GUILHERME GROSSI LOPES CANÇADO

EPIDEMIOLOGIA, ESTRATIFICAÇÃO DE RISCO E TRATAMENTO DA COLANGITE BILIAR PRIMÁRIA: ESTUDO MULTICÊNTRICO BRASILEIRO

Universidade Federal de Minas Gerais

Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto

Belo Horizonte - MG

2022

GUILHERME GROSSI LOPES CANÇADO

EPIDEMIOLOGIA, ESTRATIFICAÇÃO DE RISCO E TRATAMENTO DA COLANGITE BILIAR PRIMÁRIA: ESTUDO MULTICÊNTRICO BRASILEIRO

Tese apresentada ao Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Doutor em Ciências Aplicadas à Saúde do Adulto.

Orientadora: Profa. Dra. Cláudia Alves Couto
Co-Orientadora: Profa. Dra. Luciana Costa Faria

Belo Horizonte, MG

2022

UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS APLICADAS À SAÚDE DO ADULTO

FOLHA DE APROVAÇÃO

EPIDEMIOLOGIA, ESTRATIFICAÇÃO DE RISCO E TRATAMENTO DA COLANGITE BILIAR PRIMÁRIA: ESTUDO MULTICÊNTRICO BRASILEIRO

GUILHERME GROSSI LOPES CANÇADO

Tese de Doutorado defendida e aprovada, no dia vinte e um de setembro de dois mil e vinte e dois, pela Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Ciências Aplicadas à Saúde do Adulto da Universidade Federal de Minas Gerais constituída pelos seguintes professores doutores:

Claudia Alves Couto - Orientadora
UFMG

Luciana Costa Faria - Coorientadora
UFMG

Maria de Lourdes de Abreu Ferrari
UFMG

Paula Vieira Teixeira Vidigal
UFMG

Cristiane Alves Villela Nogueira
UFRJ

Mário Guimarães Pessôa
USP

Belo Horizonte, 21 de setembro de 2022.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Reitor
Professora Sandra Regina Goulart Almeida

Vice-Reitor
Professor Alessandro Fernandes Moreira

Pró-Reitora de Pós-Graduação
Professora Isabela Almeida Pordeus

Pró-Reitor de Pesquisa
Professor Fernando Marcos dos Reis

FACULDADE DE MEDICINA

Diretora
Professora Alamanda Kfoury Pereira

Vice-Diretora
Professora Cristina Gonçalves Alvim

Coordenador do Centro de Pós-Graduação
Professor Tarcizo Afonso Nunes

Subcoordenadora do Centro de Pós-Graduação
Professora Ana Cristina Simões e Silva

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS APLICADAS À SAÚDE DO ADULTO

Coordenadora
Professora Teresa Cristina de Abreu Ferrari

Subcoordenadora
Professora Luciana Costa Faria

Colegiado
Professora Claudia Alves Couto

Professora Gilda Aparecida Ferreira

Professora Karina Braga Gomes Borges

Professora Luciana Costa Faria

Professora Luciana Diniz Silva

Professora Melissa Orlandin Premaor

Professora Teresa Cristina de Abreu Ferrari

AGRADECIMENTOS

Meus sinceros agradecimentos a todos aqueles que, de alguma forma, doaram um pouco de si para que a conclusão deste trabalho se tornasse possível:

Aos meus pais, que, através de um amor incondicional, não mediram esforços para que eu chegasse até essa etapa da minha vida. Vocês são os principais responsáveis por quem eu sou hoje.

À minha irmã, por caminhar sempre ao meu lado, me ajudando a superar as dificuldades e comemorando comigo cada pequena vitória.

À minha orientadora, professora Cláudia Alves Couto, pela confiança em mim depositada, pelos constantes incentivos, ensinamentos e amizade. Sua dedicação, ética, conhecimento e generosidade são exemplos na minha vida. Jamais serei capaz de demonstrar minha gratidão por tudo o que fez e faz por mim. Muito obrigado!

À minha coorientadora, professora Luciana Costa Faria, por estar sempre presente, me aconselhando e contribuindo para meu crescimento profissional e pessoal. Obrigado por sempre me motivar a ir em frente e abraçar as ideias! Sem sua ajuda, esse sonho certamente não seria possível.

Aos membros das bancas de qualificação e defesa, por disponibilizarem seu tempo e conhecimento no aperfeiçoamento e enriquecimento desse trabalho.

Ao professor e amigo Paulo Bittencourt, por toda ajuda, conselhos, paciência e disponibilidade. Por me ensinar que nossa maior fraqueza está em desistir e que o caminho mais certo de vencer é tentar mais de uma vez. Essa vitória também é sua, receba minha profunda gratidão!

Ao amigo Dr. Mateus Jorge Nardelli, parceiro de pesquisas e ideias mirabolantes, por toda ajuda e dedicação. Sinto muito orgulho de ter você em nosso time!

Aos membros e colaboradores do Grupo de Estudos de Doenças Colestáticas da Sociedade Brasileira de Hepatologia e do Instituto Brasileiro do Fígado, muito obrigado pela confiança depositada em meu trabalho.

Aos professores do Instituto de Ciências Biológicas-UFMG, Faculdade de Medicina-UFMG, Instituto René Rachou-FIOCRUZ e Hospital das Clínicas-UFMG que, com dedicação, carinho e competência, transmitiram os ensinamentos que moldaram minha vida acadêmica. Destaco os Professores Ricardo Toshio Fujiwara, Lilian Lacerda Bueno, Maria de Lourdes de Abreu Ferrari e Teresa Cristina de Abreu Ferrari, para os quais registro um agradecimento especial por tudo o que fizeram por mim.

Aos amigos do Hospital da Polícia Militar de Minas Gerais, pelo constante apoio e incentivo.

À minha filha, Norah, presente de Deus, que me permitiu ver a vida com outros olhos. Você enche minha vida de alegria e amor e me faz lutar insistentemente por um mundo sempre melhor!

Por fim, dedico esta, bem como todas as minhas demais conquistas, à minha amada esposa Stael, companheira incondicional, dona de um abraço tão necessário, alicerce da nossa família. Só o amor é capaz de nos fazer passar pelas adversidades da vida com um sorriso no rosto. Juntos colhemos os frutos da nossa união e perseverança.

"A morte de um homem começa no instante em que ele desiste de aprender".

Albino Teixeira

LISTA DE ABREVIATURAS E SIGLAS

AMA – anti-mitocôndria

AUDC – ácido ursodesoxicólico

BCOADC-E2 – subunidade E2 do complexo 2-oxo-ácido desidrogenase de cadeia ramificada

CBP – colangite biliar primária

CXCR – *C-X-C motif chemokine receptors*

CXCL – *C-X-C motif chemokine ligands*

E3BP – proteína ligadora da dihidrolipoamida desidrogenase

FDA – *Food and Drug Administration*

HAI – hepatite autoimune

IgM – imunoglobulina M

IC95% – intervalo de confiança 95%

IL – interleucina

NK – *natural killer*

NNT – número necessário para tratar

OCA – ácido obeticólico

OGDC-E2 – subunidade E2 do complexo 2-ácido oxoglutárico desidrogenase

PDC-E2 – subunidade E2 do complexo piruvato desidrogenase

PPAR – receptores ativados por proliferadores de peroxissoma

RFX – receptor farnesóide X

SPPARM-α – modulador alfa seletivo de receptores ativados por proliferadores de peroxissoma

Resumo

A colangite biliar primária (CBP) é uma doença hepática colestática crônica na qual os anticorpos anti-mitocondriais (AMA) são os principais marcadores diagnósticos. Pouco se sabe sobre esta doença em populações altamente miscigenadas. O objetivo deste estudo foi avaliar as características epidemiológicas e os desfechos clínicos da CBP no Brasil. Quinhentos e sessenta e dois pacientes (95% do sexo feminino, idade média de 51 $\pm$ 11 anos) com CBP foram incluídos. O seguimento médio foi de 6,2 $\pm$ 5,3 anos. Trinta e dois porcento tiveram cirrose, 7% foram submetidos a transplante de fígado e 3% morreram de causas relacionadas ao fígado. Doenças autoimunes concomitantes e sobreposição com hepatite autoimune (HAI) ocorreram, respectivamente, em 18,9% e 14%. Indivíduos com CBP AMA-negativo foram diagnosticados significativamente mais jovens (52,2 $\pm$ 14 vs. 59,6 $\pm$ 11 anos, p = 0,001) e tiveram seu primeiro sintoma em idade mais precoce (43,2 $\pm$ 13 vs. 49,5 $\pm$ 12 anos, p = 0,005), em comparação a pacientes AMA positivo. Níveis séricos mais baixos de imunoglobulina M (272,2 $\pm$ 183 vs. 383,2 $\pm$ 378 mg/dL, p = 0,01) e triglicerídeos (107,6 $\pm$ 59,8 vs. 129,3 $\pm$ 75,7 mg/dL, p = 0,025), e mais altos de bilirrubina (3,8 $\pm$ 13,5 vs. 1,8 $\pm$ 3,4 mg/dL, p = 0,02), também foram observados neste subgrupo. Noventa e seis porcento dos pacientes foram tratados com ácido ursodesoxicólico (AUDC) e 12% necessitaram de terapia complementar com fibrato, seja bezafibrato, fenofibrato ou ciprofibrato. A resposta ao AUDC e à terapia com AUDC/fibratos variou de 39%-67% e 42-61%, respectivamente, de acordo com diferentes critérios validados. O ciprofibrato foi, aparentemente, pelo menos tão eficaz quanto o bezafibrato. Após seis meses de tratamento com AUDC, a ausência de resposta permitiu identificar adequadamente os pacientes que poderiam se beneficiar do início precoce de terapias de segunda linha, especialmente naqueles com doença avançada ou níveis basais elevados de enzimas hepáticas. Estádios histológicos avançados e não-adesão ao tratamento foram associados à não resposta primária ao AUDC, enquanto os níveis basais mais baixos de fosfatase alcalina e aspartato aminotransferase se correlacionaram com melhores respostas tanto ao AUDC quanto à combinação AUDC/fibratos.

Palavras-chave: Colangite Biliar Primária. Epidemiologia. Terapêutica. Fatores de Risco. Brasil. Dissertação Acadêmica.

Abstract

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease in which anti-mitochondrial antibodies (AMA) are the diagnostic hallmark. Little is known about this disease in highly admixed populations. The purpose of this study was to evaluate clinical features and outcomes of PBC in Brazil. 562 patients (95% females, mean age 51 ± 11 years) with PBC were included. Mean follow-up was 6.2 ± 5.3 years. 32% had cirrhosis, 7% underwent liver transplantation and 3% died of liver-related causes. Concurrent autoimmune diseases and overlap with autoimmune hepatitis occurred, respectively, in 18.9% and 14%. Subjects with AMA-negative PBC were significantly younger (52.2 ± 14 vs. 59.6 ± 11 years, $p = 0.001$) and had their first symptom at an earlier age (43.2 ± 13 vs. 49.5 ± 12 years, $p = 0.005$) in comparison to patients with AMA positive PBC. Lower Immunoglobulin M (272.2 ± 183 vs. 383.2 ± 378 mg/dL, $p = 0.01$) and triglycerides (107.6 ± 59.8 vs.129.3 ± 75.7 mg/dL, $p = 0.025$) and higher bilirubin (3.8 ± 13.5 vs. 1.8 ± 3.4 mg/dL, $p = 0.02$) levels were also observed in this subgroup. 96% of the patients were treated with ursodeoxycholic acid (UDCA) and 12% required add-on therapy with fibrate, either bezafibrate, fenofibrate or ciprofibrate. Response to UDCA and to UDCA/fibrates therapy varied from 39%-67% and 42-61%, respectively, according to different validated criteria. Ciprofibrate appears to be at least as effective as bezafibrate. After 6 months of treatment with UDCA, the absence of response could properly identify patients who could benefit from early addition of second-line therapies, especially among those with advanced disease or high baseline liver enzyme levels. Advanced histological stages and non-adherence to treatment were associated with primary non-response to UDCA, while lower baseline alkaline phosphatase and aspartate aminotransferase (AST) levels correlated with better responses to both UDCA and UDCA/fibrates.

Keywords: Primary Biliary Cholangitis. Epidemiology. Therapeutics. Risk Factors. Brazil. Academic Thesis.

SUMÁRIO

1. TÍTULO

Epidemiologia, estratificação de risco e tratamento da colangite biliar primária: estudo multicêntrico brasileiro.

2. CONSIDERAÇÕES INICIAIS

2.1. Introdução

A colangite biliar primária (CBP), previamente conhecida como cirrose biliar primária, é uma doença colestática crônica rara, imunomediada, que acomete ductos biliares intrahepáticos de pequeno e médio calibre, levando a sua destruição.[1] Dessa forma, se não tratada, pode levar a cirrose hepática e necessidade de transplante de fígado ou óbito. A epidemiologia da doença é muito heterogênea entre diferentes países. Acomete principalmente mulheres com mais de 40 anos de idade e apresenta o anticorpo anti-mitocôndria (AMA) como principal marcador sorológico.[2, 3]

Os principais sintomas da doença incluem prurido, fadiga, artralgia, desconforto abdominal, xerostomia, xeroftalmia, depressão, ansiedade e distúrbios do sono.[4] Frequentemente, a CBP se associa a outras doenças autoimunes como síndrome de Sjögren, tireoidite, doença celíaca, esclerose sistêmica, artrite reumatoide, entre outras.[5] Laboratorialmente, caracteriza-se pelo aumento persistente de fosfatase alcalina, gama-glutamiltransferase e das bilirrubinas.[6, 7] Pode, ainda, alterar o perfil lipídico, o que, muitas vezes, culmina na formação de xantomas e xantelasmas, mas sem associar-se a um aumento no risco cardiovascular.[8] Além disso, associa-se a osteopenia e osteoporose.[9, 10]

O tratamento de primeira linha da doença é feito com ácido ursodesoxicólico (AUDC), na dose de 13-15 mg/Kg/dia, o que aumenta a sobrevida livre de transplante hepático. Pacientes não respondedores ao AUDC podem se beneficiar da associação de fibratos ou ácido obeticólico (OCA).[6, 7, 9, 10] Diversos fatores parecem impactar o prognóstico na CBP, como o sexo, idade, grau de fibrose ao diagnóstico, rigidez hepática, padrão de anticorpo anti-núcleo, raça/etnia, níveis basais de fosfatase alcalina e bilirrubinas, dentre outros.[11-13]

No Brasil, o perfil clínico-epidemiológico da CBP é desconhecido. Não existiam políticas públicas de manejo da CBP no país até 2019, quando o Ministério da Saúde passou a recomendar e disponibilizar o tratamento com AUDC no Sistema Único de Saúde.[14] Diferenças na apresentação clínica e prognóstico da doença já foram previamente demonstradas em diversas populações.[12, 13, 15-17] As hepatopatias autoimunes, incluindo a CBP, correspondem a cerca de 7% das indicações de transplante hepático.[18] Dessa forma, avaliar as características da CBP no Brasil é de fundamental importância.

2.2. Antecedentes científicos

2.2.1 Aspectos históricos

Acredita-se que o primeiro caso de um paciente com sintomas semelhantes aos de CBP tenha sido descrito na literatura em 1851 por Addison e Gull.[19] No entanto, o termo cirrose biliar primária, amplamente utilizado até o ano de 2016, foi cunhado em 1949 por Dauphinee e Sinclair, quando a maioria dos casos reportados na literatura envolviam pacientes cirróticos, com icterícia, ascite e sangramento digestivo varicoso.[20] Após a descoberta do AUDC e sua aprovação para tratamento de primeira linha da CBP em 1994, observou-se uma melhora importante na sobrevida e qualidade de vida dos pacientes.[21] Paralelamente, a ampliação do acesso aos testes diagnósticos permitiu o reconhecimento mais precoce da doença. Enquanto metade dos pacientes diagnosticados com CBP na década de 1980 apresentavam um ou mais sintomas ao diagnóstico, após a década de 2000, 75-80% relatavam ser assintomáticos.[22] Diante desse novo cenário, especialistas se reuniram para propor uma nova nomenclatura para a doença, quando substituíram o termo cirrose por colangite e passaram a denominar a doença de CBP.[23-30] Além disso, a mudança do nome minimizou os estigmas associados ao termo cirrose frente a pacientes que já apresentam grande prejuízo global da qualidade de vida.[31]

2.2.2 Epidemiologia da colangite biliar primária

Todos os anos, pelo menos 100.000 pessoas em todo o mundo recebem o diagnóstico de CBP e os estudos sugerem que pelo menos uma em cada 1.000 mulheres acima da idade de 40 anos tenha a doença.[32] De maneira geral, a incidência e prevalência globais de CBP são estimadas em 17,6 por milhão de pessoas/ano e 146 por milhão, respectivamente.[33] No entanto, a prevalência e incidência da CBP apresentam grandes variações entre os diversos países, sendo aparentemente menores na região Ásia-Pacífico, exceto no Japão

e China, e maiores na América do Norte.[33] Embora essa discrepância possa ser atribuída a diferenças epidemiológicas verdadeiras, como aquelas relacionadas a diferentes etnias, background genético e exposição ambiental, é possível que variações nos desenhos dos estudos, nas definições de caso, no tamanho amostral e até mesmo relacionadas ao período estudado impactem nos resultados. Além disso, é possível que diferenças no acesso a saúde e qualidade do atendimento médico possam também interferir nessas estatísticas. Se por um lado, foi observada uma prevalência de 118,75 casos de CBP por milhão de habitantes e incidência de 8,55 casos por milhão/ano na região da Ásia-Pacífico[34], por outro, a prevalência na Europa e América do Norte foi estimada em 283-465 casos por milhão de habitantes, com uma incidência de aproximadamente 0,9-30,3 casos por milhão/ano.[32, 35, 36] Além disso, alguns estudos longitudinais realizados sempre nas mesmas regiões demonstraram um aumento consistente na prevalência, e por vezes, na incidência de CBP ao longo dos anos, o que pode se associar a melhorias no diagnóstico e tratamento da doença.[37-40]

Sabe-se ainda que a CBP acomete principalmente as mulheres, em uma proporção estimada de 9:1, com idade entre 40 e 60 anos. No entanto, alguns estudos notaram uma redução da razão mulher:homem nos últimos anos, por vezes com razões inferiores a 4:1, com maior mortalidade entre pacientes do sexo masculino.[41] A CBP apresenta como principal marcador sorológico o AMA, presente em mais de 90% dos pacientes e menos de 1% em controles.[42-44] Diferentemente, um estudo publicado na América Latina, incluindo mulheres colombianas com CBP, demonstrou uma positividade muito menor para o AMA (44,2%), sugerindo que possa haver diferenças locorregionais nos marcadores sorológicos.[45] No entanto, não existem dados sobre a epidemiologia da CBP na população brasileira, sabidamente composta por uma população altamente miscigenada e potencialmente singular no estudo dessa doença.

2.2.3 Etiopatologia da colangite biliar primária

A CBP é uma doença multifatorial, imunomediada, cuja fisiopatologia ainda não foi completamente elucidada. Acredita-se que a interação de fatores imunológicos, ambientais, genéticos e epigenéticos desempenhe um papel crucial no desenvolvimento da doença.[46] Caracteriza-se por uma perda de tolerância imunológica associada ao HLA-DR para uma enzima crucial da fosforilação oxidativa, a subunidade E2 do complexo piruvato desidrogenase.[47] O AMA reconhece uma família de enzimas localizadas na membrana interna da mitocôndria, denominada de complexo 2-oxo-ácido desidrogenase,

o qual inclui a subunidade E2 do complexo piruvato desidrogenase (PDC-E2), a subunidade E2 do complexo 2-oxo-ácido desidrogenase de cadeia ramificada (BCOADC-E2), a subunidade E2 do complexo 2-ácido oxoglutárico desidrogenase (OGDC-E2) e a proteína ligadora da dihidrolipoamida desidrogenase (E3BP).[2, 48] Acredita-se que a lesão biliar esteja relacionada a uma modificação aberrante na PDC-E2 mitocondrial de células epiteliais biliares apoptóticas, a qual resulta em um epítopo antigênico imunologicamente intacto em *blebs* apoptóticos. Este complexo imunogênico é reconhecido por anticorpos anti-mitocôndria circulantes e resulta em complexos antígeno-anticorpo.[49-51]

A resposta imune adaptativa implicada na patogênese da CBP não se restringe, no entanto, apenas aos anticorpos anti-mitocôndria altamente específicos, mas se associa ainda a infiltração de células T $CD4^+$ e células T $CD8^+$ antígeno-específicas nos tratos portais.[52, 53] Sabe-se ainda que na CBP ocorre uma redução, mediada pelo aumento do microRNA 506, na expressão do trocador de ânions Cl^-/HCO_3^- das células epiteliais biliares. Isso permite o acúmulo de bicarbonato intracelular, com entrada de sais biliares tóxicos de maneira descontrolada para o interior das células, indução de apoptose, e subsequente hiper expressão imunogênica de PDC-E2, perpetuando o processo de lesão biliar.[54-57]

Outras subpopulações de células T também exercem papéis importantes, incluindo células Th17 pró-inflamatórias, células T reguladoras que promovem auto-tolerância e células T auxiliares foliculares que facilitam a produção de anticorpos.[58-60] O receptor de quimiocina CXCR3, crítico no tráfego e diferenciação de células T, é superexpresso em células T efetoras e atua como receptor cognato para outras quimiocinas, incluindo CXCL9, CXCL10, CXCL11 e CX3CL1, as quais podem ser encontradas nos tratos portais de pacientes com CBP.[61] Subconjuntos de células T $CD8^+$ com um fenótipo de memória efetora são resistentes à apoptose, localizam-se ao redor dos tratos portais e reconhecem sequências antigênicas dentro do domínio lipoil de complexos mitocondriais, contribuindo assim para lesão biliar direcionada.[62, 63] Já no estádio avançado de fibrose, observa-se alteração do perfil de citocinas de Th1 para um perfil predominantemente Th17, dominado pelas subunidades IL-23p40 e IL-23p19, IL-6, IL-17 e TGFβ, que tem como alvo colangiócitos lesados.[60]

As células epiteliais biliares expressam receptores do tipo Toll, que quando ativados por uma variedade de ligantes, incluindo produtos microbianos como lipopolissacarídeos, podem contribuir para a lesão celular através da via mediada por NF-κB e pela liberação

de IL-8 e CX₃CL1 (fractalcina), os quais facilitam recrutamento de células linfoides efetoras para os tratos portais de pacientes com CBP.[64-66] Na presença de anticorpos anti-mitocôndria circulantes e células epiteliais biliares apoptóticas, os macrófagos regulam positivamente a expressão de citocinas pró-inflamatórias, especialmente IL-12; portanto, os macrófagos podem servir como um elo entre a lesão mediada pelo sistema imune inato e a apoptose de células epiteliais biliares.[51, 67] Além disso, foi demonstrado um aumento acentuado na frequência e número absoluto de células NK do sangue e do fígado de pacientes com CBP.[68] Dessa forma, a destruição de pequenos ductos biliares é mediada por uma resposta humoral e celular multilinhagem contra as células epiteliais biliares, envolvendo tanto o sistema imune inato quanto o adaptativo.

Fortes evidências apontam para a interação de fatores genéticos e ambientais na determinação do risco individual de se desenvolver CBP. A avaliação de risco de gêmeos e familiares, inicialmente, apoiou a ideia de que a CBP tinha uma forte predisposição genética. De fato, gêmeos monozigóticos mostram uma taxa de concordância de 0,63 para desenvolvimento de CBP, uma das mais altas relatadas em autoimunidade. Além disso, história familiar de CBP é relatada em 1,33 a 9% dos casos.[32, 69] Estudos genéticos realizados em diferentes etnias sugerem que as associações mais fortes são derivadas do HLA, em especial: DRB1*08, DR3, DPB1*0301, DRB1*08-DQA1*0401-DQB1*04.[70, 71] Além disso, loci gênicos relacionados à regulação de componentes do sistema imunológico também parecem estar envolvidos na CBP. Um estudo recém-publicado de associação genômica ampla, identificou entre 10.516 indivíduos com CBP, recrutados no Canadá, China, Itália, Japão, Reino Unido e Estados Unidos da América, 56 *loci* de interesse. Foi observada uma associação genética significativa entre CBP e outras doenças imunomediadas, incluindo lúpus eritematoso sistêmico, artrite reumatoide e doença inflamatória intestinal. Os autores demonstraram ainda que existe uma arquitetura genética muito semelhante da CBP em indivíduos de origem europeia e asiática.[72]

O papel do meio ambiente na gênese da CBP também é amplamente estudado. Evidências têm implicado vários fatores, incluindo status socioeconômico, tabagismo, história de colestase na gestação, agentes infecciosos, poluentes ambientais (ex: resíduos tóxicos, mineração de carvão, cádmio), vitamina D, nutrição, drogas, estressores físicos e psicológicos.[73-77] Alguns estudos demonstraram um relevante papel da infecção do trato urinário pela bactéria *Escherichia coli* no aumento do risco de CBP através de mimetismo molecular entre PDC-E2 humano e bacteriano.[78-80] Por fim, observa-se que pacientes com

CBP apresentam disbiose intestinal, a qual é parcialmente resolvida pelo tratamento com AUDC.[81] A alteração na microbiota intestinal pode impactar significativamente no tipo e quantidade de ácidos biliares presentes no intestino, influenciando o balanço imunológico do eixo enterohepático.[82] Além disso, altera a permeabilidade intestinal, com aumento da difusão de padrões moleculares associados a patógenos (PAMP), ácidos graxos livres e endotoxinas na vasculatura portal e sinusóides hepáticos.[83-85]

2.2.4 Apresentação clínica

A CBP pode subdividida em 4 fases evolutivas.[86] Na fase silenciosa, apenas a pesquisa do AMA é positiva. Já a fase assintomática caracteriza-se pela elevação da fosfatase alcalina na ausência de sintomas. Na fase sintomática, os sintomas mais frequentes são o prurido (20-70%) e a fadiga (50-80%) e podem impactar negativamente na qualidade de vida do paciente. Cerca de 17% dos pacientes podem apresentar dor inespecífica no hipocôndrio direito.[6] A icterícia é sinal de doença em fase avançada. A fase final caracteriza-se pelas complicações da hipertensão portal e insuficiência hepática, por vezes com indicação de transplante hepático.

A hiperlipidemia é comum em pacientes com CBP. Pode causar xantelasmas e xantomas devido à marcante elevação do colesterol, a qual parece não conferir um aumento risco de doença cardiovascular.[87] Quando comparados a pessoas saudáveis da mesma idade, pacientes com CBP são mais propensos a ter osteodistrofia hepática, como a osteoporose, a qual afeta 20 a 44% dos pacientes.[88] Pode ainda ocorrer má absorção de vitaminas lipossolúveis devido à diminuição da secreção de ácido biliar, embora deficiências significativas das vitaminas A, D, E e K sejam incomuns.[89] Além disso, alguns estudos observaram um aumento global no risco de neoplasias, enquanto a subpopulação de pacientes cirróticos apresenta maior risco de desenvolver carcinoma hepatocelular.[90]

2.2.5 Diagnóstico da colangite biliar primária

O diagnóstico de CBP deve ser suspeitado em pacientes com colestase crônica após exclusão de outras causas de doença hepática, particularmente em mulheres de meia-idade com elevação inexplicada de fosfatase alcalina sérica. A avaliação inicial dos pacientes deve ser baseada na história clínica, exame físico, exames laboratoriais e ultrassonografia abdominal. Atualmente, um número crescente de pacientes é diagnosticado em um estádio inicial da doença, ainda em fase assintomática.[22, 91] Várias doenças autoimunes extra-hepáticas podem coexistir com a CBP, como síndrome de

Sjögren, tireoidite autoimune, esclerose sistêmica, doença de Raynaud, artrite reumatoide, lúpus eritematoso sistêmico e doença celíaca.[5] A maioria dos pacientes com CBP apresenta alteração do perfil bioquímico hepático, com elevação mais importante de fosfatase alcalina e gama-glutamiltransferase, além de discreta alteração de alanina aminotransferase e/ou aspartato aminotransferase. Uma elevação policlonal de imunoglobulina M é característica da CBP e pode ser útil para corroborar o diagnóstico clínico em pacientes com características atípicas.[6, 7, 9, 10]

Os critérios diagnósticos são consensuais entre as diversas sociedades de hepatologia do mundo e são também adotados no Brasil.[92] Segundo os critérios da *American Association for the Study of Liver Diseases*, a CBP é diagnosticada pela presença de pelo menos dois de três critérios: 1) sorologia positiva para AMA ou anticorpo anti-núcleo específico para CBP (anti-sp100 ou anti-gp210); 2) aumento persistente de fosfatase alcalina sérica; 3) histologia hepática compatível com CBP, com colangite destrutiva não supurativa e destruição de ductos interlobulares.[6] Dessa forma, a biópsia hepática não deve ser realizada rotineiramente, ficando reservada para avaliação de diagnóstico diferencial ou síndrome de sobreposição (especialmente se alanina aminotransferase superior a 5 vezes o limite superior da normalidade) e para casos que não apresentam os autoanticorpos marcadores da doença.[93] Deve-se ressaltar também que a presença isolada do AMA, na ausência de alteração da fosfatase alcalina, não é suficiente para o diagnóstico, uma vez que apenas um em cada seis pacientes com AMA positivo e fosfatase alcalina normal desenvolvem CBP em cinco anos.[94] No entanto, duas coortes retrospectivas, uma suíça e outra chinesa, encontraram alterações histológicas compatíveis com CBP em pacientes com AMA positivo e fosfatase alcalina normal, um cenário não explorado pelas diretrizes internacionais vigentes.[95, 96]

2.2.6 Marcadores sorológicos na colangite biliar primária

O AMA constitui uma das características mais marcantes da CBP, sendo encontrado em mais de 90-95% dos pacientes. A reatividade do AMA na CBP está direcionada a diferentes antígenos do complexo 2-oxoácido desidrogenase da membrana mitocondrial interna.[97] Assim, sua positividade pode variar de acordo com o método de pesquisa empregado. Geralmente utiliza-se inicialmente a imunofluorescência indireta. Vários subtipos de AMA foram identificados (tradicionalmente chamado M1-M9) por métodos de imunoblotting ou ELISA.[98] Nesta classificação histórica, anticorpos dirigidos contra M2, M4, M8 e M9 estão associados a CBP.[44] Uma vez que o anticorpo anti-M2 é o mais

específico para CBP, a pesquisa do subtipo M2 de AMA por ELISA pode ser recomendada quando o AMA por IFI é negativo.[99] O nível sérico de anti-mitocôndria não reflete a gravidade ou prognóstico da doença, sendo a presença ou ausência do AMA importante somente para o diagnóstico.[100] Aproximadamente 5-10% dos pacientes com CBP podem ter AMA negativo ou em baixos títulos à imunofluorescência indireta.[101] Embora os pacientes com CBP AMA negativo sejam muito semelhantes àqueles com AMA positivo, algumas diferenças já foram descritas, como maior prevalência de anticorpos antinucleares e antimúsculo liso e níveis séricos mais baixos de IgM. Além disso, pacientes AMA negativo, aparentemente, apresentam maior prevalência de doenças autoimunes extra-hepáticas concomitantes.[101, 102] A resposta ao tratamento com AUDC parece ser similar entre os grupos, embora ainda seja controversa a existência de diferenças em relação a desfechos clínicos.[103-105] O AMA é ainda detectado em 1% ou menos das pessoas saudáveis, as quais apresentam maior risco de desenvolver CBP a longo prazo e requerem acompanhamento próximo.[43, 94]

A sensibilidade e especificidade dos anticorpos anti-mitocôndria na CBP dependem do método utilizado. Existem diversas estratégias para se detectar o AMA na prática clínica, sendo as mais comuns: imunofluorescência indireta, immunoblotting, imunoensaio enzimático, ensaio baseado em esferas magnéticas Luminex e ensaio de inibição enzimática[6]. A maioria dos laboratórios de rotina utiliza imunofluorescência indireta com título de corte de 1:40 e sensibilidade e especificidade em torno de 88,0% e 97,0%, respectivamente; no entanto, sugere-se o uso de um título de corte maior de 1:80 para o máximo benefício a ser obtido.[106-108] Sabe-se que aproximadamente 25% dos pacientes com AMA negativo por imunofluorescência indireta apresentam reatividade ao MIT3 (combinação de PDC-E2, BCOADC-E2, OGDC-E2) por ELISA, uma combinação de três antígenos mitocondriais, podendo esse valor alcançar 43% quando combinado MIT3 com gp210 e sp100.[109]

Mais da metade dos indivíduos com CBP AMA positivo apresentam anticorpo anti-núcleo, o qual é ainda mais comum entre pacientes com CBP AMA negativo (60% a 100%).[110] Vários perfis de anticorpo anti-núcleo podem ser encontrados na CBP. Os padrões mais específicos são o membrana nuclear e o nuclear pontos isolados à imunofluorescência, os quais correspondem ao anti-gp210 e anti-sp100 por ELISA, respectivamente.[111] A presença dos anticorpos anti-sp100 e anti-gp210 foi associada em diversos estudos a pior prognóstico.[112-114] Mais recentemente, outros marcadores

sorológicos anti-núcleo foram descritos em pacientes com CBP, incluindo o anti-p62, anti-*kelch-like* 12 e anti-hexoquinase 1, encontrados em 25%, 35% e 22% dos pacientes com CBP AMA negativo, respectivamente.[115, 116] O anti-hexoquinase 1 também foi implicado em menor sobrevida livre de transplante e pior resposta ao tratamento com AUDC.[117] Além disso, o anticorpo anti-músculo liso, mais comum na hepatite autoimune, é descrito em até 20%-40% dos pacientes com CBP AMA negativo, comparado a 10% daqueles com AMA positivo.[103] Nesse cenário, muitas vezes é necessária a realização de biópsia hepática para diagnóstico diferencial. Finalmente, o anticorpo anti-centrômero também tem sido ocasionalmente descrito em pacientes com CBP, sendo associado a um pior prognóstico e maior risco de desenvolvimento de doença renal crônica.[118-120]

2.2.7 Histologia

A biópsia hepática não deve ser rotineiramente realizada em pacientes com CBP na forma clássica.[6] No entanto, quando realizada, apresenta achados histológicos típicos que corroboram o diagnóstico e permitem o estadiamento da doença. Do ponto de vista histológico, a CBP é caracterizada por colangite crônica não supurativa que acomete os ductos biliares interlobulares e septais. Quando existem lesões focais exibindo intensas alterações inflamatórias e necrose ao redor dos ductos biliares, o termo "lesão ductal florida" é utilizado. O infiltrado inflamatório está em contato próximo com a membrana basal de colangiócitos em necrose e consiste em plasmócitos, macrófagos e células polimorfonucleares (especialmente eosinófilos). Granulomas epitelióides podem estar presentes, especialmente, na fase inicial da doença. Existem poucas (se houver) lesões arteriais. Em contraste, as vênulas portais são frequentemente comprimidas e ocluídas pela reação inflamatória. Ductopenia é geralmente definida quando os ductos biliares apenas podem ser identificados em menos de 50% dos tratos portais. Deve-se destacar que as alterações histológicas podem ter distribuição altamente irregular no fígado, com relatos de pacientes com todos os estádios da doença encontrados no mesmo órgão explantado no transplante hepático.[121, 122]

As lesões histológicas são classicamente divididas em 4 estádios. O estádio I é caracterizado por inflamação restrita ao espaço portal com ou sem lesões ductais floridas. A progressão da doença é caracterizada por aumento gradual das lesões periportais, as quais se estendem ao parênquima hepático, como hepatite de interface (estádio II). Pode haver ainda proliferação ductular. A gravidade da hepatite de interface é altamente preditiva do desenvolvimento de fibrose. O estádio III é caracterizado por uma distorção

da arquitetura hepática com numerosos septos fibrosos, enquanto a cirrose com nódulos de regeneração define o estádio IV.[123]

As classificações de Scheuer ou Ludwig *et al.* são as mais utilizadas para classificação histológica da CBP na prática clínica.[123, 124] No entanto, uma significativa sobreposição de achados entre os estádios limita essas classificações.[125] Dessa forma, Nakanuma *et al.*, propuseram um novo sistema de estadiamento histológico.[126] Na classificação de Nakanuma, escores de fibrose, perda de ductos biliares e deposição de grânulos positivos para orceína são utilizados para o estadiamento, enquanto atividade de colangite e atividade de hepatite são empregados para classificação. A atividade de colangite é determinada pela presença de colangite crônica ou presença de lesões ductais floridas, e a atividade de hepatite é definida pela presença de hepatite de interface ou hepatite lobular.[127] Estudos mais recentes demonstram que a sobrevida global e desfechos adversos são mais bem estratificados com a classificação de Nakanuma do que com o sistema clássico.[125, 128-130]

2.2.8 Tratamento da colangite biliar primária

2.2.8.1. Primeira linha

O AUDC é indicado como tratamento de primeira linha da CBP pelas principais diretrizes de tratamento, independentemente do estádio histológico da doença.[6, 7, 9, 10, 131] Trata-se de um ácido biliar terciário hidrofílico, epímero 7-b do ácido quenodesoxicólico, com propriedades coleréticas, citoprotetoras, imunomoduladoras e anti-inflamatórias, sendo bem tolerado pela maioria dos pacientes.[132] Deve ser administrado continuamente na dose de 13 a 15mg/kg por dia, em dose única ou duas doses. Angulo *et al.* demonstraram que o AUDC em baixa dose (5~7 mg/kg por dia) é menos eficaz, enquanto em alta dose (23~25 mg/kg por dia) não traz benefícios adicionais.[133] Efeitos colaterais são mínimos e infrequentes, havendo relatos de pequeno ganho de peso no primeiro ano de tratamento e efeitos gastrointestinais leves. Diversos ensaios clínicos randomizados e meta-análises demonstraram que o AUDC pode melhorar o perfil bioquímico hepático, interromper a progressão da doença e prolongar a sobrevida livre de transplante a longo prazo, independentemente de idade, sexo e estadiamento da doença.[134-140] Recentemente, Harms *et al.* demonstraram uma razão de risco para óbito ou transplante no tratamento da CBP com AUDC entre 3902 pacientes analisados de 0,46 (IC95% 0,40 a 0,52).[141] A sobrevida livre de transplante em 10 anos foi de 79,7% (IC 95% 78,1-81,2) entre os pacientes tratados com AUDC e 60,7% (IC 95% 58,2-63,4) entre pacientes não

tratados.[140] O número necessário para tratar (NNT) para prevenir um transplante ou morte em 5 anos foi estimado em 11 pacientes (IC95% 9 a 13), sendo maior para pacientes cirróticos [NNT = 20 (IC95% 14 a 34)], em comparação aos não-cirróticos [NNT = 4 (IC95% 3 a 5)]. Quando estratificado pelo nível de fosfatase alcalina, em baixa (≤ 2x o limite superior da normalidade), intermediária (2–4x) e alta (> 4x), o NNT foi de 26 (IC95% 15 a 70), 11 (IC95% 8 a 17) e 5 (IC95% 4 a 8), respectivamente.[141] Interessante destacar que mesmo pacientes com resposta incompleta ao tratamento com AUDC apresentam maior sobrevida livre de transplante/morte (razão de risco 0,56; IC95% 0,45-0,69) em comparação a pacientes com CBP não tratados, motivo pelo qual tratamentos de segunda e terceira linha devem ser sempre associados ao AUDC.[140]

2.8.1.2. Segunda linha

Aproximadamente 30 a 40% dos pacientes com CBP apresentarão resposta incompleta ao AUDC, o que foi associado a menor sobrevida global.[140] Ao avaliar o paciente sem resposta ao AUDC, é importante checar a adesão ao tratamento e excluir a possibilidade de associação com outras enfermidades, como doença celíaca, esteatohepatite ou síndrome de sobreposição com hepatite autoimune. Ensaios clínicos randomizados demonstraram potencial terapêutico para ácido obeticólico (OCA), diferentes fibratos e budesonida.

- Ácido obeticólico

O OCA (ácido 6α-etil-quenodesoxicólico) é um análogo de ácido biliar hidrofóbico semissintético que é altamente seletivo para o receptor farnesóide X (RFX), um receptor abundantemente expresso no fígado e enterócitos. A via de sinalização celular mediada pelo RFX, além de regular diretamente os genes envolvidos na síntese de ácidos biliares, apresenta efeitos anti-inflamatórios e anti-fibrosantes.[142] Recentemente, foi publicado um estudo de fase III do OCA em pacientes com CBP e resposta incompleta ao AUDC (POISE trial).[143] Nesse trabalho, os autores demonstraram que, após 12 meses de terapia com OCA, quase metade dos pacientes com CBP não respondedores ao AUDC alcançaram resposta terapêutica satisfatória (47% x 10% no grupo placebo). Embora o OCA tenha sido bem tolerado, prurido e fadiga foram eventos adversos comuns. OCA, na dose de 5-10mg/dia, recebeu aprovação acelerada do *Food and Drug Administration* (FDA) dos Estados Unidos em 27 de maio de 2016, mas ainda não foi aprovado no Brasil. Resposta bioquímica sustentada foi, posteriormente, confirmada em um estudo aberto de

extensão com duração de 3 anos.[144] Kowdley *et al.* demonstraram também eficácia do OCA em monoterapia de pacientes com CBP virgens ou não de AUDC, com redução de 53,9% na fosfatase alcalina em relação ao basal após 3 meses de tratamento.[145] Apesar dos resultados favoráveis do ponto de vista bioquímico, ainda não existem estudos de eficácia para desfechos clínicos de longo prazo, como sobrevida livre de transplante. Recentemente, o próprio FDA emitiu alertas quanto à contraindicação de uso do OCA em pacientes com cirrose avançada, no qual o medicamento se associou a piora clínica e morte.

- Fibratos

Os fibratos são agonistas de receptores ativados por proliferadores de peroxissoma (PPAR) e receptor pregnano X, com eficácia comprovada nas dislipidemias. Os PPARs existem em três isoformas: α, δ e γ, sendo que os fibratos apresentam diferentes afinidades por esses receptores. O PPAR-α, em particular, regula a síntese e desintoxicação de ácidos biliares, a secreção de fosfolipídios e as vias inflamatórias, enquanto o PPAR-δ e -γ tem efeitos mais profundos sobre o metabolismo de lipídios e glicose e propriedades anti-inflamatórias e anti-fibrosantes.[146]

O bezafibrato, um pan-agonista de PPAR, foi o primeiro fibrato a demonstrar eficácia no tratamento de pacientes com CBP não respondedores ao AUDC em 1999[147]. Posteriormente, em um ensaio clínico randomizado placebo controlado de fase III (BEZURSO), a combinação de AUDC e bezafibrato (400mg/dia) se associou a normalização de fosfatase alcalina em 67% dos pacientes e de toda bioquímica hepática em 31% dos pacientes com CBP não respondedora ao AUDC pelo critério de Paris II[148]. Além disso, foi observada melhora no prurido, o que foi comprovado no estudo FITCH, publicado alguns anos depois.[149] Recentemente, Tanaka *et al.* demonstraram que o uso de bezafibrato é associado a redução significativa de mortalidade por todas as causas ou necessidade de transplante hepático em pacientes com CBP (razão de risco ajustado: 0,3253, IC95% 0,1936–0,5466 e 0,2748, IC95% 0,1336–0,5655, respectivamente; p < 0,001 para ambos). Os NNTs da terapia combinada para prevenir 1 morte adicional ou transplante hepático em 5, 10 e 15 anos foram de 29 (IC95% 22–46), 14 (10–22) e 8 (6–15), respectivamente.[150]

Outro fibrato muito estudado para tratamento da CBP é o fenofibrato, um agonista de PPARα. Em um estudo piloto aberto com 20 pacientes com fosfatase alcalina superior a duas vezes o limite superior do normal após tratamento com AUDC, o uso de fenofibrato 160 mg/dia por 48 semanas reduziu a fosfatase alcalina sérica em aproximadamente 50%.[151] Posteriormente, diversos estudos retrospectivos com fenofibrato corroboraram esses achados, com redução de fosfatase alcalina em até 90% dos pacientes não respondedores ao AUDC.[152-154]

Recentemente, foram publicados alguns pequenos estudos utilizando o pemafibrato, um modulador alfa seletivo de receptores ativados por proliferadores de peroxissoma (SPPARM-α), para tratamento de pacientes com CBP não respondedores a AUDC. Assim como os demais fibratos, o pemafibrato reduziu os níveis de fosfatase alcalina e gama-glutamiltransferase em até 87% dos pacientes após 48 semanas de tratamento.[155-157] Além disso, observou-se menor alteração dos níveis de creatinina, uma vez que, diferentemente do bezafibrato e fenofibrato que são majoritariamente excretados pelos rins, o pemafibrato é metabolizado no fígado.[158]

Com relação à segurança no uso de fibratos, elevações de aminotransferases foram relatadas em pacientes com CBP tratados com fenofibrato (8%) e bezafibrato (2%). Da mesma forma, elevações de bilirrubina também foram observadas, especialmente em indivíduos em uso de fenofibrato (1,1%). Alguns relatos de caso demonstram que a injúria hepática induzida por fibrato pode ter grande período de latência.[159] Elevações de creatinina sérica também podem ocorrer durante o tratamento com fibratos e foram mais comuns em pacientes tratados com fenofibrato (2,9%) em comparação com bezafibrato (0,4%). Por fim, os fibratos aumentam o risco de miopatia na população em geral, incluindo rabdomiólise, o que parece ser mais comum em pacientes com diabetes mellitus, insuficiência renal e hipotireoidismo (comumente encontrado em pacientes com CBP). Mialgias e artralgias foram relatados em 4,8% dos tratados com bezafibrato e 2,2% dos tratados com fenofibrato (revisado em [160]).

- Budesonida

A budesonida é um glicocorticoide não halogenado, absorvido no intestino delgado e metabolizado em sua primeira passagem pelo fígado. Um possível benefício do uso de budesonida foi demonstrado em pacientes com CBP não-cirróticos e maior evidência de atividade inflamatória de interface na biópsia. Nesta subpopulação, a budesonida reduziu

em 48% dos pacientes a fosfatase alcalina em pelo menos 40%, sendo que 35% alcançaram sua normalização. No entanto, não foi observada melhora histológica significativa, comparada ao placebo, desfecho primário do estudo. Além disso, 20% dos pacientes no grupo budesonida apresentaram efeitos adversos graves não relacionados a biópsia hepática.[161]

2.8.1.2. Terceira linha

Apesar da terapia combinada de AUDC com OCA ou fibratos, um subgrupo de pacientes continuará a apresentar alterações bioquímicas e sinais de progressão da doença, incluindo aumento progressivo da rigidez hepática e/ou desenvolvimento de hipertensão portal. Estudos recentes sugerem que esses pacientes podem se beneficiar dos efeitos benéficos aditivos dos três medicamentos administrados em conjunto (terapia tripla – AUDC + OCA + fenofibrato ou bezafibrato), com melhorias significativas na fosfatase alcalina, gama-glutamiltransferase, bilirrubina e aminotransferases.[162, 163]

2.2.9 Principais critérios de resposta

Vários critérios de resposta bioquímica, divididos em critérios binários e dinâmicos (contínuos), foram propostos para ajudar na estratificação de risco de pacientes com CBP e identificar aqueles que precisam de terapia de segunda linha (**Tabela 1**). Esses escores foram originalmente desenvolvidos para avaliar a resposta bioquímica ao AUDC e, portanto, para prever a sobrevida livre de transplante a longo prazo. Existem pelo menos nove classificações binárias, incluindo os critérios de Mayo[164], Rochester[165], Rotterdam[166], Barcelona[167], Paris I[168] e II[169], POISE trial[143] e Ehime[170], a maioria deles avaliando a resposta após um ano de tratamento (variação 6 meses-24 meses). Dentre esses critérios, algumas peculiaridades devem ser destacadas. Os critérios de Paris I e Paris II são critérios bem validados e amplamente utilizados de resposta bioquímica, porém em pacientes com CBP avançada (estádio III-IV) e precoce (estádio I-II), respectivamente.[168, 169] Já o critério de Toronto foi criado com objetivo de prever a progressão do estádio histológico, mas não as complicações clínicas, após 24 meses de tratamento com AUDC.[171] Recentemente, foram também desenvolvidos sistemas de pontuação contínua, a saber o escores GLOBE[172] e o UK-PBC[173]. Esses modelos contínuos demonstraram alta especificidade e sensibilidade para predizer sobrevida livre de transplante e foram validados em uma grande coorte internacional.

Sabe-se que 30-40% dos pacientes com CBP apresentam respostas bioquímicas incompletas ao AUDC e permanecem em risco de progressão da doença para estádios avançados, incluindo cirrose. No entanto, não existe consenso entre as diferentes diretrizes internacionais sobre qual critério utilizar na avaliação de resposta ao tratamento com AUDC. Em 2014, uma metanálise foi realizada para determinar a aplicabilidade da fosfatase alcalina e bilirrubina como desfechos substitutos em ensaios clínicos.[174] Em 1 ano, fosfatase alcalina maior que 2 vezes o limite superior da normalidade foi o melhor preditor de desfecho (óbito ou transplante), enquanto bilirrubina maior que 1 vez o limite superior da normalidade o melhor preditor de sobrevida livre de transplante. Curiosamente, um estudo recente derivado do mesmo conjunto de dados sugere que os objetivos devam ser mais rigorosos, uma vez que a sobrevida melhora ainda mais em pacientes com fosfatase alcalina dentro da faixa normal e/ou bilirrubina menor que 0,6 vezes o limite superior da normalidade.[175] Por fim, recentemente, Carbone *et al.* desenvolveram um interessante modelo (UDCA Response Score) capaz de predizer a resposta pré-tratamento ao AUDC. Assim, esse escore permitiria aos médicos identificar pacientes com características basais de alto risco de resposta incompleta ao AUDC e iniciar precocemente uma droga de segunda linha em associação.[176].

Tabela 1 – Critérios de resposta ao tratamento com AUDC

Nome do Critério	Tempo para avaliação	Definição
Binários		
Barcelona	12 meses	Redução > 40% ou normalização da fosfatase alcalina
Ethime	6 meses	Redução ≥ 70% ou normalização da gama-glutamiltransferase
Mayo	6 meses	Fosfatase alcalina < 2x o limite superior da normalidade
Paris I	12 meses	Fosfatase alcalina ≤ 3x o limite superior da normalidade e aspartato aminotransferase ≤ 2x o limite superior da normalidade e normalização da bilirrubina
Paris II	12 meses	Fosfatase alcalina e aspartato aminotransferase ≤ 1,5x o limite

		superior da normalidade e normalização da bilirrubina
Rochester	12 meses	Fosfatase alcalina ≤ 1,67x o limite superior da normalidade e bilirrubina ≤ 1 mg/dL
POISE trial	12 meses	Fosfatase alcalina menor que 1,67x o limite superior da normalidade, com redução de pelo menor 15% em relação ao basal e normalização da bilirrubina
Rotterdam	12 meses	Normalização da bilirrubina e/ou albumina alteradas antes do tratamento
Toronto	24 meses	Fosfatase alcalina ≤ 1,67x o limite superior normalidade
Dinâmicos		
GLOBE	12 meses	Idade ao diagnóstico; níveis de fosfatase alcalina, bilirrubina, albumina e contagem de plaquetas aos 12 meses
UK-PBC	12 meses	Albumina e contagem de plaquetas basal; Fosfatase alcalina, bilirrubina e alanina ou aspartato aminotransferase aos 12 meses

2.2.10 Principais fatores preditores de resposta ao tratamento

O diagnóstico mais precoce e a descoberta de terapias eficazes contra a CBP foram responsáveis por uma importante melhoria no prognóstico da doença nas últimas décadas. No entanto, diversos fatores foram identificados como preditores de pior resposta ao tratamento. Alguns estudos demonstraram que pacientes com CBP do sexo masculino apresentam maior risco de doença avançada ao diagnóstico, carcinoma hepatocelular e não-resposta ao tratamento com AUDC, o que acarreta uma sobrevida livre de transplante significativamente menor em comparação às mulheres.[35, 41, 177-181] Diferenças de resposta ao tratamento com AUDC foram descritas para diferentes etnias. Levy *et al*. demonstraram que os hispânicos têm reduções menores na fosfatase alcalina em comparação com pacientes não hispânicos, além de maior frequência de síndrome de sobreposição com hepatite autoimune (31% vs 13%), porém sem diferenças na mortalidade.[15] Por outro lado, Gordon *et al*. reportaram maior mortalidade entre afro-

americanos e asiáticos-americanos com CBP não tratados com AUDC, comparados a brancos, em uma coorte retrospectiva de 4238 pacientes.[12]

A idade ao diagnóstico, especialmente para pacientes com menos de 30-35 anos, é também um importante determinante no risco de falha terapêutica, transplante hepático e morte, além de se associar a atividade inflamatória hepática.[179, 182] Além disso, o consórcio UK-PBC demonstrou que os pacientes mais jovens apresentam prurido e fadiga mais graves.[179] Alguns estudos sugeriram que a coexistência da CBP com hepatite autoimune ou síndrome de Sjögren está relacionada a menor sobrevida global.[183, 184] Híndi *et al.* observaram maior grau de lesão a ductos biliares e fibrose em pacientes portadores de CBP sobreposta a esteatohepatite não-alcoólica e índice de massa corporal ≥ 25.[185] Esse fator é de especial relevância na população brasileira, cuja prevalência de obesidade supera 25%.[186]

Do ponto de vista bioquímico, a fosfatase alcalina e bilirrubina são considerados os mais importantes marcadores de atividade e prognóstico da CBP. Murillo-Perez *et al.* relataram, recentemente, que níveis de bilirrubina ≤ 0,6 vezes o limite superior da normalidade estão associados a menor risco de transplante hepático ou morte, em comparação com pacientes com bilirrubina acima desse limiar. Além disso, a normalização da fosfatase alcalina também se associou a melhora da sobrevida[175]. De maneira similar, uma metanálise, com 4845 pacientes com CBP, revelou que níveis de fosfatase alcalina basais menores que 2 vezes o limite superior da normalidade são preditores de maior sobrevida livre de transplante em 5, 10 e 15 anos (94%, 84%, e 73%, versus 81%, 62% e 50% no subgrupo de pacientes com fosfatase alcalina superior a 2 vezes o limite superior da normalidade, respectivamente).[174] Já a elevação de gama-glutamiltransferase ao diagnóstico e após um ano de tratamento se associou a maior risco de transplante e morte em algumas coortes retrospectivas, possivelmente por refletir maior lesão colestática ao fígado.[187, 188] Do ponto de vista sorológico, pacientes com anticorpos anti-gp-210 e anti-centrômero apresentam maior risco de progressão da doença para transplante ou morte.[113]

A presença de fibrose avançada é outro marcador prognóstico importante na CBP. Corpechot *et al.* demonstraram que indivíduos com CBP e valores de rigidez hepática superiores a 9,6 kPa à elastografia transitória apresentam risco 5,1 vezes maior de desfechos adversos (descompensação, transplante ou morte). Além disso, um aumento na

rigidez hepática superior a 2,1 kPa/ano também se traduz em pior prognóstico.[189] Recentemente, Coperchot *et al.* demonstraram que os valores de corte à elastografia hepática transitória < 8, 8 a < 15 e ≥ 15 kPa são capazes de estratificar os pacientes em baixo, médio e alto risco de desfechos adversos (complicações hepáticas, transplante de fígado ou óbito).[190] Diversos estudos permitiram observar associação entre a presença de cirrose hepática ao diagnóstico e menor sobrevida livre de transplante.[191] Finalmente, do ponto de vista histológico, observa-se que a presença de hepatite de interface e ductopenia aumenta o risco de cirrotização a longo prazo[168, 171, 192].

2.3. Referências bibliográficas

1. Lleo A, Wang GQ, Gershwin ME, et al. Primary biliary cholangitis. Lancet 2020;396:1915-1926.
2. Gershwin ME, Mackay IR, Sturgess A, et al. Identification and specificity of a cDNA encoding the 70 kd mitochondrial antigen recognized in primary biliary cirrhosis. J Immunol 1987;138:3525-31.
3. Griffiths L, Dyson JK, Jones DE. The new epidemiology of primary biliary cirrhosis. Semin Liver Dis 2014;34:318-28.
4. Yagi M, Tanaka A, Abe M, et al. Symptoms and health-related quality of life in Japanese patients with primary biliary cholangitis. Sci Rep 2018;8:12542.
5. Chalifoux SL, Konyn PG, Choi G, et al. Extrahepatic manifestations of primary biliary cholangitis. Gut Liver 2017;11:771-780.
6. Lindor KD, Bowlus CL, Boyer J, et al. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. Hepatology 2019;69:394-419.
7. You H, Ma X, Efe C, et al. APASL clinical practice guidance: the diagnosis and management of patients with primary biliary cholangitis. Hepatol Int 2022;16:1-23.
8. Longo M, Crosignani A, Battezzati PM, et al. Hyperlipidaemic state and cardiovascular risk in primary biliary cirrhosis. Gut 2002;51:265-9.
9. Hirschfield GM, Dyson JK, Alexander GJM, et al. The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines. Gut 2018;67:1568-1594.
10. Lindor KD, Bowlus CL, Boyer J, et al. Primary biliary cholangitis: 2021 practice guidance update from the American Association for the Study of Liver Diseases. Hepatology 2022;75:1012-1013.
11. Goet JC, Harms MH, Carbone M, et al. Risk stratification and prognostic modelling in primary biliary cholangitis. Best Pract Res Clin Gastroenterol 2018;34-35:95-106.
12. Gordon SC, Wu KH, Lindor K, et al. Ursodeoxycholic acid treatment preferentially improves overall survival among african americans with primary biliary cholangitis. Am J Gastroenterol 2020;115:262-270.
13. Murillo Perez CF, Gulamhusein A, Carbone M, et al. Simplified care-pathway selection for nonspecialist practice: the GLOBAL Primary Biliary Cholangitis Study Group Age, Bilirubin, Alkaline phosphatase risk assessment tool. Eur J Gastroenterol Hepatol 2021;33:e266-e273.
14. Brasil. Ministério da Saúde. Secretaria de Ciência T, Inovação e Insumos Estratégicos em Saúde. Departamento de Gestão e Incorporação de Tecnologias e Inovação em Saúde. Protocolo Clínico e Diretrizes Terapêuticas da Colangite Biliar Primária [recurso eletrônico]. In: Ministério da Saúde SdC, Tecnologia, Inovação e Insumos Estratégicos em Saúde, Departamento de Gestão e Incorporação de Tecnologias e Inovação em Saúde, ed. Brasília: Ministério da Saúde, 2020:21p.
15. Levy C, Naik J, Giordano C, et al. Hispanics with primary biliary cirrhosis are more likely to have features of autoimmune hepatitis and reduced response to ursodeoxycholic acid than non-Hispanics. Clin Gastroenterol Hepatol 2014;12:1398-405.
16. Rabiee A, Polanco NAP, Vara AF, et al. Hispanic patients with primary biliary cholangitis have decreased access to care compared to non-hispanics. J Clin Transl Hepatol 2020;8:391-396.
17. Galoosian A, Hanlon C, Tana M, et al. Race/ethnicity and insurance-specific disparities in in-hospital mortality among adults with primary biliary cholangitis: analysis of 2007-2014 national inpatient sample. Dig Dis Sci 2020;65:406-415.
18. Bittencourt PL, Farias AQ, Couto CA. Liver transplantation in Brazil. Liver Transpl 2016;22:1254-8.

19.   Addison T; Gull W. On a certain affliction of the skin-vitiligoides—a planus tuberosa. Guys Hosp Rep 1851;7:265-276.
20.   Dauphinee JA, Sinclair JC. Primary biliary cirrhosis. Can Med Assoc J 1949;61:1-6.
21.   Poupon R, Chretien Y, Poupon RE, et al. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet 1987;1:834-6.
22.   Tanaka A. Current understanding of primary biliary cholangitis. Clin Mol Hepatol 2021;27:1-21.
23.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. Clin Res Hepatol Gastroenterol 2015;39:e57-9.
24.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: From 'cirrhosis' to 'cholangitis'. Dig Liver Dis 2015;47:924-6.
25.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. Am J Gastroenterol 2015;110:1536-8.
26.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. Clin Gastroenterol Hepatol 2015;13:1867-9.
27.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. J Hepatol 2015;63:1285-7.
28.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. Gastroenterology 2015;149:1627-9.
29.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. Gut 2015;64:1671-2.
30.   Beuers U, Gershwin ME, Gish RG, et al. Changing nomenclature for PBC: from 'cirrhosis' to 'cholangitis'. Hepatology 2015;62:1620-2.
31.   Montali L, Gragnano A, Miglioretti M, et al. Quality of life in patients with primary biliary cholangitis: A cross-geographical comparison. J Transl Autoimmun 2021;4:100081.
32.   Boonstra K, Beuers U, Ponsioen CY. Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review. J Hepatol 2012;56:1181-1188.
33.   Lv T, Chen S, Li M, et al. Regional variation and temporal trend of primary biliary cholangitis epidemiology: A systematic review and meta-analysis. J Gastroenterol Hepatol 2021;36:1423-1434.
34.   Zeng N, Duan W, Chen S, et al. Epidemiology and clinical course of primary biliary cholangitis in the Asia-Pacific region: a systematic review and meta-analysis. Hepatol Int 2019;13:788-799.
35.   Myers RP, Shaheen AA, Fong A, et al. Epidemiology and natural history of primary biliary cirrhosis in a Canadian health region: a population-based study. Hepatology 2009;50:1884-92.
36.   Yoshida EM, Mason A, Peltekian KM, et al. Epidemiology and liver transplantation burden of primary biliary cholangitis: a retrospective cohort study. CMAJ Open 2018;6:E664-E670.
37.   Cheung KS, Seto WK, Fung J, et al. Epidemiology and natural history of primary biliary cholangitis in the chinese: a territory-based study in Hong Kong between 2000 and 2015. Clin Transl Gastroenterol 2017;8:e116.
38.   Tanaka A, Mori M, Matsumoto K, et al. Increase trend in the prevalence and male-to-female ratio of primary biliary cholangitis, autoimmune hepatitis, and primary sclerosing cholangitis in Japan. Hepatol Res 2019;49:881-889.
39.   Marschall HU, Henriksson I, Lindberg S, et al. Incidence, prevalence, and outcome of primary biliary cholangitis in a nationwide Swedish population-based cohort. Sci Rep 2019;9:11525.
40.   French J, van der Mei I, Simpson S, Jr., et al. Increasing prevalence of primary biliary cholangitis in Victoria, Australia. J Gastroenterol Hepatol 2020;35:673-679.
41.   Lleo A, Jepsen P, Morenghi E, et al. evolving trends in female to male incidence and male mortality of primary biliary cholangitis. Sci Rep 2016;6:25906.

42. Walker JG, Doniach D, Roitt IM, et al. Serological tests in diagnosis of primary biliary cirrhosis. Lancet 1965;1:827-31.

43. Mattalia A, Quaranta S, Leung PS, et al. Characterization of antimitochondrial antibodies in health adults. Hepatology 1998;27:656-61.

44. Muratori L, Granito A, Muratori P, et al. Antimitochondrial antibodies and other antibodies in primary biliary cirrhosis: diagnostic and prognostic value. Clin Liver Dis 2008;12:261-76; vii.

45. Guatibonza-Garcia V, Gaete PV, Perez-Londono A, et al. Poor performance of anti-mitochondrial antibodies for the diagnosis of primary biliary cholangitis in female Colombian patients: A single-center study. World J Gastroenterol 2021;27:4890-4899.

46. Lleo A, Leung PSC, Hirschfield GM, et al. The Pathogenesis of Primary Biliary Cholangitis: A Comprehensive Review. Semin Liver Dis 2020;40:34-48.

47. Moteki S, Leung PS, Dickson ER, et al. Epitope mapping and reactivity of autoantibodies to the E2 component of 2-oxoglutarate dehydrogenase complex in primary biliary cirrhosis using recombinant 2-oxoglutarate dehydrogenase complex. Hepatology 1996;23:436-44.

48. Dubel L, Tanaka A, Leung PS, et al. Autoepitope mapping and reactivity of autoantibodies to the dihydrolipoamide dehydrogenase-binding protein (E3BP) and the glycine cleavage proteins in primary biliary cirrhosis. Hepatology 1999;29:1013-8.

49. Lleo A, Selmi C, Invernizzi P, et al. Apotopes and the biliary specificity of primary biliary cirrhosis. Hepatology 2009;49:871-9.

50. Lleo A, Bowlus CL, Yang GX, et al. Biliary apotopes and anti-mitochondrial antibodies activate innate immune responses in primary biliary cirrhosis. Hepatology 2010;52:987-98.

51. Gulamhusein AF, Hirschfield GM. Primary biliary cholangitis: pathogenesis and therapeutic opportunities. Nat Rev Gastroenterol Hepatol 2020;17:93-110.

52. Shimoda S, Van de Water J, Ansari A, et al. Identification and precursor frequency analysis of a common T cell epitope motif in mitochondrial autoantigens in primary biliary cirrhosis. J Clin Invest 1998;102:1831-40.

53. Kita H, Matsumura S, He XS, et al. Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. J Clin Invest 2002;109:1231-40.

54. Padgett KA, Lan RY, Leung PC, et al. Primary biliary cirrhosis is associated with altered hepatic microRNA expression. J Autoimmun 2009;32:246-53.

55. Banales JM, Saez E, Uriz M, et al. Up-regulation of microRNA 506 leads to decreased Cl-/HCO3- anion exchanger 2 expression in biliary epithelium of patients with primary biliary cirrhosis. Hepatology 2012;56:687-97.

56. Rodrigues PM, Perugorria MJ, Santos-Laso A, et al. Primary biliary cholangitis: A tale of epigenetically-induced secretory failure? J Hepatol 2018;69:1371-1383.

57. Erice O, Munoz-Garrido P, Vaquero J, et al. MicroRNA-506 promotes primary biliary cholangitis-like features in cholangiocytes and immune activation. Hepatology 2018;67:1420-1440.

58. Bettelli E, Oukka M, Kuchroo VK. T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol 2007;8:345-50.

59. Rong G, Zhou Y, Xiong Y, et al. Imbalance between T helper type 17 and T regulatory cells in patients with primary biliary cirrhosis: the serum cytokine profile and peripheral cell population. Clin Exp Immunol 2009;156:217-25.

60. Yang CY, Ma X, Tsuneyama K, et al. IL-12/Th1 and IL-23/Th17 biliary microenvironment in primary biliary cirrhosis: implications for therapy. Hepatology 2014;59:1944-53.

61. Ma HD, Ma WT, Liu QZ, et al. Chemokine receptor CXCR3 deficiency exacerbates murine autoimmune cholangitis by promoting pathogenic CD8(+) T cell activation. J Autoimmun 2017;78:19-28.

62. Ueno Y, Ambrosini YM, Moritoki Y, et al. Murine models of autoimmune cholangitis. Curr Opin Gastroenterol 2010;26:274-9.

63. Tsuda M, Ambrosini YM, Zhang W, et al. Fine phenotypic and functional characterization of effector cluster of differentiation 8 positive T cells in human patients with primary biliary cirrhosis. Hepatology 2011;54:1293-302.

64. Harada K, Isse K, Nakanuma Y. Interferon gamma accelerates NF-kappaB activation of biliary epithelial cells induced by Toll-like receptor and ligand interaction. J Clin Pathol 2006;59:184-90.

65. Yokoyama T, Komori A, Nakamura M, et al. Human intrahepatic biliary epithelial cells function in innate immunity by producing IL-6 and IL-8 via the TLR4-NF-kappaB and -MAPK signaling pathways. Liver Int 2006;26:467-76.

66. Shimoda S, Harada K, Niiro H, et al. CX3CL1 (fractalkine): a signpost for biliary inflammation in primary biliary cirrhosis. Hepatology 2010;51:567-75.

67. Lleo A, Invernizzi P. Apotopes and innate immune system: novel players in the primary biliary cirrhosis scenario. Dig Liver Dis 2013;45:630-6.

68. Chuang YH, Lian ZX, Tsuneyama K, et al. Increased killing activity and decreased cytokine production in NK cells in patients with primary biliary cirrhosis. J Autoimmun 2006;26:232-40.

69. Selmi C, Mayo MJ, Bach N, et al. Primary biliary cirrhosis in monozygotic and dizygotic twins: genetics, epigenetics, and environment. Gastroenterology 2004;127:485-92.

70. Invernizzi P. Human leukocyte antigen in primary biliary cirrhosis: an old story now reviving. Hepatology 2011;54:714-23.

71. Invernizzi P, Ransom M, Raychaudhuri S, et al. Classical HLA-DRB1 and DPB1 alleles account for HLA associations with primary biliary cirrhosis. Genes Immun 2012;13:461-8.

72. Cordell HJ, Fryett JJ, Ueno K, et al. An international genome-wide meta-analysis of primary biliary cholangitis: Novel risk loci and candidate drugs. J Hepatol 2021;75:572-581.

73. Howel D, Fischbacher CM, Bhopal RS, et al. An exploratory population-based case-control study of primary biliary cirrhosis. Hepatology 2000;31:1055-60.

74. Amano K, Leung PS, Rieger R, et al. Chemical xenobiotics and mitochondrial autoantigens in primary biliary cirrhosis: identification of antibodies against a common environmental, cosmetic, and food additive, 2-octynoic acid. J Immunol 2005;174:5874-83.

75. Ala A, Stanca CM, Bu-Ghanim M, et al. Increased prevalence of primary biliary cirrhosis near Superfund toxic waste sites. Hepatology 2006;43:525-31.

76. Corpechot C, Chretien Y, Chazouilleres O, et al. Demographic, lifestyle, medical and familial factors associated with primary biliary cirrhosis. J Hepatol 2010;53:162-9.

77. Matsumoto K, Ohfuji S, Abe M, et al. Environmental factors, medical and family history, and comorbidities associated with primary biliary cholangitis in Japan: a multicenter case-control study. J Gastroenterol 2022;57:19-29.

78. Gershwin ME, Selmi C, Worman HJ, et al. Risk factors and comorbidities in primary biliary cirrhosis: a controlled interview-based study of 1032 patients. Hepatology 2005;42:1194-202.

79. Wang JJ, Yang GX, Zhang WC, et al. Escherichia coli infection induces autoimmune cholangitis and anti-mitochondrial antibodies in non-obese diabetic (NOD).B6 (Idd10/Idd18) mice. Clin Exp Immunol 2014;175:192-201.

80. Yang Y, Choi J, Chen Y, et al. E. coli and the etiology of human PBC: Antimitochondrial antibodies and spreading determinants. Hepatology 2022;75:266-279.

81. Tang R, Wei Y, Li Y, et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy. Gut 2018;67:534-541.

82. Chen W, Wei Y, Xiong A, et al. Comprehensive Analysis of serum and fecal bile acid profiles and interaction with gut microbiota in primary biliary cholangitis. Clin Rev Allergy Immunol 2020;58:25-38.

83. Yang H, Duan Z. Bile acids and the potential role in primary biliary cirrhosis. Digestion 2016;94:145-153.

84. Li Y, Tang R, Leung PSC, et al. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. Autoimmun Rev 2017;16:885-896.

85. Ma HD, Zhao ZB, Ma WT, et al. Gut microbiota translocation promotes autoimmune cholangitis. J Autoimmun 2018;95:47-57.

86. Bittencourt PLCCA. Manual de Doenças Colestáticas: DOC Content, 2019.

87. Allocca M, Crosignani A, Gritti A, et al. Hypercholesterolaemia is not associated with early atherosclerotic lesions in primary biliary cirrhosis. Gut 2006;55:1795-800.

88. Fan J, Wang Q, Sun L. Association between primary biliary cholangitis and osteoporosis: meta-analysis. Clin Rheumatol 2017;36:2565-2571.

89. Levy C, Lindor KD. Management of osteoporosis, fat-soluble vitamin deficiencies, and hyperlipidemia in primary biliary cirrhosis. Clin Liver Dis 2003;7:901-10.

90. Liang Y, Yang Z, Zhong R. Primary biliary cirrhosis and cancer risk: a systematic review and meta-analysis. Hepatology 2012;56:1409-17.

91. Murillo Perez CF, Goet JC, Lammers WJ, et al. Milder disease stage in patients with primary biliary cholangitis over a 44-year period: A changing natural history. Hepatology 2018;67:1920-1930.

92. Couto CA, Terrabuio DRB, Cancado ELR, et al. Update of the Brazilian Society of Hepatology recommendations for diagnosis and management of autoimmune diseases of the liver. Arq Gastroenterol 2019;56:232-241.

93. Zein CO, Angulo P, Lindor KD. When is liver biopsy needed in the diagnosis of primary biliary cirrhosis? Clin Gastroenterol Hepatol 2003;1:89-95.

94. Dahlqvist G, Gaouar F, Carrat F, et al. Large-scale characterization study of patients with antimitochondrial antibodies but nonestablished primary biliary cholangitis. Hepatology 2017;65:152-163.

95. Sun C, Xiao X, Yan L, et al. Histologically proven AMA positive primary biliary cholangitis but normal serum alkaline phosphatase: Is alkaline phosphatase truly a surrogate marker? J Autoimmun 2019;99:33-38.

96. Terziroli Beretta-Piccoli B, Stirnimann G, Mertens J, et al. Primary biliary cholangitis with normal alkaline phosphatase: A neglected clinical entity challenging current guidelines. J Autoimmun 2021;116:102578.

97. Colapietro F, Lleo A, Generali E. Antimitochondrial antibodies: from bench to bedside. Clin Rev Allergy Immunol 2021.

98. Berg PA, Klein R. Antimitochondrial antibodies in primary biliary cirrhosis and other disorders: definition and clinical relevance. Dig Dis 1992;10:85-101.

99. Kouroumalis E, Samonakis D, Voumvouraki A. Biomarkers for primary biliary cholangitis: current perspectives. Hepat Med 2018;10:43-53.

100. Vergani D. Towards the serological diagnosis of primary biliary cirrhosis. Liver Int 2015;35:299-301.

101. Chascsa DM, Lindor KD. Antimitochondrial antibody-negative primary biliary cholangitis: is it really the same disease? Clin Liver Dis 2018;22:589-601.

102. Liu B, Shi XH, Zhang FC, et al. Antimitochondrial antibody-negative primary biliary cirrhosis: a subset of primary biliary cirrhosis. Liver Int 2008;28:233-9.

103. Invernizzi P, Crosignani A, Battezzati PM, et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. Hepatology 1997;25:1090-5.

104. Zhang FK, Jia JD, Wang BE. Clinical evaluation of serum antimitochondrial antibody-negative primary biliary cirrhosis. Hepatobiliary Pancreat Dis Int 2004;3:288-91.

105. Juliusson G, Imam M, Bjornsson ES, et al. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. Scand J Gastroenterol 2016;51:745-52.

106. Hasselstrom K, Nilsson LA, Wallerstedt S. The predictive value of antimitochondrial antibodies in establishing the diagnosis of primary biliary cirrhosis. Scand J Gastroenterol 1988;23:103-6.

107. Muratori P, Muratori L, Gershwin ME, et al. 'True' antimitochondrial antibody-negative primary biliary cirrhosis, low sensitivity of the routine assays, or both? Clin Exp Immunol 2004;135:154-8.

108. Hu S, Zhao F, Wang Q, et al. The accuracy of the anti-mitochondrial antibody and the M2 subtype test for diagnosis of primary biliary cirrhosis: a meta-analysis. Clin Chem Lab Med 2014;52:1533-42.

109. Bizzaro N, Covini G, Rosina F, et al. Overcoming a "probable" diagnosis in antimitochondrial antibody negative primary biliary cirrhosis: study of 100 sera and review of the literature. Clin Rev Allergy Immunol 2012;42:288-97.

110. Granito A, Muratori P, Quarneti C, et al. Antinuclear antibodies as ancillary markers in primary biliary cirrhosis. Expert Rev Mol Diagn 2012;12:65-74.

111. Granito A, Muratori P, Muratori L, et al. Antinuclear antibodies giving the 'multiple nuclear dots' or the 'rim-like/membranous' patterns: diagnostic accuracy for primary biliary cirrhosis. Aliment Pharmacol Ther 2006;24:1575-83.

112. Wesierska-Gadek J, Penner E, Battezzati PM, et al. Correlation of initial autoantibody profile and clinical outcome in primary biliary cirrhosis. Hepatology 2006;43:1135-44.

113. Nakamura M, Kondo H, Mori T, et al. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. Hepatology 2007;45:118-27.

114. Gatselis NK, Zachou K, Norman GL, et al. Clinical significance of the fluctuation of primary biliary cirrhosis-related autoantibodies during the course of the disease. Autoimmunity 2013;46:471-9.

115. Norman GL, Yang CY, Ostendorff HP, et al. Anti-kelch-like 12 and anti-hexokinase 1: novel autoantibodies in primary biliary cirrhosis. Liver Int 2015;35:642-51.

116. Bauer A, Habior A. Detection of autoantibodies against nucleoporin p62 in sera of patients with primary biliary cholangitis. Ann Lab Med 2019;39:291-298.

117. Reig A, Norman GL, Garcia M, et al. Novel anti-hexokinase 1 antibodies are associated with poor prognosis in patients with primary biliary cholangitis. Am J Gastroenterol 2020;115:1634-1641.

118. Liberal R, Grant CR, Sakkas L, et al. Diagnostic and clinical significance of anti-centromere antibodies in primary biliary cirrhosis. Clin Res Hepatol Gastroenterol 2013;37:572-85.

119. Honda A, Ikegami T, Matsuzaki Y. Anti-gp210 and anti-centromere antibodies for the prediction of PBC patients with an incomplete biochemical response to UDCA and bezafibrate. Hepatol Res 2015;45:827-8.

120. Mandai S, Kanda E, Arai Y, et al. Anti-centromere antibody is an independent risk factor for chronic kidney disease in patients with primary biliary cirrhosis. Clin Exp Nephrol 2013;17:405-10.

121. Hubscher SG, Elias E, Buckels JA, et al. Primary biliary cirrhosis. Histological evidence of disease recurrence after liver transplantation. J Hepatol 1993;18:173-84.

122. Scheuer PJ. Ludwig Symposium on biliary disorders--part II. Pathologic features and evolution of primary biliary cirrhosis and primary sclerosing cholangitis. Mayo Clin Proc 1998;73:179-83.

123. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379:103-12.

124. Scheuer P. Primary biliary cirrhosis. Proc R Soc Med 1967;60:1257-60.

125. Chan AW, Chan RC, Wong GL, et al. Evaluation of histological staging systems for primary biliary cirrhosis: correlation with clinical and biochemical factors and significance of pathological parameters in prognostication. Histopathology 2014;65:174-86.

126. Nakanuma Y, Zen Y, Harada K, et al. Application of a new histological staging and grading system for primary biliary cirrhosis to liver biopsy specimens: Interobserver agreement. Pathol Int 2010;60:167-74.

127. Hiramatsu K, Aoyama H, Zen Y, et al. Proposal of a new staging and grading system of the liver for primary biliary cirrhosis. Histopathology 2006;49:466-78.

128. Harada K, Hsu M, Ikeda H, et al. Application and validation of a new histologic staging and grading system for primary biliary cirrhosis. J Clin Gastroenterol 2013;47:174-81.

129. Kakuda Y, Harada K, Sawada-Kitamura S, et al. Evaluation of a new histologic staging and grading system for primary biliary cirrhosis in comparison with classical systems. Hum Pathol 2013;44:1107-17.

130. Nakamura M, Kondo H, Tanaka A, et al. Autoantibody status and histological variables influence biochemical response to treatment and long-term outcomes in Japanese patients with primary biliary cirrhosis. Hepatol Res 2015;45:846-55.

131. European Association for the Study of the Liver. Electronic address eee, European Association for the Study of the L. EASL Clinical Practice Guidelines: The diagnosis and management of patients with primary biliary cholangitis. J Hepatol 2017;67:145-172.

132. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology 2002;36:525-31.

133. Angulo P, Dickson ER, Therneau TM, et al. Comparison of three doses of ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a randomized trial. J Hepatol 1999;30:830-5.

134. Rudic JS, Poropat G, Krstic MN, et al. Ursodeoxycholic acid for primary biliary cirrhosis. Cochrane Database Syst Rev 2012;12:CD000551.

135. Gong Y, Huang Z, Christensen E, et al. Ursodeoxycholic acid for patients with primary biliary cirrhosis: an updated systematic review and meta-analysis of randomized clinical trials using Bayesian approach as sensitivity analyses. Am J Gastroenterol 2007;102:1799-807.

136. Shi J, Wu C, Lin Y, et al. Long-term effects of mid-dose ursodeoxycholic acid in primary biliary cirrhosis: a meta-analysis of randomized controlled trials. Am J Gastroenterol 2006;101:1529-38.

137. Poupon RE, Lindor KD, Pares A, et al. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. J Hepatol 2003;39:12-6.

138. Angulo P, Batts KP, Therneau TM, et al. Long-term ursodeoxycholic acid delays histological progression in primary biliary cirrhosis. Hepatology 1999;29:644-7.

139. Corpechot C, Carrat F, Bahr A, et al. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. Gastroenterology 2005;128:297-303.

140. Harms MH, van Buuren HR, Corpechot C, et al. Ursodeoxycholic acid therapy and liver transplant-free survival in patients with primary biliary cholangitis. J Hepatol 2019;71:357-365.

141. Harms MH, de Veer RC, Lammers WJ, et al. Number needed to treat with ursodeoxycholic acid therapy to prevent liver transplantation or death in primary biliary cholangitis. Gut 2020;69:1502-1509.

142. Fiorucci S, Di Giorgio C, Distrutti E. Obeticholic acid: an update of its pharmacological activities in liver disorders. Handb Exp Pharmacol 2019;256:283-295.

143. Nevens F, Andreone P, Mazzella G, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med 2016;375:631-43.

144. Trauner M, Nevens F, Shiffman ML, et al. Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study. Lancet Gastroenterol Hepatol 2019;4:445-453.

145. Kowdley KV, Luketic V, Chapman R, et al. A randomized trial of obeticholic acid monotherapy in patients with primary biliary cholangitis. Hepatology 2018;67:1890-1902.

146. Ghonem NS, Assis DN, Boyer JL. Fibrates and cholestasis. Hepatology 2015;62:635-43.

147. Iwasaki S; Tsuda K UHAROMSTMTOS. Bezafibrate may have a beneficial effect in pre-cirrhotic primary biliary cirrhosis. Hepatology Research 1999;16:12-18.

148. Corpechot C, Chazouilleres O, Rousseau A, et al. A placebo-controlled trial of Bezafibrate in primary biliary cholangitis. N Engl J Med 2018;378:2171-2181.

149. de Vries E, Bolier R, Goet J, et al. Fibrates for Itch (FITCH) in Fibrosing Cholangiopathies: a double-blind, randomized, placebo-controlled trial. Gastroenterology 2021;160:734-743 e6.

150. Tanaka A, Hirohara J, Nakano T, et al. Association of bezafibrate with transplant-free survival in patients with primary biliary cholangitis. J Hepatol 2021;75:565-571.

151. Levy C, Peter JA, Nelson DR, et al. Pilot study: fenofibrate for patients with primary biliary cirrhosis and an incomplete response to ursodeoxycholic acid. Aliment Pharmacol Ther 2011;33:235-42.

152. Han XF, Wang QX, Liu Y, et al. Efficacy of fenofibrate in Chinese patients with primary biliary cirrhosis partially responding to ursodeoxycholic acid therapy. J Dig Dis 2012;13:219-24.

153. Ghonem NS, Auclair AM, Hemme CL, et al. Fenofibrate improves liver function and reduces the toxicity of the bile acid pool in patients with primary biliary cholangitis and primary sclerosing cholangitis who are partial responders to ursodiol. Clin Pharmacol Ther 2020;108:1213-1223.

154. Gallucci GM, Trottier J, Hemme C, et al. Adjunct fenofibrate up-regulates bile acid glucuronidation and improves treatment response for patients with cholestasis. Hepatol Commun 2021;5:2035-2051.

155. Joshita S, Umemura T, Yamashita Y, et al. Biochemical and plasma lipid responses to pemafibrate in patients with primary biliary cholangitis. Hepatol Res 2019;49:1236-1243.

156. Dohmen K, Onohara SY, Harada S. Effects of switching from fenofibrate to pemafibrate for asymptomatic primary bliary cholangitis. Korean J Gastroenterol 2021;78:227-234.

157. Yamaguchi M, Asano T, Arisaka T, et al. Effects of pemafibrate on primary biliary cholangitis with dyslipidemia. Hepatol Res 2022.

158. Yamashita S, Masuda D, Matsuzawa Y. Clinical applications of a novel selective ppar alpha modulator, pemafibrate, in dyslipidemia and metabolic diseases. J Atheroscler Thromb 2019;26:389-402.

159. Ahmad J, Odin JA, Hayashi PH, et al. Identification and characterization of fenofibrate-induced liver injury. Dig Dis Sci 2017;62:3596-3604.

160. Carrion AF, Lindor KD, Levy C. Safety of fibrates in cholestatic liver diseases. Liver Int 2021;41:1335-1343.

161. Hirschfield GM, Beuers U, Kupcinskas L, et al. A placebo-controlled randomised trial of budesonide for PBC following an insufficient response to UDCA. J Hepatol 2021;74:321-329.

162. Smets L, Verbeek J, Korf H, et al. Improved markers of cholestatic liver Injury in patients with primary biliary cholangitis treated with obeticholic acid and bezafibrate. Hepatology 2021;73:2598-2600.

163. Soret PA, Lam L, Carrat F, et al. Combination of fibrates with obeticholic acid is able to normalise biochemical liver tests in patients with difficult-to-treat primary biliary cholangitis. Aliment Pharmacol Ther 2021;53:1138-1146.

164. Angulo P, Lindor KD, Therneau TM, et al. Utilization of the Mayo risk score in patients with primary biliary cirrhosis receiving ursodeoxycholic acid. Liver 1999;19:115-21.

165. Momah N, Silveira MG, Jorgensen R, et al. Optimizing biochemical markers as endpoints for clinical trials in primary biliary cirrhosis. Liver Int 2012;32:790-5.

166. Kuiper EM, Hansen BE, de Vries RA, et al. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. Gastroenterology 2009;136:1281-7.

167. Pares A, Caballeria L, Rodes J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. Gastroenterology 2006;130:715-20.

168. Corpechot C, Abenavoli L, Rabahi N, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology 2008;48:871-7.

169. Corpechot C, Chazouilleres O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. J Hepatol 2011;55:1361-7.

170. Azemoto N, Abe M, Murata Y, et al. Early biochemical response to ursodeoxycholic acid predicts symptom development in patients with asymptomatic primary biliary cirrhosis. J Gastroenterol 2009;44:630-4.

171. Kumagi T, Guindi M, Fischer SE, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol 2010;105:2186-94.

172. Lammers WJ, Hirschfield GM, Corpechot C, et al. Development and validation of a scoring system to predict outcomes of patients with primary biliary cirrhosis receiving ursodeoxycholic acid therapy. Gastroenterology 2015;149:1804-1812 e4.

173. Carbone M, Sharp SJ, Flack S, et al. The UK-PBC risk scores: Derivation and validation of a scoring system for long-term prediction of end-stage liver disease in primary biliary cholangitis. Hepatology 2016;63:930-50.

174. Lammers WJ, van Buuren HR, Hirschfield GM, et al. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. Gastroenterology 2014;147:1338-49 e5; quiz e15.

175. Murillo Perez CF, Harms MH, Lindor KD, et al. Goals of treatment for improved survival in primary biliary cholangitis: treatment target should be bilirubin within the normal range and normalization of alkaline phosphatase. Am J Gastroenterol 2020;115:1066-1074.

176. Carbone M, Nardi A, Flack S, et al. Pretreatment prediction of response to ursodeoxycholic acid in primary biliary cholangitis: development and validation of the UDCA Response Score. Lancet Gastroenterol Hepatol 2018;3:626-634.

177. Lucey MR, Neuberger JM, Williams R. Primary biliary cirrhosis in men. Gut 1986;27:1373-6.

178. Rubel LR, Rabin L, Seeff LB, et al. Does primary biliary cirrhosis in men differ from primary biliary cirrhosis in women? Hepatology 1984;4:671-7.

179. Carbone M, Mells GF, Pells G, et al. Sex and age are determinants of the clinical phenotype of primary biliary cirrhosis and response to ursodeoxycholic acid. Gastroenterology 2013;144:560-569 e7; quiz e13-4.

180. Trivedi PJ, Lammers WJ, van Buuren HR, et al. Stratification of hepatocellular carcinoma risk in primary biliary cirrhosis: a multicentre international study. Gut 2016;65:321-9.

181. John BV, Aitcheson G, Schwartz KB, et al. Male Sex Is Associated With Higher Rates of Liver-Related Mortality in Primary Biliary Cholangitis and Cirrhosis. Hepatology 2021;74:879-891.

182. Cheung AC, Lammers WJ, Murillo Perez CF, et al. Effects of age and sex of response to ursodeoxycholic acid and transplant-free survival in patients with primary biliary cholangitis. Clin Gastroenterol Hepatol 2019;17:2076-2084 e2.

183. Liu Y, Han K, Liu C, et al. Clinical characteristics and prognosis of concomitant primary biliary cholangitis and autoimmune diseases: a retrospective study. Can J Gastroenterol Hepatol 2021;2021:5557814.

184. Chazouilleres O, Wendum D, Serfaty L, et al. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology 1998;28:296-301.

185. Hindi M, Levy C, Couto CA, et al. Primary biliary cirrhosis is more severe in overweight patients. J Clin Gastroenterol 2013;47:e28-32.

186. Ferreira APS, Szwarcwald CL, Damacena GN, et al. Increasing trends in obesity prevalence from 2013 to 2019 and associated factors in Brazil. Rev Bras Epidemiol 2021;24:e210009.

187. Gerussi A, Bernasconi DP, O'Donnell SE, et al. Measurement of gamma glutamyl transferase to determine risk of liver transplantation or death in patients with primary biliary cholangitis. Clin Gastroenterol Hepatol 2021;19:1688-1697 e14.

188. Cortez-Pinto H, Liberal R, Lopes S, et al. Predictors for incomplete response to ursodeoxycholic acid in primary biliary cholangitis. Data from a national registry of liver disease. United European Gastroenterol J 2021;9:699-706.

189. Corpechot C, Carrat F, Poujol-Robert A, et al. Noninvasive elastography-based assessment of liver fibrosis progression and prognosis in primary biliary cirrhosis. Hepatology 2012;56:198-208.

190. Corpechot C, Carrat F, Gaouar F, et al. Liver stiffness measurement by vibration-controlled transient elastography improves outcome prediction in primary biliary cholangitis. J Hepatol 2022. Ahead of print

191. Namisaki T, Moriya K, Noguchi R, et al. Liver fibrosis progression predicts survival in patients with primary biliary cirrhosis. Hepatol Res 2017;47:E178-E186.

192. Degott C, Zafrani ES, Callard P, et al. Histopathological study of primary biliary cirrhosis and the effect of ursodeoxycholic acid treatment on histology progression. Hepatology 1999;29:1007-12.

3. OBJETIVOS

3.1. Objetivo Geral

a) Avaliar o perfil clínico-epidemiológico da colangite biliar primária no Brasil.

3.2. Objetivos Específicos

a) descrever as características demográficas e clínicas dos pacientes com colangite biliar primária;

b) avaliar a prevalência de outras doenças autoimunes concomitantes em pacientes com colangite biliar primária;

c) comparar o perfil clínico-epidemiológico de pacientes portadores de colangite biliar primária com anticorpo anti-mitocôndria positivo àqueles com anticorpo negativo;

d) avaliar a resposta terapêutica ao AUDC através de diferentes critérios reconhecidos internacionalmente;

e) avaliar a resposta terapêutica aos diferentes fibratos, incluindo o ciprofibrato, em pacientes portadores de colangite biliar primária, não-respondedores ao AUDC;

f) comparar as taxas de resposta ao tratamento com AUDC após 6 e 12 meses da introdução do medicamento;

g) avaliar marcadores clínicos de prognóstico da colangite biliar primária.

4. METODOLOGIA

4.1. Local do Estudo

Trata-se de estudo multicêntrico nacional, coordenado e financiado pela Sociedade Brasileira de Hepatologia, de coorte retrospectiva, incluindo 28 centros de referência em Hepatologia, que atendem pacientes portadores de colangite biliar primária, a saber: Universidade Federal de Minas Gerais, Universidade Federal de Juiz de Fora, Universidade Federal de Uberlândia, Universidade Federal do Triângulo Mineiro, Universidade de São Paulo, Universidade Federal de São Paulo, Universidade Estadual de Campinas, Centro Universitário Lusíada, Universidade Federal da Bahia, Hospital Português, Universidade Federal do Espírito Santo, Universidade Federal do Rio de Janeiro, Hospital Federal de Ipanema, Hospital de Base do Distrito Federal, Hospital Santa Casa de Misericórdia de Porto Alegre, Ambulatório de Hepatites de São José dos Campos, Hospital Nossa Senhora das Graças de Curitiba, Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto, Universidade Federal do Ceará, Fundação Hospital Adriano Jorge e 8 centros privados de hepatologia. Foram incluídos pacientes de todas as macrorregiões do país, sendo 80% do Sudeste, 8% do Nordeste, 5,4% do Sul, 4% do Centro-Oeste e 2,6% do Norte. Para critério de autoria nos artigos produzidos, foi utilizado o número de pacientes cadastrados por Centro.

O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (CAAE 98627218.6.1001.5149) (ANEXO 1)

4.2. Desenho do Estudo

O Grupo de Estudos de Doenças Colestáticas do Brasil, coordenado pela Sociedade Brasileira de Hepatologia, elaborou um banco de dados padronizado, o qual foi enviado a 28 centros no Brasil. Os dados foram preenchidos eletronicamente pelos coordenadores médicos de cada centro participante e conferidos por dois investigadores independentes, hepatologistas. Foram incluídos pacientes com idade ≥ 18 anos e diagnóstico estabelecido de colangite biliar primária entre 1º de janeiro de 1992 e 31 de dezembro de 2019. Para o diagnóstico de colangite biliar primária foi utilizado o critério da *American Association for the Study of Liver Diseases*, sendo necessária a presença de dois dos três critérios a seguir: 1) sorologia positiva para anticorpo anti-mitocôndria ou anticorpo anti-núcleo específico para colangite biliar primária; 2) aumento persistente de

fosfatase alcalina sérica; 3) histologia hepática compatível com colangite biliar primária. Pacientes em que o diagnóstico não pode ser confirmado ou que apresentavam outra etiologia para doença hepática, incluindo síndrome de sobreposição com hepatite autoimune, foram excluídos.

4.3. Dados coletados por revisão de prontuários

a) Dados relativos à caracterização dos pacientes: iniciais do nome, sexo, data de nascimento, data de início dos sintomas, data do diagnóstico de colangite biliar primária, data da alta ou óbito e causa *mortis.*

b) Dados relativos aos sinais e sintomas ao diagnóstico: fadiga, prurido e sua intensidade, icterícia, esplenomegalia, outros.

c) Dados relativos às comorbidades: presença de hepatite autoimune, tireoidite de hashimoto, esclerodermia (síndrome CREST), artrite reumatoide, síndrome de Sjogren, diabetes mellitus, hipertensão arterial, dislipidemia, obesidade, tabagismo, etilismo, outras, ao diagnóstico ou ao longo do seguimento.

d) Dados laboratoriais: fosfatase alcalina, gama-glutamiltransferase, alanina aminotransferase, aspartato aminotransferase, bilirrubinas e albumina ao diagnóstico e 6 meses, um, dois, três, quatro e cinco anos após início do tratamento, quando disponível; imunoglobulina M ao diagnóstico; imunoglobulina G ao diagnóstico; perfil lipídico ao diagnóstico; presença de anticorpo anti-núcleo e seu padrão ao diagnóstico; presença de anticorpo anti-mitocôndria e anti-músculo liso ao diagnóstico.

e) Dados referentes à histologia: realização de biópsia hepática ao longo do seguimento; estadiamento histológico pelos critérios de Ludwig.

f) Dados referentes à insuficiência hepática e hipertensão portal: presença de cirrose ao diagnóstico; complicações ao longo do seguimento: presença de varizes de esôfago; história de hemorragia digestiva alta varicosa; história de encefalopatia hepática; história de ascite; história de peritonite bacteriana espontânea, história de hepatocarcinoma.

g) Dados referentes à realização de transplante hepático: transplante ao longo do seguimento.

h) Dados referentes ao tratamento: início, dose e adesão ao tratamento com AUDC; início, dose, tipo e adesão ao tratamento com fibrato; outras medicações. A adesão ao tratamento foi determinada pelo médico assistente por análise retrospectiva de prontuário e subdividida em: uso regular (tomada de ≥ 80% da dose prescrita), irregular (tomada de menos de 80% da dose prescrita) ou esporádico (uso eventual).

4.4. Critérios de resposta do tratamento com AUDC

Para avaliação de resposta ao tratamento com AUDC foram adotados os seguintes critérios e definições:

Tabela 2 – Critérios de resposta e suas definições

Nome do Critério	Tempo para avaliação	Definição
Barcelona	12 meses	Redução > 40% ou normalização da fosfatase alcalina
Paris I	12 meses	Fosfatase alcalina ≤ 3x o limite superior da normalidade e aspartato aminotransferase ≤ 2x o limite superior da normalidade e normalização da bilirrubina
Paris II	12 meses	Fosfatase alcalina e aspartato aminotransferase ≤1,5x o limite superior da normalidade e normalização da bilirrubina
POISE trial	12 meses	Fosfatase alcalina menor que 1,67x o limite superior da normalidade, com redução de pelo menos 15% em relação ao basal e normalização da bilirrubina
Rotterdam	12 meses	Normalização da bilirrubina e/ou albumina alteradas antes do tratamento

Toronto	24 meses	Fosfatase alcalina ≤1,67 x o limite superior normalidade

4.5. Análise Estatística

As análises estatísticas foram realizadas utilizando-se os programas SPSS 25.0 (SPSS Inc., Chicago, IL) e GraphPad Prism 5.0 (GraphPad Prism 5.0, EUA) para Windows. As variáveis numéricas foram avaliadas quanto à normalidade (teste de Shapiro-Wilk) para a seleção da apresentação dos dados. As variáveis categóricas foram apresentadas sob a forma de percentuais. As variáveis contínuas foram expressas em média (± desvio-padrão) ou mediana (intervalo interquartílico) e comparadas utilizando-se o teste t de Student ou de Mann-Whitney de acordo com a distribuição. O teste de qui-quadrado foi utilizado para a comparação de variáveis categóricas (ou teste exato de Fisher, quando apropriado). Os coeficientes Kappa de Cohen e Kappa de Fleiss foram utilizados para avaliar a concordância entre os diferentes critérios de resposta ao tratamento. A exclusão por pares (*pairwise deletion*) foi aplicada para dados faltantes. O nível de significância foi fixado em 5%.

5. ARTIGOS

5.1. Artigo 1

Original article

Clinical features and treatment outcomes of primary biliary cholangitis in a highly admixed population

Guilherme Grossi Lopes Cançado[a,b,*], Michelle Harriz Braga[c], Maria Lúcia Gomes Ferraz[d], Cristiane Alves Villela-Nogueira[e], Debora Raquel Benedita Terrabuio[c], Eduardo Luiz Rachid Cançado[c], Mateus Jorge Nardelli[a], Luciana Costa Faria[a], Nathalia Mota de Faria Gomes[d], Elze Maria Gomes de Oliveira[f], Vivian Rotman[e], Maria Beatriz de Oliveira[g], Simone Muniz Carvalho Fernandes da Cunha[h], Daniel Ferraz de Campos Mazo[i], Liliana Sampaio Costa Mendes[j], Claudia Alexandra Pontes Ivantes[k], Liana Codes[h,l], Valéria Ferreira de Almeida e Borges[m,n], Fabio Heleno de Lima Pace[o], Mario Guimarães Pessoa[c], Izabelle Venturini Signorelli[p], Gabriela Perdomo Coral[q], Paulo Lisboa Bittencourt[l,r], Cynthia Levy[s], Cláudia Alves Couto[a], Members of the Brazilian Cholestasis Study Group Consortium

[a] Instituto Alfa de Gastroenterologia, Hospital das Clínicas da Universidade Federal de Minas Gerais, Av. Professor Alfredo Balena 110, Belo Horizonte 30130-100, Minas Gerais, Brazil
[b] Hospital da Polícia Militar de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Departamento de Gastroenterologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
[d] Disciplina de Gastroenterologia, Universidade Federal de São Paulo, São Paulo, Brazil
[e] Hospital Universitário Clementino Fraga Filho e Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[f] Centro Universitário Lusíada - UNILUS, Santos, São Paulo, Brazil
[g] Ambulatório Municipal de Hepatites Virais de São José dos Campos, São José dos Campos, São Paulo, Brazil
[h] Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil
[i] Divisão de Gastroenterologia (Gastrocentro), Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil
[j] Hospital de Base do Distrito Federal, Brasília, Distrito Federal, Brazil
[k] Serviço de Gastroenterologia, Hepatologia e Transplante Hepático, Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil
[l] Hospital Português, Salvador, Bahia, Brazil
[m] Instituto de Gastroenterologia, Endoscopia e Proctologia, Uberlândia, Minas Gerais, Brazil
[n] Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil
[o] Serviço de Gastroenterologia e Hepatologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil
[p] Hospital Universitário Cassiano Antônio Moraes, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil
[q] Irmandade da Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil
[r] Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil
[s] Division of Digestive Health and Liver Diseases, University of Miami Miller School of Medicine, Miami, Florida, United States

ARTICLE INFO

ABSTRACT

Introduction and objectives: Little is known about primary biliary cholangitis (PBC) in non-whites. The purpose of this study was to evaluate clinical features and outcomes of PBC in a highly admixed population.
Material and methods: The Brazilian Cholestasis Study Group multicentre database was reviewed to assess demographics, clinical features and treatment outcomes of Brazilian patients with PBC.
Results: 562 patients (95% females, mean age 51 ± 11 years) with PBC were included. Concurrent autoimmune diseases and overlap with autoimmune hepatitis (AIH) occurred, respectively, in 18.9% and 14%. After a mean follow-up was 6.2 ± 5.3 years, 32% had cirrhosis, 7% underwent liver transplantation and 3% died of liver-related causes. 96% were treated with ursodeoxycholic acid (UDCA) and 12% required add-on therapy with fibrates, either bezafibrate, fenofibrate or ciprofibrate. Response to UDCA and to UDCA/fibrates therapy

Abbreviations: PBC, primary biliary cholangitis; AIH, autoimmune hepatitis; UDCA, ursodeoxycholic acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AMA, anti-mitochondrial antibodies; GGT, gamma-glutamyltransferase; ULN, upper limit of normality; IQR, interquartile range; PPAR, peroxisome proliferator-activated receptor.

* Corresponding author.
E-mail address: guilhermegrossi@terra.com.br (G.G.L. Cançado).

varied from 39%-67% and 42-61%, respectively, according to different validated criteria. Advanced histological stages and non-adherence to treatment were associated with primary non-response to UDCA, while lower baseline alkaline phosphatase (ALP) and aspartate aminotransferase (AST) levels correlated with better responses to both UDCA and UDCA/fibrates.

Conclusions: Clinical features of PBC in highly admixed Brazilians were similar to those reported in Caucasians and Asians, but with inferior rates of overlap syndrome with AIH. Response to UDCA was lower than expected and inversely associated with histological stage and baseline AST and ALP levels. Most of patients benefited from add-on fibrates, including ciprofibrate. A huge heterogeneity in response to UDCA therapy according to available international criteria was observed and reinforces the need of global standardization.

© 2021 Fundación Clínica Médica Sur, A.C. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease of unknown etiology which has a variable rate of progression towards cirrhosis and end-stage liver disease. PBC predominantly affects middle-aged women and is usually associated with several extra-hepatic autoimmune disorders. The incidence and prevalence rates have been estimated at 0.9 to 5.8 and 1.91 to 40.2 per 100,000 people in Europe, North America, Asia and Australia [1]. The disease is thought to be rare in Latin America and Africa. Previous studies have shown that PBC may have a more aggressive course in Hispanics and African Americans, suggesting that genetic background may modulate biological processes related to the clinical expression and progression of the disease [2,3].

Little is known about PBC affecting subjects with heterogeneous genetic backgrounds [4]. Brazil has a population of a highly admixed origin, with varying proportions of Amerindian, African, and European genetic ancestries, shaped by local historical interactions with migrants brought by the slave trade, European settlement, and indigenous populations [5]. In order to gather data on clinical features and treatment outcomes of PBC in the country, the Brazilian Society of Hepatology sponsored a multicenter cooperative consortium named as Brazilian Cholestasis Study Group comprised by investigators from both academic and community-based institutions who manage and treat patients with cholestasis, including PBC. This paper presents the results of real-world data concerning the clinical phenotype and treatment outcomes of PBC in Brazil.

2. Material and methods

2.1. Study population

The study population included adult (≥ 18 years old) patients who were diagnosed with PBC between January 1st, 1992 and December 31st, 2019 in 28 different hepatology centers throughout the country. All procedures were conducted in accordance with the ethical standards of the Helsinki Declaration and the study was approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149). The diagnosis of PBC was considered if the patient fulfilled at least two of the following diagnostic criteria as recommended by the American Association for the Study of Liver Disease guidelines: (i) positive serology for anti-mitochondrial antibodies (AMA); (ii) persistent increase of the serum alkaline phosphatase (ALP) levels; and (iii) liver histology compatible with PBC [6]. Autoimmune hepatitis (AIH) and PBC overlap syndrome was considered if patient satisfied the Paris criteria [7]. Patients in whom the diagnosis could not be confirmed or who had another etiology of liver disease were excluded. AMA status was assessed by indirect immunofluorescence. All AMA positive patients had titers ≥ 1:40. Liver histology specimens were available for all patients with AMA negative-PBC.

2.2. Data collection

Each researcher was asked to identify all PBC patients that have been followed in their Liver Center at the time of the survey, without any selection or exclusion whatsoever, and to fill-in a standardized database provided by the Brazilian Cholestasis Study Group, which was reviewed by two independent investigators (GGLC, CAC). Clinical data obtained from medical records included: sex, age at diagnosis, year of diagnosis, year of first symptoms or first biochemical changes, last date of follow-up, liver histology (fibrosis was staged according to the Ludwig system), extra-hepatic manifestations, AMA status, serum liver biochemistry, ursodeoxycholic acid (UDCA) and/or fibrate treatment, liver decompensation (ascites, variceal bleeding, hepatic encephalopathy, spontaneous bacterial peritonitis), transplantation and death. Data on liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and ALP, were collected at diagnosis and 6 months to 5 years after treatment, when available. Biochemical results were normalized by upper limit of normality (ULN) of each laboratory in order to homogenize the interpretation of the data. The considered standardized daily dose of UDCA for PBC treatment was 13−15 mg/kg of body weight [6]. The response to treatment either to UDCA or fibrates was analyzed according to the Barcelona, Paris I and II, Toronto and POISE trial criteria [8−12]. Treatment compliance was determined at the physician's discretion as regular, irregular or sporadic use. The threshold for regular use was defined as taking ≥ 80% of pills, whereas a rate < 80% indicated irregular adherence. Sporadic use was considered in patients who randomly took the medication [13]. The duration of follow-up was defined as the interval between the diagnosis and the last visit or the date of liver transplantation or death. Baseline ALP and AST levels therapy were compared to treatment response adjusted for compliance, according to those aforementioned criteria.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 25.0 software (IBM, USA). Continuous variables distribution was assessed by Shapiro-Wilk test, and those with Gaussian distribution were expressed as mean and standard deviation, or as median and interquartile range (IQR) if skewed distribution. Categorical variables were expressed as absolute number and percentage. Univariate analysis was performed using chi-square or Fisher exact test, as appropriate, for categorical variables. Continuous variables were analyzed by the Student t-test or Mann-Whitney U-test, according to the distribution. Pairwise deletion was applied to missing data. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Patient Characteristics

A total of 562 patients with PBC were included in this study. Clinical and laboratory features of the study population are summarized in Table 1. Eighty patients (14.2%) had overlap syndrome with AIH

Table 1
Brazilian primary biliary cholangitis cohort characteristics

Variable	Overall (N = 482)
Age at diagnosis, years ± SD	51.1 ± 11.4
Age at first symptoms, years ± SD	48.6 ± 12.6
Female	95.4%
AMA-positive	82.8%
ANA-positive	72.1%
Asymptomatic	35%
Pruritus	48%
Fatigue	38.4%
Coexistent autoimmune disease	
Hashimoto's thyroiditis	18.9%
Sjogren syndrome	7.9%
CREST syndrome	5.6%
Rheumatoid arthritis	4.6%
ALP X ULN, mean ± SD	3.7 ± 3.0
GGT X ULN, mean ± SD	11.8 ± 12.3
AST X ULN, mean ± SD	2.6 ± 2.0
ALT X ULN, mean ± SD	2.8 ± 2.5
Baseline total bilirubin, mean ± SD	2.2 ± 6.3
Histological disease stage, n (%)	326 (67.6%)
Inconclusive	44 (13.5%)
Stage I	73 (22.4%)
Stage II	96 (29.5%)
Stage III	68 (20.7%)
Stage IV	45 (13.8%)
Follow up time, years ± SD	6.2 ± 5.3
Cirrhosis	32.8%
Esophageal/gastric varices	32.2%
Liver-related decompensation during follow up	
Ascites	14.8%
Variceal bleeding	7.6%
Encephalopathy	7.6%
Spontaneous bacterial peritonitis	2.7%
Treatment with UDCA	95.9%
Treatment with UCDA + fibrates	12%
Liver transplantation during follow up	6.6%
Overall deaths	7.5%
Liver-related deaths	41.6%
Deaths post-transplant	12.5%

Data are expressed as absolute number/data available (percentage). SD: standard deviation; n = number; AMA: anti-mitochondrial antibodies; ANA: anti-nuclear antibody; ALP: alkaline phosphatase; GGT: gamma-glutamyltransferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; UDCA: ursodeoxycholic acid.

varices were present in one third of the patients. Requirement for liver transplantation and liver-related deaths were observed in 6.6% and 3.2% of the patients, respectively. Hepatocarcinoma was diagnosed in 1.9% of the subjects. Hashimoto's thyroiditis (18.9%) was the most common coexistent autoimmune disease, though Sjögren syndrome (7.9%), CREST syndrome (5.6%) and rheumatoid arthritis (4.6%) were also frequently reported.

Ninety six per cent of the patients were treated with UDCA in a mean dose of 13 ± 2.6 mg/kg/day. The remaining subjects were UDCA intolerant due to bloating and diarrhea. Treatment compliance was considered regular, irregular and sporadic, respectively, in 81%, 18% and 1% of them. None of the patients required drug discontinuation due to side effects. Fifty-nine patients (12%) were treated with fibrates in association with UDCA due to an inadequate response to UDCA, 14% of them had cirrhosis. Overall, 47.5%, 47.5% and 5% of the patients used ciprofibrate (mean dose = 95.4 ± 14.6 mg/day), bezafibrate (mean dose = 358 ± 82 mg/day) and fenofibrate (mean dose = 167.7 ± 40.8 mg/day), respectively. Mean time between starting UDCA and adding fibrates was 19.7 months. Data on adverse events or temporary discontinuation were not available for fibrates analysis. Forty-five patients (92%) reported regular treatment compliance to UDCA and fibrates therapies.

3.2. Response to UDCA treatment

Response to UDCA treatment was assessed in all patients based on the availability of laboratory parameters and varied from 39 to 67% according to the different criteria (Table 2). Lower rates of response were observed using Paris II criteria. Advanced histological stages were associated with non-response according to Paris I, II and POISE criteria, while non-adherence to treatment was associated with treatment failure using the Toronto and Barcelona criteria (Table 2). We also evaluated efficacy of UDCA by different criteria stratified by pretreatment ALP levels (≥ 3 X ULN vs < 3 X ULN) at baseline. When using all available criteria, patients with baseline ALP < 3 X ULN presented a statistically better response to UDCA when compared to their counterparts with higher levels of ALP before treatment (Table 3). Similarly, we observed a strong inverse relationship between AST ≥ 2 X ULN and response to treatment to all but the Barcelona criteria (Table 3).

3.3. Response to fibrate treatment

Overall rates of response to combined therapy of UDCA and fibrates according to different criteria are reported in Table 4 and varied from 42−61%. Response rates did not differ according to stage of fibrosis, adherence to treatment and at 2 years follow-up (data not shown). Response rates were higher in those subjects with baseline AST < 2 X ULN and baseline ALP < 3 X ULN, but the difference was statistically significant only with the use of Paris I criteria (Table 3). Differently, ALT level (≥ 2 X ULN or < 2 X ULN) at baseline was not able to predict response to fibrates by any criteria (data not shown).

according to Paris criteria and were excluded from outcome analyses. The majority of subjects were middle-aged women (95%; mean age 51 ± 11 years) with classical symptoms of pruritus and/or fatigue, which were observed in 65% of the patients. Mean time to diagnosis, considering time between first symptoms or biochemical changes and definite diagnosis, was 2.5 years. The prevalence of AMA was 82.8%, while anti-nuclear antibody was found in 72.1%. Liver pathology at diagnosis was available for 326 patients (67.6%). One third of them had PBC stage III or IV according to Ludwig classification [14]. After a mean follow-up of 6.2 ± 5.3 years, 32% of the subjects had clinical, laboratory or imaging evidence of cirrhosis. Esophageal

Table 2
Response to ursodeoxycholic acid treatment according to different criteria

Response Criteria	Overall		Ludwig Staging			Adherence to Treatment		
	Available data	Cohort Response	0-2	3-4	P-value	Yes	No	P-value
Toronto	263	161 (61.2)	84/131 (64.1)	36/69 (52.2)	0.101	130/194 (67.0)	22/55 (40.0)	< 0.001
Barcelona	315	194 (61.6)	100/153 (65.4)	46/83 (55.4)	0.133	149/228 (65.4)	33/68 (48.5)	0.012
Paris I	318	190 (59.7)	103/156 (66.0)	40/83 (48.2)	0.007	140/230 (60.9)	37/69 (53.6)	0.283
Paris II	318	125 (39.3)	71/156 (45.5)	24/83 (28.9)	0.013	91/230 (39.6)	23/69 (33.3)	0.350
POISE-Trial	318	213 (67.0)	118/156 (75.6)	44/83 (53.0)	<0.001	159/230 (69.1)	41/69 (59.4)	0.133

Data are expressed as absolute number/available data (percentage). Chi-square test was performed.

Table 3

Comparison of response to ursodeoxycholic acid and fibrates by different criteria stratified by alkaline phosfatase and aspartate aminotransferase/upper limit of normality ratios at pre-treatment baseline.

	1 year follow-up after UDCA							
	Alkaline phosphatase				Aspartate aminotransferase			
Response Criteria	< 3	≥ 3	OR (95%CI)	P-Value	< 2	≥ 2	OR (95%CI)	P-Value
Toronto	135 (77.1)	40 (29.0)	3.11 (2.32-4.16)	<0.001	123 (77.4)	56 (35.4)	2.36 (1.86-2.99)	<0.001
Barcelona	95 (54.3)	97 (70.3)	0.67 (0.50-0.89)	0.004	101 (64.3)	92 (59.0)	1.12 (0.89-1.40)	0.330
Paris-I	131 (75.3)	54 (39.1)	2.27 (1.75-2.93)	<0.001	128 (81.0)	61 (38.6)	2.37 (1.88-2.93)	<0.001
Paris-II	97 (55.7)	23 (16.7)	3.12 (2.13-4.59)	<0.001	97 (61.4)	27 (17.1)	3.13 (2.21-4.43)	<0.001
POISE trial	127 (72.6)	81 (58.7)	1.39 (1.09-1.78)	0.010	125 (78.6)	87 (55.4)	1.64 (1.33-2.02)	<0.001
	1 year follow-up after UDCA + fibrates							
	Alkaline phosphatase				Aspartate aminotransferase			
Response Criteria	< 3	≥ 3	OR (95%CI)	P-Value	< 2	≥ 2	OR (95%CI)	P-Value
Toronto	16 (64.0)	2 (25.0)	3.6 (0.85-15.3)	0.101	15 (65.2)	3 (30.0)	2.8 (0.87-8.99)	0.126
Barcelona	14 (56.0)	3 (37.5)	1.8 (0.5-6.23)	0.438	13 (56.5)	4 (40.0)	1.6 (0.55-4.62)	0.465
Paris-I	20 (80.0)	1 (12.5)	12.2 (1.7-88)	0.001	18 (78.3)	3 (30.0)	4.1 (1.29-12.92)	0.016
Paris-II	14 (53.8)	1 (12.5)	5.5 (0.8-40.1)	0.053	13 (54.2)	2 (20.0)	3.2 (0.78-12.73)	0.128
POISE trial	15 (60.0)	3 (37.5)	2.0 (0.6-7)	0.418	15 (65.2)	3 (30.0)	2.8 (0.87-8.99)	0.126

OR, odds ratio; CI, confidence interval; UCDA, ursodeoxycholic acid. Data are expressed as absolute number (percentage). Chi-square test was performed.

4. Discussion

Little is known about PBC in non-white subjects who have been underrepresented historically in clinical trials. The Brazilian population presents a highly diverse ancestry with varying percentages of Caucasoid, Negroid and Amerindian ancestries not amenable to race self-classification or further characterization based on expression of morphological traits such as skin color [15]. In this study, we addressed clinical features, disease progression and real-world response to treatment in a unique highly admixed multicenter cohort of Brazil. Previous studies assessing non-whites from North America revealed that African Americans and Hispanics may have a more aggressive disease compared with their Caucasian counterparts [2,3]. Lower 1-year survival and higher in-hospital mortality was also reported in African Americans in at least two other studies, possibly due to delayed diagnosis and lower access to treatment with UDCA [16−19]. In addition, features of overlap syndrome of AIH and PBC, higher rates of decompensation over time and lower response to UDCA were also more likely to be observed in Hispanics [3]. In the present study, clinical features of PBC, including demographics and disease presentation, were similar to those previously reported worldwide, except for a higher female-to-male ratio (21:1), longer time to diagnosis, remarkable frequency of advanced PBC and lower AMA reactivity [1,6,20]. These findings may be explained by an under-recognition of the disease in Brazil, a variability of AMA testing practices throughout the country, as well as by referral bias or even a different disease phenotype in Latin America.

Treatment with UDCA has been shown to improve serum liver biochemistry, delay histological progression and increase transplant-free survival [1,9,21,22]. Response to UDCA in our cohort was lower

Table 4

Overall response to fibrates treatment at 1 year according to different criteria.

Response Criteria	Response at 1 year	
	Available data (n)	Cohort Response n (%)
Toronto	36	20 (55.5)
Barcelona	36	19 (52.7)
Paris-1	36	22 (61.1)
Paris-2	36	15 (41.7)
POISE trial	36	21 (58.3)

than expected using different international criteria, but, as previously reported, response to therapy correlated to baseline AST and ALP levels and histological stage of PBC [23]. Interestingly, the proportion of patients with symptomatic disease at diagnosis was higher than observed in other cohorts [24]. Most of our patients had baseline symptoms of fatigue and/or pruritus, which were previously associated with a more aggressive disease, lower response to UDCA, as well as higher progression to cirrhosis and its complications [24]. We also demonstrated that adherence to treatment is a crucial point to be analyzed in real life studies since it directly impacts biochemical response at all times. Furthermore, the previously reported overexpression of overlap syndrome of AIH and PBC in US non-whites was not observed in our population with a highly heterogeneous genetic background [3]. Nonetheless, the prevalence of AIH-PBC overlap syndrome was similar to the rates described in the literature (10-15%) [25]. Finally, a low rate of hepatocellular carcinoma (HCC) was observed, further confirming that there is a significant difference in HCC incidence between viral and autoimmune liver diseases [26].

Since obeticholic acid is not approved in Brazil as second-line treatment of PBC, most of the subjects with non-response to UDCA were switched to off-label add-on therapy with fibrates. Previous studies have described significant decrease in ALP associated with bezafibrate and fenofibrate treatment [27−31]. To the best of our knowledge, this is the first study to report ciprofibrate use in PBC. The majority of our patients achieved biochemical response independently of the fibrate chosen. This finding is extremely relevant, since whereas most commercially available fibrates, including fenofibrate and ciprofibrate, specifically activate PPARα, bezafibrate is a pan PPAR-agonist, activating all three PPAR subtypes (α, γ and δ) at comparable doses [32]. In this way, clinical implications of this differential PPAR selectivity in PBC remain undefined and seems not to be relevant. Furthermore, the addition of fibrates appeared to induce the strongest beneficial effect in patients with baseline ALP < 3 X ULN and AST < 2 X ULN, probably reflecting lower severity of biliary and hepatic injury. Interestingly, this difference is not observed when using obeticholic acid as an add-on treatment, which induced durable improvements in markers of hepatic injury and cholestasis, regardless of baseline ALP and total bilirubin levels, in a subanalysis of the POISE trial [33].

This is the first study to describe PBC characteristics and response to treatment in an unique large and genetically diverse Brazilian cohort. However, some limitations which are inherent of retrospective study designs should be taken into consideration. The sample

size was limited by missing data, especially for the analyses involving biochemical response. In order to optimize sample representativeness during analysis, we performed pairwise deletion of missing data. In addition, heterogeneity in the availability of AMA and its different assessment methods between participating centers may also have influenced the rates of AMA positivity and the proportion of patients with liver histology evaluation. Furthermore, data on adverse events or temporary discontinuation were not available for safety evaluation of fibrates.

5. Conclusions

In summary, clinical and laboratory features of PBC in Brazilians are similar to those previously reported in Caucasian and Asian subjects, with lower rates of AIH-PBC overlap syndrome. Response to UDCA is lower than expected and is inversely associated with histological stage, adherence to treatment and baseline AST and ALP levels. On the other hand, most of the patients unresponsive to UDCA benefited from an add-on therapy with different marketed fibrates in Brazil, including bezafibrate, fenofibrate and the less studied ciprofibrate. Finally, our work sheds light on the huge heterogeneity between prognostic scores and the need for a global standardization.

Conflicts of interest

None.

CRediT authorship contribution statement

Guilherme Grossi Lopes Cançado: Conceptualization, Visualization, Data curation, Formal analysis, Writing – original draft. **Michelle Harriz Braga:** Visualization, Data curation, Writing – original draft. **Maria Lúcia Gomes Ferraz:** Visualization, Data curation, Writing – original draft. **Cristiane Alves Villela-Nogueira:** Visualization, Data curation, Writing – original draft. **Debora Raquel Benedita Terrabuio:** Visualization, Data curation, Writing – original draft. **Eduardo Luiz Rachid Cançado:** Visualization, Data curation, Writing – original draft. **Mateus Jorge Nardelli:** Visualization, Data curation, Writing – original draft. **Luciana Costa Faria:** Visualization, Data curation, Writing – original draft, Formal analysis. **Nathalia Mota de Faria Gomes:** Visualization, Data curation, Writing – original draft. **Elze Maria Gomes de Oliveira:** Visualization, Data curation, Writing – original draft. **Vivian Rotman:** Visualization, Data curation, Writing – original draft. **Maria Beatriz de Oliveira:** Visualization, Data curation, Writing – original draft. **Simone Muniz Carvalho Fernandes da Cunha:** Visualization, Data curation, Writing – original draft. **Daniel Ferraz de Campos Mazo:** Visualization, Data curation, Writing – original draft. **Liliana Sampaio Costa Mendes:** Visualization, Data curation, Writing – original draft. **Claudia Alexandra Pontes Ivantes:** Visualization, Data curation, Writing – original draft. **Liana Codes:** Visualization, Data curation, Writing – original draft. **Fabio Heleno de Lima Pace:** Visualization, Data curation, Writing – original draft. **Mario Guimarães Pessoa:** Visualization, Data curation, Writing – original draft. **Izabelle Venturini Signorelli:** Visualization, Data curation, Writing – original draft. **Gabriela Perdomo Coral:** Visualization, Data curation, Writing – original draft. **Paulo Lisboa Bittencourt:** Conceptualization, Visualization, Data curation, Writing – original draft, Writing – review & editing. **Cynthia Levy:** Visualization, Data curation, Writing – original draft. **Cláudia Alves Couto:** Writing – review & editing, Conceptualization, Visualization, Data curation, Writing – original draft, Writing – review & editing.

Funding

References

[1] Carey EJ, Ali AH, Lindor KD. Primary biliary cirrhosis. Lancet 2015;386:1565–75.

[2] Peters MG, Di Bisceglie AM, Kowdley KV, Flye NL, Luketic VA, Munoz SJ, et al. Differences between Caucasian, African American, and Hispanic patients with primary biliary cirrhosis in the United States. Hepatology 2007;46:769–75.

[3] Levy C, Naik J, Giordano C, Mandalia A, O'Brien C, Bhamidimarri KR, et al. Hispanics with primary biliary cirrhosis are more likely to have features of autoimmune hepatitis and reduced response to ursodeoxycholic acid than non-Hispanics. Clin Gastroenterol Hepatol 2014;12:1398–405.

[4] Melchor-Mendoza YK, Martínez-Benítez B, Mina-Hawat A, Rodríguez-Leal G, Duque X, Moran-Villota S. Ursodeoxycholic acid therapy in patients with primary biliary cholangitis with limited liver transplantation availability. Ann Hepatol 2017;16:430–5.

[5] Giolo SR, Soler JM, Greenway SC, Almeida MA, de Andrade M, Seidman JG, et al. Brazilian urban population genetic structure reveals a high degree of admixture. Eur J Hum Genet 2012;20:111–6.

[6] Lindor KD, Bowlus CL, Boyer J, Levy C, Mayo M. Primary biliary cholangitis: 2018 practice guidance from the American association for the study of liver diseases. Hepatology 2019;69:394–419.

[7] Chazouillères O, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology 1998;28:296–301.

[8] Parés A, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic acid. Gastroenterology 2006;130:715–20.

[9] Corpechot C, Abenavoli L, Rabahi N, Chrétien Y, Andréani T, Johanet C, et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. Hepatology 2008;48:871–7.

[10] Kumagi T, Guindi M, Fischer SE, Arenovich T, Abdalian R, Coltescu C, et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. Am J Gastroenterol 2010;105:2186–94.

[11] Corpechot C, Chazouillères O, Poupon R. Early primary biliary cirrhosis: biochemical response to treatment and prediction of long-term outcome. J Hepatol 2011;55:1361–7.

[12] Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med 2016;375:631–43.

[13] Haynes R. Determinants of compliance: the disease and the mechanics of treatment. Johns Hopkins University Press; 1979.

[14] Ludwig J, Dickson ER, McDonald GSA. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379:103–12.

[15] Lins TC, Vieira RG, Abreu BS, Gentil P, Moreno-Lima R, Oliveira RJ, Pereira RW. Genetic heterogeneity of self-reported ancestry groups in an admixed Brazilian population. J Epidemiol 2011;21:240–5.

[16] Galoosian A, Hanlon C, Tana M, Cheung R, Wong RJ. Race/ethnicity and insurance-specific disparities in in-hospital mortality among adults with primary biliary cholangitis: analysis of 2007-2014 national inpatient sample. Dig Dis Sci 2020;65:406–15.

[17] Sayiner M, Golabi P, Stepanova M, Younossi I, Nader F, Racila A, et al. Primary biliary cholangitis in medicare population: the impact on mortality and resource use. Hepatology 2019;69:237–44.

[18] Lu M, Li J, Haller IV, Romanelli RJ, VanWormer JJ, Rodriguez CV, et al. Factors associated with prevalence and treatment of primary biliary cholangitis in united states health systems. Clin Gastroenterol Hepatol 2018;16:1333–41 e6.

[19] Gordon SC, Wu KH, Lindor K, Bowlus CL, Rodriguez CV, Anderson H, et al. Ursodeoxycholic acid treatment preferentially improves overall survival among african americans with primary biliary cholangitis. Am J Gastroenterol 2020;115:262–70.

[20] Rosa R, Cristoferi L, Tanaka A, Invernizzi P. Geoepidemiology and (epi-)genetics in primary biliary cholangitis. Best Pract Res Clin Gastroenterol 2018;34-35:11–5.

[21] Corpechot C, Carrat F, Bonnand A-M, et al. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. Hepatology 2000;32:1196–9.

[22] Harms MH, van Buuren HR, Corpechot C, Thorburn D, Janssen HLA, Lindor KD, et al. Ursodeoxycholic acid therapy and liver transplant-free survival in patients with primary biliary cholangitis. J Hepatol 2019;71:357–65.

[23] Lammers WJ, van Buuren HR, Hirschfield GM, Janssen HL, Invernizzi P, Mason AL, et al. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. Gastroenterology 2014;147:1338–49 e5.

[24] Quarneti C, Muratori P, Lalanne C, Fabbri A, Menichella R, Granito A, et al. Fatigue and pruritus at onset identify a more aggressive subset of primary biliary cirrhosis. Liv Int 2015;35:636–41.

[25] Granito A, Muratori P, Ferri S, Pappas G, Quarneti C, Lenzi M, et al. Diagnosis and therapy of autoimmune hepatitis. Mini Rev Med Chem 2009;9:847–60.

[26] Granito A, Muratori L, Lalanne C, Quarneti C, Ferri S, Guidi M, et al. Hepatocellular carcinoma in viral and autoimmune liver diseases: role of CD4+ CD25+ Foxp3+ regulatory T cells in the immune microenvironment. World J Gastroenterol 2021;27:2994–3009.

[27] Hosonuma K, Sato K, Yamazaki Y, Yanagisawa M, Hashizume H, Horiguchi N, et al. A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia. Am J Gastroenterol 2015;110:423–31.

[28] Duan W, Ou X, Wang X, Wang Y, Zhao X, Wang Q, et al. Efficacy and safety of fenofibrate add-on therapy for patients with primary biliary cholangitis and a suboptimal response to UDCA. Rev Esp Enferm Dig 2018;110:557–63.

[29] Corpechot C, Chazouillères O, Rousseau A, Le Gruyer A, Habersetzer F, Mathurin P, et al. A placebo-controlled trial of bezafibrate in primary biliary cholangitis. N Engl J Med 2018;378:2171–81.

[30] Reig A, Sesé P, Parés A. Effects of bezafibrate on outcome and pruritus in primary biliary cholangitis with suboptimal ursodeoxycholic acid response. Am J Gastroenterol 2018;113:49–55.

[31] Grigorian AY, Mardini HE, Corpechot C, Poupon R, Levy C. Fenofibrate is effective adjunctive therapy in the treatment of primary biliary cirrhosis: a meta-analysis. Clin Res Hepatol Gastroenterol 2015;39:296–306.

[32] Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. J Med Chem 2000;43:527–50.

[33] Bowlus L, Trauner M, Liberman A, Malecha E, MacConell L, Andreas E, et al. Long-term efficacy and safety of obeticholic acid in patients with PBC from the POISE trial grouped biochemically by risk of disease progression. Dig Liv Dis 2020;53(1 suppl).

5.2. Artigo 2

ORIGINAL ARTICLE

Anti-mitochondrial Antibody-Negative Primary Biliary Cholangitis Is Part of the Same Spectrum of Classical Primary Biliary Cholangitis

Guilherme Grossi Lopes Cançado[1,2] · Michelle Harriz Braga[3] · Maria Lucia Gomes Ferraz[4] ·
Cristiane Alves Villela-Nogueira[5] · Debora Raquel Benedita Terrabuio[3] · Eduardo Luiz Rachid Cançado[3] ·
Mateus Jorge Nardelli[1] · Luciana Costa Faria[1] · Nathalia Mota de Faria Gomes[4] · Elze Maria Gomes Oliveira[6] ·
Vivian Rotman[5] · Maria Beatriz Oliveira[7] · Simone Muniz Carvalho Fernandes da Cunha[8] ·
Marlone Cunha-Silva[9] · Liliana Sampaio Costa Mendes[10] · Claudia Alexandra Pontes Ivantes[11] · Liana Codes[8,12] ·
Valéria Ferreira de Almeida e Borges[13,14] · Fabio Heleno de Lima Pace[15] · Mario Guimarães Pessoa[3] ·
Izabelle Venturini Signorelli[16] · Gabriela Perdomo Coral[17] · Paulo Lisboa Bittencourt[12,18] · Cynthia Levy[19] ·
Cláudia Alves Couto[1] · Members of the Brazilian Cholestasis Study Group Consortium

Abstract

Background Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease in which anti-mitochondrial antibodies (AMA) are the diagnostic hallmark. Whether AMA-negative PBC patients represent a different phenotype of disease is highly debated.

Aims The purpose of our study was to compare AMA-positive and AMA-negative PBC patients in a large non-white admixed Brazilian cohort.

Methods The Brazilian Cholestasis Study Group multicentre database was reviewed to assess demographics, clinical features and treatment outcomes of Brazilian PBC patients, stratifying data according to AMA status.

Results A total of 464 subjects (95.4% females, mean age 56 ± 5 years) with PBC were included. Three hundred and eighty-four (83%) subjects were AMA-positive, whereas 80 (17%) had AMA-negative PBC. Subjects with AMA-negative PBC were significantly younger (52.2 ± 14 vs. 59.6 ± 11 years, $p = 0.001$) and had their first symptom at an earlier age (43.2 ± 13 vs. 49.5 ± 12 years, $p = 0.005$). Frequency of type 2 diabetes was significantly increased in subjects with AMA-negative PBC (22.5% vs. 12.2%, $p = 0.03$). Lower IgM (272.2 ± 183 vs. 383.2 ± 378 mg/dL, $p = 0.01$) and triglycerides (107.6 ± 59.8 vs. 129.3 ± 75.7 mg/dL, $p = 0.025$) and higher bilirubin (3.8 ± 13.5 vs. 1.8 ± 3.4 mg/dL, $p = 0.02$) levels were also observed in this subgroup. Response to ursodeoxycholic acid varied from 40.5 to 63.3% in AMA-positive and 34 to 62.3% in AMA-negative individuals, according to different response criteria. Outcomes such as development of liver-related complications, death and requirement for liver transplantation were similar in both groups.

Conclusions AMA-negative PBC patients are similar to their AMA-positive counterparts with subtle differences observed in clinical and laboratory features.

Keywords Primary biliary cholangitis · Anti-mitochondrial antibody · Ursodeoxycholic acid · Autoantibody · Disease phenotype

Abbreviations

PBC	Primary biliary cholangitis
AMA	Anti-mitochondrial antibodies
IIF	Indirect immunofluorescence
IB	Immunoblotting
AIH	Autoimmune hepatitis
ANA	Antinuclear antibodies
SMA	Anti-smooth muscle antibody
IgM	Immunoglobulin M
UDCA	Ursodeoxycholic acid
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyltransferase

✉ Guilherme Grossi Lopes Cançado
guilhermegrossi@terra.com.br

Extended author information available on the last page of the article

🖄 Springer

SD   Standard deviation
IQR   Interquartile range
MAFLD Metabolic dysfunction-associated fatty liver disease

Introduction

Primary biliary cholangitis (PBC) is an immune-mediated inflammatory liver disorder that affects interlobular bile ducts leading to bile duct injury, ductopenia and cirrhosis [1, 2]. It is much more common in Caucasian middle-aged women and is usually progressive without treatment toward end-stage liver disease requiring liver transplantation [1–3]. Anti-mitochondrial antibodies (AMA) are the serological hallmarks of PBC [4]. In subjects with cholestasis, their presence either by indirect immunofluorescence (IIF) or by other immunoassays such as ELISA or immunoblotting (IB) is regarded as sufficient for the diagnosis of PBC without requirement of further histological evaluation [3]. AMAs are found in 78–90% of patients when tested by IIF, and in 90–95% when more accurate immunoassays are used [4–8]. On the other hand, AMA can be detected in 0.1–0.5% of apparently healthy subjects [9–11] or in patients with other autoimmune liver diseases, mainly autoimmune hepatitis (AIH) [11, 12]. They are also considered as early markers of PBC even in the absence of cholestasis and predictors of disease development [13]. In fact, 10.2–16% of healthy AMA-positive patients have been shown to evolve to full-blown PBC during follow-up, while up to 83% of the individuals with baseline histological findings compatible with PBC developed clinical and biochemical features of PBC after the initial positive antibody test [14–17].

It is however well acknowledged that 5–15% of patients with PBC worldwide lack AMA [3, 8, 18]. This is challenging since immune-mediated damage to biliary epithelial cells in PBC is directed against the same E2 subunits of 2-oxo-acid dehydrogenase complex epitopes recognized by AMA [2]. It is also not entirely known whether the presence of AMA defines different subgroups of patients with AMA-positive and AMA-negative PBC, implying varying natural history [7, 19–28]. In the past, several authors have considered AMA-negative PBC as part of the spectrum of PBC and AIH overlap syndrome [29, 30]. Those authors coined the term autoimmune cholangitis to define AMA-negative PBC by the presence of high-titer antinuclear (ANA) and/or anti-smooth muscle (SMA) antibodies, prominent lobular and portal inflammation on liver biopsy and biochemical response to corticosteroids [31–33]. More recently, the term AMA-negative PBC has been used to define a subset of patients who lack AMA but have typical histological changes of PBC. More than half of these patients have detectable ANAs and 40–50% of these are PBC-specific

(multiple nuclear dots and rim-like membrane pattern), further supporting the diagnosis [34]. In spite of those findings, it is still unclear in the literature whether the presence of AMA could influence clinical expression and outcomes in subjects with PBC. In this respect, some [26, 27] but not all reports [19–25, 28] have described distinct clinical features in AMA-negative PBC patients including higher frequency of ANA and SMA and lower levels of serum immunoglobulin M (IgM) [20–23], reduced response to ursodeoxycholic acid (UDCA) and transplantation-free survival when compared to their AMA-positive counterparts [26, 27].

The purpose of this study was to compare clinical, laboratory and histological features of AMA-positive and AMA-negative PBC patients in a large non-white admixed Brazilian cohort.

Methods

Study Population

The study population included adult (≥ 18 years old) patients who were diagnosed with PBC between January 1st, 1992 and December 31st, 2019 in 28 different hepatology centers from all regions of the country. The diagnosis of PBC was considered if patient fulfilled at least two of the following diagnostic criteria as recommended by the American Association for the Study of Liver Disease guidelines: (i) positive serology for anti-mitochondrial antibodies (AMA); (ii) persistent increase in the serum alkaline phosphatase (ALP) levels; and (iii) liver histology compatible with PBC (3). Patients in whom the diagnosis could not be confirmed or who had another etiology of liver disease, including overlap syndrome with autoimmune hepatitis, were excluded.

Data Collection

Each investigator was asked to identify all PBC patients that have been followed in their Liver Center at the time of the survey, without any selection or exclusion whatsoever, and to fill-in a standardized database provided by the Brazilian Cholestasis Study Group to assess retrospectively demographics, real-life clinical, laboratory and histological features of PBC, as well as response to treatment with either UDCA and/or fibrates. Briefly, data obtained from medical records included sex; age at diagnosis; year of diagnosis; year of first symptoms or first biochemical changes; last date of follow-up; baseline clinical presentation, concurrent autoimmune diseases, dyslipidemia and type 2 diabetes; baseline liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), bilirubin, albumin, IgM, immunoglobulin G, glucose, triglycerides

and cholesterol levels; autoantibody profile including ANA, SMA and AMA; liver histology staged according to the Ludwig system; presence of osteopenia or osteoporosis; development of liver-related complications; response to treatment with UDCA and/or fibrates; liver transplantation and death. The response to treatment either to UDCA or fibrates was analyzed according to international validated criteria including Barcelona, Paris I and II, Toronto, Rotterdam and POISE trial criteria [34–38]. The duration of follow-up was defined as the interval between the diagnosis and the last visit or the date of liver transplantation or death.

All demographics, clinical and laboratory data including response to treatment and outcomes were compared according to AMA status assessed by IIF in two groups of patients: AMA-positive and AMA-negative PBC. All AMA-positive patients had titers ≥ 1:40. Liver histology specimens were available for all patients with AMA-negative and 256 AMA-positive PBC patients. Cirrhosis was diagnosed both histologically (when available) or clinically according to several parameters, such as (a) presence of esophagogastric varices on endoscopy; (b) suggestive imaging studies (abdominal ultrasound, computed tomography or magnetic resonance); (c) platelet count $< 150,000/mm^3$ without other possible explanations, (d) liver-related biochemical alterations, such as serum albumin < 3.5 g/dL and enlarged INR, (e) signs of liver failure on physical exam. This study was conducted in accordance with the ethical standards of the Helsinki Declaration and was approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149).

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (IBM, USA). Continuous variables distribution was assessed by Shapiro–Wilk test, and those with Gaussian distribution were expressed as mean and standard deviation (SD), or as median and interquartile range (IQR) if skewed distribution. Categorical variables were expressed as absolute number and percentage. Univariate analysis was performed using chi-square or Fisher exact test, as appropriate, for categorical variables. Continuous variables were analyzed by the Student t test or Mann–Whitney U test, according to the distribution. Pairwise deletion was applied to missing data. P values < 0.05 were considered statistically significant.

Results

Patient Characteristics

Four hundred sixty-four subjects (95.4% female, mean age 56 ± 5 years) with well-defined diagnosis of PBC were included in this study. Three hundred eighty-four (83%) subjects were AMA-positive PBC patients, whereas 80 (17.2%) had AMA-negative PBC. Demographic, clinical and laboratory features are summarized in Table 1. Subjects with AMA-negative PBC were significantly younger (52.2 ± 14 vs. 59.6 ± 11 years in AMA-positive patients, $p = 0.001$) and had their first symptom at an earlier age (43.2 ± 13 vs. 49.5 ± 12 years in AMA-positive patients, $p = 0.005$) when compared to their counterparts with AMA-positive PBC. Age at diagnosis was also lower and time to diagnosis was longer in AMA-negative patients, but the difference was not statistically significant for either variable. With respect to AMA status, no other differences in demographics and baseline clinical features were observed, with the exception of the frequency of type 2 diabetes mellitus, that was significantly increased in those subjects with AMA-negative PBC (22.5% vs. 12.2% in patients with AMA-positive PBC, $p = 0.03$). Comparison of baseline laboratory features revealed that AMA-negative patients when compared to their AMA-positive counterparts have baseline lower IgM (272.2 ± 183 vs. 383.2 ± 378 mg/dL, $p = 0.01$) and triglycerides (107.6 ± 59.8 vs. 129.3 ± 75.7 mg/dL, p = 0.025) and higher bilirubin (3.8 ± 13.5 vs. 1.8 ± 3.4 mg/dL, p = 0.02) levels. No differences were observed in ANA prevalence (Table 1). Mean dose of UDCA was 12.86 ± 2.7 and 13.3 ± 2.2 mg/Kg in AMA- positive and negative groups, respectively ($p = 0.39$). Any patient was using fibrate at the baseline. Response to UDCA varied from 40.5 to 63.3% in AMA-positive and 34 to 62.3% in AMA-negative subjects, according to different response criteria. (Table 2). No difference was observed in the frequency of treatment response in those groups of patients using different available criteria. On the contrary, paired analysis of ALP and GGT levels over 5 years of UDCA treatment showed slower decline of both ALP and GGT in those AMA-negative patients when compared to their AMA-positive counterparts, but the difference was not statistically significant (Fig. 1), with the exception of 2 years follow-up time. Outcomes such as development of liver-related complications and death and requirement for liver transplantation were similar in both groups of patients.

Discussion

The present study analyzed 464 subjects with well-defined PBC. Eighty (17%) of them lacked AMA when tested by IIF in local reference laboratories in Brazil, one of the largest cohorts of AMA-negative patients with PBC in real-world setting published thus far. Our findings support the concept that AMA-negative PBC subjects have subtle differences in baseline clinical and laboratory features but similar outcomes when compared to their AMA-positive counterparts. AMA-negative PBC subjects were shown to be significantly

Table 1 Baseline Clinical and Laboratory Features in Patients with AMA-positive and AMA-negative Primary Biliary Cholangitis

Variables	AMA-negative (n=80)	AMA-positive (n=384)	p values
Demographics			
Age (yrs.)	52.2 ± 14.1	59.6 ± 11.3	**0.001**
Age at first symptoms (yrs.)	43.2 ± 13.3	49.5 ± 11.9	**0.005**
Mean time to diagnosis (yrs.)	2.7 ± 4.5	1.9 ± 4.7	0.076
Age at diagnosis (yrs.)	47.8 ± 13.5	51.7 ± 10.9	0.056
Female sex	92.50%	96.35%	0.132
Clinical features			
Pruritus	46.7%	49.5%	0.75
Fatigue	36.4%	38.3%	0.80
Jaundice	23.1%	20.8%	0.77
Splenomegaly	7.5%	4.95%	0.41
Hepatomegaly	14.1%	14.8%	1.0
Xanthoma	5.0%	4.2%	0.76
Xanthelasma	6.33%	7.0%	1.0
Type 2 Diabetes Mellitus	22.5%	12.2%	**0.026**
Dyslipidemia	19%	22%	0.39
Concurrent autoimmune diseases			
Hashimoto's thyroiditis	13.8%	19.8%	0.27
Sjogren syndrome	8.9%	7.8%	0.93
Rheumatoid arthritis	8.9%	3.7%	0.06
Scleroderma	2.5%	6.5%	0.28
Laboratory features			
ANA	56.6%	66.3%	0.1
SMA	4.4%	3.88%	0.74
IgG (mg/dL)	1553.9 ± 515	1483.7 ± 519	0.39
IgM (mg/dL)	272.2 ± 183	383.2 ± 378	**0.01**
AST (x ULN)	2.6 ± 1.95	2.5 ± 1.9	0.66
ALT (x ULN)	3.1 ± 2.5	2.7 ± 2.5	0.09
ALP (x ULN)	3.8 ± 2.9	3.70 ± 3.0	0.27
GGT (x ULN)	13.3 ± 13.7	11.4 ± 11.6	0.16
Bilirubin (mg/dL)	3.8 ± 13.5	1.8 ± 3.4	**0.02**
Albumin (g/dL)	3.9 ± 0.5	3.9 ± 0.5	1.0
Platelets (mm^3)	216,640 ± 97,296	221,158 ± 90,536	0.74
Triglycerides (mg/dL)	107.6 ± 59.8	129.3 ± 75.7	**0.025**
Total Cholesterol (mg/dL)	230.4 ± 75.7	232.5 ± 76	0.57
Bone disease by densitometry			
Absent (n=64)	29%	33%	0.46
Osteopenia (n=82)	51.6%	40%	
Osteoporosis (n=51)	19.4%	27%	
Cirrhosis at baseline	36.6%	32%	0.53

AMA anti-mitochondrial antibody; *ANA* antinuclear antibody; *ALT* alanine aminotransferase; *ALP* alkaline phosphatase; *AST* aspartate aminotransferase; *GGT* gammaglutamyl transferase; *IgG* immunoglobulin G; *IgM* immunoglobulin M; *SMA* anti-smooth muscle antibody; *ULN* upper limit of normality; *Yrs.* years

younger at disease onset, and to have a longer time from symptoms onset to diagnosis, probably due to requirement of histological evaluation for definite diagnosis. As reported by other authors [22, 23, 29, 30], IgM levels were lower in AMA-negative patients with PBC when compared to their AMA-positive counterparts, but in contrast to other reports [21, 23, 29, 30], no increase in the frequency of either ANA or SMA was found in the former group of patients. Baseline higher bilirubin levels, usually associated with advanced disease [1–3], were more often encountered in subjects with AMA-negative PBC, indicating that those patients could have a more advanced liver disease at the time of diagnosis, possibly due to a delay in diagnosis. It is worth mentioning that a higher frequency of type 2 diabetes was also

Table 2 Outcomes and Response to Treatment in Patients with AMA-positive and AMA-negative PBC

Variables	AMA-negative (n=80)	AMA-positive (n=384)	p value
Mean follow-up time (years)	5.3±4.8	6.4±5.3	0.101
Liver-related complications patients during follow-up			
Variceal bleeding	5.6%	8.8%	0.36
Hepatic encephalopathy	5.7%	7.1%	1.0
Ascites	13.5%	15.2%	0.91
Spontaneous bacterial peritonitis	2.5%	4.0%	1.0
Hepatocellular carcinoma	0%	2.3%	0.6
Response to UDCA at 12 months			
Toronto criteria (n=316)	151 (57.6)	28 (51.9)	0.435
Barcelona criteria (n=312)	164 (63.3)	29 (54.7)	0.240
Paris-1 criteria (n=315)	158 (60.3)	30 (56.6)	0.616
Paris-2 criteria (n=315)	106 (40.5)	18 (34.0)	0.377
POISE trial criteria (n=315)	179 (68.3)	33 (62.3)	0.391
Rotterdam criteria (n=272)	150 (65.2)	23 (54.8)	0.195
Liver transplantation	5%	6.8%	0.74
Liver-related deaths	1.5%	3.6%	0.39

AMA anti-mitochondrial antibody; *PBC* primary biliary cholangitis; *UDCA* ursodeoxycholic acid

identified in those AMA-negative patients. Interestingly, Hindi et al. [40] have reported more advanced PBC in subjects with risk factors for metabolic syndrome and metabolic dysfunction-associated fatty liver disease (MAFLD) that is closely associated with type 2 diabetes mellitus. It is, thus, possible that those AMA-negative patients could have competing risks for advanced or progressive disease such as younger age at disease onset, higher bilirubin levels and associated MAFLD. On the other hand, lower levels of triglycerides were observed in this subgroup, a finding that might be linked to the use of hypoglycemic medications and/or insulin and reflect satisfactory glycemic control.

Differently from Sakauchi et al. [22], who reported a significantly higher prevalence of Sjogren's syndrome, rheumatoid arthritis, autoimmune thyroiditis, and scleroderma in AMA-negative patients, a similar distribution of concurrent autoimmune diseases was observed in AMA-positive and negative subjects in the present study. Furthermore, although ANA has been reported with extremely high proportions
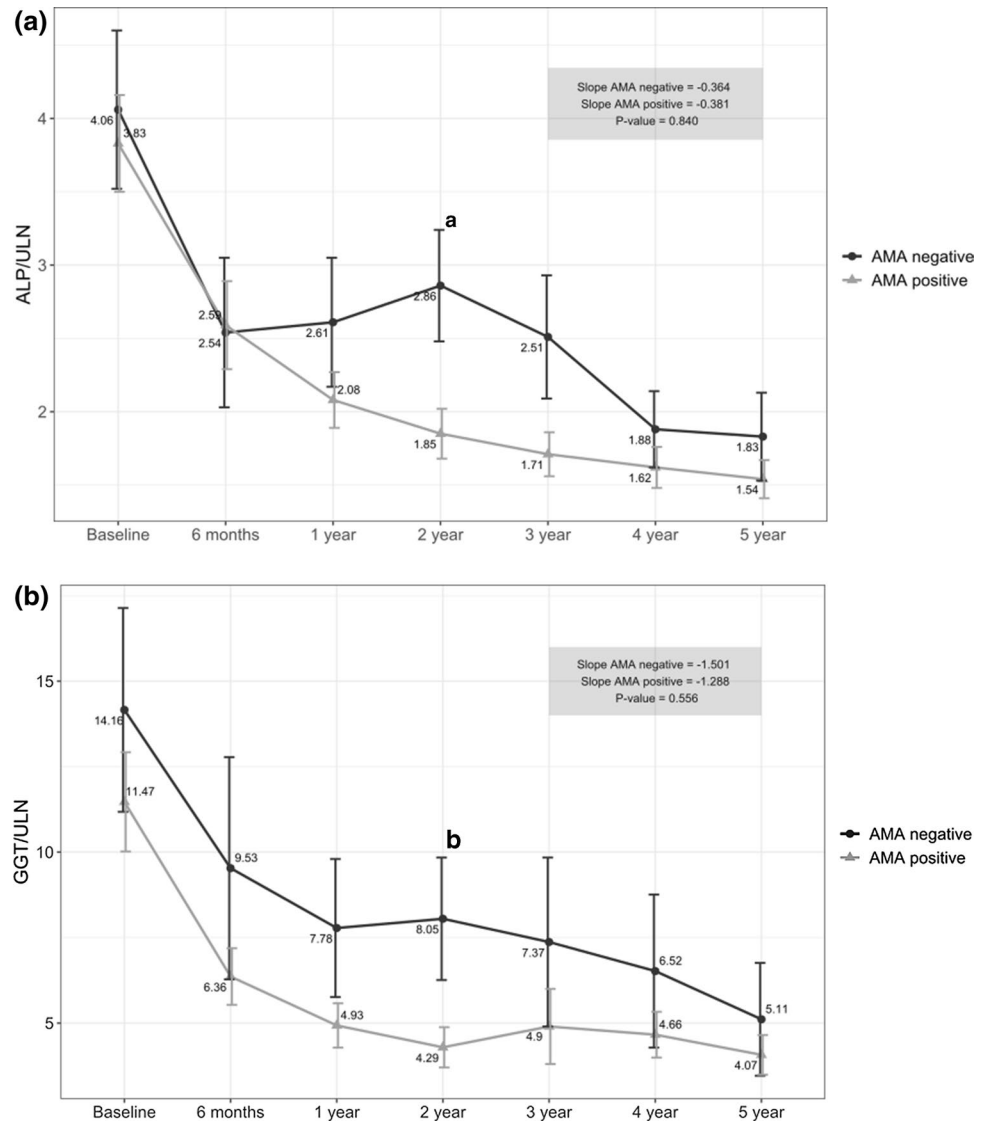
in AMA-negative PBC, in our study, we observed a relatively lower prevalence, but still very significant levels [21, 23]. This may be ascribed to differences in genetics and/or environmental factors related to each population or even to diverse methodology employed in each study [41, 42].

Several investigators have reported similar outcomes [21, 28] and treatment responses to UDCA [19, 24, 25] in patients with PBC irrespective of AMA status, whereas others reported conflicting results [26, 27]. Koulentani et al. [27] evaluated a very small cohort of patients with AMA-positive and AMA-negative PBC and suggested a lower effect of UDCA treatment in subjects with advanced disease and AMA-negative PBC. Juliusson et al. [26], on the other hand, reviewed 71 AMA-negative PBC matching them on year of diagnosis to the same number of AMA-positive counterparts. The authors reported reduced survival free of liver-related complications in the former group of patients. In the present study, the response to UDCA treatment was assessed using various internationally validated criteria with similar rates of response observed in AMA-negative PBC patients when compared to their AMA-positive counterparts. However, a slower decline in ALP and GGT levels was observed in AMA-negative PBC patients over 5-years, indicating that normalization or near normalization of ALP and GGT may take longer to achieve in AMA-negative patients. No difference in other outcomes such as liver-related complications, liver-related mortality or liver transplantation was noticed.

Our study has some limitations, including its retrospective design and lack of data regarding variable methods to test AMA (IB, beads and/or ELISA). It also important to highlight that our cohort presented a high prevalence of cirrhotic patients at baseline. This might reflect a referral bias to specialized hepatology centers or late diagnosis of PBC in Brazil. On the other hand, it has to be recognized that it reflects real-life practices of AMA detection that is currently based in IIF in large parts of the world.

In conclusion, our data show that AMA-negative PBC patients are remarkably similar to AMA-positive subjects in clinical and laboratory features, as well as in treatment responses and outcomes with very subtle differences. Even though treatment responses to UDCA are similar irrespective of AMA status, subjects with AMA-negative PBC may have a slower decline in ALP and GGT levels over time.

Fig. 1 ALP and GGT levels in subjects with PBC according to AMA status. Shown are the mean values of ALP and GGT at each time point of the follow-up. Bars indicate the standard deviation. [a]$p = 0.02$; [b]$p = 0.03$; *ALP* alkaline phosphatase; *AMA* anti-mitochondrial antibody; *GGT* gammaglutamyl transferase; *PBC* primary biliary cholangitis

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Lleo A, Wang G-Q, Gershwin ME, Hirschfield GM. Primary biliary cholangitis. *Lancet* (London, England) 2020; 396:1915–1926.

2. Leung KK, Deeb M, Hirschfield GM. Review article: pathophysiology and management of primary biliary cholangitis. *Aliment Pharmacol. Ther.* 2020; 52:1150–1164.

3. Lindor KD, Bowlus CL, Boyer J et al. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2019; 69:394–419.

4. Sebode M, Weiler-Normann C, Liwinski T, Schramm C. Autoantibodies in Autoimmune Liver Disease—Clinical and Diagnostic Relevance. *Front Immunol*. 2018. https://doi.org/10.3389/fimmu.2018.00609.

5. Hurlburt KJ, McMahon BJ, Deubner H et al. Prevalence of autoimmune liver disease in Alaska Natives. *Am J Gastroenterol*. 2002; 97:2402–7.

6. Arbour L, Rupps R, Field L et al. Characteristics of primary biliary cirrhosis in British Columbia's First Nations population. *Can. J. Gastroenterol*. 2005; 19:305–10.

7. Muratori P, Muratori L, Gershwin ME et al. "True" antimitochondrial antibody-negative primary biliary cirrhosis, low sensitivity of the routine assays, or both? *Clin Exp Immunol*. 2004; 135:154–8.

8. Selmi C, Zuin M, Bowlus CL, Gershwin ME. Anti-mitochondrial antibody-negative primary biliary cirrhosis. *Clin Liver Dis*. 2008; 12:173–85, ix.

9. Mattalia A, Quaranta S, Leung PS et al. Characterization of anti-mitochondrial antibodies in health adults. *Hepatology*. 1998; 27:656–61.

10. Cunha LM, Bittencourt PL, Abrantes-Lemos CP et al. Prevalence of non-organ-specific autoantibodies in a rural community from northeastern Brazil: a population-based study. *Hum Immunol*. 2012; 73:70–4.

11. Shibata M, Onozuka Y, Morizane T et al. Prevalence of antimitochondrial antibody in Japanese corporate workers in Kanagawa prefecture. J *Gastroenterol*. 2004; 39:255–9.

12. Farias AQ, Goncalves LL, Bittencourt PL et al. Applicability of the IAIHG scoring system to the diagnosis of antimitochondrial/anti-M2 seropositive variant form of autoimmune hepatitis. *J Gastroenterol Hepatol*. 2006; 21:887–893.

13. Sun C, Xiao X, Yan L et al. Histologically proven AMA positive primary biliary cholangitis but normal serum alkaline phosphatase: Is alkaline phosphatase truly a surrogate marker? *J Autoimmun*. 2019; 99:33–38.

14. Metcalf J V., Mitchison HC, Palmer JM et al. Natural history of early primary biliary cirrhosis. *Lancet* (London, England) 1996; 348:1399–402.

15. Mitchison HC, Bassendine MF, Hendrick A et al. Positive antimitochondrial antibody but normal alkaline phosphatase: Is this primary biliary cirrhosis? *Hepatology*. 1986. https://doi.org/10.1002/hep.1840060609.

16. Dahlqvist G, Gaouar F, Carrat F et al. Large-scale characterization study of patients with antimitochondrial antibodies but non established primary biliary cholangitis. *Hepatology*. 2017; 65:152–163.

17. Zandanell S, Strasser M, Feldman A, Tevini J, Strebinger G, Niederseer D, Pohla-Gubo G, Huber-Schönauer U, Ruhaltinger S, Paulweber B, Datz C, Felder TK, Aigner E. Low rate of new-onset primary biliary cholangitis in a cohort of anti-mitochondrial antibody-positive subjects over six years of follow-up. J Intern Med. 2020 Apr;287:395-404.

18. Oertelt S, Rieger R, Selmi C et al. A sensitive bead assay for antimitochondrial antibodies: Chipping away at AMA-negative primary biliary cirrhosis. *Hepatology*. 2007; 45:659–65.

19. Liu B, Shi XH, Zhang FC et al. Antimitochondrial antibody-negative primary biliary cirrhosis: a subset of primary biliary cirrhosis. Liver Int. 2008; 28:233–9.

20. Mendes F, Lindor KD. Antimitochondrial antibody-negative primary biliary cirrhosis. *Gastroenterol Clin North Am*. 2008; 37:479–84, viii.

21. Invernizzi P, Crosignani A, Battezzati PM et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. *Hepatology*. 1997. https://doi.org/10.1002/hep.510250507.

22. Sakauchi F, Mori M, Zeniya M, Toda G. Antimitochondrial antibody negative primary biliary cirrhosis in Japan: utilization of clinical data when patients applied to receive public financial aid. *J Epidemiol*. 2006; 16:30–4.

23. Zhang F-K, Jia J-D, Wang B-E. Clinical evaluation of serum antimitochondrial antibody-negative primary biliary cirrhosis. *Hepatobiliary Pancreat Dis Int*. 2004; 3:288–291.

24. Kim WR, Poterucha JJ, Jorgensen RA et al. Does antimitochondrial antibody status affect response to treatment in patients with primary biliary cirrhosis? Outcomes of ursodeoxycholic acid therapy and liver transplantation. *Hepatology*. 1997; 26:22–6.

25. Joshi S, Cauch-Dudek K, Heathcote EJ et al. Antimitochondrial antibody profiles: are they valid prognostic indicators in primary biliary cirrhosis? *Am J Gastroenterol*. 2002; 97:999–1002.

26. Juliusson G, Imam M, Björnsson ES et al. Long-term outcomes in antimitochondrial antibody negative primary biliary cirrhosis. *Scand J Gastroenterol*. 2016; 51:745–52.

27. Koulentaki M, Moscandrea J, Dimoulios P et al. Survival of anti-mitochondrial antibody-positive and -negative primary biliary cirrhosis patients on ursodeoxycholic acid treatment. *Dig Dis Sci*. 2004. https://doi.org/10.1023/B:DDAS.0000037811.48575.da.

28. Zandanell S, Strasser M, Feldman A et al. Similar clinical outcome of AMA immunoblot-M2-negative compared to immunoblot-positive subjects over six years of follow-up. *Postgrad Med*. 2021:1–8.

29. Michieletti P, Wanless IR, Katz A et al. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. *Gut*. 1994; 35:260–265.

30. Lacerda MA, Ludwig J, Dickson ER et al. Antimitochondrial antibody-negative primary biliary cirrhosis. *Am J Gastroenterol*. 1995; 90:247–249.

31. Kadokawa Y, Omagari K, Ohba K et al. Does the diagnosis of primary biliary cirrhosis of autoimmune cholangitis depend on the "phase" of the disease? *Liver Int*. 2005. https://doi.org/10.1111/j.1478-3231.2005.01078.x.

32. Sánchez-Pobre P, Castellano G, Colina F et al. Antimitochondrial antibody-negative chronic nonsuppurative destructive cholangitis. Atypical primary biliary cirrhosis or autoimmune cholangitis? *J. Clin. Gastroenterol*. 1996; 23:191–198.

33. Vierling JM. Primary biliary cirrhosis and autoimmune cholangiopathy. Clin Liver Dis. 2004. https://doi.org/10.1016/S1089-3261(03)00132-6.

34. Levy C, Bowlus CL. Role of Antinuclear Antibodies in Primary Biliary Cholangitis. *Am J Gastroenterol*. 2020; 115:1604–1606.

35. Parés A, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. *Gastroenterology*. 2006; 130:715–20.

36. Corpechot C, Abenavoli L, Rabahi N et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology*. 2008; 48:871–7.

37. Kumagi T, Guindi M, Fischer SE et al. Baseline ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. *Am J Gastroenterol*. 2010; 105:2186–94.

38. Corpechot C, Chazouillres O, Poupon R. Early primary biliary cirrhosis: Biochemical response to treatment and prediction of long-term outcome. *J Hepatol*. 2011. https://doi.org/10.1016/j.jhep.2011.02.031.

39. Nevens F, Andreone P, Mazzella G et al. A Placebo-Controlled Trial of Obeticholic Acid in Primary Biliary Cholangitis. *N Engl J Med*. 2016. https://doi.org/10.1056/nejmoa1509840.

40. Híndi M, Levy C, Couto CA et al. Primary biliary cirrhosis is more severe in overweight patients. *J Clin Gastroenterol*. 2013. https://doi.org/10.1097/MCG.0b013e318261e659.

41. Bittencourt PL, Palácios SA, Farias AQ et al. Analysis of major histocompatibility complex and CTLA-4 alleles in Brazilian patients with primary biliary cirrhosis. *J Gastroenterol Hepatol*. 2003; 18:1061–1066.

42. Tanaka A, Leung PSC, Gershwin ME. The genetics of primary biliary cholangitis. *Curr Opin Gastroenterol*. 2019. https://doi.org/10.1097/MOG.0000000000000507.

Authors and Affiliations

Guilherme Grossi Lopes Cançado[1,2] · Michelle Harriz Braga[3] · Maria Lucia Gomes Ferraz[4] ·
Cristiane Alves Villela-Nogueira[5] · Debora Raquel Benedita Terrabuio[3] · Eduardo Luiz Rachid Cançado[3] ·
Mateus Jorge Nardelli[1] · Luciana Costa Faria[1] · Nathalia Mota de Faria Gomes[4] · Elze Maria Gomes Oliveira[6] ·
Vivian Rotman[5] · Maria Beatriz Oliveira[7] · Simone Muniz Carvalho Fernandes da Cunha[8] ·
Marlone Cunha-Silva[9] · Liliana Sampaio Costa Mendes[10] · Claudia Alexandra Pontes Ivantes[11] · Liana Codes[8,12] ·
Valéria Ferreira de Almeida e Borges[13,14] · Fabio Heleno de Lima Pace[15] · Mario Guimarães Pessoa[3] ·
Izabelle Venturini Signorelli[16] · Gabriela Perdomo Coral[17] · Paulo Lisboa Bittencourt[12,18] · Cynthia Levy[19] ·
Cláudia Alves Couto[1] · Members of the Brazilian Cholestasis Study Group Consortium

[1] Instituto Alfa de Gastroenterologia, Hospital das Clínicas, Universidade Federal de Minas Gerais, Av. Professor Alfredo Balena 110, Belo Horizonte, Minas Gerais 30130-100, Brazil

[2] Hospital da Polícia Militar de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

[3] Departamento de Gastroenterologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil

[4] Disciplina de Gastroenterologia, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

[5] Hospital Universitário Clementino Fraga Filho e Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal Do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

[6] Centro Universitário Lusíada—UNILUS, Santos, São Paulo, Brazil

[7] Ambulatório Municipal de Hepatites Virais de São José Dos Campos, São José dos Campos, São Paulo, Brazil

[8] Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil

[9] Divisão de Gastroenterologia (Gastrocentro), Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

[10] Hospital de Base do Distrito Federal, Brasília, Distrito Federal, Brazil

[11] Serviço de Gastroenterologia, Hepatologia e Transplante Hepático, Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil

[12] Hospital Português, Salvador, Bahia, Brazil

[13] Instituto de Gastroenterologia, Endoscopia e Proctologia, Uberlândia, Minas Gerais, Brazil

[14] Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

[15] Serviço de Gastroenterologia e Hepatologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

[16] Hospital Universitário Cassiano Antônio Moraes, Universidade Federal do Espírito Santo, Vitória, Espírito Santo, Brazil

[17] Irmandade da Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

[18] Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

[19] Division of Digestive Health and Liver Diseases, University of Miami Miller School of Medicine, Miami, FL, USA

5.3. Artigo 3

Fibrates for the Treatment of Primary Biliary Cholangitis Unresponsive to Ursodeoxycholic Acid: An Exploratory Study

Guilherme Grossi Lopes Cançado [1,2]*, Cláudia Alves Couto [1], Laura Vilar Guedes [3], Michelle Harriz Braga [3], Débora Raquel Benedita Terrabuio [3], Eduardo Luiz Rachid Cançado [3], Maria Lucia Gomes Ferraz [4], Cristiane Alves Villela-Nogueira [5], Mateus Jorge Nardelli [1], Luciana Costa Faria [1], Elze Maria Gomes de Oliveira [6], Vivian Rotman [5], Daniel Ferraz de Campos Mazo [7], Valéria Ferreira de Almeida e Borges [8,9], Liliana Sampaio Costa Mendes [10], Liana Codes [11,12], Mario Guimarães Pessoa [3], Izabelle Venturini Signorelli [13], Cynthia Levy [14] and Paulo Lisboa Bittencourt [12,15] on behalf of Members of the Brazilian Cholestasis Study Group Consortium

[1]Instituto Alfa de Gastroenterologia, Hospital das Clínicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Hospital da Polícia Militar de Minas Gerais, Belo Horizonte, Brazil, [3]Departamento de Gastroenterologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil, [4]Disciplina de Gastroenterologia, Universidade Federal de São Paulo, São Paulo, Brazil, [5]Hospital Universitário Clementino Fraga Filho e Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, [6]Centro Universitário Lusíada—UNILUS, Santos, Brazil, [7]Divisão de Gastroenterologia (Gastrocentro), Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, Brazil, [8]Instituto de Gastroenterologia, Endoscopia e Proctologia, Uberlândia, Brazil, [9]Universidade Federal de Uberlândia, Uberlândia, Brazil, [10]Hospital de Base do Distrito Federal, Brasília, Brazil, [11]Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Brazil, [12]Hospital Português, Salvador, Brazil, [13]Hospital Universitário Cassiano Antônio Moraes, Universidade Federal do Espírito Santo, Vitória, Brazil, [14]Division of Digestive Health and Liver Diseases, University of Miami Miller School of Medicine, Miami, FL, United States, [15]Escola Bahiana de Medicina e Saúde Pública, Salvador, Brazil

Aim: Up to 40% of patients with primary biliary cholangitis (PBC) will have a suboptimal biochemical response to ursodeoxycholic acid (UDCA), which can be improved by the addition of fibrates. This exploratory study aims to evaluate the long-term real-life biochemical response of different fibrates, including ciprofibrate, in subjects with UDCA-unresponsive PBC.

Methods: The Brazilian Cholestasis Study Group multicenter database was reviewed to assess the response rates to UDCA plus fibrates in patients with UDCA-unresponsive PBC 1 and 2 years after treatment initiation by different validated criteria.

Results: In total, 27 patients (100% women, mean age 48.9 ± 9.2 years) with PBC were included. Overall response rates to fibrates by each validated criterion varied from 39 to 60% and 39–76% at 12 and 24 months after treatment combination, respectively. Combination therapy resulted in a significant decrease in ALT and ALP only after 2 years, while GGT significantly improved in the first year of treatment. Treatment

---

response rates at 1 and 2 years appear to be comparable between ciprofibrate and bezafibrate using all available criteria.

Conclusion: Our findings endorse the efficacy of fibrate add-on treatment in PBC patients with suboptimal response to UDCA. Ciprofibrate appears to be at least as effective as bezafibrate and should be assessed in large clinical trials as a possibly new, cheaper, and promising option for treatment of UDCA-unresponsive PBC patients.

Keywords: bezafibrate, ciprofibrate, fibrate, primary biliary cholangitis, treatment failure, ursodeoxycholic acid

INTRODUCTION

Primary biliary cholangitis (PBC) is a cholestatic liver disorder of unknown cause that may progress to cirrhosis and liver failure (Lleo et al., 2020). Treatment with ursodeoxycholic acid (UDCA) has been shown to improve transplantation-free survival, particularly in subjects with biochemical response assessed 1 year after treatment (Harms et al., 2019; Montano–Loza and Corpechot, 2020). However, more than one-third of the patients with PBC do not respond to UDCA (Montano–Loza and Corpechot, 2020). Recently, add-on therapy with fibrates was shown to improve treatment responses to UDCA in refractory patients (Ghonem and Boyer, 2013; Grigorian et al., 2015; Corpechot et al., 2018; Reig et al., 2018). Fibrates are peroxisome proliferator–activated receptor (PPAR) agonists and are FDA approved for treatment of dyslipidemia. PPARs are a family of ligand-dependent transcription factors composed of three subtypes PPARα, PPARβ/δ, and PPARγ with different functions, distributions, affinities, and specificities for their ligands. Each of them has distinct pleiotropic roles in the modulation of energy, lipid, cholesterol, and bile acid homeostasis (Ghonem et al., 2015; Tanaka et al., 2017; Monroy–Ramirez et al., 2021). In this regard, it has been demonstrated that PPARα activation is capable of modulating bile acid metabolism due to activation of genes involved in bile acid synthesis and transportation. Fenofibrate and pemafibrate, PPARα ligands, and bezafibrate, a pan-PPAR agonist, were shown to improve treatment response to UDCA in several uncontrolled randomized controlled trials (RCTs) (Ghonem and Boyer, 2013; Grigorian et al., 2015; Reig et al., 2018) and at least one RCT (Corpechot et al., 2018). Pruritus was also significantly improved in subjects with PBC (Reig et al., 2018) and primary sclerosing cholangitis (de Vries et al., 2021) treated with bezafibrate. Most of the studies evaluating the use of fibrates in cholestatic liver diseases employed either one of those drugs; the use of ciprofibrate, another PPARα agonist, has not yet been evaluated in patients with PBC. The purpose of this exploratory study was to evaluate the long-term real-life biochemical response of different fibrates in subjects with PBC unresponsive to UDCA.

METHODS

Study Population

The Brazilian Cholestasis Study Group (BCSG) is a multicenter collaborative consortium of investigators from academic institutions and community-based sites that treat patients with PBC in Brazil. The study population included adult (aged ≥18 years) patients diagnosed with PBC between January 1st 1992 and December 31st' 2019 in 28 hepatology centers across the country. All study procedures were conducted in accordance with the ethical standards of the Helsinki Declaration. The present study was approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149), and individual informed consent was waived as this study was retrospective in design. Diagnosis of PBC was considered if patients fulfilled at least two of the following three diagnostic criteria for PBC as recommended by the American Association for the Study of Liver Diseases guidelines: 1) positive serology for anti-mitochondrial antibodies (AMA) or PBC-specific antinuclear antibodies (ANA); 2) persistent increase in the serum alkaline phosphatase (ALP) level; and 3) liver histology compatible with PBC (Lindor et al., 2019). Patients in whom the diagnosis could not be confirmed or who had another etiology of liver disease were excluded.

Data Collection

Each investigator was asked to identify all PBC patients followed up in their center at the time of the survey, without any selection or exclusion whatsoever, and to fill in a standardized database provided by the BCSG. Patients unresponsive to UDCA after at least 1 year of treatment were identified in the database and those individuals treated with fibrates enrolled in this study. Data on liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and ALP, were collected at baseline and 12 and 24 months after fibrate add-on therapy for paired analysis. Biochemical results were normalized by upper limit of normal (ULN) to homogenize data interpretation. The considered standardized daily dose of UDCA for PBC treatment was 13–15 mg/kg of body weight. Lack of response to UDCA treatment was analyzed according to local investigator discretion using either one of the following criteria: Barcelona, Paris 1 and 2, Toronto, Rotterdam, and POISE trial at different time points. The duration of follow-up was defined as the interval between the diagnosis and the last visit or the date of liver transplantation or death. Advanced PBC was defined by the presence of moderate to severe fibrosis (Ludwig stage III or IV) on liver histology (when available) or clinical evidence of cirrhosis. All patients with cirrhosis were Child–Pugh A and had compensated disease.

TABLE 1 | Baseline characteristics of patients with primary biliary cholangitis using fibrates.

Variable	*N* = 27
Age at diagnosis (years ± SD)	48.9 ± 9.2
Female	100%
Autoantibody	
AMA-positive	88.9%
ANA-positive	85.2%
Symptoms at diagnosis	
Asymptomatic	33.3%
Pruritus	44.4%
Fatigue	44.4%
Coexistent autoimmune diseases	
Hashimoto thyroiditis	14.8%
Sjogren syndrome	11.1%
CREST syndrome	3.7%
Histological disease stage, n (%)	20 (74.1)
Stage I	25%
Stage II	40%
Stage III	25%
Stage IV	10%
Follow-up time (months ± SD)	67 ± 35
Advanced PBC	29.6%
Liver transplantation during follow-up	3.7%
Death	7.4%

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (IBM, United States). Continuous variables distribution was assessed by the Shapiro–Wilk test, and those with Gaussian distribution were expressed as mean and standard deviation, or as median and interquartile range (IQR) in case of skewed distribution. Categorical variables were expressed as absolute number and percentage. Univariate analysis was performed using chi-square, Fisher's exact, or McNemar's test, as appropriate, for categorical variables. Continuous variables were analyzed by the Student *t*-test or Mann–Whitney *U*-test, according to the distribution. A *p*-value < 0.05 was considered significant.

RESULTS

Patient Characteristics

The clinical and laboratory features and treatment outcomes of the entire cohort of 482 Brazilian patients with PBC were previously described (Cançado et al., 2022). Fifty-nine patients with inadequate response to UDCA received add-on therapy with fibrates. Twenty-seven of the 59 patients had paired results of liver enzymes at baseline and 1 and 2 years after treatment with bezafibrate (*n* = 9) or ciprofibrate (*n* = 18) and were included in this analysis (**Table 1**). Briefly, all patients were women, with a mean age at diagnosis of 48.9 ± 9.2 years. Based on histological or clinical and laboratory findings, 29.6% of them had advanced PBC disease. The mean time of UDCA treatment before add-on therapy with fibrates was 19.7 ± 10.6 months. All patients were followed up for a mean period of 67 ± 35 months. The mean dose of bezafibrate was 358.3 ± 82.1 mg/day, while that of ciprofibrate was 100 mg/day. Two (7.4%) patients

died and 1 (3.7%) required liver transplantation during the follow-up.

Response to Fibrates

Overall response rates to fibrates by each validated criterion at 12 and 24 months are shown in **Figure 1A**. The proportion of nonresponders to treatment continued to reduce after 1 year of treatment with fibrates, reaching lower values at 24 months. ALP levels diminished at any degree in 59.4% of the patients after 12 months and in 66.7% after 24 months. Combination therapy resulted in a statistically significant decrease in AST/ULN and ALP/ULN only after 2 years, while GGT/ULN significantly improved in the first year of treatment (**Table 2**). Treatment response rates at 1 and 2 years for the ciprofibrate and bezafibrate groups are shown in **Figure 1B**.

No differences in response rates by different criteria at 12 and 24 months of therapy were observed when comparing patients with AMA-positive *vs* AMA-negative PBC for response to treatment, except for the Barcelona criteria at 24 months, in which AMA-positive patients were more likely to achieve response to treatment (71.4% *vs* 28.6%, *p* = 0.042). Biochemical changes stratified by the presence and absence of advanced PBC are presented in **Supplementary Table S1**.

DISCUSSION

About 40% of the patients will not have an optimal response to UDCA and are at a higher risk for disease progression to cirrhosis and liver failure. In this study, we have shown that more than half
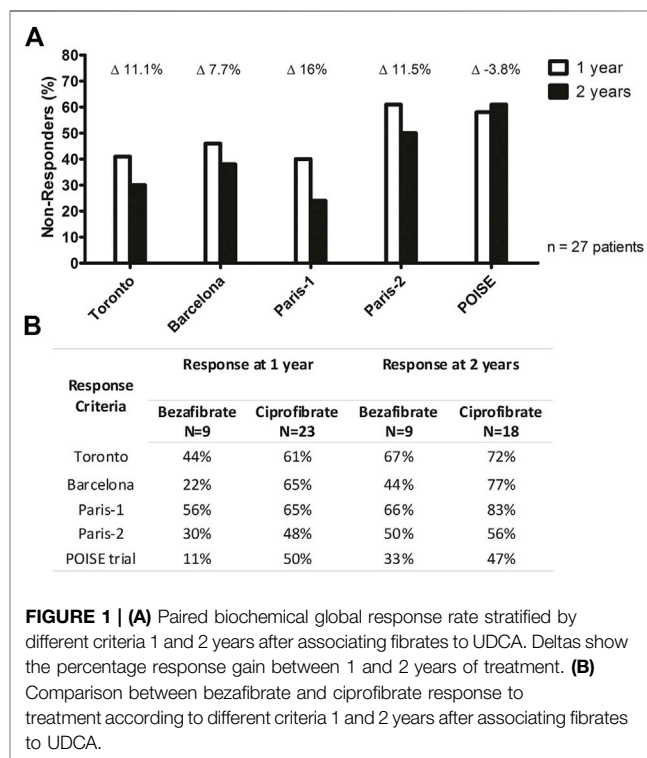

Response Criteria	Response at 1 year		Response at 2 years	
	Bezafibrate N=9	Ciprofibrate N=23	Bezafibrate N=9	Ciprofibrate N=18
Toronto	44%	61%	67%	72%
Barcelona	22%	65%	44%	77%
Paris-1	56%	65%	66%	83%
Paris-2	30%	48%	50%	56%
POISE trial	11%	50%	33%	47%

FIGURE 1 | (A) Paired biochemical global response rate stratified by different criteria 1 and 2 years after associating fibrates to UDCA. Deltas show the percentage response gain between 1 and 2 years of treatment. **(B)** Comparison between bezafibrate and ciprofibrate response to treatment according to different criteria 1 and 2 years after associating fibrates to UDCA.

TABLE 2 | Median paired biochemical changes overtime after the introduction of fibrates

Time of measurement	AST/ULN (n = 27)	AST/ULN percentage difference from baseline	p-value Comparison with baseline values	p-value Comparison with last measurement
Baseline	1.42	—	—	—
1 year	1.10	−25.4%	0.353	0.353
2 years	1.06	−26.9%	0.052	0.010
Time of measurement	ALT/ULN (n = 27)	ALT/ULN percentage difference from baseline	p-value Comparison with baseline values	p-value Comparison with last measurement
Baseline	1.52	—	—	—
1 year	1.13	−25.7%	0.287	0.287
2 years	1.06	−30%	0.030	0.101
Time of measurement	ALP/ULN (n = 27)	ALP/ULN percentage difference from baseline	p-value Comparison with baseline values	p-value Comparison with last measurement
Baseline	1.73	—	—	—
1 year	1.61	−7%	0.304	0.304
2 years	1.36	−21.4%	0.021	0.225
Time of measurement	GGT/ULN (n = 27)	GGT/ULN percentage difference from baseline	p-value Comparison with baseline values	p-value Comparison with last measurement
Baseline	4.60	—	—	—
1 year	3.22	−30%	0.048	0.048
2 years	3.08	−33%	0.036	0.278
Time of measurement	TB/ULN (n = 27)	TB/ULN percentage difference from baseline	p-value Comparison with baseline values	p-value Comparison with last measurement
Baseline	0.54	—	—	—
1 year	0.55	+1.8%	0.647	0.647
2 years	0.43	−20.4%	0.820	0.386

AST, aspartate aminotransferase; ULN, upper limit of normal; UDCA, ursodeoxycholic acid; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TB, total bilirubin. Data are expressed as ratio between serum measurement and ULN. Wilcoxon test was performed.

of those patients with PBC previously unresponsive to UDCA using different criteria had 1-year biochemical response with add-on therapy with either ciprofibrate or bezafibrate. Most of the patients with PBC were treated with ciprofibrate because this drug is currently offered free of charge by Brazil's unified health system (Sistema Único de Saúde, SUS) to treat dyslipidemia. To our knowledge, this is the first report on the use of ciprofibrate in subjects with PBC, suggesting that treatment response to those drugs is not restricted to bezafibrate or fenofibrate (Ghonem and Boyer, 2013; Grigorian et al., 2015; Corpechot et al., 2018; Reig et al., 2018) and may in fact be due to a class effect. This is in accordance with a recent pilot study evaluating the use of another fibrate in patients with PBC, which reported more than 50% reduction in ALP associated with the use of pemafibrate (Joshita et al., 2019).

Recently, the combination of UDCA with bezafibrate was associated with a lower risk of all-cause and liver-related mortality or need for liver transplantation (Tanaka et al., 2021). In contrast to the BEZURSO trial (Corpechot et al., 2018), which showed up to 60% reduction in ALP after only 3 months of add-on bezafibrate therapy, biochemical response in the present study was much slower, with only 21.4% reduction in ALP after 2 years of add-on fibrate therapy. Although other studies (Kurihara et al., 2000; Nakai et al., 2000; Itakura et al.,

2004) also reported a greater reduction in ALP over time with the use of UDCA associated with fibrates, some observed a much lower reduction (Liberopoulos et al., 2010; Cheung et al., 2016). This may be explained by different baseline alkaline phosphatase levels and by the proportion of patients with advanced PBC included in the aforementioned studies, which may impact the frequency and timing of biochemical response to treatment.

Each fibrate differs in its specificity for the different PPAR subtypes, α, β/δ, and γ. The mechanism(s) by which fibrates reduce biochemical markers of cholestasis remains unclear, but experimental studies have shown that they may have different roles in the regulation of bile acid (BA) synthesis and secretion. Ciprofibrate, a PPARα agonist, has been previously shown to downregulate the mRNA expression of BA-synthesizing enzymes—cytochrome P450 (CYP) cholesterol 7A1-hydroxylase (CYP7A1) and cytochrome sterol 27-hydroxylase (CYP27A1). Furthermore, it induces the promoter activity of the human apical sodium-dependent bile salt transporter (ASBT) gene in Caco-2 cells and upregulates hepatic mRNA Mdr1a/b in wild-type mice. On the other hand, bezafibrate, a dual PPAR and pregnane receptor X agonist, increases the mRNA expression of sodium taurocholate cotransporting polypeptide (NTCP), CYP3A4, multidrug resistance proteins 1 and 3, and multidrug resistance–associated protein 2 (MRP2), while downregulating

the expression of CYP7A1 and CYP27A1 in human hepatoma cells [reviewed in Ghonem et al. (2015)].

Our study has limitations, including its retrospective design, lack of data regarding adverse effects, and limited number of patients. Although safety and tolerability have been previously described for bezafibrate and fenofibrate in patients with PBC and primary sclerosing cholangitis (Carrion et al., 2021), safety data regarding ciprofibrate use in humans have only been described in patients with dyslipidemia (Betteridge and O'Bryan-Tear, 1996).

In summary, our findings support the efficacy of fibrate add-on treatment in PBC patients with suboptimal response to UDCA. Although we cannot conclude on the effectiveness of ciprofibrate for UDCA-unresponsive PBC, nor confirm its safety, this investigation provides a proof of concept of a new and possibly cheaper alternative for treating these patients, since ciprofibrate appears to be at least as effective as bezafibrate. Ciprofibrate should be assessed in large prospective clinical trials as a promising option for the treatment of UDCA-unresponsive PBC patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GC: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and statistical analysis; CC: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and study supervision; LG: acquisition of data, MB: acquisition of data, DB: study concept and design, acquisition of data, and critical revision of the manuscript for important intellectual content; EC: study concept and design, acquisition of data, and critical revision of the manuscript for important intellectual content; MF: study concept and design, acquisition of data, and critical revision of the manuscript for important intellectual content; CV-N: study concept and design, acquisition of data, and critical revision of the manuscript for important intellectual content; MN: drafting of the manuscript, critical revision of the manuscript for important intellectual content, and statistical analysis; LF: study concept and design, acquisition of data, and critical revision of the manuscript for important intellectual content; EO: acquisition of data, and critical revision of the manuscript for important intellectual content; VR: acquisition of data, and critical revision of the manuscript for important intellectual content; DM: acquisition of data, and critical revision of the manuscript for important intellectual content; VB: acquisition of data, and critical revision of the manuscript for important intellectual content; LM: acquisition of data, and critical revision of the manuscript for important intellectual content; LC: acquisition of data, and critical revision of the manuscript for important intellectual content; MP: acquisition of data, and critical revision of the manuscript for important intellectual content; IS: acquisition of data, and critical revision of the manuscript for important intellectual content; CL: study concept and design, drafting of the manuscript, and critical revision of the manuscript for important intellectual content; and PB: study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, and obtaining funding.

FUNDING

ACKNOWLEDGMENTS

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.818089/full#supplementary-material

REFERENCES

Betteridge, D. J., and O'Bryan-Tear, C. G. (1996). Comparative Efficacy and Safety of Ciprofibrate and Sustained-Release Bezafibrate in Patients with Type II Hyperlipidaemia. *Postgrad. Med. J.* 72 (854), 739–743. doi:10.1136/pgmj.72.854.739

Cançado, G. G. L., Braga, M. H., Ferraz, M. L. G., Villela-Nogueira, C. A., Terrabuio, D. R. B., Cançado, E. L. R., et al. (2022). Clinical Features and Treatment Outcomes of Primary Biliary Cholangitis in a Highly Admixed Population. *Ann. Hepatol.* 27, 100546. doi:10.1016/j.aohep.2021.100546

Carrion, A. F., Lindor, K. D., and Levy, C. (2021). Safety of Fibrates in Cholestatic Liver Diseases. *Liver Int.* 41 (6), 1335–1343. doi:10.1111/liv.14871

Cheung, A. C., Lapointe-Shaw, L., Kowgier, M., Meza-Cardona, J., Hirschfield, G. M., Janssen, H. L., et al. (2016). Combined Ursodeoxycholic Acid (UDCA) and Fenofibrate in Primary Biliary Cholangitis Patients with Incomplete UDCA Response May Improve Outcomes. *Aliment. Pharmacol. Ther.* 43 (2), 283–293. doi:10.1111/apt.13465

Corpechot, C., Chazouillères, O., Rousseau, A., Le Gruyer, A., Habersetzer, F., Mathurin, P., et al. (2018). A Placebo-Controlled Trial of Bezafibrate in Primary Biliary Cholangitis. *N. Engl. J. Med.* 378, 2171–2181. doi:10.1056/NEJMoa1714519

de Vries, E., Bolier, R., Goet, J., Parés, A., Verbeek, J., de Vree, M., et al. (2021). Fibrates for Itch (FITCH) in Fibrosing Cholangiopathies: A Double-Blind, Randomized, Placebo-Controlled Trial. *Gastroenterology* 160 (3), 734–743. e6. doi:10.1053/j.gastro.2020.10.001

Ghonem, N. S., Assis, D. N., and Boyer, J. L. (2015). Fibrates and Cholestasis. *Hepatology* 62 (2), 635–643. doi:10.1002/hep.27744

Ghonem, N. S., and Boyer, J. L. (2013). Fibrates as Adjuvant Therapy for Chronic Cholestatic Liver Disease: Its Time Has Come. *Hepatology* 57 (5), 1691–1693. doi:10.1002/hep.26155

Grigorian, A. Y., Mardini, H. E., Corpechot, C., Poupon, R., and Levy, C. (2015). Fenofibrate Is Effective Adjunctive Therapy in the Treatment of Primary Biliary Cirrhosis: A Meta-Analysis. *Clin. Res. Hepatol. Gastroenterol.* 39 (3), 296–306. doi:10.1016/j.clinre.2015.02.011

Harms, M. H., van Buuren, H. R., Corpechot, C., Thorburn, D., Janssen, H. L. A., Lindor, K. D., et al. (2019). Ursodeoxycholic Acid Therapy and Liver Transplant-Free Survival in Patients with Primary Biliary Cholangitis. *J. Hepatol.* 71, 357–365. doi:10.1016/j.jhep.2019.04.001

Itakura, J., Izumi, N., Nishimura, Y., Inoue, K., Ueda, K., Nakanishi, H., et al. (2004). Prospective Randomized Crossover Trial of Combination Therapy with Bezafibrate and UDCA for Primary Biliary Cirrhosis. *Hepatol. Res.* 29, 216–222. doi:10.1016/j.hepres.2004.04.001

Joshita, S., Umemura, T., Yamashita, Y., Sugiura, A., Yamazaki, T., Fujimori, N., et al. (2019). Biochemical and Plasma Lipid Responses to Pemafibrate in Patients with Primary Biliary Cholangitis. *Hepatol. Res.* 49 (10), 1236–1243. doi:10.1111/hepr.13361

Kurihara, T., Niimi, A., Maeda, A., Shigemoto, M., and Yamashita, K. (2000). Bezafibrate in the Treatment of Primary Biliary Cirrhosis: Comparison with Ursodeoxycholic Acid. *Am. J. Gastroenterol.* 95, 2990–2992. doi:10.1111/j.1572-0241.2000.03220.x

Liberopoulos, E. N., Florentin, M., Elisaf, M. S., Mikhailidis, D. P., and Tsianos, E. (2010). Fenofibrate in Primary Biliary Cirrhosis: a Pilot Study. *Open Cardiovasc. Med. J.* 4, 120–126. doi:10.2174/1874192401004010120

Lindor, K. D., Bowlus, C. L., Boyer, J., Levy, C., and Mayo, M. (2019). Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology* 69 (1), 394–419. doi:10.1002/hep.30145

Lleo, A., Wang, G. Q., Gershwin, M. E., and Hirschfield, G. M. (2020). Primary Biliary Cholangitis. *Lancet* 396 (10266), 1915–1926. doi:10.1016/S0140-6736(20)31607-X

Monroy-Ramirez, H. C., Galicia-Moreno, M., Sandoval-Rodriguez, A., Meza-Rios, A., Santos, A., and Armendariz-Borunda, J. (2021). PPARs as Metabolic Sensors and Therapeutic Targets in Liver Diseases. *Int. J. Mol. Sci.* 22 (15), 8298. doi:10.3390/ijms22158298

Montano-Loza, A. J., and Corpechot, C. (2020). Definition and Management of Patients with Primary Biliary Cholangitis and an Incomplete Response to Therapy. *Clin. Gastroenterol. Hepatol.* (11), 2241–2251. [Epub ahead of print]. doi:10.1016/j.cgh.2020.06.062

Nakai, S., Masaki, T., Kurokohchi, K., Deguchi, A., and Nishioka, M. (2000). Combination Therapy of Bezafibrate and Ursodeoxycholic Acid in Primary Biliary Cirrhosis: A Preliminary Study. *Am. J. Gastroenterol.* 95, 326–327. doi:10.1111/j.1572-0241.2000.01667.x

Reig, A., Sesé, P., and Parés, A. (2018). Effects of Bezafibrate on Outcome and Pruritus in Primary Biliary Cholangitis with Suboptimal Ursodeoxycholic Acid Response. *Am. J. Gastroenterol.* 113 (1), 49–55. doi:10.1038/ajg.2017.287

Tanaka, A., Hirohara, J., Nakano, T., Matsumoto, K., Chazouillères, O., Takikawa, H., et al. (2021). Association of Bezafibrate with Transplant-Free Survival in Patients with Primary Biliary Cholangitis. *J. Hepatol.* 75, 565–571. doi:10.1016/j.jhep.2021.04.010

Tanaka, N., Aoyama, T., Kimura, S., and Gonzalez, F. J. (2017). Targeting Nuclear Receptors for the Treatment of Fatty Liver Disease. *Pharmacol. Ther.* 179, 142–157. doi:10.1016/j.pharmthera.2017.05.011

Supplementary Table 1: Biochemical changes overtime during fibrate treatment stratified by the presence of advanced PBC.

	Initial disease (n = 19)			Advanced disease (n = 8)		
	Baseline	12 mo.	24 mo.	Baseline	12 mo.	24 mo.
AST/ULN	1.43	1.10	1.13	1.12	1.13	0.83
ALT/ULN	1.74	1.03	1.11	0.97	0.9	0.77
ALP/ULN	1.60	1.19	1.10	2.0	1.82	1.07
GGT/ULN	3.85	2.42	2.66	4.22	3.02	3.08
TB/ULN	0.54	0.51	0.51	0.52	0.73	0.43

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gammaglutamyl-transferase; Mo., months; TB, total bilirubin; ULN, upper limit of normal. Data are expressed as median serum levels divided by the upper limit of normal.

5.4. Artigo 4

ORIGINAL ARTICLE

Response to Ursodeoxycholic Acid May Be Assessed Earlier to Allow Second-Line Therapy in Patients with Unresponsive Primary Biliary Cholangitis

Guilherme Grossi Lopes Cançado[1,2] · Cláudia Alves Couto[1] · Debora Raquel Benedita Terrabuio[3] ·
Eduardo Luiz Rachid Cançado[3] · Cristiane Alves Villela-Nogueira[4] · Maria Lucia Gomes Ferraz[5] ·
Michelle Harriz Braga[3] · Mateus Jorge Nardelli[1] · Luciana Costa Faria[1] ·
Nathalia Mota de Faria Gomes[5] · Elze Maria Gomes Oliveira[6] · Vivian Rotman[4] · Maria Beatriz Oliveira[7] ·
Simone Muniz Carvalho Fernandes da Cunha[8] · Marlone Cunha-Silva[9] · Liliana Sampaio Costa Mendes[10] ·
Claudia Alexandra Pontes Ivantes[11] · Liana Codes[8,12] · Valéria Ferreira de Almeida e Borges[13,14] ·
Fabio Heleno de Lima Pace[15] · Mario Guimarães Pessoa[3] · Laura Vilar Guedes[3] · Izabelle Venturini Signorelli[16] ·
Gabriela Perdomo Coral[17] · Cynthia Levy[18] · Paulo Lisboa Bittencourt[12,19] · Members of the Brazilian Cholestasis
Study Group Consortium

Abstract

Background Response to ursodeoxycholic acid (UDCA) in primary biliary cholangitis (PBC) has been traditionally assessed 1 to 2 years after treatment initiation. With the development of new drugs, some patients may benefit from an earlier introduction of second-line therapies.

Aims This study aims to identify whether well-validated response criteria could correctly identify individuals likely to benefit from add-on second-line therapy at 6 months.

Methods Analysis of a multicenter retrospective cohort which included only patients with clear-cut PBC.

Results 206 patients with PBC (96.6% women; mean age 54 ± 12 years) were included. Kappa concordance was substantial for Toronto (0.67), Rotterdam (0.65), Paris 1 (0.63) and 2 (0.63) criteria at 6 and 12 months, whereas Barcelona (0.47) and POISE trial (0.59) criteria exhibited moderate agreement. Non-response rates to UDCA was not statistically different when assessed either at 6 or 12 months using Toronto, Rotterdam or Paris 2 criteria. Those differences were even smaller or absent in those subjects with advanced PBC. Mean baseline alkaline phosphatase was 2.73 ± 1.95 times the upper limit of normal ($\times$ULN) among responders versus $5.05 \pm 3.08 \times$ULN in non-responders ($p < 0.001$).

Conclusions After 6 months of treatment with UDCA, the absence of response by different criteria could properly identify patients who could benefit from early addition of second-line therapies, especially in patients with advanced disease or high baseline liver enzymes levels.

Keywords Primary biliary cholangitis · Treatment · Second-line therapy · Ursodeoxycholic acid

Abbreviations

UDCA	Ursodeoxycholic acid	ALP	Alkaline phosphatase
PBC	Primary biliary cholangitis	ALT	Alanine aminotransferase
ULN	Upper limit of normal	AST	Aspartate aminotransferase
BCSG	Brazilian Cholestasis Study Group	GGT	Gamma-glutamyl transferase
AMA	Anti-mitochondrial antibodies	IQR	Interquartile range
		ANA	Antinuclear antibodies
		SD	Standard deviation

✉ Guilherme Grossi Lopes Cançado
guilhermegrossi@terra.com.br

Extended author information available on the last page of the article

Introduction

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease of unknown etiology with a variable rate of progression toward cirrhosis and end-stage liver disease. Ursodeoxycholic acid (UDCA) is currently the standard of care first-line therapy and numerous studies have confirmed its efficacy in retarding histological progression and improving long-term transplant-free survival [1–3]. However, up to 40% of patients with PBC will have a suboptimal biochemical response to UDCA, presenting with increased risk of progression to liver failure or liver transplantation [4]. Recently, add-on therapy with either fibrates or obeticholic acid in association with UDCA has been shown to improve biochemical markers of cholestasis even further [5–9]. Furthermore, in a recently published large retrospective cohort study the addition of bezafibrate to UDCA was associated with improved long-term prognosis [10]. Therefore, reliable early identification of non-responders could be of great importance. Despite the fact that several risk stratification scoring systems have been developed to assess therapeutic response in PBC, most of them considers clinical and biochemical variables obtained only after at least 1 year of UDCA therapy. The aim of this study was to identify whether well-validated response criteria could correctly identify individuals likely to benefit from add-on second-line therapy at 6 months to anticipate dual therapy and mitigate liver damage and disease progression.

Patients and Methods

Study Population

The Brazilian Cholestasis Study Group (BCSG) is a multicenter collaborative consortium of investigators from academic institutions and community-based sites that treat patients with PBC in Brazil. The study population included adult ($\geq$ 18 years old) patients diagnosed with PBC between January 1st 1992 and December 31st 2019 in 28 hepatology centers across the country. All study procedures were conducted in accordance with the ethical standards of the Helsinki Declaration. The present study was approved by the Federal University of Minas Gerais Ethics Committee Board (CAAE 98627218.6.1001.5149) and individual informed consent was waived as this study was retrospective in design. Diagnosis of PBC was considered if patients fulfilled at least two of the following three diagnostic criteria for PBC as recommended by

the American Association for the Study of Liver Disease guidelines: (i) positive serology for anti-mitochondrial antibodies (AMA); (ii) persistent increase of the serum alkaline phosphatase (ALP) level; and (iii) liver histology compatible with PBC [11]. Patients in whom the diagnosis could not be confirmed or who had another etiology of liver disease were excluded.

Data Collection

Each investigator was asked to identify all PBC patients followed in their Center at the time of the survey, without any selection or exclusion whatsoever, and to fill-in a standardized database provided by the BCSG. Clinical data obtained from medical records included: sex, age at diagnosis, year of diagnosis, last date of follow-up, liver histology (fibrosis was staged according to the Batts–Ludwig system) [12], serum liver biochemistry, UDCA treatment, liver decompensation, transplantation and death. Data on liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) and ALP, were collected at diagnosis, 6, 12 and 24 months after treatment, when available. Biochemical results were normalized by upper limit of normal (ULN) of each laboratory to homogenize data interpretation. The considered standardized daily dose of UDCA for PBC treatment was 13–15 mg/kg of body weight [11]. The response to treatment with UDCA was analyzed according to: (1) the Barcelona definition (decrease in ALP level > 40% of the baseline level or a normal level at 12 months); (2) Paris 1 (ALP level $\leq$ 3 $\times$ ULN, together with AST level $\leq$ 2 $\times$ ULN and a normal bilirubin level at 12 months); (3) Paris 2 (ALP and AST $\leq$ 1.5 $\times$ ULN with normal bilirubin at 12 months) (4) Toronto (ALP level < 1.67 $\times$ ULN after 2 years of UDCA); (5) Rotterdam (normal bilirubin and albumin concentrations when one or both parameters are abnormal before treatment, or normal bilirubin or albumin concentrations after treatment when both are abnormal at entry) and (6) POISE trial criteria (ALP level < 1.67 times ULN, with a reduction of at least 15% from baseline, and a total bilirubin level at or below the ULN at 12 months), at different time points [2, 8, 13–15]. Treatment compliance was determined at the physician's discretion as regular or irregular. The threshold for proper compliance was defined as use of more or equal to 80% of the pills, whereas a rate less than 80% indicated irregular compliance to therapy [16]. Advanced PBC was defined by presence of moderate to severe fibrosis (Metavir stage III or IV) on liver histology or clinical evidence of cirrhosis. The duration of follow-up was defined as the interval between the diagnosis and the last visit or the date of liver transplantation or death.

Statistical Analysis

Statistical analysis was performed using SPSS 25.0 software (IBM, USA). Continuous variables distribution was assessed by Shapiro–Wilk test, and those with Gaussian distribution were expressed as mean and standard deviation, or as median and interquartile range (IQR) if skewed distribution. Categorical variables were expressed as absolute number and percentage. Univariate analysis was performed using chi-square, Fisher´s exact test or McNemar test, as appropriate, for categorical variables. Continuous variables were analyzed by the Student t test or Mann–Whitney U-test, according to the distribution. Cohen's and Fleiss' kappa were used to access agreement between different response to treatment criteria. A p value < 0.05 was considered significant.

Results

Patient Characteristics

Five hundred and sixty-two patients with PBC were included in the BCSG database. Eighty (14%) were subsequently excluded due to the diagnosis of overlap syndrome of PBC and autoimmune hepatitis. Clinical and laboratory features of the global cohort have already been reported elsewhere [17]. Ninety six percent of the subjects were treated with UDCA, mean dose 13 ± 2.6 mg/kg/day. The remaining subjects (4%) were UDCA intolerant due to bloating and/or diarrhea. Treatment compliance was considered satisfactory in 373 (81%) of them. Only 206 out the 373 subjects were included in the study due to availability of paired results of ALP, AST, total bilirubin, and albumin levels for assessment of biochemical treatment response six and 12 months after starting UDCA. The clinical and laboratory features of those patients are depicted in Table 1. Briefly, 96.6% were women, with a mean age at diagnosis of 54 ± 12 years. Based on histological or clinical and laboratory findings, 34% of them had advanced PBC. Eight patients underwent liver transplantation and 21 subjects died after a mean follow-up time to death of 6.25 [3.5–10.25] years, 41.6% from liver-related causes.

Biochemical Pattern of Response to UDCA

Biochemical response to UDCA was assessed in those 206 PBC patients with paired laboratory results six and twelve months after starting therapy. A heat map was elaborated to evaluate heterogeneity between different available response criteria in those two distinct time points (Fig. 1). Fleiss' kappa concordance between different criteria at six months and one year was 0.46 and 0.47, respectively, which is considered moderate. Concordance was stable at six and twelve

Table 1 Clinical, laboratory and histological features of patients with primary biliary cirrhosis ($n = 206$)

Variables	
Age at diagnosis (years)	54 ± 12
Female sex	199 (96.6)
AMA-positive patients	176 (85.4)
ANA-positive patients	149 (72.3)
Asymptomatic subjects	74 (35.9)
Pruritus	97 (47.1)
Fatigue	75 (36.4)
Concurrent autoimmune diseases	94 (45.6)
Hashimoto's thyroiditis	45 (21.8)
Sjögren syndrome	21 (10.2)
Others	28 (13.6)
ALP × ULN	2.76 (1.82–5.08)
GGT × ULN	8.95 (4.68–14.55)
AST × ULN	2.13 (1.22–3.73)
ALT × ULN	1.97 (1.22–3.73)
Bilirubin (mg/dL)	0.87 (0.56–1.40)
Histological disease stage (n = 130)	
Stage I	32 (15.5)
Stage II	45 (21.8)
Stage III	30 (14.6)
Stage IV	23 (11.2)
Follow-up time (years)	6.25 (3.5–10.25)
Cirrhosis	70 (34.0)
Decompensation of cirrhosis	31 (15.0)
Liver transplantation	8 (3.9)
Overall deaths	21 (10.2)

Data are expressed as absolute number (percentage), mean $\pm$ standard deviation or median (interquartile range)

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *AMA* anti-mitochondrial antibodies; ANA, anti-nuclear antibody, *AST* aspartate aminotransferase, *GGT* gamma-glutamyl transferase, × *ULN* number of times the upper limit of normal

months after stratifying the analysis by presence or absence of advanced liver disease (0.41 vs 0.45 and 0.41 vs 0.43, at 6 and 12 months, for individuals with advanced PBC and non-cirrhotics patients, respectively). Overall Cohen´s kappa concordance was substantial for Toronto (0.67), Rotterdam (0.65), Paris 1 (0.63) and 2 (0.63) criteria at six and 12 months, whereas Barcelona (0.47) and POISE trial (0.59) criteria exhibited only moderate agreement. Concordance was higher for patients with advanced PBC, compared with those with early disease (Supplementary Table 1). Different from other treatment prediction models, non-response to UDCA was not statistically different when assessed either at six or 12 months using Toronto, Rotterdam or Paris 2 criteria (Fig. 2). Interestingly, although all criteria showed only a small variability when assessed either at 6 or 12 months, those differences were even smaller or absent in those subjects with advanced

Fig. 1 Concordance heat map of different PBC response to treatment criteria overtime. Each column represents a different patient, while each row represents a different criterion. This heat map graphically shows the heterogeneity of response to UDCA treatment according to different criteria. Red color = non-responder; green color = responder
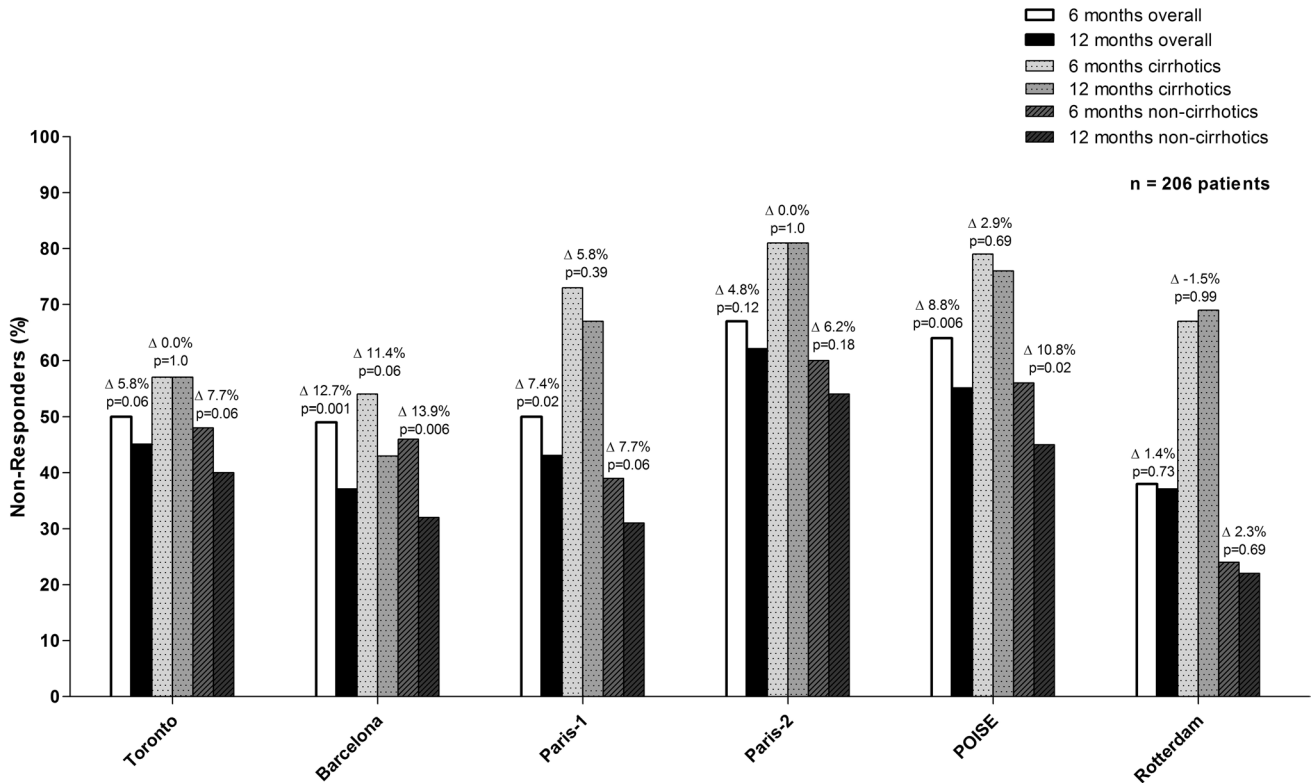

Fig. 2 Paired biochemical response at 6 and 12 months after introducing UDCA treatment stratified by different criteria. Solid columns demonstrate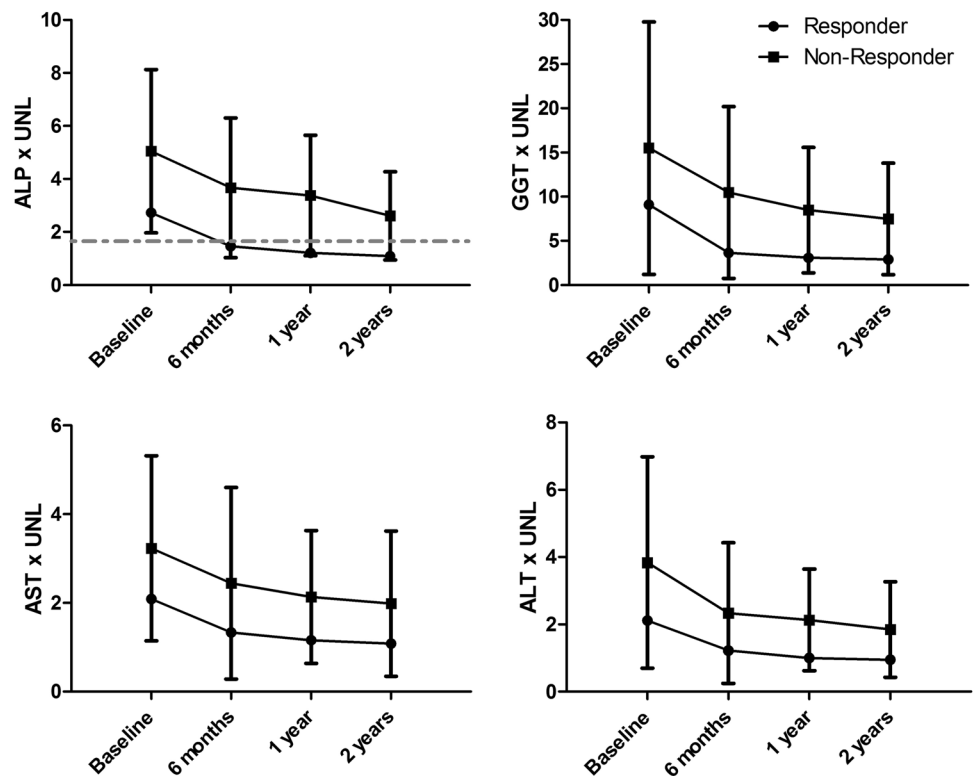 overall response to UCDA; dotted columns show the response rate among cirrhotic patients. Hatched columns depict the response rate among non-cirrhotic patients. Deltas show the difference between response rates at different timepoints

PBC (Fig. 2). In fact, response gain between 6 and 12 months assessments was smaller than 5.6% for patients with cirrhosis and 13.9% for individuals without cirrhosis independently of the chosen response criteria. A stratified analysis of liver enzymes by response to treatment using Toronto criteria revealed that non-responders presented higher baseline levels of liver enzymes. Mean baseline ALP was $2.73 \pm 1.95 \times$ ULN among responders vs $5.05 \pm 3.08 \times$ ULN in non-responders [$p < 0.001$] (Fig. 3).

Discussion

Treatment with UDCA has been shown to improve serum liver enzymes, to delay histological progression of PBC and improve transplant-free survival [1–3, 18]. The biochemical response at 12 months of treatment with UDCA, defined by numerous criteria, accurately predicts PBC long-term outcomes [13]. Since PBC is a

Fig. 3 Paired biochemical response to UDCA treatment stratified by Toronto criteria ($n=206$). Responders were defined by reduction in alkaline phosphatase to less than or equal to 1.67 times the upper limit of normal by 2 years of treatment, whereas non-responders had alkaline phosphatase values greater than 1.67 times the upper limit of normal. Dotted gray line shows Toronto criteria cutoff. Mean values $\pm$ SD are expressed for all data

slowly progressive disease, non-invasive surrogate end points are needed to guide decisions on clinical therapy. About 40% of patients do not have an optimal response to UDCA and will progress more rapidly to cirrhosis or end-stage liver disease. Studies suggest that if UDCA is started in the early stages of the disease, it may extend life expectancy for people with PBC, while little effects are observed in the late stages [19, 20]. In this study, we sought to determine whether an earlier evaluation of biochemical response at 6 months, using previously validated criteria, could properly identify patients who could benefit from early introduction of second-line therapies or participation in clinical trials.

Our findings suggest that different validated PBC response criteria might be used at the sixth month of UDCA treatment to predict response and determine new therapeutic approaches. Less than 13% of the patients unresponsive to UDCA at 6 months fulfilled criteria for treatment response at one year. Variability in treatment response over time was shown to be negligible using either Toronto or Rotterdam criteria, probably due to the fact that a large proportion of patients included in our study had advanced liver disease, with abnormal baseline levels of albumin and/or bilirubin. Using other criteria, treatment response showed a small variability when assessed at six or twelve months. In this respect, levels of ALP, GGT, AST and ALT significantly decreased within the first 6 months of UDCA therapy and then stayed relatively stable. As previously shown,

non-responders to UDCA in the present study had higher baseline levels of liver enzymes, pointing out to the role of add-on therapies in those patients as early as possible [4]. ALP $> 2.7 \times$ ULN was identified as an interesting cut off to be used at 6 months to recognize those in higher risk to non-respond. In fact, Angulo et al. also demonstrated that PBC patients with ALP $< 2 \times$ ULN following 6 months of UDCA were more likely to sustain response at 2 years of treatment [21]. Interestingly, a recent study suggested that bilirubin levels $\leq 0.6 \times$ ULN and ALP normalization are associated with a decreased risk for liver transplantation or death, thus indicating that a more aggressive therapy could be related to better outcomes [22]. The addition of bezafibrate to UDCA was also associated with a significantly lower risk of all cause and liver-related mortality or need for liver transplantation in a Japanese retrospective cohort, further confirming that second-line therapies can impact long-term survival of UDCA primary non-responders [10].

Although this study has some limitations, including its retrospective design, our findings suggest that non-response to UDCA can be assessed at 6 months without overlooking too many late UDCA responders. In fact, only three, 10, 12, 15, 18 and 26 non-responders at 6 months according to Rotterdam, Paris II, Toronto, Paris I, POISE trial and Barcelona criteria, respectively, turned out to be late responders at 12 months. Therefore, we propose that evaluation of treatment response at 6 months would be of benefit especially in those patients with predictors of non-response

to UDCA, such as advanced liver disease or high baseline liver enzymes or when using Rotterdam, Toronto or Paris II criteria.

In conclusion, evaluation of treatment response should not be limited to a predetermined 12-month interval. In this respect, assessment at 6 months may be attempted in subjects with more severe disease who may benefit from early add-on therapy with either fibrates, obeticholic acid or even promising new drugs in clinical trials.

Data availability Data is available upon request to corresponding author.

Declarations

Conflict of interest None declared.

References

1. Corpechot C, Carrat F, Bonnand AM et al. The effect of ursodeoxycholic acid therapy on liver fibrosis progression in primary biliary cirrhosis. *Hepatology.* 2000;32:1196–1199.
2. Corpechot C, Abenavoli L, Rabahi N et al. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology.* 2008;48:871–877.
3. Harms MH, van Buuren HR, Corpechot C et al. Ursodeoxycholic acid therapy and liver transplant-free survival in patients with primary biliary cholangitis. *J Hepatol.* 2019;71:357–365.
4. Lammers WJ, van Buuren HR, Hirschfield GM et al. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. *Gastroenterology.* 2014;147:1338-1349.e5.
5. Itakura J, Izumi N, Nishimura Y et al. Prospective randomized crossover trial of combination therapy with bezafibrate and UDCA for primary biliary cirrhosis. *Hepatol Res.* 2004;29:216–222.
6. Lens S, Leoz M, Nazal L et al. Bezafibrate normalizes alkaline phosphatase in primary biliary cirrhosis patients with incomplete response to ursodeoxycholic acid. *Liver Int.* 2014;34:197–203.
7. Corpechot C, Chazouillères O, Rousseau A et al. A placebo-controlled trial of bezafibrate in primary biliary cholangitis. *N Engl J Med.* 2018;378:2171–2181.
8. Nevens F, Andreone P, Mazzella G et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N Engl J Med.* 2016;375:631–643.
9. Trauner M, Nevens F, Shiffman ML et al. Long-term efficacy and safety of obeticholic acid for patients with primary biliary cholangitis: 3-year results of an international open-label extension study. *Lancet Gastroenterol Hepatol.* 2019;4:445–453.
10. Tanaka A, Hirohara J, Nakano T et al. Association of bezafibrate with transplant-free survival in patients with primary biliary cholangitis. *J Hepatol.* 2021;75:565–571.
11. Lindor KD, Bowlus CL, Boyer J et al. Primary biliary cholangitis: 2018 Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2019;69:394–419.
12. Ludwig J, Dickson ER, McDonald GSA. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol.* 1978;379:103–112.
13. Parés A, Caballería L, Rodés J. Excellent long-term Survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic acid. *Gastroenterology.* 2006;130:715–720.
14. Corpechot C, Chazouillères O, Poupon R. Early primary biliary cirrhosis: Biochemical response to treatment and prediction of long-term outcome. *J Hepatol.* 2011;55:1361–1367.
15. Kumagi T, Guindi M, Fischer SE et al. Baseline Ductopenia and treatment response predict long-term histological progression in primary biliary cirrhosis. *Am J Gastroenterol.* 2010;105:2186–2194.
16. Haynes R. Determinants of compliance: The disease and the mechanics of treatment. Compliance in health care. 1979.
17. Cançado GGL, Braga MH, Ferraz MLG et al. Clinical features and treatment outcomes of primary biliary cholangitis in a highly admixed population. *Ann Hepatol.* 2022;27:100546.
18. Carey EJ, Ali AH, Lindor KD. Primary biliary cirrhosis. *Lancet (London, England).* 2015;386:1565–1575.
19. Corpechot C, Carrat F, Bahr A et al. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology.* 2005;128:297–303.
20. Lammert C, Juran BD, Schlicht E et al. Biochemical response to ursodeoxycholic acid predicts survival in a North American cohort of primary biliary cirrhosis patients. *J Gastroenterol.* 2014;49:1414–1420.
21. Angulo P, Lindor KD, Therneau TM et al. Utilization of the Mayo risk score in patients with primary biliary cirrhosis receiving ursodeoxycholic acid. *Liver.* 1999;19:115–121.
22. Perez CFM, Harms MH, Lindor KD et al. Goals of treatment for improved survival in primary biliary cholangitis: treatment target should be bilirubin within the normal range and normalization of alkaline phosphatase. *Am J Gastroenterol.* 2020;115:1066–1074.

Authors and Affiliations

Guilherme Grossi Lopes Cançado[1,2] · **Cláudia Alves Couto**[1] · **Debora Raquel Benedita Terrabuio**[3] ·
Eduardo Luiz Rachid Cançado[3] · **Cristiane Alves Villela-Nogueira**[4] · **Maria Lucia Gomes Ferraz**[5] ·
Michelle Harriz Braga[3] · **Mateus Jorge Nardelli**[1] · **Luciana Costa Faria**[1] ·
Nathalia Mota de Faria Gomes[5] · **Elze Maria Gomes Oliveira**[6] · **Vivian Rotman**[4] · **Maria Beatriz Oliveira**[7] ·
Simone Muniz Carvalho Fernandes da Cunha[8] · **Marlone Cunha-Silva**[9] · **Liliana Sampaio Costa Mendes**[10] ·
Claudia Alexandra Pontes Ivantes[11] · **Liana Codes**[8,12] · **Valéria Ferreira de Almeida e Borges**[13,14] ·
Fabio Heleno de Lima Pace[15] · **Mario Guimarães Pessoa**[3] · **Laura Vilar Guedes**[3] · **Izabelle Venturini Signorelli**[16] ·
Gabriela Perdomo Coral[17] · **Cynthia Levy**[18] · **Paulo Lisboa Bittencourt**[12,19] · **Members of the Brazilian Cholestasis
Study Group Consortium**

1   Instituto Alfa de Gastroenterologia, Hospital das Clínicas da Universidade Federal de Minas Gerais, Av. Professor Alfredo Balena 110, Belo Horizonte, Minas Gerais 30130-100, Brazil

2   Hospital da Polícia Militar de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

3   Departamento de Gastroenterologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, São Paulo, Brazil

4   Hospital Universitário Clementino Fraga Filho e Departamento de Clínica Médica da Faculdade de Medicina, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil

5   Disciplina de Gastroenterologia, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

6   Centro Universitário Lusíada - UNILUS, Santos, São Paulo, Brazil

7   Ambulatório Municipal de Hepatites Virais de São José dos Campos, São José dos Campos, São Paulo, Brazil

8   Hospital Universitário Professor Edgard Santos, Universidade Federal da Bahia, Salvador, Bahia, Brazil

9   Divisão de Gastroenterologia (Gastrocentro), Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

10   Hospital de Base do Distrito Federal, Brasília, Distrito Federal, Brazil

11   Serviço de Gastroenterologia, Hepatologia e Transplante Hepático, Hospital Nossa Senhora das Graças, Curitiba, Paraná, Brazil

12   Hospital Português, Salvador, Bahia, Brazil

13   Instituto de Gastroenterologia, Endoscopia e Proctologia, Uberlândia, Minas Gerais, Brazil

14   Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

15   Serviço de Gastroenterologia e Hepatologia, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

16   Hospital Universitário Cassiano Antônio Moraes, Universidade Federal do Espírito Santo, Vitoria, Espírito Santo, Brazil

17   Irmandade da Santa Casa de Misericórdia de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

18   Division of Digestive Health and Liver Diseases, University of Miami Miller School of Medicine, Miami, FL, USA

19   Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil

6. CONSIDERAÇÕES FINAIS

A CBP é uma doença hepática crônica de etiologia autoimune pouco estudada na América Latina, em especial no Brasil. Nesse estudo, as características clínicas da CBP em brasileiros foram semelhantes às relatadas em caucasianos e asiáticos, mas com taxas inferiores de síndrome de sobreposição com HAI. Comprovou-se que os pacientes com CBP AMA-negativos são semelhantes aos AMA-positivos, com diferenças sutis em algumas características clínicas e laboratoriais de menor relevância clínica. De maneira geral, a resposta ao AUDC foi menor que o esperado e inversamente associada ao estádio histológico e aos níveis basais de transaminases. A maioria dos pacientes não respondedores ao AUDC se beneficiaram da associação de fibratos, incluindo ciprofibrato, o qual teve seu uso em CBP descrito pela primeira vez na literatura. Além disso, demonstrou-se que após 6 meses de tratamento com AUDC, a ausência de resposta por diferentes critérios validados foi capaz identificar adequadamente os pacientes que se beneficiariam da associação precoce de terapias de segunda linha, especialmente em pacientes com doença avançada ou com níveis basais elevados das enzimas hepáticas.

Trata-se de um estudo multicêntrico, construído, de maneira pioneira, com o esforço e união de vários centros de Hepatologia do Brasil, motivo pelo qual agradeço a todos os colaboradores dessa pesquisa. Embora os dados tenham sido obtidos de maneira retrospectiva, os resultados devem ser valorizados, uma vez que estudos prospectivos são extremamente complexos e caros de serem realizados no contexto de uma doença rara e lentamente progressiva. Por outro lado, devemos reconhecer as limitações, como a presença de dados faltantes e ausência de padronização na coleta e técnica de exames, incluindo o AMA e perfil hepático. Cabe ainda destacar que o AMA pode positivar ao pela simples troca da metodologia de análise ou mesmo espontaneamente ao longo do seguimento, o que não foi mensurado nesse estudo. Dessa forma, é possível que a prevalência de pacientes AMA negativo esteja superestimada. Além disso, as dificuldades de acesso aos centros de referência e, especialmente, ao tratamento regular com AUDC podem ter impactado negativamente nas taxas de resposta observadas.

Certamente, novos estudos são necessários para avaliar o perfil epidemiológico da CBP no Brasil e em outros países da América Latina. O uso de ciprofibrato em não-

responsedores ao AUDC deve ser submetido a ensaio clínico randomizado controlado com uma droga promissora para tratamento de segunda linha da CBP. Por fim, destaca-se a necessidade de um consenso global sobre os escores prognósticos de resposta, com intuito de padronizar as indicações de medicações de segunda e terceira linha na CBP e permitir coleta de dados uniformes em pesquisas clínicas.

7. ANEXOS

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: Colangite biliar primária e colangite esclerosante primária: Inquérito brasileiro
Pesquisador: Claudia Alves Couto
Área Temática:
Versão: 8
CAAE: 98627218.6.1001.5149
Instituição Proponente: Hospital das Clínicas - Universidade Federal de Minas Gerais
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.950.689

Apresentação do Projeto:

Trata-se EMENDA ao projeto de pesquisa que propõe estudo retrospectivo de revisão de prontuários com coleta de dados clínicos e laboratoriais conforme protocolo realizado pelos centros clínicos participantes. Serão recrutados todos os pacientes com diagnóstico clínico de CBP (colangite biliar primaria) e CEP (colangite esclerosante primaria) nos serviços de hepatologia. Conforme descrito, a CBP e a CEP são doenças colestáticas crônicas do fígado, de origem autoimune presumível, que podem evoluir para doença hepática terminal e complicações graves. Além disso, os pacientes frequentemente apresentam sintomas de difícil manejo clínico, complicações clinicas e doenças autoimunes associadas que podem impactar na qualidade de vida. São doenças pouco frequentes. Neste contexto, estudos de casuísticas e estudos multicêntricos são de fundamental importância e tem permitido o melhor conhecimento das doenças hepáticas colestáticas, a identificação de marcadores sorológicos de desfechos clínicos importantes para a abordagem dos pacientes e também a avaliação de tratamento no longo prazo e impacto na sobrevida. Vários aspectos do quadro clínico tais como evolução, mortalidade, morbidades e resposta ao tratamento não foram avaliados no nosso meio. Além disso, a frequência de associação entre doenças colestatica hepáticas e doença inflamatória intestinal não é conhecida no nosso meio. Esse inquérito brasileiro deverá trazer informações importantes, possibilitando ampliar o conhecimento das particularidades no nosso meio e contribuindo para estabelecer diretrizes diagnósticas, terapêuticas e prognósticas para esses pacientes.

Objetivo da Pesquisa:

Conforme Formulário de Informações Básicas: Objetivo Primário: • O objetivo deste estudo é determinar as características clinico-epidemiológicas e de resposta ao tratamento dos pacientes com CBP e CEP atendidos em centros de referência em Hepatologia no Brasil.

Objetivo Secundário: • Investigar variáveis clínicas e epidemiológicas em pacientes com CBP e CEP e sua correlação com os marcadores imunológicos e a resposta terapêutica. • Correlacionar os diversos critérios de resposta terapêutica propostos na literatura com sobrevida na população brasileira. • Caracterizar o perfil do prurido, a prevalência de prurido refratário e resposta ao tratamento.

Avaliação dos Riscos e Benefícios:

Conforme Formulário de Informações Básicas: Riscos: Não estão previstos riscos para os participantes. Não serão realizadas intervenções Os resultados da pesquisa serão tornados públicos, sejam eles favoráveis ou não. O sigilo na pesquisa e a continuidade do acompanhamento e do tratamento, independente da participação na pesquisa dos indivíduos selecionados, serão garantidos. Benefícios: O melhor conhecimento da cirrose biliar primária e colangite esclerosante primária poderá trazer benefícios futuros para o conhecimento sobre essas doenças. Não são previstos benefícios imediatos para os participantes da pesquisa

Comentários e Considerações sobre a Pesquisa:

A emenda propôs a inclusão de 4 centros participantes,

- Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – USP
- Hospital Universitário Cassiano Antônio Moraes, UFES-Vitória – ES
- HOSPITAL DE BASE DO DISTRITO FEDERAL
- Hospital Universitário Walter Cantídeo da Universidade Federal do Ceará

Esse último foi incluído na resposta à diligência, em que a pesquisadora informa que estaria substituindo o Hospital Nossa Senhora das Gracas anteriormente mencionado na primeira versão da emenda. Todos tem CEP próprio.

Considerações sobre os Termos de apresentação obrigatória:

Endereço: Av. Presidente Antonio Carlos, 6627 ¿ 2º. Andar ¿ Sala 2005 ¿ Campus Pampulha
Bairro: Unidade Administrativa II   **CEP:** 31.270-901
UF: MG   **Município:** BELO HORIZONTE
Telefone: (31)3409-4592   **E-mail:** coep@prpq.ufmg.br

Página 02 de 04

Continuação do Parecer: 4.950.689

Para a emenda foram apresentados:

- Formulário de informações básicas, no qual consta jiustificativa de emenda como a seguir.

Justificativa da Emenda: Solicito a inclusão de mais 4 centros no Brasil. Trata-se de estudo multicêntrico nacional. O centro "Hospital Nossa Senhora das Graças" por estar com pendência até obtenção de anuência da Instituição foi temporariamente excluído desta emenda, sendo substituído pelo centro Hospital Universitário Walter Cantidio. Não serão necessários aprovação de quaisquer documentos, procedimento ou aumento de amostras.

-Carta resposta à pendência para esclarecimentos quanto a existencia ou nao de CEP proprio no Hospital Nossa Senhora das Gracas, com a seguinte reposta "O Hospital Nossa Senhora das Graças foi retirado dos centros participantes do Projeto. Ademais, adicionamos como novo centro participante o Hospital Universitário Walter Cantídeo da Universidade Federal do Ceará, que possui Comitê de Ética acessível ao site da instituição".

Recomendações:

-

Conclusões ou Pendências e Lista de Inadequações:

SMJ, a emenda está aprovada

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente (Resolução CNS 466/12), o CEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1526991_E5.pdf	27/08/2021 10:31:39		Aceito
Outros	RespostaParecer4004091.pdf	27/08/2021	Claudia Alves	Aceito

Continuação do Parecer: 4.950.689

Outros	RespostaParecer4004091.pdf	10:31:14	Couto	Aceito
Outros	inclusaodeoutroscentros.pdf	07/05/2019 20:21:03	Claudia Alves Couto	Aceito
Outros	Solicitacao_Alteracao_CNPJ_CristianeNogueiraVillela.pdf	11/02/2019 10:20:51	Claudia Alves Couto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEmodificadoCOEP.pdf	30/10/2018 11:32:47	Claudia Alves Couto	Aceito
Outros	respostaparecer2980424.pdf	30/10/2018 11:32:09	Claudia Alves Couto	Aceito
Projeto Detalhado / Brochura Investigador	InqueritoSBHCBPCEP.pdf	11/09/2018 14:03:44	Claudia Alves Couto	Aceito
Folha de Rosto	folharosto.pdf	11/09/2018 14:02:53	Claudia Alves Couto	Aceito
Outros	aprovacaoCLM.pdf	11/09/2018 14:00:08	Claudia Alves Couto	Aceito
Outros	Anexo1.pdf	11/09/2018 13:58:06	Claudia Alves Couto	Aceito
Outros	GEP.pdf	11/09/2018 13:54:28	Claudia Alves Couto	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	11/09/2018 13:53:35	Claudia Alves Couto	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BELO HORIZONTE, 02 de Setembro de 2021

_____

Assinado por:
Críssia Carem Paiva Fontainha
(Coordenador(a))