: OCA treatment resulted in a meaningful improvement in a biochemical response criteria shown to be associated with transplant-free survival across a broad range of patient baseline characteristics supporting its potential utility in the treatment of PBC.

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Table: POISE PBC Response Criteria (ALP <1.67x ULN & ≥15% reduction, and bilirubin ≤ ULN) at End of Study

	Treatment				
	Placebo	10 mg OCA	25 mg OCA	50 mg OCA	Total OCA
	n % (95% CI) p value	n % (95% CI) p value	n % (95% CI) p value	n % (95% CI) p value	n % (95% CI) p value
POISE PBC Response Criteria†					
Pooled Overall (n=187)	53 8 (2-18)	46 41 (27-57) p < 0.0001	39 44 (28-60) p < 0.0001	49 43 (29-59) p < 0.0001	134 43 (34-52) p < 0.0001
Monotherapy (n=51)	21 5 (0-24)	16 44 (20-70) p < 0.02	NA	14 50 (23-77) p < 0.004	30 47 (28-66) p < 0.002
In combination with UDCA (n=136)	32 9 (2-25)	30 40 (23-59) p < 0.004	39 44 (28-60) p < 0.001	35 40 (24-58) p < 0.003	104 41 (32-51) p < 0.0003
Age < 50 years at Diagnosis (n=114)	31 10 (2-26)	32 34 (19-53) p < 0.05	16 31 (11-59) p = NS	35 43 (27-62) p < 0.01	83 37 (27-49) p < 0.01
Age ≥ 50 years at Diagnosis (n=73)	22 5 (0-23)	14 57 (29-82) p < 0.01	23 52 (31-73) p < 0.001	14 43 (18-71) p < 0.01	51 51 (37-65) p < 0.002
Female (n=174)	49 8 (2-20)	42 40 (26-57) p < 0.001	36 42 (26-59) p < 0.001	47 40 (27-57) p < 0.001	125 41 (25-39) p < 0.0001
Male (n=13)	4 0 (0-60)	4 50 (7-93) p = NS	3 67 (9-99) p = NS	2 100 (16-100) p = NS	9 67 (30-93) p = NS
BMI < 30 kg/m2 (n=134)	33 9 (2-24)	35 37 (21-55) p < 0.01	25 48 (28-69) p < 0.002	41 49 (34-66) p < 0.0005	101 45 (35-55) p < 0.0002
BMI ≥ 30 kg/m2 (n=53)	20 5 (0-25)	11 55 (23-83) p < 0.004	14 36 (13-65) p = NS	8 13 (0-53) p = NS	33 36 (20-55) p < 0.02

†ALP < 1.67x ULN and ≥ 15% reduction and total bilirubin ≤ ULN
P values based on pairwise comparisons (vs placebo) - Fisher's Exact Test were exploratory

NOTES

Poster P16

CLINICAL OUTCOMES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS TREATED WITH THE FXR AGONIST OBETICHOLIC ACID: RETROSPECTIVE ANALYSIS OF POOLED CLINICAL DATA STRATIFIED BY GENDER AND AGE AT DIAGNOSIS

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Introduction: Obeticholic acid (OCA), a derivative of chenodeoxycholic acid and potent farnesoid-X receptor agonist, is being developed for the treatment of Primary Biliary Cirrhosis (PBC). A variety of factors can influence clinical endpoints of drug therapies. Carbone et. al. (2013) reported that both gender and age at presentation are important determinants of the response to UDCA therapy in patients with PBC.

Aim: The purpose of this post hoc pooled analysis was to examine the efficacy and safety of OCA stratified by gender and age at diagnosis in patients with PBC.

Methodology: Two 12-week, double-blind, placebo-controlled Phase 2 trials evaluated the safety and efficacy of OCA in PBC patients with persistently high ALP (≥1.5 -10x ULN) and bilirubin < 2x ULN, ±UDCA (n=224). All OCA doses showed highly significant decreases in ALP and bilirubin versus placebo. OCA was generally well-tolerated; dose-related pruritus, generally mild, was common. Pooled efficacy and safety data were retrospectively evaluated based on age at diagnosis of PBC (<50 years or ≤50 years) and gender.

Results: See Table for results. Patients treated with OCA experienced improvements in liver biochemistry across patient subgroups. OCA was generally well-tolerated; increased HDL was observed with no meaningful change in LDL.

Conclusions: This post-hoc pooled analysis suggests that neither gender nor age at diagnosis of PBC are important determinants of response to OCA therapy in patients with PBC.

Table:

	Age at Diagnosis				Gender			
	<50 years		>/= 50 years		Male		Female	
	Placebo (n=35)	OCA 10 mg (n=39)	Placebo (n=26)	OCA 10 mg (n=19)	Placebo (n=5)	OCA 10 mg (n=6)	Placebo (n=56)	OCA 10 mg (n=52)
Baseline ALP Δ (%)	328.3 (27.5) -0.8 (2.7)	360.0 (35.9) -30.8 (3.5) **p < 0.0001**	321.6 (36.0) -2.3 (2.0)	336.0 (53.3) -30.3 (5.5) **p < 0.0001**	364.3 (120.7) 4.7 (3.5)	307.4 (47.0) -44.0 (9.0) **p = 0.001**	322.0 (21.6) -2.1 (1.9)	357.2 (32.5) -29.1 (3.1) **p < 0.0001**
Baseline GGT Δ (%)	246.5 (30.8) 2.1 (4.7)	372.3 (53.4) -50.9 (5.3) **p < 0.0001**	357.0 (65.2) 4.7 (4.7)	378.5 (83.6) -67.6 (3.0) **p < 0.0001**	595.4 (201.7) 9.5 (4.3)	587 (127.2) -76.0 (5.7) **p < 0.0001**	266.7 (30.0) 2.6 (3.6)	349.8 (46.9) -54.2 (4.1) **p < 0.0001**
Baseline ALT Δ (%)	59.7 (6.9) 3.7 (6.4)	72.0 (6.8) -30.0 (5.3) **p < 0.0002**	60.8 (10.1) -8.7 (6.7)	45.7 (4.7) -33.8 (5.5) **p < 0.01**	121.3 (41.0) 6.0 (31.4)	71.7 (15.5) -39.3 (14.6) **p = NS**	54.7 (4.7) -2.1 (4.4)	62.5 (5.4) -30.3 (4.1) **p < 0.0001**
Baseline AST Δ (%)	53.8 (5.6) 1.7 (4.2)	60.4 (4.8) -18.0 (4.4) **p < 0.01**	51.6 (5.8) -9.8 (4.6)	43.1 (3.7) -15.4 (6.0) **p = NS**	81.3 (20.3) -2.6 (20.2)	51.5 (10.4) -22.2 (12.0) **p = NS**	50.3 (3.9) -3.1 (3.1)	55.2 (3.9) -16.4 (3.7) **p < 0.01**
Baseline Bilirubin Δ (%)	12.2 (1.2) 13.1 (5.3)	14.2 (1.4) -8.3 (5.3) **p < 0.01**	10.8 (0.9) 2.1 (4.0)	12.9 (1.2) -11.7 (5.9) **p = NS**	15.6 (3.1) -4.4 (9.1)	12.9 (3.1) -8.2 (17.7) **p = NS**	11.3 (0.8) 9.7 (3.8)	13.9 (1.1) -9.6 (4.0) **p < 0.001**
Baseline HDL Δ (%)	1.8 (0.1) 4.3 (2.3)	1.7 (0.1) -16.1 (3.2) **p < 0.0001**	1.8 (0.1) 1.7 (3.2)	1.7 (0.1) -13.1 (4.6) **p < 0.01**	1.6 (0.1) 4.2 (3.5)	1.4 (0.1) -5.4 (9.7) **p = NS**	1.8 (0.1) 3.1 (2.0)	1.8 (0.1) -16.3 (2.7) **p < 0.0001**
Baseline LDL Δ (%)	3.4 (0.2) 0.3 (2.5)	3.4 (0.2) 8.4 (3.5) **p = NS**	3.6 (0.2) 3.1 (2.0)	3.5 (0.2) 5.5 (3.8) **p = NS**	3.6 (0.5) -3.0 (5.1)	3.1 (0.2) 2.1 (5.8) **p = NS**	3.5 (0.1) 1.8 (1.8)	3.5 (0.1) 8.1 (2.8) **p = NS**

Baseline and Δ values are mean (SE).
P values based on pairwise comparisons (vs placebo) - Fisher's Exact Test were exploratory

Poster P17 - YI

PRIMARY BILIARY CIRRHOSIS AND AUTOIMMUNE HEPATITIS: AN AUTOIMMUNE ENIGMA IN 2 CASES

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Introduction: Autoimmune cholangitis (AIC) is a rare constellation of clinical, biochemical, serological and histological characteristics shared with Primary Biliary Cirrhosis (PBC) and Autoimmune Hepatitis (AIH).

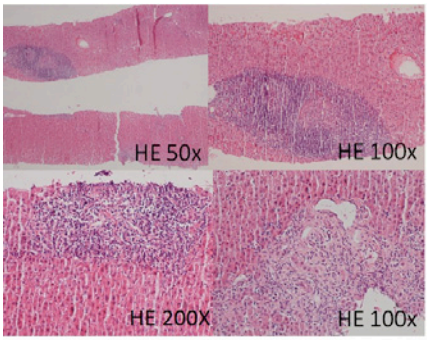
Aim: To present the variability of manifestations of AIC

Methodology: We analized the epidemiological, clinical, analytical, ultrasonographic, histological and therapeutic data from the AIC patients followed in our department.

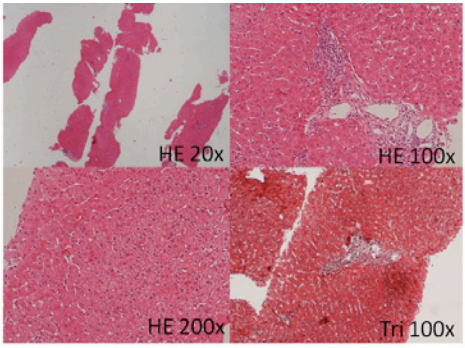
Results: We describe 2 female patients, both in their fourth-fifth decade, previously healthy, asymptomatic in one case, the other reporting intense pruritus and fatigue. Their laboratory tests showed marked increase in alkaline phosphatase (155-523U/L) and gammaGT levels (299-339U/l) and elevated serum levels of liver aminotransferases (117-198U/L), cholesterol (211-268mg/dL) and immunoglobulin M (3,35-3,71g/L). Abdominal ecography showed no focal lesions or biliary tract dilatation. Antinuclear antibodies were strongly positive, while antimitochondrial and anti-smooth-muscle antibodies were negative. Iron and cooper studies and markers for hepatitis B and C came all negative. Liver biopsy was compatible with primary autoimmune cholangitis, revealing significant lesions of the bile ducts. Combination therapy with immunosuppressive and choleuretic agents was given, with liver tests showing a rapid normalization in the first case. The patient with pruritus showed, however, clinical and biochemical refractoriness to multiples therapies and is now referred to hepatic transplantation.

Conclusions: These patients can be considered as presenting the clinical extremes of what is known as the AIC, an enigmatic entity, still evolving in its etiopathogenesis and comprehension. Some agree it is a variant of PBC (AMA-negative PBC), while others authors consider AIC as a result of transitional stage of AIH and PBC or even a separate entity with varying manifestations. The diagnostic differentiation from those two diseases it is though important to tailor treatment strategies. Our cases thus highlight the importance of the clinician awareness of the autoimmune spectrum of biliary pathologies.

Figure:



patient 1



patient 2

Poster P18

NGM282 IS A POTENT MODULATOR OF BILE ACID SYNTHESIS IN HUMANS VIA SUPPRESSION OF CYP7A1 ACTIVITY

Stephen Rossi [1,*], Michael Elliott [1], Stephenson Nkinin [2], Kenneth D. Setchell [2], Janakan Krishnarajah [3], Jian Luo [1], Lei Ling [1], Krishna Allamneni [1], Hui Tian [1], Alex DePaoli [1]
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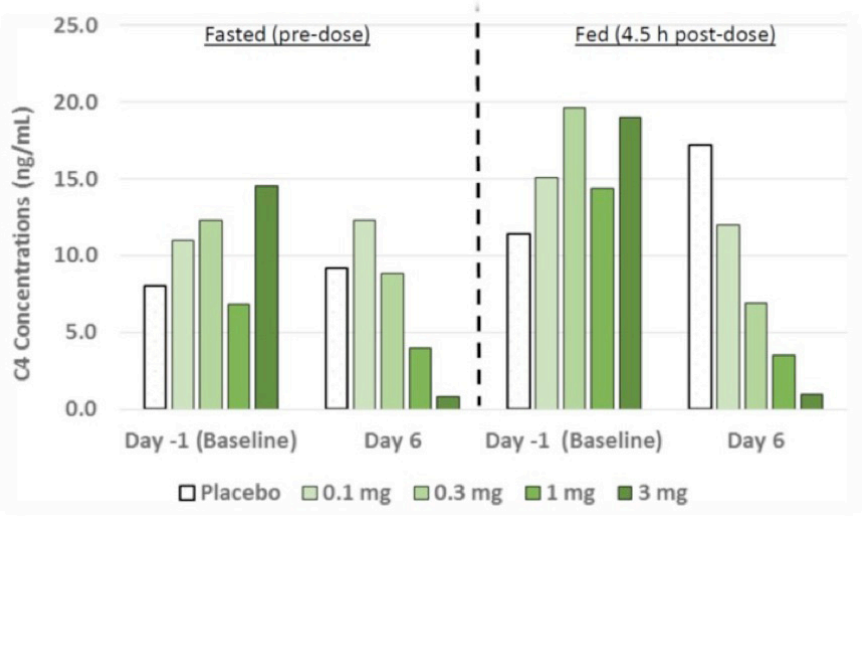
Introduction: NGM282 is an engineered recombinant protein variant of the ileal hormone human fibroblast growth factor 19 (FGF19) which down-regulates the classical pathway of bile acid (BA) synthesis by specifically suppressing hepatic CYP7a1. In-vitro and in-vivo data demonstrate a potent reduction in BA synthesis-with NGM282 while eliminating the abnormal proliferative activity of FGF19 seen in mice. Primate animal models have shown that both FGF19 and NGM282 decrease serum concentrations of 7a-hydroxy-4-cholesten-3-one (C4), a surrogate biomarker for BA synthesis. The direct activity of NGM282 on BA synthesis in humans was evaluated by measuring serum C4 concentrations in a Phase 1 clinical study in healthy volunteers.

Methodology: NGM282 was dosed daily in the morning (AM) over 6 consecutive days at 0.1, 0.3, 1 and 3 mg vs placebo. Serum C4 concentrations were measured off drug at Baseline (Day -1) pre-meal (fasted) then again at 4.5 hours post-meal (fed). Measurements were repeated at the same time points and feeding conditions at Day 6 before and after NGM282 dosing. Serial NGM282 levels were collected for pharmacokinetic (PK) calculations and evaluation of the relationship to pharmacodynamic markers. Quantification of C4 was performed using liquid chromatography electrospray ionization tandem mass spectrometry with stable-isotope dilution analysis.

Results: NGM282 significantly decreased serum C4 concentrations in a dose-dependent manner from Baseline to Day 6 in both fasted and fed states at the 0.3, 1 and 3 mg dose (Figure 1). Median % change from Baseline in C4 ranged from a 28-95% decrease when fasted and a 64-95 % decrease when fed vs a 16-51% increase with placebo. The magnitude of change was consistent with those observed in primates. Suppression of C4 was achieved even in the post-prandial setting where baseline C4 concentrations were higher. The dose-dependent decreases in C4 was consistent with the observed dose proportional PK of NGM282. Maximal biologic activity was seen in all subjects dosed with 3 mg (fasted or fed) where as "no effect" dose was at 0.1 mg.

Conclusions: Administration of NGM282 resulted in a rapid and potent suppression of C4 in healthy human subjects, reflective of decreased BA synthesis via the classical pathway. These data support the potential therapeutic activity of NGM282 in BA-related cholestatic disorders. An exploratory study is currently underway in patients with primary biliary cirrhosis.

Figure:



Poster P19 - YI

PROTECTIVE ROLE OF AZATHIOPRINE ON RECURRENCE OF PRIMARY BILIARY CIRRHOSIS AFTER LIVER TRANSPLANTATION.

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Introduction: Patients with primary biliary cirrhosis (PBC) can develop recurrent disease (rPBC) after liver transplantation (LT). It has been suggested that tacrolimus rather than cyclosporine is associated with rPBC. These reports did not evaluate the role of azathioprine.

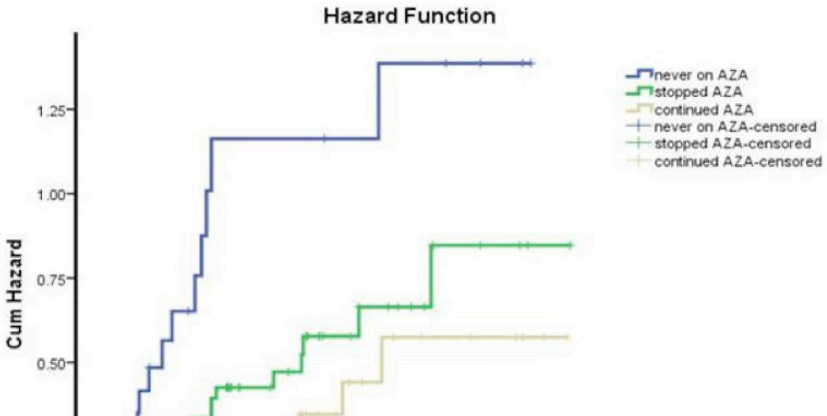
Aim: Aim of this study was to evaluate the impact of azathioprine on rPBC.

Methodology: We retrospectively evaluated 154 consecutive patients who underwent LT for PBC (median follow-up 100 (1-285) months) with protocol biopsies (n=356), scheduled at 1, 3, 5, 10, and 15 years post-LT and additional biopsies when clinically indicated (i.e. abnormal cholestatic markers indicating rPBC in absence of any other cause). Cox regression was used to evaluate factors associated with rPBC: MELD score pre-LT, recipient/donor age and gender, cold/warm ischemia time, HLA mismatches, rejection episodes and treatment, CMV infection and immunosuppression (initial/maintenance).

Results: rPBC occurred in 50 (32%) patients at a median time of 75.8 (8-200) months post-LT. Cyclosporine was used in 55 (22 monotherapy) and tacrolimus in 93 (26 monotherapy) patients. 154 were started on steroids and 28 were maintained > 6 months post-LT. Mycophenolate mofetil was used in 44 patients. Azathioprine (1 mg/Kg) was used in 120 (43 with cyclosporine, 77 with tacrolimus): 61 discontinued at a median of 19.1m. Histologically confirmed rPBC was independently associated with non-use/discontinuation of azathioprine (p=0.001, OR=3.6, 95%CI=1.7-7.6) and mantainance of steroids (>6 m) (p=0.024, OR=0.49, 95%CI=0.3-0.9). rPBC occurred at a median of 71m, 58m and 40m in patients who were treated, discontinued or never used azathioprine respectively.

Conclusions: In our study, treatment with azathioprine was independently protective against rPBC after LT. Larger numbers are needed to show if the combination with cyclosporine or tacrolimus results in further benefit.

Figure:



Poster P20 - YI

CHEMOKINE RECEPTOR 5 (CCR5) DELETION POLYMORPHISM IN NORTH INDIAN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS (PBC)

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Introduction: CC-chemokine receptor 5 (CCR5) polymorphisms have been studied as genetic marker of primary biliary cirrhosis (PBC). CCR5 is critical in regulating T cell functions by mediating recruitment, polarization, activation and differentiation of type 1 cytokine secreting T helper and cytotoxic T cells.

Aim: Data on association of the CCR5 deletion variant in etiology of PBC are conflicting. In the present study we tested genetic association between CCR5 Delta32polymorphism and PBC among North Indians.

Methodology: CCR5Delta32 polymorphism was determined by PCR in 192 PBC patients and 200 matched controls from North India. Genotype/allele frequencies were compared in patients and controls using the chi-square test.

Results: The frequency of heterozygosity of CCR5 Delta32 was higher in primary biliary cirrhosis than controls. the genotype distribution comparing patients and controls (wt/wt 95% vs 96%; Delta32/Delta32 1.5% vs 3.5%; wt/Delta32 3.1% vs 0.5% P=0.04, chi square 3.8, Odds ratio 6.4, CI 0.7-53.82).there was significant differences in the genotype CCR5 Delta32 when compared between patients and controls.

Conclusions: CCR5 Delta32 heterozygosity was associated with susceptibility to primary biliary cirrhosis. These results affirm an important role of immunogenetic factors in the outcome of primary biliary cirrhosis.

NOTES

Poster P21 - YI

LIVER TRANSPLANTATION FOR PRIMMARY BILIARY CIRRHOSIS

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Introduction: Primary biliary cirrhosis (PBC) is a chronic and slowly progressive cholestatic liver disease characterized by destruction of the interlobular bile ducts, which, if untreated, leads to fibrosis, biliary cirrhosis, and liver failure. Orthotropic liver transplant (OLT) remains the only definitive therapy for end-stage liver disease.

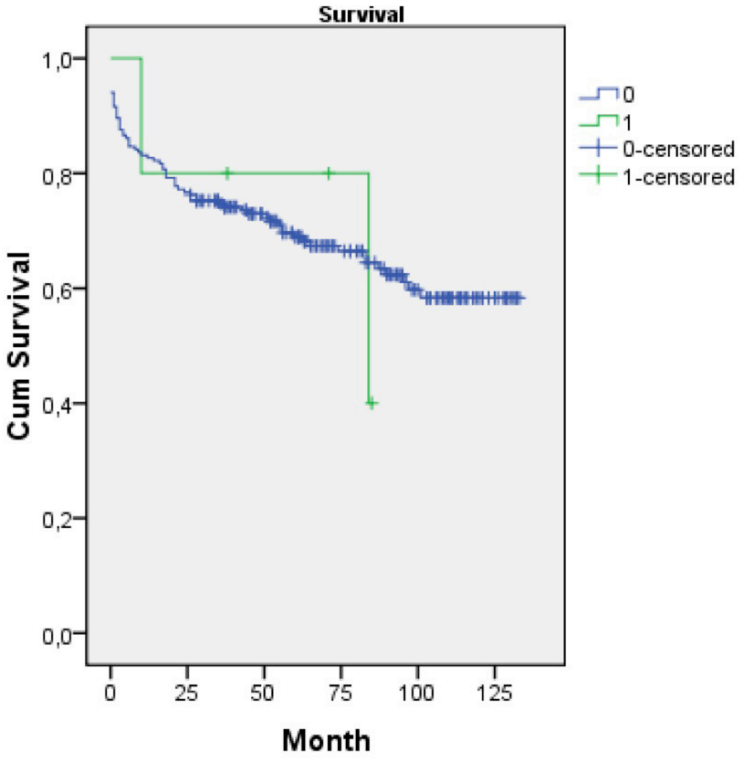
Aim: The aim of this study was to analyze patient survival in transplantation for PBC.

Methodology: We retrospectively analyze 209 patients who underwent OLT from January 2001 to December 2009 in Rome "La Sapienza" center. PBC was in 2.8% (n=6) of cases OLT indication. We stratified the cohort according to OLT indication: Group 1 (no PBC, n=203) and Group 2 (PBC, n=3). The two groups were compared according to surgical time, and survivals.

Results: Cumulative patient survival on the entire cohort is 65.2% (n=135), while cumulative graft survival is 64.7% (n=134).
In Group 1, 34.8% (70/201) of deaths was reported, 24 of them being observed within the first 3 months.
35.3% (71/201) of graft losses was observed in this group.
In Group 2, 33.3% (2/6) of deaths was observed, with no patients deceased within 3 months from OLT. no grafts were lost.
The median survival was 74 months for group 1 and 82 months for Group 2. No significant difference was found between the two groups (figure 1)

Conclusions: PBC have same survival and graft survival rates than other OLT indication. OLT for PBC remain the unique therapeutic treatment with good results.

Figure:


Survival
Cum Survival
1,0
0,8
0,6
0,4
0,2
0,0
0
25
50
75
100
125
Month
0
1
0-censored
1-censored

Poster P22 - YI

ASSOCIATION OF TUMOR NECROSIS FACTOR (TNF)-ALPHA POLYMORPHISMS WITH PRIMARY BILIARY CIRRHOSIS AND AUTOIMMUNE LIVER DISEASES IN NORTH INDIAN POPULATION

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Introduction: Primary biliary cirrhosis (PBC) and Autoimmune hepatitis (AIH) are two autoimmune diseases of unknown etiology. Genetic factors appear to be involved in the pathogenesis of both the diseases. Tumor necrosis factor TNF-α is a cytokine with a wide range of inflammatory, apoptotic and immunomodulatory activities is implicated in the pathogenesis of PBC.

Aim: In this study, we studied the association between North Indian patients with PBC, AIH and the polymorphisms in promoter-region polymorphisms of the TNF-alpha gene at position -308.

Methodology: We have investigated the candidate gene loci in 15 patients with AIH, 25 cases with PBC and 40 healthy controls. TNF-α genes were amplified using amplification refractory mutation systems (ARMS)-PCR methodology to detect any polymorphism involved at position -308 of TNF-α.

Results: We found the frequencies of TNF-alpha-308 G/G polymorphisms were significantly higher in both AIH and PBC when compared with those of healthy controls (P = 0.001; OR, 4.33; 95% CI, 1.69–11.06) respectively. The frequency of TNF-alpha -308 G/G polymorphism was significantly higher in the patients than those of the healthy controls. The frequencies of TNF-alpha -308 G/G, G/A, A/A genotypes were 65.00%, 32.50% and 2.50% in AIH and PBC and 30.00%, 60.00%, 10.00% in healthy controls. The genotypic frequency of G/G in AIH & PBC were 27.50%, 25.00%,12.50% respectively.

Conclusions: Our findings suggest that the TNF-alpha promoter-region polymorphisms distribution is different between differe of ethnic groups; there are no genetic links of the TNF-alpha promoter-region polymorphisms to AIH and PBC in North Indian Population.

Poster P23

PRIMARY BILIARY CIRRHOSIS AND AUTOIMMUNE HEPATITIS OVERLAP SYNDROME: CHARACTERISTIC FEATURES AND OUTCOMES.

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Introduction: The most frequent reported association occurs between autoimmune hepatitis and primary biliary cirrhosis (AIH-PBC). It remains unclear whether this overlap syndrome (OS) form distinct entities or they are only variants of the major autoimmune liver diseases. Diagnostic criteria for PBC-AIH have not been standardized so far.

Aim: The aims of this study were to investigate and to compare the clinical and laboratory features and responses to therapy in primary PBC and OS.

Methodology: Retrospective study including patients with PBC admitted to our department between January 2011 and December 2013. PBC was diagnosed according to EASL guidelines. OS was defined by the presence in the same patient of at least 2 of 3 accepted criteria of PBC and AIH. Clinical, biological, and histological features and treatment response were compared between patients with OS and PBC.

Results: We enrolled 14 PBC and 9 OS. No significant difference was found between the two groups concerning the age, the sex ratio and the main initial symptoms. Patients with OS had serum aminotransferase significantly higher than patients with PBC (ALT 154 UI/l vs 44 UI/l; p=0.01; AST 152 UI/l vs 64 UI/l; p=0.03) and lower alkaline phosphatase level (222 UI/l vs 455 UI/l,p=0,05). No difference was found regarding gamma-glutamyl-transpeptidase, gammaglobulin and IgM, IgG levels and antibodies. Histological analysis showed more often severe lymphocytic piecemeal necrosis in the OS group. Among 9 patients with OS, 7 patients were treated: 2 patients with ursodeoxycholic acid (UDCA) and 5 patients received combined therapy. Complete response was noted in 71.4%. Biochemical response and survival was similar in the two groups.

Conclusions: In this Tunisian cohort, OS is not rare and accounts for 39.1% of patients with PBC. Higher aminotransferase, lower alkaline phosphatase level and severe lymphocytic piecemeal necrosis were significantly more frequent in OS.

Poster P24

DOES ANTI-GP210 ANTIBODY MIRRORS DISEASE SEVERITY IN PRIMARY BILIARY CIRRHOSIS ?

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Introduction: Several types of antinuclear antibodies have been associated with primary biliary cirrhosis (PBC). PBC-specific antinuclear antibodies (anti-gp210, SP100) have been characterized and associated with disease progression and outcome.

Aim: The aim of our study was to investigate the autoantibody profile and its clinical implication in the patients with PBC.

Methodology: Retrospective study including patients with PBC admitted to our department between January 2011 and December 2013. The autoantibody profile was tested for each patient by using immunoblotting and indirect immunofuorescence. According to the presence of anti-gp210, PBC patients were subdivided into two groups: Group 1: PBC patients with anti-gp210; Group2: PBC patients without anti-gp210. Epidemiological, biochemical, clinical, histological features and prognosis were compared.

Results: Of 23 patients with anti mitochondrial M2 antibodies (AMA), 2 patients had anti-gp210. Only one patient had PBC-AMA negative with anti-gp210 positive. No patient had anti-sp100 antibodies. Anti-gp 210 negative/positive patients were remarkably similar in terms of clinical manifestations, liver biochemistries, frequency of anti-nuclear antibodies, anti-smooth-muscle antibody and histological findings. Outcome was comparable for the two groups.

Conclusions: In our cohort, anti-gp210 negative/positive PBC patients are similar in clinical, laboratory, liver biopsy features and outcome. The anti-gp 210 positivity was helpful for making a diagnosis of PBC-AMA negative patients which can be considered as a variant of AMA-positive PBC rather than a separate clinical entity.

Poster P25

CONCURRENT AUTOIMMUNE DISEASES IN PRIMARY BILIARY CIRRHOSIS

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Introduction: Autoimmune disease are frequently associated to primary biliary cirrhosis.

Aim: To assess the prevalence of concurrent autoimmune diseases in patients with primary biliary cirrhosis (PBC).

Methodology: Retrospective study including patients with PBC admitted to our department between January 2011 and December 2013. We analyzed our cohort of 23 patients with PBC for concurrent autoimmune diseases.

Results: In the study population, 12 patients had concurrent autoimmune diseases. A total of 16 autoimmune disease diagnoses were found in our patients. Among them, 2 patients had two diagnosed autoimmune diseases. Nine cases of overlap syndrome were found. Concurrent autoimmune diseases including autoimmune thyroid diseases were found in 2 cases and Sjögren syndrome in 3 cases. Raynaud syndrome and scleroderma were present in one case respectively. No case of celiac disease or psoriasis was found.

Conclusions: Concurrent autoimmune diseases are common in patients with PBC and suggest common genetic and immunological background. Therefore, extended screening for existing autoimmune diseases during the routine assessment of these patients is recommended.

Poster P26

BONE MINERAL DENSITY IN TUNISIAN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Introduction: Loss of bone mass is frequently identified in primary biliary cirrhosis (PBC), leading to fragility fractures and significant morbidity. The pathogenesis is not completely elucidated.

Aim: The aim of this study is to assess the prevalence of bone loss in PBC patients.

Methodology: Bone mineral density (BMD) was retrospectively performed in patients with PBC seen in our department between January 2010 and December 2013, by X-ray absorptiometry at both lumbar spine and femoral neck sites. Other diseases disturbing the bone density were excluded. Osteopenia was considered if T score < -1.5 DS and osteoporosis if T-score <-2.5 DS.

Results: Twenty three patients were included in our study. They were 20 women and three men with a mean age of 55 years [25 - 80]. Bone mineral density was low in 6 patients (26%): osteopenia in 3 cases and osteoporosis in 3 cases. No predictive factor of osteopenia or osteoporosis was found.

Conclusions: In our study, prevalence of low bone mineral density was 26 %. This high prevalence suggests that bone status should be evaluated routinely in patients with PBC, regardless to the severity of the liver disease.

NOTES

Poster P27 - YI

ADAPTIVE MECHANISMS IN CHRONIC CHOLESTASIS IN HUMANS: CHANGES IN THE EXPRESSION OF NUCLEAR RECEPTORS AND SULPHOTRANSFERASE 2A1

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Introduction: Nuclear receptors (NRs) such as farnesoid-X-receptor (FXR), pregnane-X-receptor (PXR) and constitutive androstane receptor (CAR) play a pivotal role in regulation of genes involved in responses to cholestasis/detoxification processes. Sulphation catalyzed by sulphotransferase 2A1 (SULT2A1) is a phase II detoxification process.

Aim: To analyze liver expression (both protein and mRNA) of FXR, PXR, CAR and SULT1 in patients with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC).

Methodology: Western blot analysis and expression of mRNA for NRs and SULT2A1 were performed on explanted livers from cirrhotic patients with PSC (n=11), PBC (n=14) and control tissues (n=16). Expression of mRNA was additionally estimated in 26 biopsies from non-cirrhotic PBC.

Results: Increased expression of FXR, CAR and PXR protein was seen in cirrhotic PSC (p<0.0001; p<0.0001 and p=0.002 respectively) and PBC (p=0.04; p<0.0001 and p=0.02 respectively). Expression of FXR mRNA decreased with progression of fibrosis in non-cirrhotic PBC and was decreased in cirrhotic PBC. Both SULT2A1 mRNA and protein was significantly overexpressed in PBC (p=0.0009 and$p$=0.0002, respectively) but was not upregulated in PSC.

Conclusions: Nuclear receptors are overexpressed in PBC and PSC. In PSC, unlike in PBC, an activation of nuclear receptors PXR and CAR is not associated with increased expression of SULT2A1. This finding may suggest that impaired sulphation contributes to the pathogenesis of PSC.

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Poster P28

NONINVASIVE ASSESSMENT OF LIVER FIBROSIS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Introduction: In the last decade, numerous noninvasive methods for assessment of liver fibrosis have been developed and evaluated. Ideally they should be reliable, fast, reproducible, easily applicable in every day clinical practice, acceptable for patients and robust for both prognosis and staging of liver disease. However, most of those methods have been extensively studied in viral hepatitis but not much has been done on patients with PBC

Aim: The aim of this work was to compare the diagnosis accuracy of liver stiffens (transient elastography) with simple and routinely available blood markers (APRI, Forns, AST/ALT ratio) using liver biopsy as the reference in patients with PBC.

Methodology: Hundred-forty patients underwent both liver biopsy and blood work-up on the same day and transient elastography in the following month. Liver biopsies were reviewed by a single pathologist using the METAVIR scoring system for assessment of liver fibrosis. APRI and Forns scores, AST/ALT ratio and transient elastography were compared with liver fibrosis stage in order to define the best non-invasive marker of liver fibrosis

Results: A statistically significant difference ($p<0.05$) was found for APRI score, Forns index, transient elastography according to stages of liver fibrosis. Transient elastography showed better diagnostic performances comparing to other investigated surrogate markeris of liver fibrosis. Optimal cut offs for TE were 4.25 and 5.9, respectively, for diagnosing presence of fibrosis and distinguishing mild/moderate and advance stages of fibrosis. The AUROCs of TE were 0.963 and 0.865, resepctively.

Conclusions: Based on our investigation we concluded that APRI score, Forns index and transient elastography adequately display fibrosis stage in patients with primary biliary cirrhosis, but the most sensitive and specific parameter revealed to be transient elastography. Using noninvasive markers and methods in evaluation of patients in everyday clinical practice may reduce, but not eliminate, the need for invasive diagnostic procedures.

NOTES

Poster P29

AUTOANTIBODY STATUS AND HISTOLOGICAL VARIABLES INFLUENCE BIOCHEMICAL RESPONSE TO TREATMENT AND LONG-TERM OUTCOMES IN JAPANESE PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

Minoru Nakamura [1,*] on behalf of Gp210 working in Intractable Hepatobiliary Disease Study Group supported by the Ministry of Health, Labour and Welfare of Japan, Hisayoshi Kondo [2], Atsushi Tanaka [3], Atsumasa Komori [4], Kazuhide Yamamoto [5], Hiromasa Ohira [6], Mikio Zeniya [7], Etsuko Hashimoto [8], Shuichi Kaneko [9], Yoshiyuki Ueno [10], Shinji Shimoda [11], Masanori Abe [12], Shotaro Sakisaka [13], Morikazu Onji [12], Hajime Takikawa [3], Hirohito Tsubouchi [14], Hiromi Ishibashi [15], Kenichi Harada [16], Masahiro Ito [1], Yasuni Nakanuma [16]

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Introduction: Biochemical response to ursodeoxycholic acid (UDCA) therapy is a good biomarker to predict long-term outcomes of primary biliary cirrhosis(PBC). However, this criterion still needs further validation and the relationship between treatment response and other known risk factors associated with disease-progression remains to be elucidated.

Aim: The aim of the present study is to evaluate the factors influencing biochemical response to treatment and the value of biochemical response for predicting long-term outcomes in Japanese patients with PBC.

Methodology: A total of 306 needle liver biopsy specimens from 287 PBC patients (Scheuer stage I, n=159; II, n=92; III, n=43; IV, n=12) were re-evaluated according to the new staging and grading system by Nakanuma et al (Pathol Int 2010;60:167-74). Biochemical response to ursodeoxycholic acid (UDCA) or UDCA plus bezafibrate was defined as good (≦ULN), fair (≦1.5 x ULN), or poor (<1.5 x ULN) at 2 years after initiation of UDCA treatment. Associations between various factors (age, sex, autoantibody status, and histological variables at baseline), biochemical response, and long-term outcomes were evaluated in 164 PBC patients.

Results: Anti-gp210 positivity and a higher bile duct loss score were significant risk factors for non-good ALP response (OR, 2.78 and 1.85, respectively). Age, anti-gp210 positivity, and anti-centromere positivity were significant risk factors for non-good ALT response (OR, 1.05, 4.0, and 2.77, respectively). Anti-gp210 positivity and a higher hepatitis score were significant risk factors for non-good IgM response (OR, 2.10 and 2.06, respectively). Non-good ALP and IgM response were significant risk factors for progression to late-stage disease without jaundice (clinicasl stage II) (OR, 2.27 and 2.32, respectively). Non-good ALT response was a significant risk factor for progression to late-stage disease with persistent jaundice (clinical stage III) (OR, 11.11). When these risk factors were analyzed at the same time, non-good ALP and ALT response remained significant risk factors for progression to clinical stage II (OR, 4.23) and III (OR, 10.86), respectively.

Conclusions: Biochemical response to treatment at 2 years, which is influenced by autoantibody status and histological variables at baseline, can predict long-term outcomes in Japanese patients with PBC. To better understand the mechanism of treatment response and progression of PBC, further studies are needed in association with analysis of genetic and environmental factors.

Poster P30

TREATMENT OF INTRACTABLE PRURITUS WITH ARTIFICIAL LIVER MARS

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Introduction: Intractable Pruritus (IP) is a frequent feature of chronic liver disease, especially when cholestasis is present. Albumin dialysis, using the Molecular Adsorbent Recirculating System (MARS), allows one to achieve a high clearance of albumin-bound substances, such as bile acids and bilirubin, and to diminish cholestatic pruritus.

Aim: Our aim was to examine improvement in the patients' pruritus after the treatment.

Methodology: Fourteen patients affected by primary biliary cirrhosis were enrolled in the study: 9 famale and 5 male (mean age 46 yrs). Two patients had been transplanted; the other ten were on the waiting list for liver transplantations. Each patient underwent one daily treatment of 8 hours length for a mean of 5 days with the MARS, using a standard dialysis machine. Pruritus was greater than 7 according to the visual analog scale (VAS). In each patient, liver function tests, kidney function tests, and hemodynamic targets were evaluated before and after the treatment. Blood and albumin circuit flows, membrane pressure, ratio albumin concentration between patient and circuit were adapted to each patient. Adsorbtion efficency value (η) and Stanton number (St) were used to value the detoxification capacity of MARS.

Results: The MARS treatments were well tolerated in all patients. There were no significant hemodynamic changes and no bleeding events. A decrease in total bilirubin, creatinine, and bile acids together with a significant improvement in VAS was observed. Assesing the real detoxification on η and St, nine treatments were need for three patients whom two after liver transplant. In addition, five treatments for nine and six treatments for two patients were performed. Follow-up in 11 cases showed sustained improvement of pruritus lasting for more than 12 months, while in 3 cases for 9 months.

Conclusions: The MARS may be used for treatment of pruritus in patients where all the other therapeutic options have failed. We should consider the fact that MARS is not a definitive treatment, but one that improves the quality of life both of patients on the waiting list for liver transplantation and of already transplanted patients.

Poster P31 - YI

ATTENUATION OF BILE ACID-INDUCED HEPATOTOXICITY BY OMEGA-3 FATTY ACIDS.

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Introduction: The impaired biliary secretion from the cholestatic livers of primary biliary cirrhosis (PBC) patients results in the retention of toxic bile acids (BA) in hepatocytes. BA accumulation can lead to cirrhosis or fibrosis and eventually to liver failure, their reduction is therefore an important target for anti-cholestatic therapies. Our laboratory recently observed that the omega-3 (ω-3) polyunsaturated fatty acid docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids inhibit the expression of genes involved in BA synthesis and uptake, while activating those encoding BA detoxification and excretion.

Aim: The goal of the present study was therefore to evaluate the hepatoprotective consequences of such regulatory effects.

Methodology: Human hepatoma HepG2 cells were pretreated with increasing dose of EPA and/or DHA (10 to 50μM) for duration varying from 3 to 24H, before being exposed to a BA mixture consisted of 100μM CDCA, CA, DCA and LCA for 0.5 to 24H. Following these treatment apoptotic and/or necrotic cells were detected using a caspase-3 detection kit (Invitrogen) and/or through flow cytometry analysis (FACS) of cell membrane permeability. Finally, how these treatments affected the expression of inflammatory markers (IL-8, PDCD4, PAI-1, PTEN, ICAM1) was analyzed by qRT-PCR.

Results: When compared to control cells, pre-treatment with ω-3 caused a 3-fold reduction in BA-induced Caspase 3 activity. In FACS analyses, EPA and DHA used alone and in combination, time- and dose-dependently prevented the accumulation of apoptotic cells caused by BA exposure, but exerted pro-necrotic effects. Finally, the pre-treating HepG2 cells with EPA/DHA also reduced the BA-dependent activation of IL-8, PAI-1 or PDCD4 mRNA expression.

Conclusions: The present study indicates that ω-3 can protect liver cells against BA-induced apoptosis and inflammation, and thus could be efficient in protecting PBC patients from BA-induced liver damages.

Poster P32

ASSESSMENT OF LIVER FIBROSIS STAGE IN PBC

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Introduction: The accuracy of non-invasive methods for the quantification of liver fibrosis in patients with PBC is still debated.

Aim: We analyzed the accuracy of a number of indirect markers of liver fibrosis in the detection of different histological stage of PBC, in comparison with the histomorphometrical measurement of fibrotic tissue.

Methodology: Histomorphometrical measurement of fibrotic tissue was performed on sirius red stained sections of liver biopsies obtained from 50 patients with PBC (mean age, 57±12.30 years; 43 F and 7 M; 8 AMA negative, 42 AMA positive). Area percentage measures of fibrotic tissue were ranked into 4 groups reflecting Ludwig's staging and compared with values of the following serum markers of liver fibrosis: APRI, LOK, FORNS, FIB-4. The percentage of fibrosis was calculated with ImageJ. All results were expressed as mean ± standard deviation. The numerical comparison of continuous data was performed using the Wilcoxon signed-ranks test applied to two-sample. Statistical significance was set at a value of $p<0.05$.

Results: In the entire cohort, the mean morphometric fibrosis values were 8.79% ± 6.03. There were 19 (38%) patients in histological stage I, 14 (28%) in stage II, 12 (24%) in stage III and 5 (10%) in stage IV. The morphometric values of fibrotic tissue were significantly different in the various stage of PBC: stage I versus stage II (0.74% ± 0.65 vs 3.87% ± 1.5, respectively; p=0.03); stage II vs stage III (3.87% ± 1.5 vs 6.15% ± 1.68, respectively; p=0.003); stage III vs stage IV (6.15% ± 1.68 vs 14.06% ± 8.45, respectively; p=0.05). No significant difference was found in the values of APRI, FORNS, FIB-4 and LOK scores.

Conclusions: Indirect markers of liver fibrosis in PBC do not reflect the histological stage.

Poster P33

EARLY RESPONSES TO BEZAFIBRATE PREDICT LONG-TERM OUTCOME OF PATIENTS WITH PRIMARY BILIARY CIRRHOSIS REFRACTORY TO UDCA

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Introduction: Bezafibrate (BF) is reported to have biological effect for patients with PBC refractory to ursodeoxycholic acid (UDCA). In Japan, nationwide surveys have been performed every three year since 1980.

Aim: In the current study, we evaluated whether early response to BF was associated with improvement of long-term outcome of patients with PBC refractory to UDCA.

Methodology: The patients who met these criteria were selected and included in this retrospective study; 1) registered in the database after 2000 when BF administration for UDCA non-responders was started in Japan, 2) UDCA was administrated, 3) followed up for at least 2 years, and 4) the prognosis was clearly documented.

Results: Overall, 1,121 patients with PBC met these criteria. The followed-up period was 6.1+/-3.4 years. BF was administrated in 284 UDCA refractory patients (25.3%). Among them, early response to BF (ALT levels within 3 years from the commencement of BF) was documented in 106 cases; 43 elevated ALT (≥31 U/L) and 63 normal. At the end of observation, 18 of elevated ALT (42%) and 9 of normal ALT (14%) had symptoms, respectively, indicating that patients with elevated ALT had liver-related symptoms at significantly higher rates (p=0.023, Log-rank test). Among 837 BF untreated cases, 167 had symptoms at the end of observation, and the complication rates were significantly lower than BF treated cases with elevated ALT (p=0.006), while comparable to BF treated cases with normal ALT.

Conclusions: The normalization of ALT with BF treatment within 3 years was associated with improvement of long-term outcome of UDCA-refractory PBC patients.

Poster P34 - YI

MOUSE MODELS FOR CHOLESTATIC ITCH

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Introduction: In patients with cholestasis, including intrahepatic cholestasis of pregnancy (ICP), serum autotaxin (ATX) activity is increased and correlates with itch intensity.[1,2] We hypothesize that ATX causes itch by formation of lysophosphatidate, activating sensory nerve endings.[1,3-5]

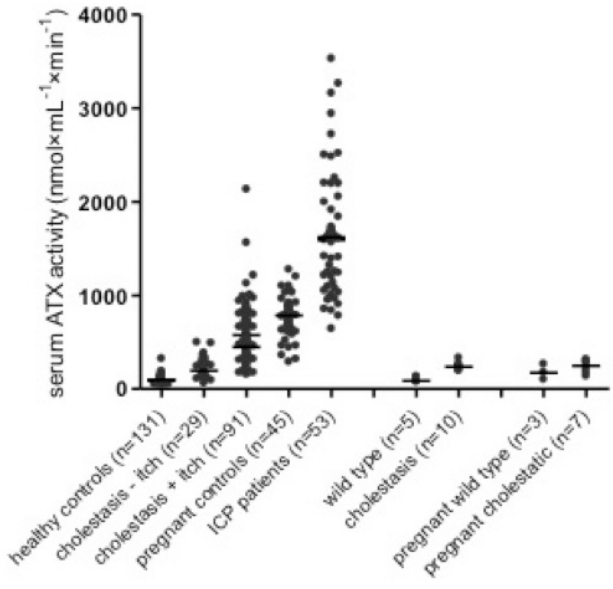
Aim: To establish a mouse model to investigate the causality of increased serum ATX on itch.

Methodology: In mice we studied the effect of cholestasis by 1) bile duct ligation (BDL), 2) a 0.1% cholate diet in Atp8b1 mutant mice and 3) during cholestatic pregnancy. We measured serum ATX activity weekly, and scratch activity during 12 hours at night, for 4 consecutive nights per condition. Immunohistochemistry for ATX and chromogranin A was performed on mouse and human tissues.

Results: Serum ATX was very mildly induced by BDL (1.5-fold; $p=0.003$), cholate diet in Atp8b1 mutant mice (2-fold; $p<0.000$) and pregnancy (2.5-fold; $p<0.000$). Cholestasis during pregnancy did not further enhance serum ATX. Scratch activity remained unaffected in all groups. Immunohistochemical analysis revealed strong ATX expression in human small intestinal enteroendocrine cells (EECs), which was absent in mice.

Conclusions: Cholestasis induces serum ATX much less in mice than in humans (see figure 1). Interestingly, this correlated with the absence of scratch behaviour in cholestatic mice. ATX expression in EECs as observed in humans seems to be absent in mice, addressing a possible explanation for the much lower plasma ATX activity during cholestasis. Alternative methods to further increase serum ATX activity in mice are sought for in order to investigate the causality of serum ATX in cholestatic itch.

Figure:



Poster P35 - YI

INCREASED PREVALENCE OF GALLBLADDER&PANCREAS AND LOWER GI TRACT ABNORMALITIES IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

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Introduction: It was reported that involvement of the gallbladder (GB) & pancreas and lower gastrointestinal (GI) tract is rare in patients with primary biliary cirrhosis (PBC).

Aim: Our aim was to find the prevalance of these involvements by using transabdominal ultrasound (US) and lower GI tract endoscopy in patients with PBC. Because of both disease, inflammatory bowel disease (IBD) and PBC, have autoimmune background, the occurence of IBD was also questioned in these patients by endoscopy with/or without biopsy.

Methodology: We evaluated our PBC clinic's records. We used US to show GB and pancreas abnormalities. Lower GI tract abnormalities were evaluated by colonoscopy and rectoscopy. Patients with recent onset dyspepsia were used as a control group.

Results: Of the 82 patients with PBC, 62 had documented US results. There were also 61 patients without PBC as a control group. All of the patients, 8.1% in PBC and 8.1% in control were male ($p > 0.05$). The prevalence of abnormalities as follows: gallbladder polip, 1.6% in PBC vs 4.9% in controls ($p > 0.05$); gallbladder sludge & stone, 14.5% in PBC vs 9.8% in controls ($p > 0.05$); gallbladder operation, 22.6% in PBC vs 9.8% in controls ($p = 0.05$); gallbladder wall tickness, 6.5% in PBC vs 1.6% in controls ($p > 0.05$); all gallbladder abnormalities 43.5% in PBC vs 26.2% in controls ($p < 0.05$); pancreas abnormalities 6.5% in PBC vs 0% in controls ($p < 0.05$). Of the 4 patients with pancreas abnormalities, 0ne was dead due to the liver impairment; echoendoscopy examination in 2 patients showed minor 2 criteria. Lower GI tract examination was performed by colonoscopy with ileum entubation (IE) in 5 patients and without IE in 8 patients, and rectoscopy in 5 patients. Colon polyps (size from 1 mm to 15 mm) were found in 5 patients as follows: 3 with adenomatous, 1 with tubulovillous and 1 with inflammatory changes. Ileum biopsies showed normal ileum mucosa in 5 patients.

Conclusions: Our results showed that GB and pancreas abnormalities are not frequent, but also not rare in patients with PBC. Although the colon adenomatous polyps occurence was increased in PBC, none of the patients had IBD.

NOTES

Poster P36

PATTERN OF LIPIDS DERANGEMENTS AND RISK EVALUATION OF CARDIOVASCULAR EVENTS IN PRIMARY BILIARY CIRRHOSIS PATIENTS.

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Introduction: Primary biliary cirrhosis (PBC) is a chronic cholestatic autoimmune disease that disrupts cholesterol metabolism in several ways, in clinical practice derangements in the serum lipids pattern are frequently observed in PBC patients.

Aim: Our aim was to investigate the frequency of dyslipidemias and to evaluate the risk of cardiovascular events in PBC patients.

Methodology: All patients with histological diagnosis of PBC from 2000 to 2009 at our hospital were included; patients were compared with healthy organ donors attended during those years (control group). Total cholesterol, c-HDL, c-LDL and triglycerides serum concentrations were measured in both groups, and the frequency of dyslipidemias was recorded. Patients were followed for the development of cardiovascular events (ischemic cardiopathy or cerebrovascular event). The cardiovascular risk was calculated using the Framingham tables.

Results: Forty two PBC patients were included in the study and were compared with one-hundred healthy controls. There were differences in total cholesterol (controls 199.6 mg/dl $\pm$ 40.1 vs 270.3$\pm$124.5 mg/dl in CBP patients, p=0.0001), triglycerides (118.5$\pm$43.3 mg/dl vs 151.5$\pm$58.2 mg/dl in CBP patients, p=0.001), HDL-cholesterol (48$\pm$12.2 mg/dl vs 59.4$\pm$30 mg/dl in CBP patients, p=0.002) and in LDL-cholesterol (127.7$\pm$34.5 vs 183.3$\pm$122.1 mg/dl in CBP patients, p=0.0001).

Hypercholesterolemia (≥ 240 mg/dl) was found in 52.4% of CBP patients vs 11% in healthy controls, high LDL-cholesterol was found in 45.2% of PBC patients vs 10% of controls and hyper-alphalipoproteinemia (HDL-cholesterol ≥ 60 mg/dl) was found in 45.2% of PBC patients vs 16% of controls. The 10-years cardiovascular risk were 5.6 % ± 6.45 in the PBC patients and 5.23 % ± 6.37 in the control group (p=0.97, IC 95% -2.69 - 1.96). There were not differences in the cardiovascular risk when analyzing only the patients with LDL-cholesterol higher than 130 mg/dl, in whom the risk was 7.036 % ± 7.06 for the PCB patients (n=28) and 7.042 % ± 7.12 in the healthy controls (n=48, p=0.92, IC 95% -3.36 – 3.37). In a mean follow up time of 57.9 ± 36.5 months there were only one cardiovascular event (stroke) in the PBC group and none in the control group (72.17 ± 51 months).

Conclusions: There are marked derangements in serum lipids and a high frequency of dyslipidemias in PBC patients but these does not increase the cardiovascular- events risk.

Poster P37 - YI

CLINICAL SIGNIFICANCE OF OCASIONALLY DETECTED PRIMARY BILIARY CIRRHOSIS-LINKED AUTOANTIBODIES IN NORMAL INDIVIDUALS

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Introduction: Nowadays, the clinical meaning and subsequent risk of progression to a full blown form of chronic liver disease in asymptomatic individuals in whom PBC (Primary Biliary Cirrhosis)-linked autoantibodies have been detected remains controversial and in many aspects it is unknown whether or not this represent the so called, "early" PBC.

Aim: We aimed to compare the clinical and laboratorial course of patients with definite PBC with an otherwise healthy population of patients with PBC-linked autoantibodies in the sera.

Methodology: Retrospectively, we have collected demographic, clinical and laboratorial data from a population with PBC-linked autoantibodies detected in the serum and compared with our "classical" PBC cohort patients.

Results: We identified 28 cases of definite PBC (G1) and 60 cases of individuals with PBC-linked autoantibodies detected in the serum without a PBC diagnosis (G2). Female gender was dominant in both groups but there was a superior frequency of female individuals in G1 (93% vs 74%; $p < 0.05$). There were no differences regarding age at diagnosis/detection. We observed a higher frequency of other autoimmune disorders in G2 (43% vs 18%; $p < 0.05$) and a higher frequency of endocrine & metabolic disorders in G1 group (61% vs 12%; $p < 0.05$). None of the G2 group reported symptoms related/typical of PBC. Cholestatic laboratory features were significantly more proeminent in G1. Alkaline Phosphatase mean±SD G1: 227±185 (G2: 119±80; Ref value < 126 IU/L; $p < 0.05$) and Gama glutamyl transpeptidase mean±SD G1: 247±235 (G2: 104±112; Ref value < 43 IU/L; $p < 0.05$).

No differences were detected regarding serum aminotransferase, albumin and IgM levels. The prevalence of AMA, AMA subtype M2, ANA (antinuclear), PML (Promyelocytic Leukemia Protein) and gp-210 was similar between groups. The only autoantibody that could differentiate the groups was the AMA subtype M2 BPO (3E) (95% vs 59%; $p < 0.05$), more prevalent in G1. Regarding cirrhosis development, it was observed more frequently in the G1 group (25%; n=7) and in only 1.7% (n=1) of the patients in the G2 group ($p < 0.05$).

Conclusions: Our small retrospective observational study suggests that patients with PBC-linked autoantibodies detected by chance tend to display other autoimmune disorders, usually have no clinical or laboratorial features of PBC and seem to harbor a low risk of disease progression into cirrhosis. Albeit whether the early detection of such individuals has a clinical benefit remains to be determined.

Poster P38 - YI

NATURAL HISTORY AND PREDICTORS OF CIRRHOSIS IN PRIMARY BILIARY CIRRHOSIS – A PORTUGUESE SINGLE-CENTER EXPERIENCE

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Introduction: Primary biliary cirrhosis (PBC) is an autoimmune liver disease that generally affects middle-aged women and is characterized by ongoing inflammatory destruction of the intralobular bile ducts, which leads to chronic cholestasis and biliary cirrhosis, with consequent complications such as portal hypertension and liver failure.

Aim: We aimed to analyze our PBC cohort and find risk factors for the development of cirrhosis.

Methodology: Retrospective, observational and single-center study. Demographic data, symptoms at admission, laboratorial data, ultrassonografic findings, Mayo risk score, development of cirrhosis and history of decompensation as well as therapeutic measures and clinical/laboratorial response was assessed and factors associated to cirrhosis development were calculated.

Results: We identified 28 cases, mean (±SD) age at diagnosis was 66 (±12) y/o and 93% (n=26) were female. The majority was referred for abnormal liver biochemistry (61%) and the main symptom was pruritus (25%). Other autoimune disorder was present in 18% and a metabolic disorder in 61%. Regarding autoimune panel: AMA (75%), AMA-M2 (96%), AMA-M2 BPO (95%), Ro-52 (32%), PML (9%), gp-210 (17%), sp-100 (5%). An hepatic biopsy was done in 11% (n=3). The median Mayo risk score was 5.1 (P^{25} = 4.4; P^{75} = 5.3). ¼ of the patients (25%; n=7) displayed features of cirrhosis, of them, 71% (n=5) had, at least, one previous decompensation episode. Abnormal ultrassonographic findings were present in 2/3 of them. Therapy with ursodesoxicolic acid (UDCA), at the recommended dose range, was given to almost all of them (93%), albeit that, only 57% displayed a full laboratorial response.

We identified a positive correlation between the Mayo risk score (r = + 0.513) and albumin serum concentration (r = 0.536) and the development of cirrhosis. An albumin cut-off value of < 3.5 g/dL was the only independent risk factor for cirrhosis (OR = 7.000; CI95%: 2.455-19.957; $p < 0.05$). Advanced age at diagnosis, presence of other autoimune/metabolic disorder, IgM levels, positive sp-100/gp-210 or absence of response to UDCA were not predictors of cirrhosis.

Conclusions: In our study, the prevalence of cirrhosis development among PBC patients is high and an absence/presence of response to UDCA doesn't seem to be related to its development. Serum albumin level still is the best marker of disease progression.

Poster P39

FATIGUE IN PRIMARY BILIARY CIRRHOSIS IS ASSOCIATED WITH COMORBIDITIES AND SEVERITY OF CHOLESTASIS.

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Introduction: The pathogenesis of fatigue in patients with primary biliary cirrhosis (PBC) is unknown, and previous studies are uncertain for elucidating the specificity of this symptom and the association with comorbidities in PBC.

Aim: To assess the prevalence and severity of fatigue and the potential factors related to this symptom in a series of patients with PBC.

Methodology: A multidomain disease-specific quality of life responses and clinical findings questionnaire for PBC (PBC-40) was distributed to a consecutive series of 108 patients (7 men, age 58.4 ± 12.6 years) during a 4-month period. Clinical, laboratory, histological, duration and biochemical response to ursodeoxycholic acid (UDCA), and comorbidities (depression, thyroid disease, Sjogren's syndrome) were recorded.

Results: The mean PBC-40 domains were: fatigue: 23.5 ± 1.0, social: 19.1 ± 0.8, symptoms: 18.0 ± 0.5, emotional: 7.0 ± 0.3, cognitive: 11.9 ± 0.6, and itching 4.6 ± 0.2. 35 patients (32%) had moderate or severe fatigue, 13 patients had no fatigue and 60 patients showed mild changes. Depending on the fatigue severity patients were divided into two groups: severe/moderate fatigue and no/mild fatigue. There were no significant differences in age, sex, biochemical variables, duration of UDCA treatment and histology at diagnosis between the two groups. Patients with severe/moderate fatigue had lower hemoglobin levels (12.4 ± 1.9 vs 13.12 ± 1.3 g/l, p = 0.002), higher levels of total bilirubin (1.1 ± 0.5 vs. 0.3 ± 0.5 mg/dl, p = 0.001) and (gGT 210 ± 45 vs 129 ± 20 U/ml, p = 0.005) as compared with patients with no/mild fatigue. There was no relationship between fatigue and hypothyroidism, sicca syndrome or with response to UDCA. Diagnosis or therapeutic intervention for depression was found in 18 of 78 patients (23%), and depression was significantly associated with fatigue (OR: 5.8, 95% CI: 1.9 - 17.9, p = 0.003).

Conclusions: Fatigue is a common manifestation of PBC and is related to the presence of comorbidities such as depression and lower haemoglobin levels. The symptom is dependent on the severity of PBC, but independent of their duration and therapeutic response to ursodeoxycholic acid.

Poster P40

SERUM METABOLOMIC PROFILING IN PATIENTS WITH CHOLESTATIC PRURITUS. EFFECTS OF ALBUMIN DIALYSIS

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Aim: This study assesses changes in the serum metabolomic profile before and after treatment with albumin dialysis using the molecular adsorbents recirculating system (MARS), to identify metabolites potentially associated with the pathogenesis of pruritus.

Methodology: Samples from sera before and after MARS and from albumin dialyzate were taken from 9 patients (7 women) with cholestasis and resistant pruritus. Metabolite extraction was accomplished by fractionating the samples into pools of species with similar physicochemical properties, and three different profiles were used: a) polar lipids, non-esterified fatty acids, and bile acids; b) amino acids, and c) apolar lipids. The analyses were performed by UPLC-ESI-QTOF-MS, random forests and multivariate and univariate analysis.

Results: More than 470 metabolites were identified. Most metabolites decreased in sera after MARS and the differences were particularly significant for sterols, N-acyl ethanolamines, 1-ether,2–acylglycerophosphoetanolamine and free sphingoid bases. A significant decrease of primary bile acids ($p = 0.04$) mainly taurine and glycine conjugates, and secondary bile acids, were found after MARS. The amino acid profile did not change, except for a significant decrease in cystine, taurine and tyrosine, and an increase in glutamine. A significant reduction of pregnenolone sulfate, androsterone sulphate isomer, and some glycerophospholipids and kynurenine were also found. Four of these serum metabolites and bile acids were particularly high in the albumin dialysate.

Conclusions: Albumin dialysis is associated with a decrease of circulating metabolites especially phospholipids, primary bile acids and sterols. This metabolomic analysis identifies a panel of biomarkers that could participate into the pathogenesis of cholestatic pruritus.

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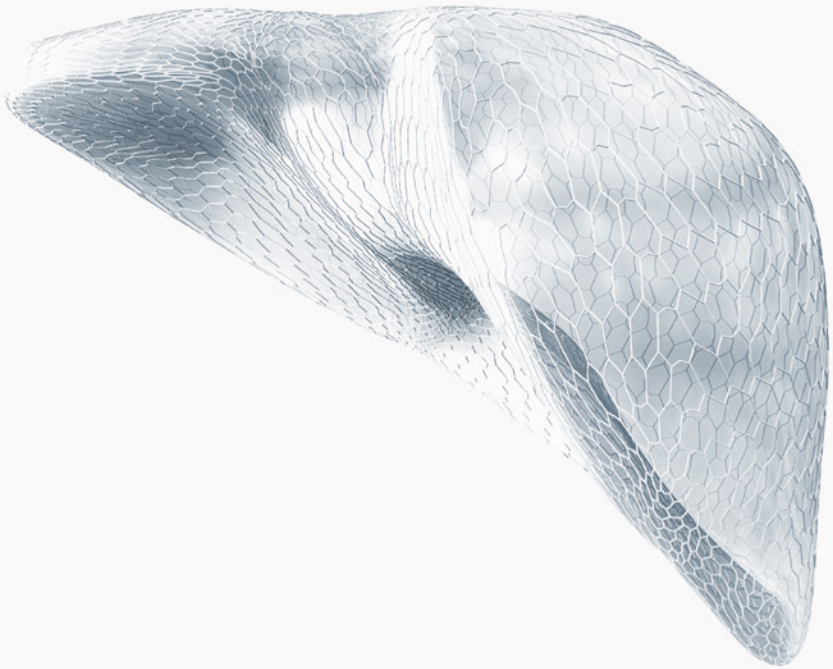

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