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PROGRAMA DE PÓS-GRADUAÇÃO EM PEDIATRIA: ATENÇÃO À SAUDE DA CRIANÇA E DO ADOLESCENTE

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Avaliação do HLA em doadores e receptores de transplante hepático pediátrico e a presença de anticorpo contra doador e seus desfechos clínicos.

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RESUMO

Introdução: A importância do antígeno leucocitário humano (HLA) nos transplantes (Tx) de órgãos sólidos é um assunto bastante debatido, e a presença de anticorpo contra o doador (DSA) circulante no soro está associada à lesão e à perda do enxerto. O desenvolvimento de fibrose e rejeição são desfechos importantes no pós-transplante hepático e definir quais são os seus fatores de risco é fundamental para uma melhor evolução. **Objetivo:** Analisar a compatibilidade HLA do doador e do receptor e suas compatibilidades, tentando identificar quais são os fatores de risco para essa população no desenvolvimento de anticorpos anti-HLA do doador (DSA) e sua relação com rejeição e fibrose. Objetivos Secundários: identificar as características demográficas e cirúrgicas dos pacientes que desenvolveram anticorpos circulantes contra o doador, além da rejeição e fibrose hepática; Analisar a presença de C4d nos pacientes que desenvolveram rejeição; Determinar a quantidade de *Mismatch* (MM) nos HLA de classe I (A; B) e classe II (DR) correlacionando com o desfecho de rejeição ou fibrose; Correlacionar o valor de PRA pré e pós-transplante com os desfechos de rejeição e fibrose; Identificar o valor de MFI que representa um fator de risco para o desenvolvimento de rejeição ou fibrose do enxerto.

Métodos: Os dados foram coletados entre dezembro de 2013 e dezembro de 2023**.** Foram analisados: HLA Classe I (A/B) e classe II (DR); reatividade contra painel classe I e II (sangue periférico). O soro foi coletado no receptor, para detecção de *"de novo"* DSA a partir de 3 meses após o transplante. Os critérios de inclusão foram crianças transplantadas até 18 anos de idade; com sobrevida maior que 30 dias após o transplante hepático e com pelo menos uma coleta de DSA pós-transplante. Todas as rejeições foram comprovadas por biópsia hepática. A decisão de biopsiar os pacientes decorreu da alteração laboratorial de disfunção hepática. Para identificar DSA foi usada a tecnologia de citometria com utilização do Luminex ® pela metodologia Single Antigen Beads (SAB) (One *Lambda*), considerando intensidade média de fluorescência > 1000 como positivo para anticorpos anti-HLA. **Resultados:** A presença de qualquer DSA no pós-transplante está associada ao desenvolvimento de rejeições e fibrose nos pacientes pediátricos submetidos a transplante hepático,

mas principalmente quando há DSAs dirigidos contra *locus* DQ. A prevalência de qualquer DSA Classe I ou II no pré-transplante (Tx) é de 28,3%, enquanto no pós-transplante a presença de DSA Classe I ou II foi de 43,1% nesta amostra. A mediana de seguimento dos pacientes foi de 3 anos. Dos 13 pacientes que apresentaram rejeição, o C4d esteve positivo em 5 pacientes. Um dado observado foi que a presença de uma ou mais incompatibilidade HLA no *locus* A entre doador e receptor acarreta um risco 16x superior de desenvolver trombose da artéria hepática. O tempo de isquemia também parece estar relacionado ao desfecho desses pacientes. **Conclusão:** Houve um risco aumentado de trombose da artéria hepática em pacientes com incompatibilidade HLA no *locus* A. Doadores mais jovens apresentaram uma maior propensão ao desenvolvimento de anticorpos anti-HLA "*de novo"* DSA (dnDSA). A característica dos pacientes que desenvolveram rejeição foi os que tinham doadores mais jovens, transplante com doador falecido, doadores não aparentados e a presença de dnDSA. No grupo de pacientes que desenvolveram fibrose e rejeição, foi documentado uma relação com dnDSA anti-HLA-DQ. Não se encontrou uma associação significativa entre a presença de C4d e nem de incompatibilidade HLA A-B ou DR nos desfechos avaliados. Houve uma tendência de maior incidência no desenvolvimento de fibrose e rejeição em pacientes com valores mais elevados de PRA de classe II. Os resultados indicaram uma relação estatisticamente significativa entre valores mais elevados de MFI e maior risco de desenvolver esses desfechos.

Descritores: Compatibilidade HLA; DSA; Transplante hepático pediátrico, Rejeição.

ABSTRACT

Introduction: The importance of the human leukocyte antigen (HLA) in solid organ transplants is a much debated subject, and the presence of circulating donor antibody (DSA) in the serum is associated with graft injury and loss. The development of fibrosis and rejection are important post-liver transplant outcomes and defining their risk factors is fundamental for a better outcome. **Objective:** to analyze the HLA profile of the donor and recipient and their compatibility, trying to identify the risk factors for this population in the development of donor anti-HLA antibodies (DSA) and their relationship with rejection and fibrosis. Secondary Objectives: to identify the demographic and surgical characteristics of patients who developed circulating antibodies against the donor, in addition to rejection and hepatic fibrosis; to analyze the presence of C4d in patients who developed rejection; to determine the amount of Mismatch (MM) in class I (A; B) and class II (DR) HLA correlating with the outcome of rejection or fibrosis; to correlate the pre- and post-transplant PRA value with the outcomes of rejection and fibrosis; to identify the MFI value that represents a risk factor for the development of graft rejection or fibrosis. **Methods:** Data was collected between December 2013 and December 2023. The following were analyzed: HLA class I A/B/ and class II DR; reactivity against class I and II panels (peripheral blood). Serum was collected from the recipient to detect “de novo” DSA 3 months after the transplant. The inclusion criteria were transplanted children up to 18 years of age; with survival of more than 30 days after liver transplantation and with at least one post-transplant DSA collection. All rejections were proven by liver biopsy. The decision to biopsy the patients was made due to laboratory changes in liver dysfunction. The LABScreen Single Antigen (SAB) test using Luminex® technology was used to detect anti-HLA antibodies from the recipient, considering mean fluorescence intensity > 1000 as positive for anti-HLA antibodies. **Results:** The presence of any DSA in the post-transplant period is associated with the development of rejections and fibrosis in pediatric patients undergoing liver transplantation, but especially when we have DSAs directed against DQ locus. The prevalence of any Class I or II DSA pre-transplant (Tx) is 28.3%, while post-transplant the presence of Class I or II DSA was 43.1% in this sample. The median follow-up was 3 years. Of the 13 patients who rejected, C4d was positive in 5 patients. The presence of one or more HLA incompatibilities at the A locus between donor and recipient entails a 16x higher risk of developing hepatic artery

thrombosis. Ischemia time also seems to be related to the outcome of these patients. **Conclusion:** There was an increased risk of hepatic artery thrombosis in patients with HLA incompatibility at locus A. Younger donors showed a higher propensity for the development of de novo anti-HLA antibodies (dnDSA). The characteristic of patients who developed rejection was younger donors, deceased donor transplantation, unrelated donors, and the presence of dnDSA. In the group of patients who developed fibrosis and rejection, a relationship with dnDSA anti-HLA-DQ was documented. No significant association was observed between the presence of C4d and HLA A-B or DR incompatibility in the assessed outcomes. There was a trend towards a higher incidence of fibrosis and rejection in patients with higher class II PRA values. The results indicated a statistically significant relationship between higher MFI values and a greater risk of developing these outcomes.

Keywords: HLA compatibility; DSA; Pediatric liver transplantation, Rejection.

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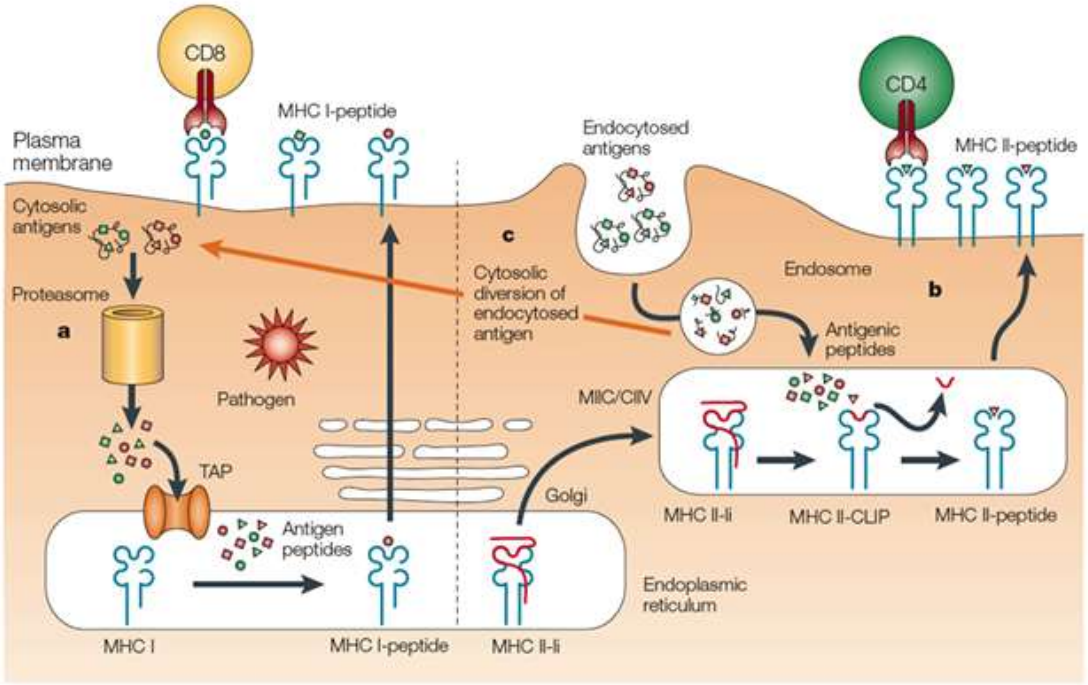
AC	Anticorpo
DSA	Anticorpo específico do doador, do inglês, *Donor Specific Antibodies*
dnDSA	anticorpo contra o doador *de novo*, do inglês *de novo donor-specific antibodies*
EBV	Epstein-Barr Vírus
HLA	Antígeno leucocitário humano, do inglês *human leukocyte antigen*
IVIG	Imunoglobulina humana
MELD	Doença hepática em estágio terminal, do inglês M*odel for End-Stage Liver Disease*
MFI	Intensidade média de fluorescência, do inglês *median fluorescence intensity*
MHC	Complexo principal de histocompatibilidade, do inglês *major histocompatibility complex*
MM	do inglês *Mismatch*
MMe	do inglês *Mismatch de Eplets*
PRA	Reatividade contra painel, do inglês *Panel Reactive Antibody*
PTLD	Distúrbio linfoproliferativo pós-transplante, do inglês *post-transplant lymphoproliferative disorder*
PELD	Doença hepática em estágio terminal pediátrica, do inglês *Pediatric End-stage Liver Disease*
RMA	Rejeição mediada por anticorpos
RMCT	Rejeição mediada por células T
SAB	Esferas de antígenos únicos, do inglês, *single-antigen beads*
SclgG	Clivagem única de IgG, do inglês *single cleavage IgG*
THA	Trombose da artéria hepática
Tx	Transplante hepático

SUMÁRIO

1. Introdução

A importância do antígeno leucocitário humano (HLA) nos transplantes de órgãos sólidos é um assunto bastante debatido[1]. O HLA é o principal complexo de histocompatibilidade com um polimorfismo importante. A região HLA contém 6 genes clássicos que são classificados em genes de classe I: HLA A; B e C; e genes de Classe II: HLA DR, DQ, DP. Em cada grupo, existem muitos alelos. As moléculas de HLA vão para superfície das células ligadas a um antígeno proteico para que possam ativar os linfócitos T. As moléculas de classe I são expressas tem todas as células nucleadas que irão apresentar os peptídeos antigênicos para os linfócitos T CD8+, responsável pela resposta citotóxica. As moléculas da classe II estão presentes nas células apresentadoras de antígenos (células dendríticas, linfócitos B, macrófagos) que irão apresentar os peptídeos antigênicos para os linfócitos T CD4+, iniciando uma resposta imune[2], conforme representado na figura 1[3].

Figura 1 - Representação do complexo MHC realizando o reconhecimento dos peptídeos e apresentando-os aos linfócitos



Fonte: Heath, W. R e col; 2001

Nos transplantes renal, cardíaco, pulmonar, intestinal e transplante de medula óssea (TMO), já é aceito que a presença dos anticorpos específicos contra o doador (DSA) são fatores de risco para a diminuição da sobrevida do enxerto[1,4]. Os pacientes mais jovens ou com uma baixa aderência à imunossupressão parecem ter um risco maior de desenvolver *de novo* DSA (dnDSA). No entanto, nem todos os DSA são patogênicos. Alguns autores sugerem a realização de ensaios clínicos a fim de procurar não apenas anticorpo contra-HLA, mas também realizar uma análise de subclasses de DSA não-HLA[5-6].

Nos últimos tempos, este assunto tem sido bastante debatido e, até recentemente, acreditava-se que o aloenxerto hepático haveria uma resistência aos anticorpos específicos de doadores, pois o fígado apresenta alguns mecanismos para se proteger desse insulto[7-8]. Por tal motivo, a maioria dos centros transplantadores dava pouca atenção para a presença ou a persistência dos DSA nesse tipo de transplante[9].

A presença de DSA no receptor, além de rejeição mediada por anticorpo, parece estar relacionada com hepatite de células plasmáticas, estenose biliar e complicações a longo prazo como rejeição crônica, ductopenia e fibrose hepática, principalmente nos pacientes em que o *crossmatch* é positivo[6;10].

No transplante renal, a presença do anticorpo HLA pode levar a uma rejeição hiperaguda [9]. Muitos estudos prospectivos mostraram que a sobrevida do enxerto foi menor, mesmo em quem tinha níveis baixos de DSA circulante. Acredita-se que os mecanismos pelos quais o anticorpo HLA causa lesão sejam a ativação do complemento pelo caminho clássico que leva à formação do complexo de ataque à membrana; pela lesão direta ao endotélio capilar por interações de anticorpos com antígenos de superfície celular e, por fim, através da ativação de células pró-inflamatórias, como células Natural Killer (NK), macrófagos e neutrófilos[9;11].

Um estudo mostrou que a presença de DSA pré-formado com uma alta intensidade de fluorescência, independentemente de classe, estava associada à mortalidade dos receptores após um ano de transplante hepático[8].

O transplante de fígado pediátrico apresenta uma resposta imunológica mais robusta ao aloenxerto do que os de adultos, tendo uma prevalência de DSA circulante maior na população pediátrica. Portanto, definir as características desencadeadas pelo DSA será um progresso importante para pacientes transplantados. Esse é um

fato ainda relativamente pouco frequente, e essa análise não é realizada de rotina entre os serviços[12,13].

O objetivo de avaliar a presença de DSA no receptor é útil tanto para o diagnóstico quanto para um início de tratamento precoce, podendo evitar-se uma rejeição mediada por anticorpo, inclusive em pacientes que apresentam testes de função hepática normal, resistência a esteroides ou características histológicas para rejeição [4].

2. Revisão da literatura

2.1 Fígado

O fígado, que representa de 1 a 3% do peso corporal total, é o maior órgão sólido do corpo. Ele desempenha diversas funções essenciais, incluindo excreção, síntese e metabolização, exercendo, assim, uma multifuncionalidade crucial no organismo[14]. Devido a sua grande multifuncionalidade e importância, problemas nele podem desenvolver significativos impactos na saúde geral do indivíduo[15]. O desenvolvimento hepático infantil é um processo contínuo que envolve a maturação das funções hepáticas à medida que a criança cresce. Certas lesões hepáticas podem ocorrer em diferentes estágios do desenvolvimento do fígado, tanto durante a formação quanto após a sua maturação. Muitas vezes, essas lesões são irreversíveis, sendo o transplante hepático a única alternativa de tratamento[15].

A função imunológica do fígado é desempenhada pelas células de Kupffer, que compõem cerca de 80-90% da população fixa de macrófagos do sistema retículo endotelial. Essas células têm um papel crucial como filtro na circulação sistêmica, removendo partículas exógenas, como bactérias, endotoxinas e parasitas, além de partículas endógenas, como eritrócitos senescentes.

O fígado possui uma notável capacidade de regeneração, permitindo que pacientes que sobrevivem a lesões hepáticas agudas graves se recuperem completamente. No entanto, em casos de lesão crônica, o fígado frequentemente responde com um processo contínuo que pode levar à cirrose, resultando em uma perda progressiva da função hepática. Esse processo é quase sempre irreversível e pode evoluir para uma falência hepática incompatível com a vida [15].

2.2 Transplante hepático

O transplante hepático (Tx) é um procedimento terapêutico aplicado a doenças graves do fígado quando outras alternativas falham ou não estão disponíveis[16]. Tal cirurgia salva vidas de crianças com doença hepática aguda ou crônica[17].

A decisão de realizar um transplante hepático em crianças é bastante complexa, envolvendo diversas considerações, incluindo a gravidade da doença, o diagnóstico etiológico, a disponibilidade de doador vivo ou falecido, o momento ideal

para o procedimento cirúrgico, o estado de saúde da criança, seu peso e a presença de outras comorbidades[18].

É fundamental que uma equipe médica multidisciplinar esteja envolvida no processo. O primeiro transplante de fígado pediátrico ocorreu em 1963, seguido pelo primeiro transplante bem-sucedido em um lactente com atresia biliar em 1967. A partir de 1985, com melhorias no tratamento imunossupressor e avanços nas técnicas cirúrgicas, o procedimento passou a apresentar resultados satisfatórios[18].

O transplante hepático com doador vivo teve seu início nos anos 1990. Na América Latina, ainda enfrentamos uma alta taxa de recusa familiar de doadores falecidos, destacando a necessidade de promover uma cultura de doação. A escassez de doadores falecidos levou ao uso de enxertos de doadores vivos, que atualmente representam cerca de 15% dos transplantes[17].

Em 1995, os Estudos de Transplante de Fígado Pediátrico (SPLIT) serviram como um registro multicêntrico, observacional e longitudinal para coletar dados prospectivos de transplante hepático pediátrico nos Estados Unidos e Canadá. Esse registro foi fundamental para várias publicações importantes sobre o tema e contribuiu significativamente para o desenvolvimento do escore para alocação de órgãos[19].

No ano de 2002, foi implementado esse escore, o qual é chamado de PELD (Doença Hepática em Estágio Terminal Pediátrica), para pacientes com menos de 12 anos, bem como o escore MELD (Doença Hepática em Estágio Terminal) para pacientes com mais de 12 anos[17]. Pacientes que recebem uma alta pontuação de MELD/PELD apresentam urgência para realização do transplante devido à gravidade da doença de base. No entanto, dado a crescente demanda, não há órgãos suficientes para atender a todos, o que eleva ainda mais essa urgência. No Brasil, ainda há uma taxa elevada de mortalidade de pacientes em lista de espera[20]. Aproximadamente um terço dos transplantes de fígado pediátricos são realizados em pacientes com menos de 12 meses, e metade dos receptores tem menos de 24 meses. Isso ocorre principalmente devido a doenças colestáticas, sendo a atresia biliar a mais comum (responsável por 38,5% das indicações de transplante hepático pediátrico). Outras indicações compreendem tumores hepáticos, infecciosas, distúrbios metabólicos, síndromes genéticas, doenças hepáticas pós-oncológicas, insuficiência hepática fulminante e doença hepática da fibrose cística[18].

Os transplantes pediátricos constituem cerca de 7% a 8% do número total de transplantes realizados nos Estados Unidos[17]. No Brasil, 22-25% em Tx adultos e 4-5% nos Tx pediátricos[21]. A criação de programas de transplante específicos para pacientes pediátricos contribuiu para melhorar os resultados e a disponibilidade de órgãos. A modernização geral, incluindo exames de imagem mais sensíveis, regimes de antibióticos aprimorados e um acompanhamento meticuloso, também impactam esses resultados[22].

Ao redor do mundo, foram realizados mais de dez mil transplantes hepáticos. O Brasil tem o maior sistema público de transplantes de órgãos e, em 2021, chegou ao quarto lugar entre os países que mais realizaram transplante hepático, ficando atrás dos Estados Unidos, da China e Índia[20,23]. A sobrevida dos pacientes pós-transplante em 1 e 5 anos é de 95% e 85%, respectivamente[24]. Hoje, o transplante hepático é uma intervenção bem-sucedida quando o fígado nativo não é mais uma opção[21].

Dados do Canadá mostram taxas de sobrevida após Tx pediátrico em 10 anos acima de 90%[25]. Esses resultados superam o transplante hepático em adultos, que apresenta uma sobrevida em um ano de 81%. Historicamente, os resultados no transplante pediátrico eram ligeiramente piores em comparação com os adultos, mas melhoraram de forma drástica nos últimos tempos. As razões por trás disso incluem inovação técnica, seleção de enxertos, melhoria na técnica de anastomose, a opção de transplante intervivo, gerenciamento perioperatório e terapia imunossupressora aprimorada a longo prazo[26].

2.3 Imunossupressão

Os agentes imunossupressores atuam inibindo ou diminuindo a resposta do sistema imunológico aos aloantígenos do enxerto. Há diversos medicamentos e agentes biológicos que são utilizados para a imunossupressão do paciente transplantado de fígado. Esses medicamentos podem ser classificados de acordo com seus sítios de atuação na cascata das células T[27]. O avanço da imunossupressão melhorou significativamente a sobrevida do enxerto, marcando um ponto importante na realização dos transplantes a partir da década de 1980, com a introdução da ciclosporina[28]. A compreensão crescente sobre a rejeição dos órgãos transplantados

e a importância de uma equipe multidisciplinar para o cuidado desses pacientes também se tornaram evidentes a partir da década de 1990[17].

Um estudo realizado entre 1968 e 2009 utilizando dados de diferentes centros do Registro Europeu de Transplante de Fígado mostrou que a sobrevida na população pediátrica foi de 81% em cinco anos[18].

O ajuste da dose do imunossupressor, no paciente transplantado, varia com o tempo decorrido desde o transplante e apresenta suas próprias dificuldades e particularidades conforme a idade da criança ou adolescente. A necessidade de manter doses mais elevadas ocorre principalmente nos primeiros meses após o transplante. Contudo, também pode causar reações adversas, como o aumento de infecções oportunistas devido ao comprometimento do sistema imunológico, nefrotoxicidade, hepatotoxicidade, hipertensão arterial, distúrbios gastrointestinais, distúrbios hematológicos e distúrbios neurológicos, bem como o aumento do risco de neoplasias, especialmente linfomas, carcinomas de pele e o distúrbio linfoproliferativo pós-transplante (PTLD) associado à infecção pelo Vírus Epstein-Barr (EBV)[17,29-33].

Encontrar o equilíbrio da imunossupressão é fundamental, considerando que pouco imunossupressor gera lesão no enxerto, e seu excesso pode acarretar em complicações sistêmicas[34].

2.4 Complicações pós-transplante

Clinicamente, os pacientes transplantados podem apresentar complicações médicas tanto a curto quanto a longo prazo[24]. Essas complicações envolvem o tamanho do enxerto, sobretudo no transplante parcial, especialmente em casos com doador vivo, de adulto para criança[35]. Problemas vasculares como trombose ou estenose da artéria hepática, veia porta ou das veias hepáticas, complicações biliares e as rejeições também são comuns[18,36].

Complicações relacionadas à rejeição mediada por anticorpo ainda são obstáculos significativos que precisam ser compreendidos para aumentar ainda mais a sobrevida do paciente e do enxerto[37]. Na tentativa de evitar ou amenizar as complicações, alguns centros realizam biópsias de rotina do enxerto, mesmo sem alterações laboratoriais. As possíveis rejeições subclínicas são vistas nessas biópsias de protocolo, mas seu efeito no receptor não é claramente compreendido[38]. Em locais

em que são realizadas biópsias protocolares, observou-se um aumento da hepatite e fibrose no enxerto, ainda que na ausência de alterações nos resultados laboratoriais prévios[17].

2.4.1 Trombose de Artéria Hepática

Nos pacientes transplantados de fígado, a trombose da artéria hepática gera consequências catastróficas. A artéria hepática corresponde a 20% do fluxo sanguíneo hepático total. Esse fenômeno leva a um aumento da morbidade e mortalidade após o transplante de fígado e é uma das principais causas de perda do enxerto[39]. A incidência desse evento varia conforme os centros, mas pode chegar a 25%[40].

Crianças constituem o grupo de maior risco de uma trombose da artéria hepática, sendo a incidência quase quatro vezes maior em comparação com os adultos. Por muito tempo acreditou-se que a técnica cirúrgica era o principal fator de risco para o desenvolvimento da trombose de artéria hepática (TAH). Atualmente, sabe-se que existem outros fatores de risco, como o tamanho do vaso do receptor, uso de enxerto, diferença de calibre entre o doador e o receptor[41,42]. Sabe-se também que a transfusão excessiva de plasma fresco congelado, a preservação do enxerto, distúrbios de coagulação, incompatibilidade ABO, aumento do tempo de isquemia, hipotensão severa, aumento do hematócrito pós-transplante, infecções por citomegalovírus e fatores imunológicos podem estar associados ao desenvolvimento de TAH[43,44]. Após um transplante, a ocorrência de trombose na artéria hepática pode desencadear complicações nas vias biliares, como fístulas ou estenoses devido à vascularização da artéria hepática nos ductos biliares[43].

O manejo da TAH difere conforme cada centro transplantador, podendo ir de intervenções cirúrgicas, radiológicas, medicamentosas até um retransplante. Infelizmente, a revascularização falha em até 33% dos casos, geralmente resultando na perda do enxerto[40].

Para reduzir o risco de trombose da artéria hepática, diversas estratégias podem ser adotadas. Essas incluem aprimoramentos nos cuidados médicos e nas técnicas cirúrgicas, como a realização de anastomose microvascular por cirurgiões experientes utilizando microscópios, a técnica *“no touch”* durante a dissecção dos

tratos arteriais hepáticos nativos e do doador, a utilização de ultrassonografia com Doppler intraoperatória após a anastomose dos vasos antes do fechamento do abdome, a ligadura da artéria esplênica em casos nos quais o índice de resistividade arterial na ultrassonografia intraoperatória seja superior a um, o que sugere um refluxo portal, além da monitorização para evitar níveis de hematócrito acima de 30% após o transplante hepático[41].

Como destacado por Seda e col. (2016), o diagnóstico precoce desempenha um papel crucial no sucesso da revascularização dessa artéria. Identificar previamente os pacientes com maior predisposição ao desenvolvimento de trombose da artéria hepática pode contribuir significativamente para a prevenção e redução da incidência desse evento[39,43,44].

2.4.2 Rejeição

A rejeição é a complicação mais frequente do transplante hepático. Pode estar associada ou não a alterações laboratoriais ou sintomas clínicos[15]. Ela pode ser classificada em hiperaguda, aguda ou crônica, como demonstrado na Tabela 1.

Tabela 1 - Conceitos de rejeição no transplante hepático

Termos Antigos	**Termos Novos**	
Rejeição Humoral ou hiperaguda	Rejeição mediada por anticorpos (RMA)	Aguda Aguda de início tardio Crônica Subclínica
Rejeição Celular Aguda	Rejeição mediada por células T (RMCT)	Aguda (precoce <6m) Crônica (tardia >6m)
Hepatite autoimune *de novo*	Rejeição rica em plasmócitos	

Fonte: Adaptado de Demetris e col., 2016.

2.4.2.1 Rejeição Mediada por Anticorpo (RMA)

A RMA é uma forma de rejeição que ocorre após o transplante hepático e está relacionada aos DSA circulantes. Pode se manifestar de forma aguda ou crônica, exibindo diversas características histológicas, e ambas têm o potencial de levar à disfunção do enxerto se não forem tratadas adequadamente[45].

Os enxertos de fígado têm uma resistência bem documentada à RMA. Anteriormente, acreditava-se que esse tipo de rejeição não ocorria no enxerto hepático, não havia critérios diagnósticos histológicos específicos e seu impacto na sobrevida do enxerto era considerado mínimo. No entanto, há um entendimento crescente sobre o fígado como um órgão "privilegiado imunologicamente", sustentado pelos seguintes fatos [9]:

1) O fígado possui um suprimento sanguíneo duplo, resultando em um baixo fluxo dentro do sistema sinusoidal, o que permite interações entre os produtos microbianos intestinais, antígenos e células imunes.
2) Devido ao seu grande parênquima, o fígado tem uma superfície endotelial aumentada para diluir as interações com anticorpos.
3) Antígenos HLA de classe I são expressos em todas as células do fígado, enquanto a expressão da classe II é relativamente baixa.
4) Os sinusoides hepáticos são revestidos pelas células de Kupffer e células endoteliais, que eliminam os complexos imunes circulantes.
5) O fígado possui uma notável capacidade de regeneração.

Mas sabe-se, hoje, também, que apesar desse privilégio, os pacientes pós-transplante hepático continuam suscetíveis a lesão mediada pelo sistema imunológico[12,46].

Em 2013, uma pesquisa foi conduzida em 41 centros dos EUA, onde 91% dos entrevistados acreditavam na existência da RMA, porém apenas 46% consideravam-na uma causa de lesão no enxerto hepático[37]. Devido a essa percepção, poucos programas realizavam investigações rotineiras de DSA ou coloração com C4d nas biopsias hepáticas, pois não a consideravam custo-efetivas[47].

A hipótese sobre o desenvolvimento da RMA no fígado sugere que este sofra um insulto inicial, podendo ser uma rejeição mediada por células T (RMCT), hepatite viral ou lesão por isquemia-reperfusão. Após esse insulto, há um aumento na

expressão de HLA classe II, facilitando a ligação de DSA e ativando a cascata clássica de complemento, juntamente com a facilitação da citotoxicidade celular por meio de interações com os receptores Fc, resultando em lesão do enxerto[46].

Identificar, diagnosticar e tratar precocemente esse tipo de rejeição mediada por anticorpo é bastante desafiador, em decorrência da falta de protocolos de tratamento e dos desfechos incertos a longo prazo. Em um estudo publicado em 2023 por Goto e col., foi apresentado um protocolo de tratamento aplicado desde 2015 para pacientes com DSA positivo pós-transplante e de alto risco. Isso incluía ter um MFI >= 10.000 e um escore de fibrose > 2 na histologia. A imunossupressão que esses pacientes receberam foi: rituximab, tacrolimus (nível sérico > 5ng/ml) associado a micofenolato (MMF). Nos casos intermediários, o tratamento de imunossupressor se manteve com tacrolimus associado a MMF. Nos casos de baixo risco com escore de fibrose 0-1 e DSA com MFI < 10.000, o tratamento não foi modificado, apenas biópsias anuais foram realizadas. Tal estudo concluiu que independentemente do tratamento realizado, não foram observadas diferenças significativas no desfecho clínico entre os grupos de pacientes[48].

O'Leary e col. (2014) desenvolveram uma coorte de treinamento para validar histologicamente a RMA. As características mais sugestivas de RMA aguda foram identificadas como eosinofilia portal (OR=4.37, p<0.001), hipertrofia de células endoteliais da veia porta (OR=2.88, p<0.001) e venulite central eosinofílica (OR=2.48, p=0.003). A presença de um escore >1.0 proporciona uma sensibilidade de 81% e uma especificidade de 71% para o diagnóstico de RMA aguda[47].

O Grupo de Trabalho de Banff sobre Patologia de Enxerto de Fígado revisou e discutiu, em 2016, as evidências da literatura sobre a rejeição mediada por anticorpos em transplantes de fígado em suas reuniões desde 2011. Seu objetivo era estabelecer critérios diagnósticos para RMA no enxerto após transplante hepático[12].

Os quatro critérios para definir RMA são os seguintes: (1) Histologia caracterizada por hipertrofia das células endoteliais, dilatação capilar portal, microvasculite com infiltrado de monócitos, eosinófilos e neutrófilos, além de edema portal/periportal, (2) aumento de DSA circulantes, (3) presença difusa de C4d na microvasculatura ou no estroma portal e (4) exclusão de outras doenças hepáticas ou complicações que possam resultar em padrões semelhantes de lesão (por exemplo, rejeição mediada por células T concomitante)[47]. Na RMA aguda os pacientes podem

manifestar uma disfunção do órgão transplantado, como trombocitopenia refratária, hiperbilirrubinemia, elevação significativa das aminotransferases, níveis reduzidos de complemento sérico e altos títulos de DSA. Além disso, pode ocorrer rejeição aguda de início tardio, quando a presença de DSA é detectada após seis meses ou mais do transplante[8,49].

A rejeição crônica mediada por anticorpos parece ocorrer em aproximadamente 8-15% dos receptores que mantêm ou desenvolvem novamente DSA, sendo mais comum contra o HLA de Classe II, especialmente o *locus* DQ. No entanto, a verdadeira prevalência é desconhecida, pois a maioria dos pacientes podem apresentar aminotransferases normais, apesar de evidências histológicas de lesão do enxerto. O mecanismo fisiopatológico da RMA crônica, em todos os enxertos de órgãos, não é completamente compreendido, mas as vias efetoras mediadas por anticorpos e a ativação do complemento aparentemente conduzem à inflamação e fibrose[12].

A RMA crônica desencadeia uma vasculopatia, processo atualmente irreversível com impacto significativo na morbidade e mortalidade do paciente. Manifesta-se tardiamente, após o transplante, e muitas de suas características se assemelham às da RMA aguda, incluindo alterações nas células endoteliais, inflamação microvascular e a presença de C4d com anticorpos específicos do doador, sendo a fibrose também uma característica importante da RMA crônica. Em biópsias de rotina, essa vasculopatia pode não ser visualizada devido ao seu envolvimento predominante em vasos maiores, que raramente são observados, exceto em amostras de tecido profundo. Embora haja forte evidência clínica apoiando a associação entre RMA crônica, vasculopatia e HLA-DSAs em vários órgãos sólidos, os mecanismos precisos pelos quais a proliferação neointimal e fibrose ocorrem não são claros[49].

Os critérios diagnósticos para RMA crônica incluem histologia compatível, caracterizada por fibrose portal e perisinusoidal densa do enxerto, inflamação portal e perivenular não específica e venopatia portal obliterativa, presença de DSA positivo dentro de 3 meses da biópsia, positividade focal de C4d (> 10% dos tratos porta), bem como exclusão de outros insultos hepáticos. Além disso, a RMA pode estar associada à rejeição mediada por células T concomitante e/ou à rejeição crônica ductopênica[46].

A tipagem HLA de rotina do doador e do receptor facilita o diagnóstico de RMA e fornece dados de referência para determinações de DSA. O monitoramento rotineiro de DSA pode ajudar na administração dos imunossupressores[12].

2.4.2.2 Rejeição Mediada por Células T (RMCT)

Rejeição mediada por células T (RMCT) é comum no primeiro mês pós-transplante de fígado, com uma prevalência variando de 20% a 50%, a depender da definição utilizada. Dentre os fatores de risco associados ao seu desenvolvimento estão doadores falecidos, aumento do tempo de isquemia fria e receptores com cirrose biliar primária ou hepatite C[50].

Na histologia da RMCT aguda precoce (< 6 meses), é evidente a apresentação direta de aloantígenos, o que desencadeia danos inflamatórios nos ductos biliares e inflamação portal caracterizada por linfócitos, macrófagos, eosinófilos e outros. Por outro lado, na RMCT tardia ou crônica (> 6 meses), a apresentação indireta de aloantígenos é mais prevalente, com infiltrados linfoplasmocíticos e histiocíticos mais homogêneos, além de menos colangite linfocítica e atividade de interface perivenular de baixo grau do tipo necroinflamatória[12].

Embora a definição do Grupo de Trabalho de Banff exija todos os quatro critérios para um diagnóstico definitivo de RMA aguda, casos suspeitos podem não apresentar todos os quatro critérios simultaneamente, sobretudo dada a apresentação ubíqua com RMCT[46,49].

Em relação ao tratamento, sabe-se que a rejeição aguda mediada por células T geralmente responde bem ao aumento da imunossupressão, enquanto a RMA é pouco responsiva à terapia padrão[51].

2.4.2.3 Rejeição Rica em Células Plasmáticas

Também conhecida como “hepatite rica em células plasmáticas” ou “hepatite autoimune *de novo*”, está associada à presença da coloração C4d nos capilares da área portal e parece ser uma manifestação de rejeição do enxerto. É pouco compreendida e incomum, ocorrendo em cerca de 3–5% dos receptores adultos com disfunção tardia (>6 meses), semelhante à hepatite autoimune, frequentemente em receptores positivos para o vírus da hepatite C tratados com interferon[12].

Ainda não está claro se essa condição representa uma apresentação atípica de RMA aguda/crônica ou se é uma entidade separada. Portanto, é fundamental sempre analisar DSA e C4d para avaliar a presença de RMA subjacente nesses

casos. Alguns pacientes diagnosticados com rejeição rica em células plasmáticas podem apresentar rápida progressão da fibrose, culminando em cirrose do enxerto, e também podem ser resistentes à otimização do tratamento imunossupressor[46].

2.5 Aspectos Imunológicos

2.5.1 C4d e rejeição

Em 2003, a coloração para C4d no transplante renal foi incorporada aos critérios de Banff para rejeição mediada por anticorpos e começou a ser analisada no transplante hepático. O C4d é um produto resultante da ativação do complemento sem função biológica conhecida, que se liga aos tecidos covalentemente, sendo assim mais fácil de ser reconhecido histologicamente[9]. Sua coloração linear/granular nas células endoteliais microvasculares é aceita como evidência de ativação do complemento no tecido, devendo ser realizada na suspeita de RMA aguda ou crônica. Cada laboratório deve validar suas reações anti-C4d contra controles positivos e negativos para monitorar o efeito de tempos de fixação, técnicas de processamento, automação e seleção de anticorpos. A deposição de C4d tem predominância na veia porta e capilar sinusoidal nas fases agudas da RMA, enquanto na RMA crônica pode se estender para outros locais, como o endotélio da veia central e sinusoidal[12,52].

Anteriormente, era possível obter-se a coloração de C4d apenas por imunofluorescência de espécimes de tecido congelado, o que podia ser difícil devido a problemas de preservação, juntamente com coloração de fundo não específica. Entretanto, um estudo recente confirmou a viabilidade de utilizar tecido hepático fixado em formalina e embutido em parafina para detectar C4d na RMA. Ao analisar a positividade do C4d, os seguintes compartimentos estruturais devem ser avaliados: veia porta, capilar sinusoidal, estroma portal, endotélio sinusoidal e veia central[52,53].

Musat e col. (2011) avaliaram 43 receptores com suspeita de rejeição aguda e crônica e encontraram uma frequência aumentada de DSA com coloração C4d naqueles com diagnóstico de rejeição celular aguda, especialmente quando havia ductopenia e um aumento no risco de rejeição resistente a esteroides[54,55].

Por outro lado, alguns estudos sugerem que a coloração de hepatócitos com anticorpos anti-C4d é observada como resultado de lesão por isquemia/reperfusão, não sendo um marcador específico de lesão mediada por anticorpos. A coloração

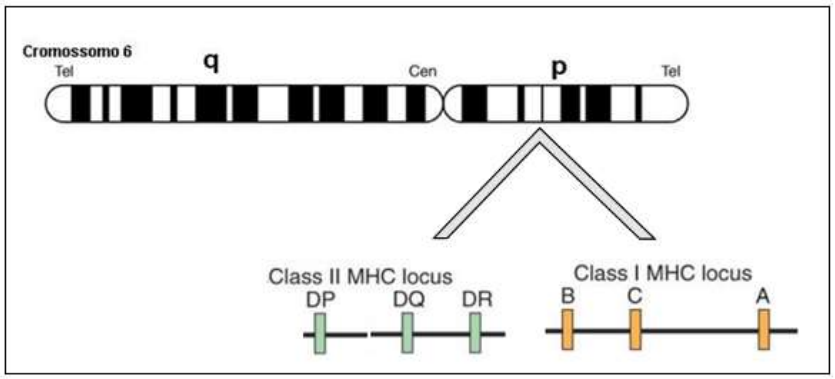
difusa de C4d pode ser vista em até dois terços das amostras de biópsia com rejeição. Foi observado que os locais em que o C4d pode ser visualizado incluem o endotélio arterial, venoso portal e sinusoidal, sendo mais frequente em receptores com *crossmatch* positivo. Quando não há DSA circulante, a deposição de C4d provavelmente não é específica para anticorpos HLA e foi encontrada em aloenxertos hepáticos com outras comorbidades, como rejeição crônica, recorrência do vírus da hepatite C, hepatite autoimune, obstruções ou estenoses biliares, lesão de preservação, trombose vascular e lesão isquêmica. Isso levou à hipótese de que a ativação de C4d possa ser desencadeada por DSA e não HLA[9].

Outros estudos também descrevem que ter um DSA contra HLA não está automaticamente associado a uma detecção de C4d, assim como uma detecção de C4d também pode estar presente em pacientes sem DSA[56,57].

2.5.2 Antígeno leucocitário humano (HLA)

O sistema HLA foi descrito por J. Dausset em 1958, trabalho pelo qual recebeu o Prêmio Nobel em 1980. Localizado no braço curto do cromossomo 6, o HLA compreende um complexo gênico que codifica um grupo altamente polimórfico de proteínas de superfície celular, conhecidas como complexo principal de histocompatibilidade (MHC, do inglês “major histocompatibility complex”), divididas em classe I e classe II (fig.1) [58]. Essas proteínas são responsáveis por reconhecer a atividade não própria do organismo e iniciar respostas imunológicas. Existem três *loci* que codificam as moléculas de classe I, denominadas HLA-A, HLA-B e HLA-C, e três *loci* gênicos do MHC de classe II, denominados HLA-DP, HLA-DQ e HLA-DR[55].

Figura 2 – Cromossomo 6 – Mapa genético da região HLA



Modificado de: Berlingerio et al., 2009.

Herda-se duas cópias de cada *locus* gênico, uma de cada progenitor, resultando em três *loci* de classe I e três *loci* de classe II. Todos esses *loci* apresentam um alto grau de polimorfismo, com múltiplos alelos na população. As moléculas HLA de classe I estão presentes em todas as células nucleadas e têm a função de ligar peptídeos antigênicos processados e apresentá-los aos linfócitos T citotóxicos ou CD8. Por outro lado, os genes HLA de classe II estão presentes nas células apresentadoras de antígenos, nas quais sua função é apresentar peptídeos exógenos para as células T auxiliares ou CD4[59].

A tipagem de HLA é utilizada para parear os determinantes mais importantes conhecidos de histocompatibilidade entre doador e receptor. Com mais de 38.975 alelos determinando os 6 antígenos HLA (HLA-A, -B, -C, -DP, -DQ, -DR), a compatibilidade representa um desafio significativo[60]. O pareamento do maior número possível de antígenos HLA tem sido associado a uma significativa melhoria na sobrevida funcional dos enxertos, especialmente em transplantes de rins e células-tronco hematopoiéticas. Ainda que o pareamento de HLA também melhore a sobrevida em transplantes hepáticos, essa melhora é mais modesta devido às múltiplas diferenças de histocompatibilidade não detectadas[61].

A alo sensibilização aos HLA ocorre após a exposição a tecidos não próprios, seja por meio de gravidez, implantes de dispositivos de assistência mecânica cardíaca, transfusões, transplantes de órgãos sólidos, enxertos de tecidos ou infecções[62].

Apesar de uma prova cruzada negativa ser um indicador importante, ela não garante a segurança contra incompatibilidades[61].

O papel patogênico de aloanticorpos no transplante de órgãos sólidos foi reconhecido por volta de 1969, quando Patel e Terasaki demonstraram que anticorpos específicos do doador, detectados por prova cruzada por citotoxicidade, estavam associados à falha imediata de aloenxertos renais[63,64].

Um maior grau de incompatibilidade de HLA tem sido associado a resultados desfavoráveis no transplante de fígado de doador vivo. No entanto, algumas evidências contrastantes sugeriam que a presença e o grau de compatibilidade HLA não impactariam na sobrevida do aloenxerto hepático ou do receptor a longo prazo[7].

A principal estratégia para reduzir a imunogenicidade dos enxertos transplantados é buscar a máxima compatibilidade entre os aloantígenos do doador e do receptor[65].

Com o avanço no tratamento imunossupressor, a elegibilidade para transplantes foi ampliada, e as incompatibilidades de HLA não desqualificam automaticamente o paciente para o transplante hepático, uma vez que a terapia imunossupressora se tornou mais eficaz[61].

2.5.3 DSA (anticorpos específicos contra o doador)

São anticorpos que direcionam sua ação contra os antígenos do doador durante um transplante. Atualmente, os DSAs anti-HLA são considerados biomarcadores essenciais para prever lesões e a perda de aloenxertos. Contudo, não existe consenso na definição de sua patogenicidade nem um padrão estabelecido para sua avaliação que oriente a tomada de decisões clínicas no transplante hepático[66].

Historicamente, os DSAs eram classificados em DSAs pré-formados (pré-existentes) (pDSAs) e DSAs de novo (dnDSAs). Os pDSAs são detectados no soro pré-transplante e se desenvolvem como resultado de eventos de sensibilização anteriores, como transfusões de sangue, gravidez, transplantes anteriores ou infecções. Eles podem desencadear rejeição mediada por anticorpos logo após o transplante. Já os dnDSAs se desenvolvem após o transplante, como resultado do primeiro encontro com antígenos HLA do doador, e são responsáveis pela rejeição mediada por anticorpos tardia. Um novo termo, DSAs precoces (eDSAs), foi cunhado para descrever uma categoria de DSAs não detectados antes do transplante, mas que se desenvolveram logo após o transplante devido à ativação da resposta de memória. Os eDSAs estão associados à rejeição mediada por anticorpos precoce[67].

Um estudo conduzido por Taner e col. (2012) foi um dos primeiros estudos de análise sistemática e prospectiva da prevalência e impacto de DSA pré-formados na função do aloenxerto hepático. Os resultados indicaram que eles eram comuns, sendo encontrados em 22,2% dos receptores analisados, com incidências variando de 5,2% a 24,2%. Observou-se uma redução nos níveis de DSA imediatamente após a cirurgia na maioria desses receptores, tornando-se indetectáveis por até 4 meses. Essa

diminuição tem sido atribuída à absorção de anticorpos circulantes pelo fígado, embora o mecanismo exato não seja conhecido[68].

Acredita-se que o fígado apresente uma capacidade de resistência à rejeição mediada por anticorpos (RMA) em seu transplante. Todavia, há também outros fatores que aumentam a suscetibilidade a lesões relacionadas à RMA, como a sensibilização em nível elevado, anticorpos IgG (especialmente subclasses que fixam eficientemente o complemento e mediadores da citotoxicidade celular dependente de anticorpos), enxertos de tamanho reduzido, inativação ou depleção de células de Kupffer, assim como o direcionamento de anticorpos aos antígenos endoteliais[7].

Hoje, já está definido que pacientes transplantados com DSA pré-formados têm um risco maior de dano imunológico através da RMA aguda, rejeição crônica e perda de aloenxertos em todos os órgãos sólidos[67,69].

A prevalência de RMA entre pacientes pré-sensibilizados é superior a 20%[51]. Mas nem todos os DSA são patogênicos e levam à lesão do enxerto[70]. Para definir se um DSA é patogênico, outros parâmetros precisam ser analisados, como a intensidade média de fluorescência (MFI, em inglês), o título, subclasse, epítopo alvo e ligação ao complemento (usando ensaios de ligação ao C1q, C3d ou C4d). Acredita-se que eles causem lesões no endotélio capilar pela ativação da cascata do complemento, ativando o recrutamento de células inflamatórias mediado pelo receptor Fc, incluindo macrófagos e células *natural killer*, podendo também provocar a ativação direta do endotélio[67].

Em 1999, Kasahara e col. analisaram, através do *crossmatch* por citometria de fluxo, que 21% dos receptores de transplante de fígado de doador vivo desenvolveram DSA no primeiro mês após o transplante, e que essa parcela (12 pacientes) foram diagnosticados com rejeição aguda, em comparação com apenas 17% daqueles sem DSA[71].

Estudos mais recentes sobre receptores pediátricos de transplante de fígado de doador vivo mostraram que os DSA estavam presentes em 32 de 67 (48%) dos sobreviventes de longo prazo. Na biópsia hepática, esses pacientes apresentavam uma maior probabilidade de fibrose e inflamação. Observou-se que pacientes com DSA positivo pós-transplante estavam sob imunossupressão de manutenção ou submetidos à redução da imunossupressão[72].

As tecnologias atuais para detectar os DSAs podem fornecer um meio mais eficaz de gerenciar os pacientes após o transplante de forma individual[69].

As técnicas de teste de anticorpos anti-HLA e de cruzamento evoluíram nos últimos anos. Pacientes que produzem anticorpos anti-HLA são denominados “sensibilizados ao HLA”, e a sensibilização é medida como parte da avaliação padrão no transplante renal. A amplitude da sensibilização é refletida no escore de anticorpo reativo ao painel calculado (cPRA) e na prova cruzada[73].

O PRA é um indicador crucial que reflete a probabilidade de um receptor de transplante ser compatível com um doador adequado. Ele estima a proporção da população geral cujos HLA, de um potencial candidato a transplante, resultariam em uma prova cruzada positiva devido à presença de anticorpos específicos do doador pré-formados (DSA). Um PRA elevado pré-transplante (relatado como percentual) está associado a uma menor probabilidade de compatibilidade com um doador adequado. Os escores de PRA variam de 0%, que indica ausência de anticorpos anti-HLA, a 100%, que indica uma amplitude de anticorpos que prediz incompatibilidade com 100% do pool de doadores. É importante ressaltar que ter um PRA elevado pode ser prejudicial independentemente da presença de DSA[73,74].

O método de detecção de anticorpos anti-HLA pré-existentes no candidato por meio de tecnologia de fase sólida envolve a incubação do soro com esferas de triagem revestidas com moléculas de HLA purificadas. Essas esferas contém um único antígeno HLA recombinante, conhecido como “esferas de antígenos únicos” (SAB), ou um aglomerado de antígenos nativos de classe I ou II purificados de linhagens de células B transformadas. A interação entre o soro e as esferas é então detectada por citometria de fluxo em um analisador Luminex. Esses ensaios de fase sólida têm alta capacidade de determinar rapidamente a presença, especificidade e potencial força dos anticorpos anti-HLA no soro do receptor[74].

Por outro lado, a prova cruzada por citometria de fluxo (FCXM) consiste na incubação de linfócitos do doador com o soro do receptor, seguida pela adição de um anticorpo anti-IgG marcado com fluoresceína. Este anticorpo se ligará especificamente aos linfócitos T e B ligados aos anticorpos do receptor. As células T, que expressam HLA de Classe I, e as células B, que também expressam HLA de Classe I e II, são identificadas através desse processo. Um resultado "positivo" na prova cruzada por citometria de fluxo é observado quando ocorre um deslocamento

na fluorescência além de um limiar predeterminado. Em termos de sensibilidade, o método de citometria de fluxo é considerado mais confiável, capaz de detectar a grande maioria dos DSA[74].

Outra alternativa, conhecida como “prova cruzada virtual”, consiste na comparação dos anticorpos anti-HLA do receptor, detectados por tecnologia de fase sólida, com os antígenos HLA expressos pelo potencial doador. Nesse método, uma prova cruzada virtual é interpretado como “positivo” se os anticorpos anti-HLA detectados atingirem limiares que indicam uma possível resposta imunológica adversa. Dependendo da precisão da tipagem de HLA, prova cruzada pode ser considerado um método superior de avaliação. Ele tem a capacidade de identificar quantitativamente DSA específicos por meio da medida do MFI (intensidade média de fluorescência), especialmente DSA anti-HLA de classe II, que têm sido mais associados a resultados negativos no transplante. Além disso, a prova cruzada pode prever não apenas a possibilidade de uma prova cruzada por citometria de fluxo negativa, mas também quantificar a probabilidade de um teste positivo real. Sua importância reside na redução do tempo necessário para o teste, o que consequentemente diminui o tempo de isquemia fria[65,74]. O uso do *crossmatch* virtual pode representar uma melhoria na avaliação pré-transplante do risco imunológico, ampliando a aplicação desses protocolos[65].

Apesar de uma prova cruzada positiva não impedir a realização de um transplante hepático, vale destacar que, com ela, tem-se um maior risco de rejeição associado a esse resultado, sugerindo a necessidade de atenção especial à imunossupressão do paciente. Conforme estudos recentes, pacientes que apresentam um *crossmatch* positivo após o transplante hepático têm um risco maior de perda precoce do enxerto[68,75,76].

Alguns autores sugerem que um *crossmatch* positivo de linfócitos T pode resultar em baixa sobrevida do enxerto e rejeição aguda, enquanto outros concluem que um *crossmatch* positivo de linfócitos T não tem efeito sob o enxerto[75,76].

Takaya e col. (1999) conduziram um estudo que investigou o desfecho de aloenxertos hepáticos provenientes de doadores com *crossmatch* positivo, focando nas complicações biliares e na rejeição crônica ductopênica. Comparando-os com um grupo controle de *crossmatch* negativo, o estudo constatou que a rejeição crônica foi encontrada em 26,3% dos pacientes com *crossmatch* positivo versus 4,9% nos

pacientes com *crossmatch* negativo. Além disso, as complicações biliares significativas ocorreram em 36,8% dos pacientes com *crossmatch* positivo, comparado a 10,2% no grupo de controle negativo[77].

Em uma coorte em que a prova cruzada por citometria de fluxo foi positiva, a incidência de rejeição renal aguda foi de 28%, comparada a 10% nos casos de prova cruzada negativa. Ademais, a perda precoce do enxerto aumentou em pacientes com a prova cruzada positiva, especialmente quando apresentavam DSA alta com MFI >10.000[78]. Apesar disso, devido à percepção geral de não haver rejeição hiperaguda em pacientes com prova cruzada positiva, o transplante continuou sendo realizado mesmo quando a prova cruzada era positiva[55].

A tecnologia de esferas de antígenos únicos (SAB), que detecta DSA por citometria de Luminex, revolucionou o diagnóstico nos últimos anos, permitindo a avaliação da presença de anticorpos anti-HLA em níveis muito baixos no soro dos pacientes, por meio da leitura da intensidade média de fluorescência (MFI)[62,79].

Estudos identificaram que a presença de dnDSAs após qualquer transplante de órgãos sólidos é um fator de risco importante para a diminuição da sobrevida do enxerto a longo prazo[51].

Dentre os fatores de risco para a produção de dnDSA estão receptores de etnia afro-americana, episódios de rejeição aguda mediada por células no primeiro ano após o transplante, incompatibilidade do doador-receptor para alelo DQ, sensibilização pré-transplante ao HLA, doador falecido, má adesão ao tratamento, rejeição celular até os 6 meses pós-transplante e *mismatch* (MM) DQ[51].

O *mismatch* (MM) indica o grau de incompatibilidade entre os alelos DQ do doador e do receptor, podendo resultar em três diferentes cenários: 0MM, indicando compatibilidade total em DQ, 1MM, representando uma compatibilidade de 50% em DQ e 2MM, sugerindo que o receptor é totalmente incompatível em DQ com o doador.

O transplante de fígado pediátrico desencadeia uma resposta imunológica mais intensa ao aloenxerto em comparação com adultos. Uma meta-análise revelou uma prevalência de 41% (IC 95% 29-54%) de DSA pós-transplante em receptores menores de 18 anos, uma taxa maior do que em receptores adultos. Essa condição está associada ao desenvolvimento de fibrose e inflamação no aloenxerto a longo prazo[7].

No entanto, não há evidências de uma redução na taxa de sobrevida após o transplante ou um aumento na necessidade de retransplante[80]. Estudos adicionais

também observaram que o escore de fibrose foi mais elevado entre os pacientes com DSAs, o que destaca a importância de se monitorar o desenvolvimento de DSAs em pacientes submetidos a transplante de fígado para identificar aqueles com maior risco de desenvolver fibrose e deterioração da função do enxerto[8].

Um estudo observacional recente, realizado ao longo de oito anos, sugere que a presença de DSA no soro pode estar correlacionada ao desenvolvimento de fibrose em 89 receptores pediátricos de transplante hepático [69].

Com base em estudos recentes, é definido que os dnDSAs estão associados à rejeição, fibrose, inflamação e sobrevida mais curta após o transplante de fígado. Fatores clínicos como imunossupressão, infecção e grau de lesão de isquemia-reperfusão, nível de expressão do antígeno leucocitário humano (HLA), afinidade do DSA e persistência do anticorpo contribuem para o desenvolvimento de disfunção do enxerto[69].

Sabe-se também que valores elevados de intensidade média de fluorescência (MFI), sobretudo contra a classe II do HLA, e mais especificamente os dnDSAs e alelos DQ, estão fortemente associados a piores resultados da função do enxerto após o transplante de fígado. Contudo, ainda não está definido qual é o ponto de corte de MFI para este ser considerado um fator de risco no desenvolvimento dos DSAs. Pacientes com DSAs pré-formados têm um risco aumentado de rejeição hiperaguda e RMA nas primeiras semanas após o procedimento. Além disso, os DSAs estão associados à rejeição crônica, fibrose acelerada e estenoses biliares anastomóticas[67,70].

Os DSAs que ativam o complemento, tendem a ser, os DSA contra alelos DQ com alta MFI (> 13.000). No entanto, a força e a capacidade de ativar o complemento por si só não parecem ser os únicos recursos discriminatórios entre DSAs patogênicos e não patogênicos[72].

A patogenicidade do HLA-DQ pode ser atribuída a vias intracelulares distintas após interações com células T ou aloanticorpos. As diferenças estruturais dos heterodímeros HLA-DQ, com duas cadeias alfa e beta polimórficas, também podem contribuir para sua imunogenicidade. Até quatro heterodímeros (ou mais) podem ser expressos na superfície celular, potencialmente aumentando a probabilidade de alo-reconhecimento e subsequente ativação imunológica. Os anticorpos HLA-DQ são, com frequência, os mais observados após o transplante de órgãos sólidos e estão

associados aos piores desfechos adversos do enxerto em comparação com todos os outros anticorpos HLA. Porém, a explicação biológica para essa observação ainda não é conhecida. Os efeitos clínicos da incompatibilidade do doador-receptor no HLA-DQ, o risco de geração de anticorpos de novo que levam à rejeição e os desfechos inferiores do enxerto indicam imunogenicidade e patogenicidade aumentadas únicas para esse antígeno HLA[81,82].

2.5.4 *Mismatches* de *eplets*

Com o avanço da tecnologia, os exames de compatibilidade se tornaram mais precisos, levando a resultados mais específicos e sensíveis. Um dos métodos utilizados é a análise da compatibilidade epitôpica[83].

Eplets são epítopos funcionais reconhecidos pelos anticorpos anti-HLA, e a determinação do número de *eplets* diferentes entre doador e receptor é denominada *mismatch* de *eplets* (MME), também conhecida como incompatibilidade de *eplets*[62]. Esses *eplets* são formados por 20-25 aminoácidos polimórficos e apresentam regiões estruturais e funcionais, sendo esta última conhecida como o local onde o anticorpo se encaixa, com uma conformação de até três aminoácidos.

Quanto maior o número de *eplets* incompatíveis, maior a incompatibilidade entre doador e receptor. Alguns epítopos são mais imunogênicos que outros. A análise baseada na compatibilidade em epítopos de HLA pode fornecer resultados mais precisos, e a análise molecular de MME pode ser aplicada para se adaptar a imunossupressão de acordo com os riscos individuais[50].

Nas últimas revisões, o HLA Matchmaker tem sido utilizado como um algoritmo baseado em epítopos verificados experimentalmente por anticorpos definidos por aminoácidos polimórficos em configurações chamadas de *eplets*. Esse algoritmo calcula uma pontuação de risco representada pelo número de incompatibilidades de *eplet*[50].

Em um estudo de transplante hepático pediátrico conduzido por Shin et al. (2022), foi observado que quanto maior o valor de MME, menor a curva de sobrevida livre de dnDSA (*de novo donor-specific antibodies*). Além disso, um ponto de corte foi encontrado para determinar o desenvolvimento de dnDSA e definir grupos de risco. Quanto mais *mismatch* de *eplets* em alelo DR e DQ menor é a sobrevida livre de dnDSA, indicando maior risco para rejeição. Seria ideal que esses pacientes tivessem

um nível sérico de tacrolimus mais elevado, mas essa análise no Tx hepático pediátrico precisa ser mais estudada[84].

Outro algoritmo, o PIRCHE II, foi desenvolvido para prever o número de peptídeos que podem ser apresentados pelo HLA de classe II. Esse algoritmo parece ser eficaz como preditor independente de formação de dnDSA, com um valor maior que 9 sendo estatisticamente significativo para a incidência de dnDSA após o transplante renal[83,85].

2.5.5 Tratamento para reduzir DSA

Alguns estudos que desenvolveram protocolos de coleta sérica de anticorpos contra o doador sugerem que todos os pacientes devem ser monitorados para DSA, visando identificar o início de novos DSAs ou a persistência deles após o transplante hepático. Esses estudos destacam que, em todos os cenários, a presença de algum tipo de DSA enfatiza a importância da instituição do tratamento, pois este pode diminuir o risco de complicações e melhorar a sobrevida do enxerto hepático[75].

Existem diversos protocolos para a remoção ou redução de DSA, embora ainda não existam condutas específicas para cada tipo de anticorpo. O tratamento pode incluir altas doses de imunoglobulina com ou sem rituximabe, plasmaferese terapêuticas com ou sem baixas doses de imunoglobulina (IVIG), bortezomibe ou carfilzomibe em combinação com plasmaferese/IVIG, rituximabe ou eculizumabe. No entanto, são necessários mais estudos para esclarecer quais características do DSA podem ser usadas para decidir o regime de tratamento[67].

Por exemplo, o C4d é um marcador de ativação do complemento, enquanto o eculizumabe, um anticorpo monoclonal que se liga ao fator C5, seria ineficaz em um contexto de rejeição C4d-negativa. Esse conhecimento pode fornecer subsídios para a seleção do regime de tratamento mais eficaz de forma oportuna[67].

Goto e col. (2023) descreveram um protocolo de tratamento para pacientes com alto risco e com DSA circulante pós-transplante desde 2015. Pacientes com DSA-MFI ≥ 10.000 e achados histológicos de fibrose do enxerto ≥2 foram tratados com 50 mg/kg de rituximabe e tacrolimus, visando um nível mínimo de ≥5 ng/ml, além de MMF. Pacientes com DSA-MFI ≥ 10.000, mas sem fibrose do enxerto, foram observados durante o curso de tratamentos adicionais. Alguns indivíduos com alto DSA-MFI e baixa fibrose foram tratados com tacrolimus e MMF adicionais por 2,15 ± 1,04 anos, enquanto outros foram observados sem imunossupressores adicionais por 2,58 ± 1,51

anos. Independentemente dos tratamentos adicionais, não foram observadas diferenças significativas nos pacientes com alto DSA-MFI e baixa fibrose[48].

Em um centro de pacientes transplantados de pulmão desde 2013, aqueles que desenvolveram DSA precocemente (eDSA) após o transplante foram submetidos a um protocolo de tratamento baseado em infusões de imunoglobulinas humanas intravenosas enriquecidas com IgA e IgM. Os resultados demonstraram uma boa depuração de eDSA e melhora na sobrevida do enxerto a curto prazo, comparável à sobrevida de pacientes sem eDSA[86].

Para pacientes com doença renal em lista de espera para transplante e altamente sensibilizados (com cPRA > 99%), as abordagens variam entre os países. Contudo, reconhece-se a importância de melhorar a oferta de órgãos para esses pacientes. Nesse sentido, um novo medicamento capaz de converter um *crossmatch* positivo em negativo, permitindo assim a realização do transplante renal entre pares doador-receptor previamente incompatíveis por HLA, está sendo cada vez mais estudado[87].

Em 2001, foi descoberta uma protease denominada IdeS (enzima degradante de imunoglobulina G do Streptococcus pyogenes), que possui uma especificidade única para IgG e é um potente fator de virulência produzido por S. pyogenes. Essa protease remove a região Fc da IgG do hospedeiro, o que essencialmente anula a imunidade humoral, pois a IgG clivada não pode mais ativar o complemento ou mediar citotoxicidade celular dependente de anticorpos. Além disso, a enzima cliva receptores de células B dos linfócitos B circulantes, resultando na inibição das respostas de IgG de células B específicas de antígenos *in vitro*[87].

Essa enzima bacteriana, chamada Imlifidase (Idefirix®, Hansa Biopharma AB), inibe a função efetora mediada por Fc, consequentemente bloqueando a citotoxicidade celular dependente de complemento (CDC) e a citotoxicidade celular dependente de anticorpos (ADCC) horas após a administração[87].

Um estudo de fase I, duplo-cego e randomizado avaliou a segurança e tolerabilidade do IdeS em homens saudáveis, administrando doses únicas crescentes de 0,01mg/kg até 0,24mg/kg. Dos 29 indivíduos participantes, 20 receberam o medicamento, enquanto o restante foi tratado com placebo. Minutos após a administração, o primeiro momento o Ides cliva a molécula de IgG convertendo a IgG plasmática em scIgG (clivagem única de IgG, do inglês *single cleavage IgG*), cujas

funções efetoras foram comprometidas e em um segundo momento se transforma em um produto todo clivado que não pode mediar citotixinas dependentes do complemento nem por células mediadas por anticorpo. O IdeS foi considerado seguro, sem eventos adversos graves ou toxicidade limitante de dose. A remoção completa, rápida, porém temporária, de IgG oferece uma nova e potente oportunidade terapêutica em condições patogênicas mediadas por IgG[88].

A imlifidase difere da plasmaferese ou IVIg em sua velocidade de ação e na extensão em que remove a IgG total do corpo. A dessensibilização com imlifidase já foi utilizada em pacientes receptores de transplante renal, demonstrando eficácia e segurança a curto prazo em receptores previamente incompatíveis[89].

Estudos relataram que o Imlifidase reduziu ou eliminou os anticorpos específicos do doador, permitindo o transplante de órgãos incompatíveis ao HLA em pacientes altamente sensibilizados[87].

Em um estudo conduzido por Lonze e col. (2018), pacientes altamente sensibilizados receberam IdeS como monoterapia para dessensibilização, resultando na conversão de prova cruzada positiva para negativa e permitindo o sucesso do transplante de rim em todos os casos, sem ocorrência de efeitos adversos[73].

Em resumo, a terapia com IdeS desativa rapidamente a IgG de humanos com uma única dose, mantendo seu efeito por várias semanas. Esta abordagem isolada ou combinada com medicamentos que afetam as células B, como bortezomibe ou rituximabe, mostra-se promissora para tratar condições impulsionadas pela IgG. Embora o IdeS possa ser imunogênico e não permitir um tratamento crônico, doses repetidas anuais ou semestrais podem ser viáveis. Quando combinado com outras terapias, como adsorção imune ou plasmaferese, o IdeS pode manter baixos níveis de anticorpos patogênicos por um período prolongado[88].

O Imlifidase cliva as quatro subclasses humanas de IgG, inativando-as por 1 a 2 semanas até que uma nova síntese de IgG seja detectada. No entanto, é crucial entender como prevenir a recidiva do DSA entre os dias 5 e 15 pós-transplante. Além disso, o medicamento está sendo estudado para tratar a rejeição mediada por anticorpos em receptores de transplante renal altamente sensibilizados ao HLA, representando um avanço significativo nesse campo[90].

A avaliação da compatibilidade HLA entre doador e receptor é fundamental para otimizar os resultados pós-transplante. É essencial aumentar a conscientização

e o reconhecimento oportuno da rejeição mediada por anticorpos após o transplante de fígado, incentivando assim futuras pesquisas e o desenvolvimento de estratégias diagnósticas e de tratamento protocoladas.

3. Justificativa

A maioria dos centros de transplante de fígado não tem rotina de avaliar o HLA do doador e do receptor, nem a presença de anticorpos antes ou após o transplante. O procedimento é realizado independentemente dessas informações. No entanto, o conhecimento desses dados pode servir como um importante marcador para a evolução dos pacientes pós-transplante hepático e permitir um melhor manejo personalizado da imunossupressão.

4. Objetivos

4.1 Objetivo Geral

Analisar a compatibilidade HLA do doador e do receptor, bem como suas compatibilidades, a fim de identificar os fatores de risco no desenvolvimento de DSA, rejeição e outros desfechos.

4.2 Objetivos Secundários

- Identificar as características demográficas e cirúrgicas dos pacientes que desenvolveram anticorpos circulantes contra o doador, além da rejeição e fibrose hepática.
- Analisar a presença de C4d nos pacientes que desenvolveram rejeição.
- Determinar a quantidade de MM nos HLA de classe I (A; B) e classe II (DR) correlacionando com o desfecho de rejeição ou fibrose.
- Correlacionar o valor de PRA pré e pós-transplante com os desfechos de rejeição e fibrose.
- Identificar o valor de MFI que representa um fator de risco para o desenvolvimento de rejeição ou fibrose do enxerto.

5. Hipóteses

Hipótese alternativa: a presença de anticorpos contra o doador está associada à disfunção do enxerto hepático.

Hipótese nula: a presença de anticorpos contra o doador não está relacionada à lesão do enxerto hepático.

6 Método Ampliado

6.1 Delineamento do estudo

Coorte prospectiva de transplante hepático pediátrico ABO compatível.

6.2 Local de realização do estudo

O estudo foi realizado no ambulatório de imunologia do transplante no complexo Santa Casa de Misericórdia de Porto Alegre/ RS.

6.3 População do estudo

Foram incluídos pacientes com até 18 anos de idade submetidos a transplante de fígado no Serviço de Transplante Hepático Infantil do Hospital da Criança Santo Antônio no período de dezembro de 2013 a dezembro de 2023. A coleta de dados de HLA foi realizada em todos os pacientes e seus doadores. Foram excluídos os pacientes que faleceram e aqueles que não tiveram tempo hábil para a coleta de anticorpos após o transplante.

6.4 Imunossupressão

Entre dezembro de 2013 e 2017, os pacientes transplantados receberam imunossupressão com inibidores da calcineurina e corticoides. De 2017 a 2019, além desses medicamentos, pacientes com insuficiência hepática e menores de 18 meses também passaram a receber basiliximab na imunossupressão de indução. A partir de 2020, todos os pacientes passaram a receber uma combinação de basiliximab, prednisolona e inibidores da calcineurina na imunossupressão de indução. Durante o

acompanhamento ambulatorial, a imunossupressão com tacrolimus seguiu um protocolo de redução gradual dos níveis sanguíneos: de 10-12 µg/L no primeiro mês, 8-10 µg/L até o terceiro mês e, em seguida, 6-8 µg/L até o final do primeiro ano. A dose foi reduzida para valores menor de 6-8 µg/L em pacientes que não apresentaram intercorrências de rejeição ou aqueles que apresentaram infecção persistente por CMV ou EBV.

Com relação aos esteroides, a dose é de 10mg/kg na cirurgia do transplante, no momento da reperfusão hepática, seguida por 2mg/kg/dia do 1° ao 6° dia pós-operatório. Do 7° ao 13° dia, a dose é reduzida para 1mg/kg/dia, momento em que ocorre a troca para prednisolona por via oral. A dose é então ajustada para 0,75mg/kg/dia do 14° ao 20° dia; 0,5mg/kg/dia do 21° ao 28° dia e 0,25mg/kg/dia nos 2º e 3º meses pós-transplante, sendo gradualmente reduzida para dias intercalados a partir do 6º mês.

O basiliximab é administrado na dose de 10mg/dose em pacientes com menos de 30kg e 20mg/dose em pacientes com mais de 30kg. A primeira dose é administrada 6 horas após a reperfusão hepática e a segunda dose no quarto dia pós-operatório.

Em casos de reações alérgicas ou outros efeitos adversos aos imunossupressores, a ciclosporina pode ser utilizada como alternativa. Já para pacientes que desenvolvem doença linfoproliferativa pós-transplante, o sirolimo é empregado como imunossupressor.

No caso de rejeição com disfunção hepática confirmada por biópsia, é realizado um regime de pulsoterapia com esteroides por 5 dias, juntamente com terapia de resgate, mantendo os níveis séricos de tacrolimus em torno de 10-15 µg/L. Frequentemente, um segundo imunossupressor, como micofenolato, é associado. Em casos de falta de resposta ou recidiva, a timoglobulina pode ser utilizada.

6.5 Critérios de inclusão

Foram selecionados pacientes submetidos a transplante hepático de fígado ABO compatível com idade inferior a dezoito anos, no intervalo de dezembro de 2013 a dezembro de 2023. A inclusão foi restrita aos pacientes que sobreviveram por mais de 30 dias após o transplante e que tiveram pelo menos uma coleta de DSA.

6.6 Critérios de exclusão

Foram excluídos do estudo os seguintes grupos:

- Pacientes que faleceram após o transplante.
- Pacientes que não puderam ser submetidos à coleta do painel de reatividade, DAS, após o transplante dentro do prazo estabelecido.
- Pacientes cujos responsáveis legais não consentiram com a coleta de sangue para a pesquisa de DSA após um ano do transplante.

6.7 Considerações éticas

O estudo recebeu aprovação do Comitê de Ética e Pesquisa da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) e do Complexo Hospitalar da Irmandade da Santa Casa de Misericórdia de Porto Alegre (ISCMPA), sob os números 3805918 e 3938979, respectivamente. Além disso, foi registrado na plataforma Registro Brasileiro de Ensaios Clínicos (ReBec) com o número RBR-3gtcvjU111112367585.

Todos os pacientes, ao assinarem o Termo de Consentimento Livre e Esclarecido (TCLE) para o transplante hepático, também consentiram para a realização das coletas. Tanto os doadores quanto os receptores concordaram em participar da coleta prospectiva de amostras no momento do transplante e após o transplante para os receptores. Eles foram devidamente informados de que as amostras e dados coletados seriam utilizados para fins de pesquisa (Anexo 1).

6.8 Variáveis estudadas

As variáveis estudadas incluíram a tipagem de HLA do receptor e do doador, bem como a verificação de compatibilidade e a determinação de anticorpos anti-HLA antes e após o transplante.

A tipagem HLA do doador e do receptor, e a coleta de reatividade contra o painel de classe I e classe II foram realizadas através de uma triagem sistemática de coleta de soro antes do transplante.

Posteriormente ao transplante, a coleta do painel DSA foi realizada em momentos diferentes conforme os eventos clínicos de cada paciente no período pós-transplante.

Essas coletas foram feitas no laboratório central do Hospital da Criança Santo Antônio, no momento da coleta de rotina de cada paciente, supervisionadas pela enfermeira responsável pelo ambulatório.

Os testes de anticorpos de antígenos leucocitários humanos (HLA) classe I e classe II foram realizados com amostra de sangue coletado em tubo sem anticoagulante. Para identificar DSA foi usada a tecnologia de citometria com utilização do Luminex ® pela metodologia *Single Antigen Beads (SAB*) (One *Lambda*), considerando intensidade média de fluorescência > 1000 como positivo para anticorpos anti-HLA.

Os DSA foram classificados como pré-formados se estivessem presentes antes do transplante, dnDSA (*de novo*) se tivessem se desenvolvido após o transplante e persistentes quando os DSA pré-formados permanecessem após o transplante. Para pacientes com mais de um DSA, o critério considerado foi o DSA com o valor de MFI mais elevado, ou então, foi calculado o valor acumulado de cada classe de DSA.

Em relação a rejeição do enxerto: todas as incidências de rejeição foram confirmadas por meio de biópsias hepáticas, seguindo os critérios estabelecidos pela classificação de Banff (2016). A decisão de realizar as biópsias foi tomada com base em alterações laboratoriais que indicavam disfunção hepática, como elevações nas aminotransferases, após a exclusão de causas infecciosas durante o acompanhamento ambulatorial, como infecção por citomegalovírus. Todas as biópsias foram interpretadas pela mesma patologista, e os valores normais de aminotransferases foram baseados nas referências do nosso laboratório: Aspartato aminotransferase sérica (AST) com valor de referência (VR) abaixo de 79 U/L; alanina aminotransferase (ALT) com VR de 3-35 U/L; bilirrubina total com VR de 0,2-1 mg/dL; e glutamiltranspeptidase (GGT) com VR abaixo de 38 U/L.

Fibrose do enxerto: ao analisar a presença de disfunção do enxerto em biópsias hepáticas, também se avaliou qualquer grau de fibrose presente. Consideramos qualquer tipo de fibrose presente no enxerto. Utilizamos o sistema de pontuação histológica METAVIR para o estadiamento e os critérios modificados de Dixon para avaliar a fibrose sinusoidal ao redor das veias centrais.

C4d na biópsia hepática: em todos os pacientes que apresentaram rejeição, a coloração para C4d foi revisada. A coloração imuno-histoquímica para C4d dos blocos de tecido incluídos em parafina foi realizada utilizando anticorpos policlonais humanos

anti-C4d (BI-RC4D; Biomédica, Viena, Áustria). A presença de coloração C4d nas células endoteliais microvasculares, variando de linear a granular, foi diagnosticada como um resultado positivo.

6.9 Análise de dados

As variáveis quantitativas foram descritas por média e desvio padrão e as categóricas por frequências absolutas e relativas.

A comparação de médias foi realizada pelo teste t-*student*.

A comparação de proporções foi realizada pelos testes qui-quadrado de Pearson ou exato de Fisher.

O nível de significância adotado foi de 5% ($p<0,05$) e as análises foram realizadas no programa *Statistical Package for the Social Sciences* (SPSS), versão 21.0.

6.10 Limitações do trabalho

Nem todos os pacientes transplantados tiveram amostras de DSA coletadas antes do transplante.

A medição do painel de DSA após o transplante não foi realizada em tempos pré-estabelecidos em todos os pacientes. Da mesma forma, as biópsias hepáticas não foram conduzidas seguindo um protocolo padronizado; em vez disso, eram realizadas somente quando havia suspeita clínica de rejeição. Essa abordagem pode levar a casos em que os enxertos já apresentam algum grau de fibrose, mesmo na ausência de alterações laboratoriais persistentes, que poderiam não ser diagnosticadas devido à falta de biópsias.

A revisão de C4d nas biópsias hepáticas foi realizada apenas nos pacientes que apresentaram rejeição.

Não foi possível analisar os MM de HLA DQ, apesar de serem os mais associados à formação de dnDSA e, consequentemente, a um maior risco de rejeição.

7. Conclusão

Ao analisar a compatibilidade HLA entre o doador e receptor, foi observado um risco aumentado de trombose da artéria hepática em pacientes com incompatibilidade

HLA no locus A. Outra característica foi que doadores mais jovens e um tempo menor de isquemia fria apresentaram maior propensão ao desenvolvimento de anticorpos anti-HLA "*de novo"* DSA (dnDSA).

O desenvolvimento de rejeição foi mais comumente identificado em pacientes que tinham doadores mais jovens, doador falecido, doadores não aparentados e a presença de dnDSA em qualquer momento.

No grupo de pacientes que desenvolveram fibrose e rejeição, foi documentado uma relação com dnDSA anti-HLA-DQ.

Não foi encontrado uma associação significativa entre a presença de C4d nos desfechos avaliados. Da mesma forma, não foi identificado uma maior prevalência de rejeição ou fibrose em pacientes com incompatibilidade HLA A-B ou DR.

Embora tenha ocorrido uma tendência de maior incidência de fibrose e rejeição em pacientes com valores mais elevados de PRA, principalmente de classe II, essa associação não alcançou significância estatística.

Não foi possível estabelecer um ponto de corte no valor de MFI de classe II para prever o desenvolvimento de fibrose ou rejeição, nossos resultados indicam uma relação estatisticamente significativa entre valores mais elevados de MFI e maior risco de desenvolver esses desfechos.

8. Considerações finais

Este estudo demonstrou a importância da detecção de qualquer DSA, especialmente os anti-HLA-DQ em pacientes pediátricos transplantados de fígado. Sua presença pode orientar um melhor manejo no uso dos imunossupressores, podendo assim evitar uma disfunção do enxerto. Pode também orientar para uma melhor escolha de um doador vivo mais compatível e de menor risco.

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9. Artigo 1

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Retrospective Cohort Study

Human leukocyte antigen compatibility and incidence of donor-specific antibodies in pediatric liver transplant recipients

Melere MU et al. HLA compatibility and DSA in pediatric liver transplant recipients

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Abstract

BACKGROUND

Antibody-mediated rejection following liver transplantation (LT) has been increasingly recognized, particularly with respect to the emergence of de novo donor-specific antibodies (DSA) and their impact on graft longevity. While substantial evidence for adult populations exists, research focusing on pediatric LT outcomes remains limited.

AIM

To investigate the prevalence of human leukocyte antigen (HLA) mismatches and DSA and to evaluate their association with rejection episodes after pediatric LT.

METHODS

A cohort of pediatric LT recipients underwent HLA testing at Santa Casa de Porto Alegre, Brazil, from December 2013 to December 2023. Only patients who survived for >30 days after LT with at least one DSA analysis were included. DSA classes I and II and cross-matches were analyzed. The presence of de novo DSA (dnDSA) was evaluated at least 3 months after LT using the Luminex® single antigen bead method, with a positive reaction threshold set at 1000 MFI. Rejection episodes were confirmed by liver biopsy.

RESULTS

Overall, 67 transplanted children were analyzed; 61 received grafts from living donors, 85% of whom were related to recipients. Pre-transplant DSA (class I or II) was detected in 28.3% of patients, and dnDSA was detected in 48.4%. The median time to DSA detection after LT was 19.7 (interquartile range [IQR]: 4.3–35.6) months. Biopsy-proven rejection occurred in 13 patients at follow-up, with C4d positivity observed in 5/13 liver biopsies. The median time to rejection was 7.8 (IQR: 5.7–12.8) months. The presence of dnDSA was significantly associated with rejection (36% vs. 3%, P<0.001). The rejection-free survival rates at 12 and 24 months were 76% versus 100% and 58% versus 95% for patients with dnDSA anti-DQ versus those without, respectively.

CONCLUSION

Our findings highlight the importance of incorporating DSA assessment into both pre- and post-transplantation protocols for pediatric LT recipients. Future implications may include immunosuppression minimization strategies based on this analysis in pediatric LT recipients.

Key words: Human leukocyte antigens; Donor-specific antibodies; Liver transplantation; Pediatric; Rejection

Core tip: HLA and DSA assessment is becoming of crucial importance in pediatric liver transplantation. This cohort study demonstrates the relation of DSAs with rejection episodes after liver transplantation.

INTRODUCTION

The role of human leukocyte antigens (HLA) in determining the outcomes of solid organ transplantation has been the subject of extensive debate [1]. In the last decade, antibody-mediated rejection, detection of circulating anti-HLA donor-specific antibodies (DSA), and their influence on the outcomes of pediatric liver transplantation (LT) have been extensively studied [2–6]. Few single-center studies have been conducted, and their impact on long-term graft and recipient survival and on immunosuppression protocols has been surrounded with controversy [4,5,7,8].

Rejection remains one of the most common complications of LT [6]; nonetheless, our understanding of antibody-mediated rejection remains limited. Some centers have adopted protocols for liver biopsies in an effort to detect early signs of rejection despite normal liver function test results. In antibody-mediated rejection, B lymphocytes and C4d deposits dominate in the periportal vessels. However, C4d staining is not routinely performed in liver biopsy, and it is still unclear how this detection could affect patient care [9,10].

It is widely believed that children exhibit a more vigorous immunological response than adults. A meta-analysis showed a higher prevalence of circulating DSAs in pediatric LT recipients than in adults [8]. Furthermore, a previous study reported that the presence of circulating DSAs was linked to rejection and long-term liver fibrosis development [5]. HLA typing and circulating DSA analysis have the potential to facilitate early rejection diagnosis, guide personalized treatment approaches, and aid in the selection of more compatible donors. Therefore, the current study aimed to investigate the prevalence of class I and class II HLA mismatches and DSA and to evaluate their association with rejection episodes after pediatric LT.

MATERIALS AND METHODS

A cohort of pediatric LT recipients aged <18 years underwent pre- and post-LT HLA testing and DSA detection at Santa Casa de Porto Alegre, Brazil, from December 2013 to December 2023. Only patients who survived for >30 days after LT with at least one DSA analysis were included. DSA classes I (A/B/C) and II (DQ/DR) and cross-matches were analyzed before and after LT. The presence of de novo DSA (dnDSA) was evaluated at least 3 months after LT using the Luminex® single antigen bead method, with a positive reaction threshold set at 1000 MFI. Rejection episodes were confirmed by liver biopsy.

HLA collection and analysis

HLA typing of both the donor and recipient, collection of reactivity against the panel of class I and class II antigens, and cross-match testing were performed through systematic serum collection screening prior to LT. After transplantation, DSAs were collected at various time points according to clinical events in each patient, starting from 3 months after LT. Such collection was performed during routine patient visits at the central laboratory of the Hospital da Criança Santo Antônio (Porto Alegre, Brazil) under the supervision of an outpatient clinic nurse. Donor samples were collected at the immunology laboratory under the supervision of the same technician.

HLA class I and II antibody tests were performed on sera. The presence of recipient HLA antibodies was detected using the Luminex® Mixed single antigen bead technology by One Lambda, with MFI >1000 being considered positive for anti-HLA antibodies.

DSAs were classified as "preformed" if they were present before LT, "dnDSAs" if they developed after LT, or "persistent" if preformed DSAs remained after LT. For patients with more than one DSA, the highest MFI value was used (or the cumulative value for each class was calculated). All anti-HLA antibody assays were conducted at the Transplant Immunology Laboratory located at Hospital Dom Vicente Scherer, within the Santa Casa de Misericórdia de Porto Alegre Complex, RS, Brazil.

Liver biopsy

All rejections were confirmed by liver biopsy following the Banff classification criteria. The decision to perform biopsy was made based on laboratory abnormalities indicative of hepatic dysfunction such as elevated aminotransferase levels. Normal aminotransferase values were based on our laboratory's reference values, as follows: serum aspartate aminotransferase with a reference value below 79 U/L, alanine aminotransferase with a reference value below 35 U/L, total bilirubin with a reference value of 0.2–1 mg/dL, and glutamyl transpeptidase with a reference value below 38 U/L. Infectious causes such as cytomegalovirus infection during outpatient follow-up were excluded, and all biopsies were interpreted by the same pathologist. C4d staining was performed retrospectively after confirmation of rejection; thus, this was only searched for patients with biopsy-proven rejection.

Graft fibrosis

Fibrosis in any grade within the liver graft was considered indicative of positive fibrosis status.

Statistical analysis

Quantitative variables were expressed as means and standard deviations, whereas categorical variables were presented as absolute and relative frequencies. Mean comparisons were conducted using Student's t-test. Proportional comparisons were performed using Pearson's chi-square test or Fisher's exact test. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA), with the significance level set at 5% ($P<0.05$). The study was reviewed by our expert Biostatistic Alvaro Rosler.

RESULTS

During the study period, 91 LTs were performed at the Santa Casa de Porto Alegre Hospital Complex; of these, 73 were from living donors, whereas three were re-transplants. Fifteen patients died at <30 days after LT, and six patients were excluded because they did not reach the minimum follow-up. Finally, a total of 67 patients were analyzed in this study (Figure 1).

With respect to the general characteristics of the analyzed patients, there was a nearly equal distribution of sex, with 37 (55%) being male. Furthermore, the median age at transplantation was 11.9 (IQR: 7.7–24.6) months, and the median weight was 7.4 (IQR: 6.4–11.5) kg. LT from living donors was the most prevalent modality, accounting for 90% (n=60). Biliary atresia was the primary etiology of disease, leading to an indication for LT in 38 patients (57%) (Table 1).

As for the donors, 40 (59.7%) were male, with fathers, mothers, unrelated persons, and other grades of relationships being the donors in 30 (45%), 17 (25%), 10 (15%), and 10 (15%) cases, respectively. The mean donor age was 30 ± 11.5 years.

In 60 patients, HLA typing was performed prior to LT: DSA was directed against HLA class I in 9 (15%) patients, HLA class II in 11 (18.3%) patients (Figure 2), and both classes I and II in 3 (5%) patients In 66 patients, HLA typing was performed after LT at a median of 19.7 (IQR: 4.3–35.6) months: DSA was directed against HLA class I in eight (12%) patients, HLA class II in 28 (43%) patients (Figure 2), and both classes I and II in four (6%) patients.

Overall, 33 (50%) patients had dnDSA. The predominant distribution was at the DQ locus (dnDSA/DQ), which was present in 26 (39%) patients (Figure 3). Figure 4 illustrates DQ and DR locus distributions. A total of 12 (17.9%) patients exhibited biopsy-proven rejection; of these, five (41.6%) tested positive for C4d.

The rejection-free survival rates after dnDSA/DQ detection were 76% at 12 months and 58% at 24 months. In comparison, for the group that did not develop dnDSA/DQ, the rejection-free survival rates were 100% at 12 months and 95% at 24 months (P<0.001) (Figure 5).

The persistence of positive antibody levels for DAS/DQ after LT was associated with a graft rejection rate of 60%, indicating that this population had a more unfavorable outcome than others (Figure 6).

Table 2 shows the HLA mismatch value (MM) analysis in patients who developed rejection versus those who did not. Higher MM values were observed in the rejection group, particularly at locus A. However, these differences were not statistically significant.

DISCUSSION

Donor selection for LT has traditionally prioritized clinical urgency, ABO compatibility, and donor size, with HLA compatibility and the presence of DSAs being often overlooked. Nonetheless, recent studies have highlighted the significance of DSA in liver graft outcomes. Considering that DSA is a major contributor to allograft dysfunction and loss, monitoring patients positive for antibodies is crucial for improved post-transplant rejection and fibrosis-free survival (11, 12).

In the present study, the prevalence of preformed DSAs was low (15% for class I and 18% for class II). In contrast, previous studies found dnDSAs in 50% of recipients [13–16]. Most of dnDSAs were directed against HLA class II, with the predominance of dnDSA/DQ present in 39% of patients evaluated after transplantation. The predominance of the DQ locus has been described in previous studies [16]

However, it is difficult to determine when dnDSA could be detected after LT. Most studies on pediatric recipients are small series, collected without a specific protocol, and no evaluation of preformed DSA was conducted. There are also clues from renal transplantation that the prevalence of dnDSAs tends to increase over time [17]. Variations from 50 months to 10 years following LT have been described in studies on children [18,19]

The prevalence of DSA has been consistently reported to be higher in children [20]. Several hypotheses in the literature explain the higher prevalence of DSA in pediatric patients. These include a heightened immune response in the developing immune system, susceptibility to viral illnesses inducing non-specific HLA antibodies, and frequent blood transfusions leading to sensitization [1].

The occurrence of dnDSA in LT recipients was lower than that in kidney transplant recipients, possibly because of the ability of the liver to absorb anti-HLA antibodies. Although this study

did not analyze the immunosuppression levels, it is hypothesized that inadequate immunosuppression results in the emergence of dnDSA and T-cell-mediated rejection. Given the significant association between CD4 + T cells and B cell immunity, inadequate immunosuppression may result in insufficient blockade of CD4 + T cell helper activity against B cells, thereby facilitating DSA development [13].

The development of dnDSA could serve as a biomarker for the risk of T-cell-mediated rejection, potentially informing treatment decisions. dnDSA directed towards HLA class II was previously related to the development of rejection [4,20]. In the present study, the DQ locus played a major role in the development of rejection and was associated with lower rejection-free survival. In the group with persistent DSA/DQ, 60% developed rejection. The DQ locus antibody has been described as a risk factor for decreased survival in solid organ transplant recipients. [17]. Patient survival was not analyzed in our study because all mortalities occurred at <30 days after LT and these patients were not included in the study.

Recent studies have shown that dnDSAs are associated with rejection, fibrosis, inflammation, and shorter survival after LT. Clinical factors, such as immunosuppression, infection, degree of ischemia-reperfusion injury, and level of human leukocyte antigen (HLA) expression, contribute to the development, affinity for DSA, and persistence of the antibody after transplantation, leading to graft dysfunction [13].

Despite these insights, this study had several limitations. Pretransplant DSA samples were not collected from all patients, and posttransplant DSA measurements were not uniform. Liver biopsies were performed based on clinical suspicion, potentially leading to undiagnosed fibrosis. The C4d review was limited to patients with rejection, and HLA anti-DQ MM analysis was not feasible.

CONCLUSION

The presence of DSA significantly increases the risk of rejection, with the highest incidence observed in patients with persistent DSA/DQ. The predominance of class II DSA targeting the DQ locus underscores its significance. DSA detection can guide personalized immunosuppressive therapy and influence living-donor selection, highlighting the need for continuous surveillance and personalized management to optimize LT outcomes.

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Footnotes

Institutional review board statement: This study was approved by the Ethics and Research Committee of the Federal University of Health Sciences of Porto Alegre (UFCSPA) and the Santa Casa de Misericórdia de Porto Alegre Complex (ISCMPA) (approval numbers 3805918 and 3938979, respectively). This study was also registered at the Brazilian Clinical Trials Registry (ReBec) under number RBR-3gtcvjU111112367585.

Informed consent statement: Upon signing the informed consent form for LT, the patients also provided signed authorization for sample collection.

Conflict-of-interest statement: All the Authors have no conflict of interest related to the manuscript.

Data sharing statement: The original anonymous dataset is available on request from the corresponding author at flavia.feier@gmail.com

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

TABLES

Table 1. Patients' characteristics

Number of patients included	67
Gender, male, n (%)	37 (55)
Age at transplant (months), median (IQR)	11.9 (7.7 to 24.6)
Weight at transplant (kg), median (IQR)	7.4 (6.4 to 11.5)
Z-score BMI, median (IQR)	- 0.4 (-1.3 to – 1.0)
PELD score, median (IQR)	14 (6 to 22)
GRWR (%), median (IQR)	3.2 (2.5 to 4.0)
Transplant modality, n (%)	
Living Donor	60 (90)
Deceased Donor	7 (10)
Baseline disease, n (%)	
Biliary Atresia	38 (57)
Acute Liver Failure	3(4,4)
Alagille Syndrome	6(9)
Primary Liver Tumor	4 (6)
Other	16(24)

IQR, interquartile range; BMI, body mass index; GRWR, graft-to-recipient weight ratio.

Table 2. Analysis of HLA A, B, and DR Mismatch (MM) in the groups with and without rejection.

	With Rejection	*Without Rejection*	*p*
	N=12	N=53	
HLA A MM, n (%)			0.08
0	1(8.3)	3(5.7)	
1	7(58.3)	45(84.9)	
2	4(33.3)	5 (9.4)	
HLA B MM, n (%)			0.12
0	0	5 (9.4)	
1	7 (58.3)	39 (73.6)	
2	5 (41.7)	9 (17)	
HLA DR MM, n (%)			0.9
0	1 (8.3)	4 (7.5)	
1	9 (75)	41 (77.4)	
2	2 (16.7)	8 (15.1)	
CLASS I MM, n (%)			
1	1 (8.3)	8 (15.1)	0.16
2	6 (50)	37 (69.8)	
3	1(8.3)	3 (5.7)	
4	4 (33.3)	5 (9.4)	
TOTAL MM, n (%)			0.52
2	2 (16.7)	11 (20.8)	
3	5 (41.7)	32 (60.4)	
4	1 (8.3)	3 (5.7)	
5	2 (16.7)	3 (5.7)	
6	2 (16.7)	4 (7.5)	

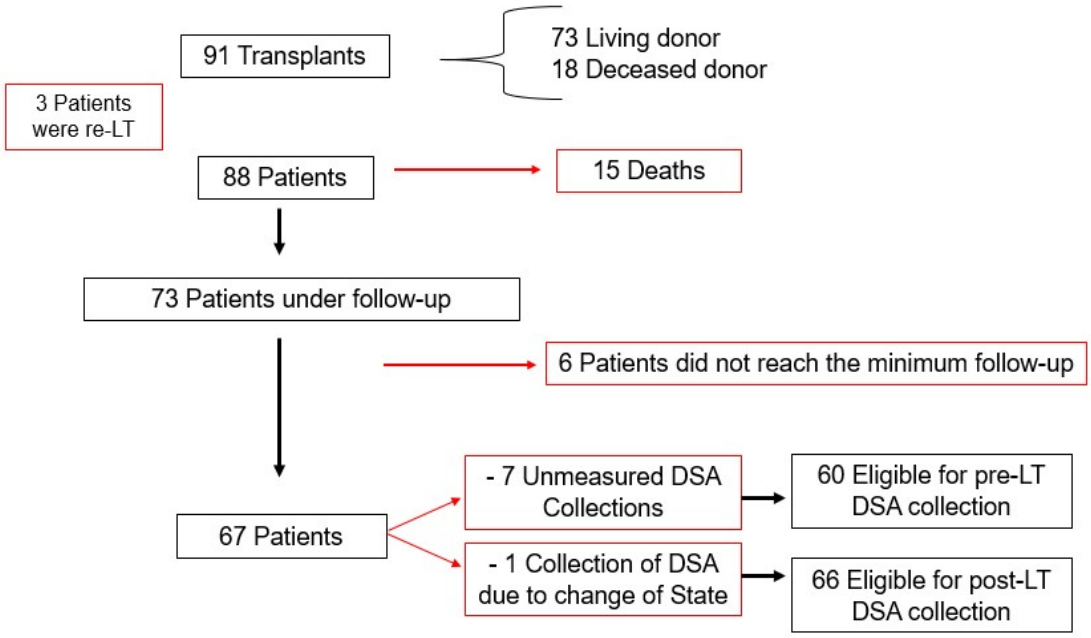

Figure 1. Study design and patient inclusion and exclusion criteria.

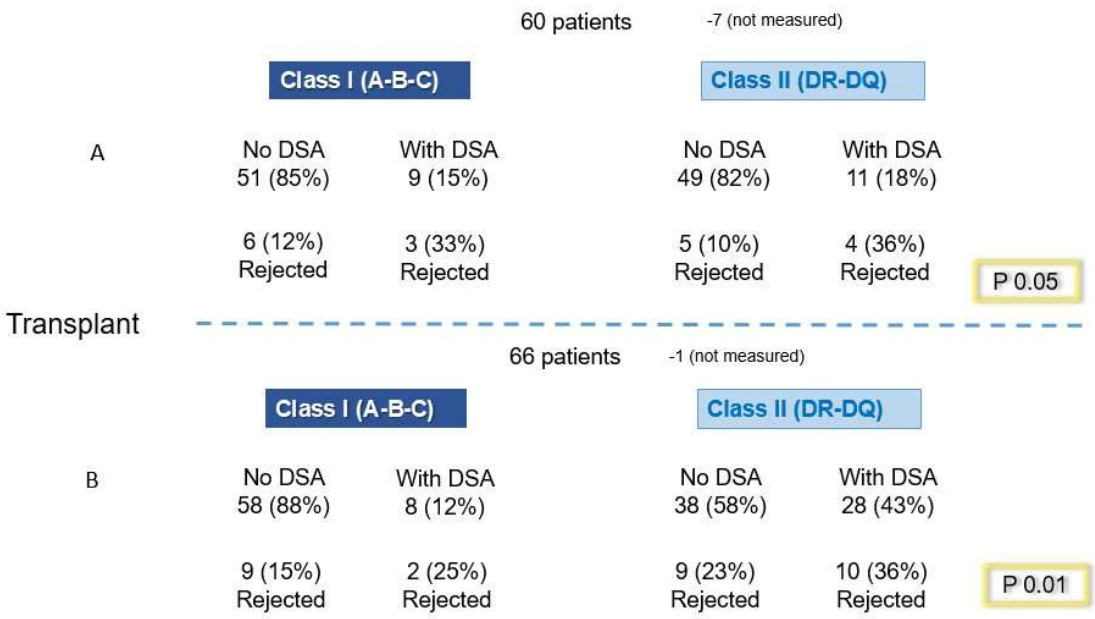

Figure 2. Prevalence of class I and II DSA pre-transplant (A) and post-transplant (B) correlated with the prevalence of rejection.

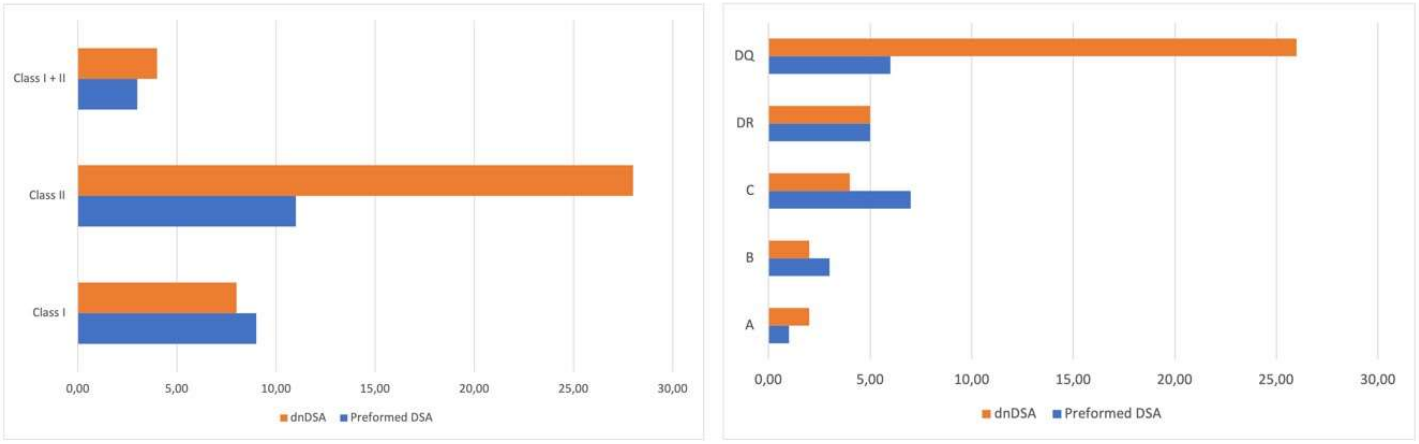

Figure 3. HLA typing in patients with preformed and dnDSA. X-axis represents number of patients.

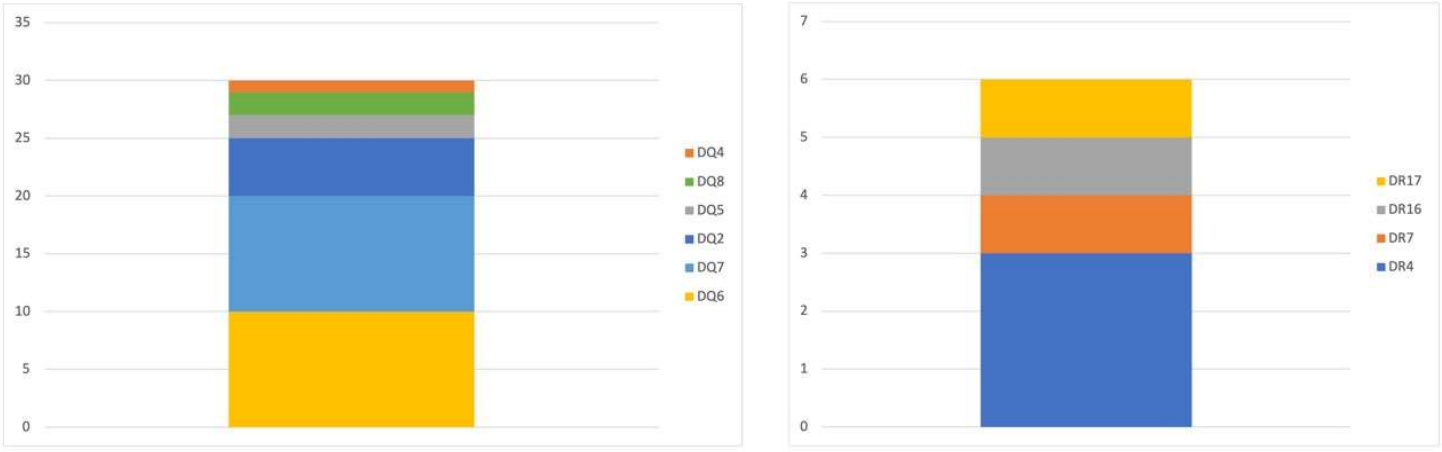

Figure 4. Pre and post LT DSA frequencies – (A) locus distribution (B) DQ and DR alleles.

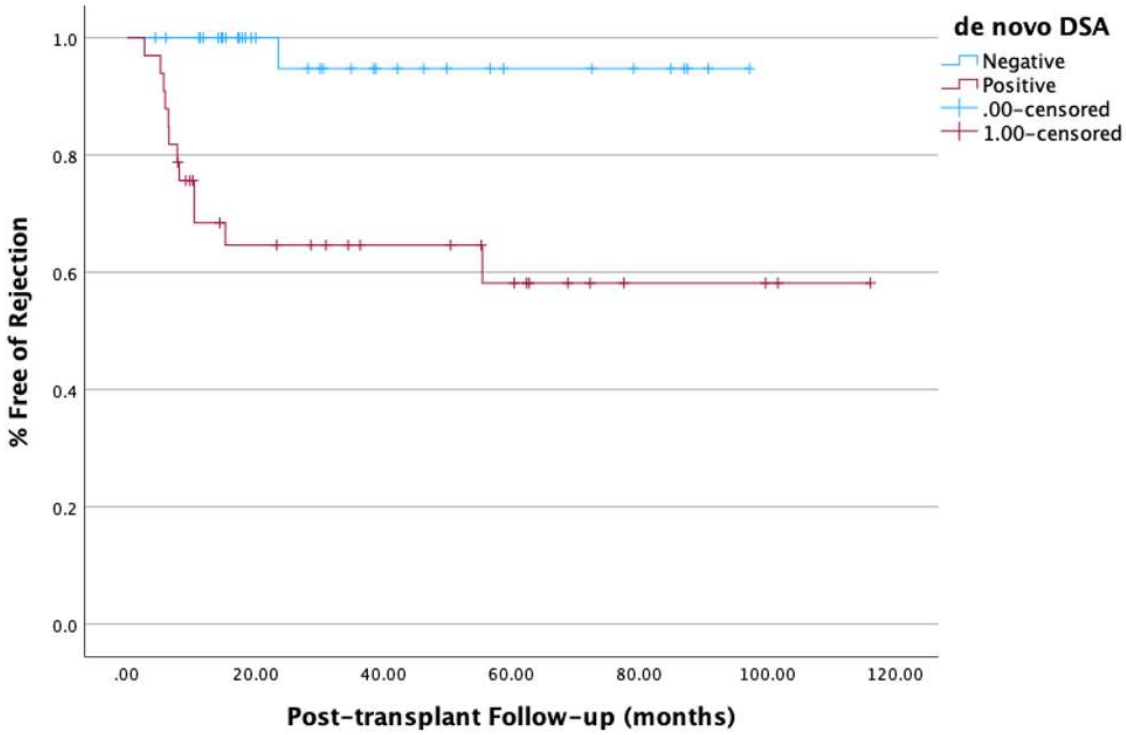

Figure 5. Rejection-free survival according to dnDSA.

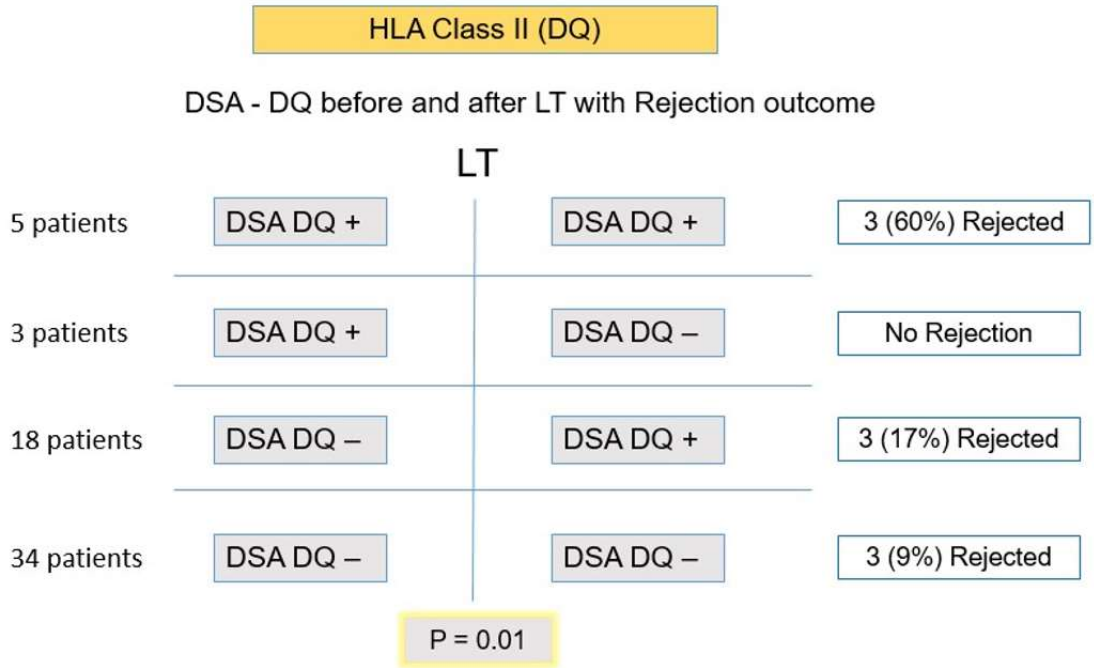

Figure 6. Pre-transplant DSA, DSA persistence, dnDSA-DQ, and graft rejection.

10. Artigo 2

Impact of Donor Specific Antibodies on the Outcomes of Pediatric Liver Transplant Recipients

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List of Abbreviations:

alanine aminotransferase, ALT

Antibody-mediated rejection, AMR

aspartate aminotransferase, AST

de novo DSA, dnDSA

donor-specific antibodies, DSAs

glutamyl transpeptidase, GGT

hepatic artery thrombosis, HAT

human leukocyte antigens, HLA

liver transplantation, LT

mean fluorescence intensity, MFI

mismatches, MM

Model for End-Stage Liver Disease, MELD

Abstract

The role of human leukocyte antigens (HLA) in solid organ transplantation, particularly liver transplantation, is extensively debated. Donor-specific antibody (DSA) detection is associated with an increased risk of graft loss, making it crucial to identify modifiable factors to prevent rejection. In this study, we sought to identify DSAs in pediatric liver transplant recipients and assess their correlation with rejection and post-transplant outcomes. Pediatric liver transplant recipients aged <18 years who underwent transplantation between December 2013 and December 2023 were included. Only patients who survived for >30 days post-transplantation were considered. DSA analysis included HLA class I (A/B/C) and class II (DQ/DR) using Luminex®-Single Antigen Beads (SAB), with a mean fluorescence intensity (MFI) >1000 indicating positivity. Rejection episodes were confirmed by liver biopsy. The prevalence of DSAs class I and II was 28% pre-transplantation and 43% post-transplantation de novo DSA (dnDSA). Detection of dnDSA, particularly at the DQ locus, correlated with graft rejection. HLA-A incompatibility correlated with increased risk of hepatic artery thrombosis. DSA detection plays a significant role in pediatric liver transplantation. Identifying high-risk patients allows for targeted interventions such as increased immunosuppression, intensified anticoagulation, or seeking a more compatible donor pre-transplant. Although further studies are needed, routine DSA testing in pediatric liver transplant recipients is strongly recommended.

Key words: Human leukocyte antigen; donor-specific antibodies; pediatric liver transplantation; rejection; hepatic artery thrombosis.

Introduction

Liver transplantation is currently acknowledged as a safe intervention for addressing various medical conditions, including liver failure, hepatic malignancies, metabolic disorders, and other pediatric conditions. With continuous advancements in the field, culminating in current survival rates exceeding 90% among pediatric liver transplant recipients[1], emphasis has shifted towards mitigating complications and enhancing post-transplantation quality of life.

Rejection has emerged as a prominent complication following liver transplantation (LT), occurring at rates ranging from 20% to 50%.[2–4] Notably, children exhibit a heightened immunological response compared with adults, resulting in a heightened propensity for the development of donor-specific antibodies (DSAs).[5,6] The emergence of post-transplantation donor-specific antibodies (dnDSA) has been correlated with rejection episodes and diminished graft longevity. However, routine screening for DSAs, both pre- and post-transplantation, remains inconsistent across many liver transplant centers.[7]

Antibody-mediated rejection (AMR) is closely associated with the presence of DSAs, and can manifest acutely or chronically, potentially resulting in graft dysfunction.[8] Initially, it was presumed that AMR plays a negligible role in LT because of several inherent characteristics of the liver, including its dual blood supply, substantial parenchymal mass, low expression of Class II antigens, presence of Kupffer and endothelial cells, and remarkable regenerative capacity.[9] However, it is now recognized that, despite these characteristics, AMR can indeed inflict damage upon the liver graft.[10,11]

Chronic AMR can arise in 8%–15% of liver transplant recipients who either maintain or develop DSAs. These antibodies are considered biomarkers for predicting the risk of liver allograft loss. However, there is currently no consensus on the precise definition of pathogenicity.[12]

C4d is a biomarker used to identify sites of tissue injury due to antibody binding, linking it to the presence of anti-donor antibodies. Since 2003, C4d staining was incorporated into the Banff criteria for assessing AMR in renal transplant recipients and subsequently extended to liver evaluation.[9] C4d deposition primarily occurs in the endothelium of portal veins and capillary sinusoids during the acute phase, whereas involvement of the central vein endothelium may occur in chronic phases.[13] Nonetheless, certain studies have posited that anti-C4d antibodies

may arise from ischemia/reperfusion injury rather than serving as specific markers for AMR.[14,15]

Adverse outcomes of living-donor LT have previously been associated with human leukocyte antigens (HLA) incompatibility.[7] Therefore, seeking optimal DSA compatibility where feasible represents a prudent strategy for mitigating graft immunogenicity.[16]

The objective of this study was to ascertain the prevalence of DSAs among pediatric liver transplant recipients at our institution and to evaluate the correlation between DSA presence and outcomes, such as rejection and other parameters pertinent to LT.

Methods

Patients

All pediatric patients (<18 years-old) who underwent LT at Santa Casa de Porto Alegre Hospital, Brazil between December 2013 and December 2023 were enrolled in this study. The eligibility for analysis was restricted to patients with at least one DSA collection. Individuals who did not survive beyond 30 days post-transplantation were excluded from the analysis, as were those for whom DSA analysis was not feasible. This was a prospective cohort study. The major outcomes examined included liver transplant vascular complications, rejection episodes, and graft fibrosis.

Liver biopsy

All suspected rejection episodes were confirmed by liver biopsy and subsequent classification according to the Banff criteria. Rejection episodes were identified based on alterations in liver function tests, including elevated levels of aspartate aminotransferase (AST) (>79U/L), alanine aminotransferase (ALT) (>35U/L), total bilirubin (>1 mg/dL), and glutamyl transpeptidase (GGT) (>38 U/L). Prior to the liver biopsy, any infections potentially causing these alterations were excluded. Consistency in biopsy analysis was ensured by assigning the same pathologist to examine all biopsies. Fibrosis of any grade within the liver graft was considered indicative of positive fibrosis status.

HLA typing

HLA typing was conducted for both the donor and recipient at the Immunology Laboratory of Santa Casa de Porto Alegre Hospital in Brazil. Following transplantation, DSAs were collected at various intervals, commencing 3 months post-transplantation. Recipient anti-HLA antibodies were detected utilizing the LABScreen Single Antigen (SAB) test by Luminex®, with an mean fluorescence intensity (MFI) >1000 indicating positivity.

DSAs that were present before transplantation were categorized as preformed. Patients who developed DSAs after transplantation were classified as having de novo DSA (dnDSA), whereas patients in whom DSAs persisted post-transplantation were classified as having persistent DSA. In cases in which recipients exhibited multiple positive DSAs, the highest MFI value was considered.

All HLA typing procedures were conducted at the Immunology Laboratory of Santa Casa de Porto Alegre Hospital, Brazil.

Ethical considerations

Approval for this study was obtained from the Research and Ethics Committee of the Institution, and the study was registered on the Brazilian Clinical Trials Platform under the registration number RBR-3gtcvjU111112367585.

Informed consent was obtained from all donors and recipients or their legal guardians before their participation in the study.

Statistical analyses

Quantitative variables are expressed as means and standard deviations, whereas categorical variables are presented as absolute and relative frequencies. Mean comparisons were conducted using the Student's t-test. Proportional comparisons were performed using Pearson's chi-squared test or Fisher's exact test. Statistical analyses were performed using the IBM Statistical Package for the Social Sciences for Windows version 21.0 (IBM Corp., Armonk, NY, USA), with the significance level set at 5% ($p < 0.05$). This study was analytically reviewed by our expert Biostatistician, Alvaro Rosler.

Results

During the study period, 91 liver transplants were performed at the Santa Casa de Porto Alegre Hospital Complex, among which, 73 were from living donors and three were re-transplants. Fifteen patients died <30 days after LT, and six patients were excluded because they did not reach the minimum follow-up. Ultimately, 67 patients were included in this study. None of these patients underwent re-transplantation, and there was no mortality during follow-up.

Pre-transplant DSA analysis of 60 recipients revealed that 15% were positive for class I and 18% for class II, while three recipients tested positive for both classes. The median time for post-transplant DSA analysis was 19.7 months (range: 4.3 to 35.6 months). Post-transplant DSA analysis was feasible for 66 recipients, with 12% testing positive for class I, 43% for class II, and 6% for both.

Recipients who underwent post-transplantation DSA analysis were categorized into two groups: DSA-positive (DSA+; n = 33) and DSA-negative (DSA–; n = 34). The two groups showed significant differences in terms of donor age (27.9 vs. 32.9 years, respectively; p = 0.04) and median time for DSA analysis (23.8 vs. 13.5 months, respectively; p = 0.04) (Table 1). Moreover, the DSA+ group showed a higher frequency of rejection episodes (36% vs. 3%, p < 0.001), and liver graft fibrosis (33% vs. 12%, p = 0.04) (Table 2).

The MFI intensity of class II DSAs demonstrated a correlation with the development of rejection (exceeding 45%) when the MFI values exceeded 10,000 (Figure 1).

To investigate the correlation between post-transplant DSA positivity and rejection occurrence, we conducted a subgroup analysis comparing patients with and without rejection (Table 3). The median time to the first rejection episode was 7.8 months (range: 2.6 to 55.4 months).

Significant differences were observed between patients who experienced rejection (rejection+) and those who did not (rejection–): donor age (23.6 vs. 32 years, p = 0.01), frequency of living donor LT (69.2% vs. 96.3%, p = 0.001), frequency of whole liver graft (30.8% vs. 5.6%, p = 0.009), and frequency of related donor (61.5% vs. 88.9%, p = 0.03), respectively.

In terms of post-transplantation outcomes, the rejection+ group had a higher incidence of Epstein-Barr virus and Cytomegalovirus infections, as well as a higher prevalence of fibrosis, as indicated by liver biopsy (Table 3).

Further analysis of the DSA profile according to the rejection status revealed a higher prevalence of DSA positivity at any time point among patients who experienced rejection. Notably, dnDSA and DAS-class II proteins were particularly prominent at the DQ locus (Table 5).

Positive post-transplant DSA status remained a significant risk factor for rejection, even after adjusting for other factors, presenting a hazard-ratio of 20.3 (Table 6).

Analysis of the 32 recipients who underwent liver biopsy revealed that 15 of 32 (46.8%) presented with fibrosis, 13 of 32 (40%) with rejection, and 9 of 32 (28%) presented with both. Notably, five (38.5%) of 13 patients who experienced rejection tested positive for C4d according to the liver biopsy results.

The number of mismatches (MM) influenced the occurrence of hepatic artery thrombosis (HAT) after LT (Table 7). All six recipients who developed HAT had at least one MM at loci A, B, or DR. A higher frequency of HAT was observed as the number of MM increased, with a significant trend observed for HLA-A (Figure 2). Including HLA MM in a logistic regression

model for the occurrence of HAT revealed that having two MMs increased the risk of HAT by almost 16-fold (Table 8).

Discussion

It is well established that recipients with DSAs face an increased risk of immunological damage, leading to acute antibody-mediated rejection, chronic rejection, and diminished graft survival.[17,18]

Several factors have been associated with DSA formation in the adult population, including inadequate immunosuppression, younger age at transplantation, lower Model for End-Stage Liver Disease (MELD) scores, and re-transplantation. However, in pediatric patients, only insufficient immunosuppression and low weight at transplantation have been identified as contributing factors.[19]

Kaneku et al. (2013) demonstrated a correlation between younger donors and the development of DSAs in recipients. Notably, no significant difference was observed based on donor sex or whether the transplant was performed with a living or deceased donor. These findings underscore the importance of considering donor-related factors when assessing the risk of DSA formation and the subsequent immunological complications in transplant recipients.[19]

In our study, we found that younger donors and a longer duration from transplantation to DSA collection were associated with the development of DSAs after transplantation. The presence of dnDSAs has previously been identified as a biomarker for T cell-mediated rejection in transplanted children.[20] Consistent with these findings, our study revealed that DSA positivity after transplantation increased the risk of rejection.

Elevated MFI values, particularly against the HLA class II locus DQ, are strongly correlated with unfavorable post-transplantation outcomes. Recipients with DSAs exhibit a heightened risk of rejection in the initial weeks following the procedure.[20,21] DSAs that activate the complement cascade, particularly DQ alleles with a high MFI (>13,000), are strongly associated with a non-tolerant phenotype, indicating a more aggressive rejection response.[22]

Other studies conducted in lung and heart recipients have demonstrated that high MFI values are associated with antibody-mediated rejection (AMR).[21,23] Similarly, our study on pediatric liver transplant recipients indicated an association between MFI values and risk of rejection.

In renal transplant recipients, MFI values exceeding 5,000 have been shown to have impact on rejection and graft survival. However, a specific cutoff value for liver transplant recipients has

not yet been clearly defined.[24] Our findings suggest that the risk of rejection increases when MFI values exceed 5,000 for the DQ allele.

Recent studies focusing on pediatric living-donor liver transplant recipients have identified DSAs in 32 of 67 (48%) long-term survivors. Analysis of liver biopsies from these patients revealed a higher probability of fibrosis and inflammation. Consequently, these patients opted to received reduced immunosuppression.[22]

Liver biopsy protocols play a crucial role in the early detection of liver graft injuries. O'Leary et al. evaluated 79 protocol liver biopsies from liver transplant recipients and observed a significantly higher prevalence of fibrosis in DSA-positive patients compared to DSA-negative patients (88% vs. 17%, $p < 0.001$)[19] Similarly, Kivela et al. detected a higher prevalence of fibrosis (50%) in DSA-positive patients when compared to DSA-negative ones.[25] In our study, 33% of the DSA-positive patients exhibited fibrosis on liver biopsy. Notably, not all patients, but only those with abnormalities in liver function tests underwent liver biopsy. Consequently, there is a possibility that some cases of fibrosis may have been missed. Nonetheless, these findings underscore the importance of centers being vigilant about the heightened risk for DSA-positive patients, and the necessity for close monitoring of this patient population.

dnDSA-DQ was the most prevalent allele in our population, identified in 26 patients (39%). Additionally, the group of patients who experienced rejection demonstrated a higher prevalence of DSA-DQ6 after transplantation (38.5% vs. 9.4%, $p = 0.02$). However, there is divergence in the literature regarding which DQ alleles are most strongly associated with rejection and fibrosis, with some studies implicating DQ6 and DQ7, and others pointing to DQ2.[26]

Recent evidence suggests that the DQ locus has higher pathogenicity than other loci, with the presence of two polymorphic alpha and beta chains thought to contribute to its immunogenicity. However, a clear biological explanation of its role in poor outcomes following solid organ transplantation remains elusive.[27]

The relationship between DSA positivity and C4d deposits has been a relatively recent subject of investigation. C4d deposits can result from various factors besides DSAs, such as autoimmune hepatitis, biliary complications, ischemia/reperfusion injury, and vascular thrombosis. C4d deposits may also be caused by DSAs that are not associated with HLA.[9] Previous retrospective studies have reported contradictory results regarding the relationship between C4d deposits and DSAs.[15,28] In our study, C4d positivity was observed in 38.5% of the patients who experienced rejection. However, as C4d staining was only performed in patients who experienced rejection, we cannot draw further conclusions regarding the relationship

between C4d positivity and rejection beyond this observation. Further studies examining C4d staining in larger patient cohorts, including patients without rejection, are necessary to elucidate the precise relationship between C4d positivity and rejection in liver transplant recipients.

Although pre-transplant HLA typing is not commonly conducted due to inconsistent evidence regarding its benefits, a study involving 1042 adult liver transplant recipients revealed a significant association between having more than one HLA-A MM and reduced graft and patient survival rates. Specifically, this study found that at least one HLA-A MM was present in all cases of graft loss attributed to HAT, at least one HLA-A MM was present.[29]These findings align with our own observations that all patients who developed HAT had at least one HLA MM, and that the incidence of HAT increased with the number of HLA-A MM. Moreover, in multivariate analysis, having two HLA-A MMs was associated with an almost 16-fold increase in the risk of HAT.

In conclusion, our findings underscore the importance of routine HLA typing in pediatric patients of LT patients. Post-transplantation and persistent DSA positivity, particularly targeting the DQ locus, strongly correlate with rejection development. In the context of living-donor LT, the diagnosis of HLA-A MM before transplantation should prompt consideration of seeking a more compatible donor, if available. Tailored adjustments in immunosuppression protocols based on DSA profiles should be explored in future prospective multicenter studies.

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Table 1. Recipient characteristics according to post-transplant DSA status

	DSA -	DSA +	P value
	N=34	N=33	
Recipient sex, male, n(%)	18 (52.9)	19 (57.6)	0.8
Donor sex, male, n(%)	20 (48.8)	20 (60.3)	1,0
Recipient age at transplant, months, median (IQR)	12.8 (7.37 to 53)	10.8 (7.9 to 17.4)	0.5
Donor age, years, mean ± SD	32.9 ± 11.5	27.9 ± 11.6	0.04
Z score BMI, median (IQR)	-0.8 (-1.4 to - 0.95)	- 0.2 (-1.0 to -1.1)	0.3
Z score WA, median (IQR)	-1.1 (-3 to -0.09)	-0.9 (-2.3 to -0.3)	0.6
Z score HA, median (IQR)	-1.8 (-3 to -0.2)	-1.6 (-2.4 to -0.7)	0.7
Weight, kg, median (IQR)	7.3 (6.1 to 17)	7.6 (6.5 to 11.2)	0.6
PELD, median (IQR)	13 (1.7 to 21.2)	15 (8.5 to 24)	0.2
Graft weight, g, median (IQR)	270 (231.5 to 317)	285 (244.5 to 347)	0.2
GRWR, %, mean ± SD	3.2 ± 1.6	3.4 ± 1.2	0.2
Time from transplant to DSA collection, median (IQR)	13.5 (3.5 to 29.6)	23.8 (8.2 to 45.4)	0.04
Related donor, n(%)	29 (85)	27 (82)	0.7
Living Donor Transplantation, n(%)	33 (97)	28 (85)	0.1
Type of graft, n(%)			0.29
LLS	31(91)	28 (85)	
LL	1 (3)	0	
Whole liver	2(6)	5 (15)	
Biliary atresia, n(%)	17 (50)	21 (64)	0.26

IQR – interquartile range; SD – standard deviation; W/A – weight/age; H/A – heigh/age; BMI – body mass index; GRWR – graft-to-recipient-weight-ratio; BA – biliary atresia; DSA – donor specific antibodies; LLS – left lateral segment; LL – left lobe.

Table 2. Recipient outcomes according to post-transplant DSA status.

	DSA -	DSA +	P value
	N=34	N=33	
CMV infection, n(%)	20 (61)	22 (67)	0.8
EBV infection, n(%)	13 (38)	18 (54.5)	0.2
Bile leak, n(%)	10 (30)	11 (33)	1
Biliary stricture, n(%)	11 (33)	9 (27)	0.8
Portal vein thrombosis, n(%)	4 (12)	4 (12)	1
Portal vein stenosis, n(%)	7 (21)	4 (12)	0.5
Hepatic artery thrombosis, n(%)	2 (6)	4 (12)	0.4
Graft rejection, n(%)	1 (3)	12 (36)	<0.001
Graft rejection, >1 episode, n(%)	0 (0)	6 (18)	0.01
Fibrosis, n(%)	4 (12)	11 (33)	0.04

DSA – donor specific antibodies; CMV – citomegalovirus; EBV – Epstein-baar virus

Table 3. Recipient characteristics in the group of patients who experienced rejection compared to those who didn't.

	Rejection +	Rejection -	P value
	N=13	N=54	
Recipient sex, male, n(%)	7 (53.8)	30 (55.6)	0.5
Donor sex, male, n(%)	8 (61.5)	32 (59.6)	0.57
Recipient age at transplant, months, median (IQR)	10.8 (8 to 20.6)	12 (7.6 to 28.9)	0.97
Donor age, years, mean ± SD	23.6 ± 11.8	32 ± 11.2	0.01
Z score BMI, median (IQR)	- 0.37 (-1.2 to 1.3)	-0.4 (-1.3 to 0.9)	0.82
Z score WA, median (IQR)	-1.0 (-1.9 to 0.6)	-1,1 (-2,9 a -0,8)	0.44
Z score HA, median (IQR)	-1.3 (-1.8 to -0.3)	-1,6 (-3,0 a -0,7)	0.29
Weight, kg, median (IQR)	7.4 (6.6 to 12.5)	7,3 (6,2 a 12,8)	0.57
PELD, median (IQR)	14 (10.5 to 26)	14 (3,75 a 22)	0.32
Graft weight, g, median (IQR)	250 (220 to 402)	286,5 (242,5 a 317)	0.61
GRWR, %, mean ± SD	3.4 ± 1.2	3.3 + 1.5	0.4
Related donor, n(%)	8 (61.5)	48 (88.9)	0.03
Living Donor Transplantation, n(%)	9 (69.2)	52 (96.3)	0.01
Type of graft, n(%)			0.009*
SLE	9 (69.2)	50 (92.6)	
LE	0 (0)	1 (1.9)	
Whole liver	4 (30.8)	3 (5.6)	
Biliary atresia, n(%)	10 (76.9)	28 (51.9)	0.06

* Pearson's linear association 0,02

IQR – interquartile range; SD – standard deviation; W/A – weight/age; H/A – heigh/age; BMI – body mass index; GRWR – graft-to-recipient-weight-ratio; BA – biliary atresia; DSA – donor specific antibodies; LLS – left lateral segment; LL – left lobe.

Table 4. Recipient outcomes in the group of patients who experienced rejection compared to those who didn't.

	Rejection +	Rejection -	p
	N=13	N=54	
CMV infection, n(%)	12 (92.3)	30 (56.6)	0.02
EBV infection, n(%)	10 (76.9)	21 (38.9)	0.02
Bile leak, n(%)	3 (23.1)	18 (34)	0.52
Biliary stricture, n(%)	6 (46.2)	14 (26.4)	0.14
Portal vein thrombosis, n(%)	2 (15.4)	6 (11.3)	0.65
Portal vein stenosis, n(%)	0 (0)	11 (20.8)	0.1
Hepatic artery thrombosis, n(%)	2 (15.4)	4 (7.4)	0.32
Fibrosis, n(%)	9 (69.2)	6 (11.1)	<0.001

Table 5. DSA analysis in the group of patients who experienced rejection compared to those who didn't.

	Rejection +	Rejection -	p
	N=13	N=54	
DSA+ (at any time point), n (%)	12 (93)	27 (50)	0.005
DSA+ (before liver transplant), n (%)			
Class I	3 (33)	6 (12)	0.12
Class II	4 (44)	7 (14)	0.05
DSA+ after liver transplant (dnDSA), n (%)	12 (93)	21 (39)	<0.001
Class I	2 (15)	6 (11)	0,6
Class II	10 (77)	18 (34)	0.01
DSA-DQ+ after liver transplant, n (%)	9 (69)	17 (32)	0.02
DSA-DQ+ persistent, n (%)	3 (33)	2 (4)	0.003
DSA-DQ6+ after liver transplant, n (%)	5 (38.5)	5 (9.4)	0.02
DSA-DQ7+ after liver transplant, n (%)	4 (30.8)	6 (11.3)	0.09

Table 6. Multivariate logistic regression analysis for rejection

	HR	95%CI	p
Donor age (years)	0.98	(0.91 to 1.06)	0.7
Living donor liver transplantation	0.69	(0.01 to 25.75)	0.8
Related donor	0.22	(0.01 to 5.2)	0,35
DSA+ after liver transplant	20.3	(1.96 to 210)	0.01

HR – *hazard-ratio*; CI95% - confidence interval; DSA – *donor specific antibodies.*

Table 7. Number of HLA mismatchs (MM) before the transplant in patients who developed HAT

	HAT +	HAT -	P value
	N=6	N=61	
HLA-A			0.02 (0.01)*
0 MM	0 (0)	4 (6.8)	
1 MM	3 (50)	49 (83.1)	
2 MM	3 (50)	6 (10.2)	
HLA-B			0.18 (0.07)*
0 MM	0	5(8.5)	
1MM	3 (50)	43 (72.9)	
2MM	3 (50)	11 (18.6)	
HLA-DR			0.37 (0.16)*
0MM	0 (0)	5 (8.5)	
1MM	4 (66,7)	46 (78)	
2MM	2 (33,3)	8 (13.6)	
HLA CLASS I - 4MM	3 (50)	6 (10.7)	0.03

Table 8. Multivariate logistic regression analysis for hepatic artery thrombosis (HAT)

	HR	95% CI	P value
HLA-A, 2MM	15.9	(1.8 to 140.3)	0.01
Living donor liver transplant	3.78	(0.14 to 101.4)	0.42
Recipient age, months	0.96	(0.89 to 1.03)	0.32
Recipient weight, kg	1.15	(0.87 to 1.53)	0.3

HR – *hazard-ratio*; 95CI% - confidence interval; MM – mismatch

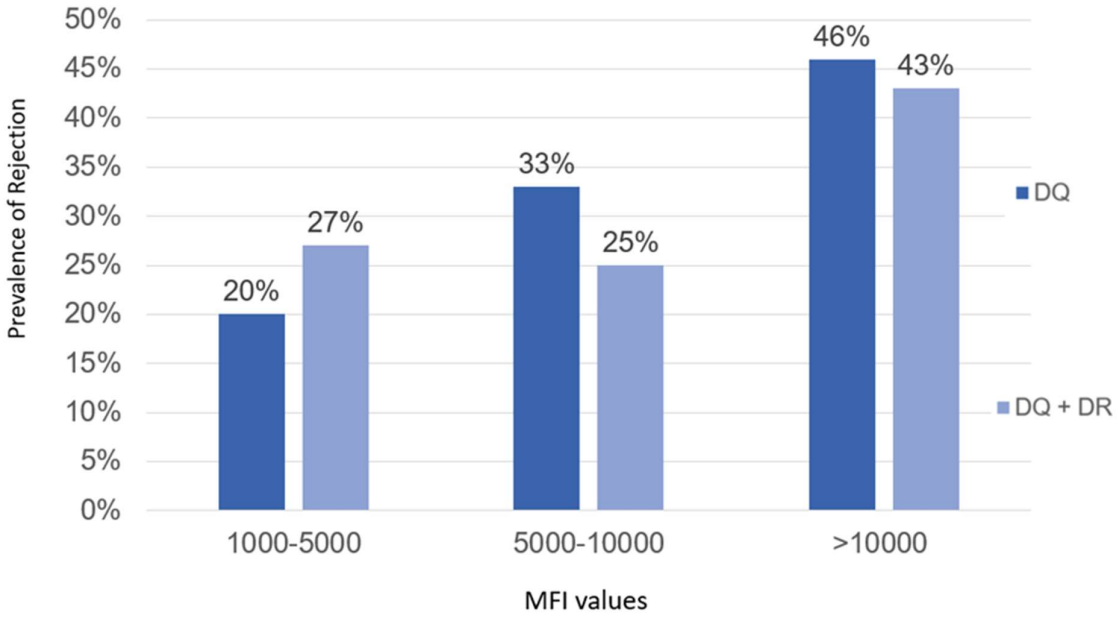

Fig 1. Graft rejection rates according to MFI value at DQ and DR loci.

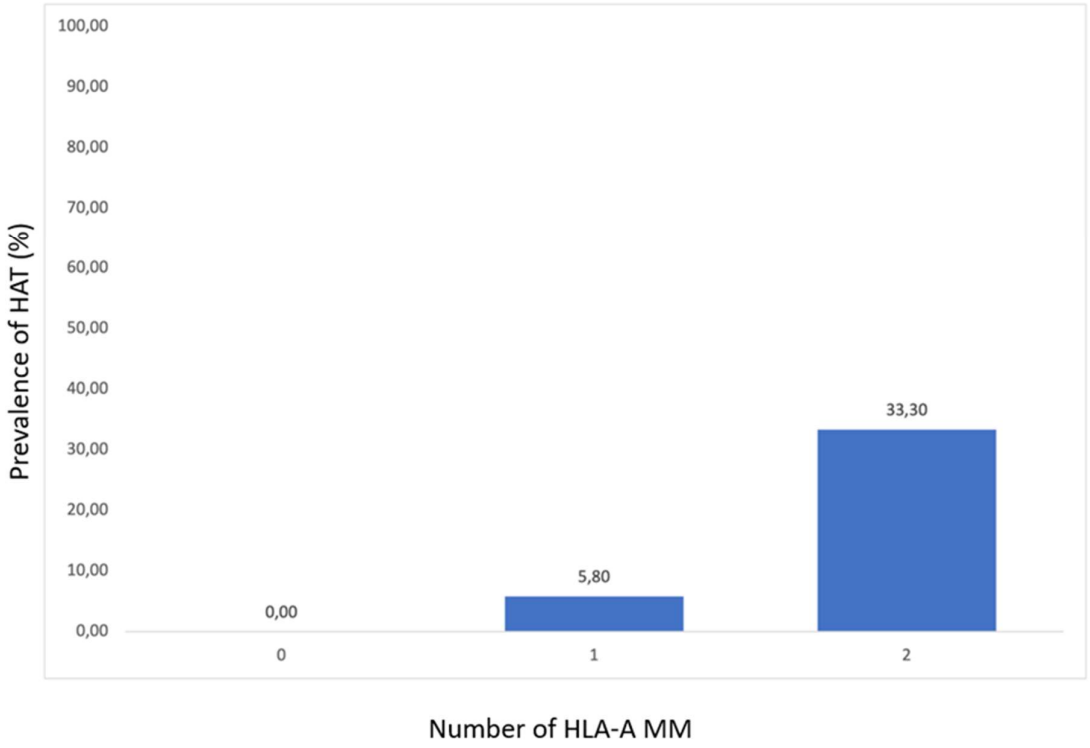

Fig 2. Incidence of hepatic artery thrombosis according to the number of HLA-A mismatchs

Apêndice A – TERMO DE CONSENTIMENTO ESCLARECIDO - Doador

Eu_____RG _____

Doador de fígado declaro, por meio deste termo, que concordei em participar na pesquisa de campo referente ao projeto de doutorado da Dra. Melina Utz Melere cujo título é: “Avaliação do HLA e do DSA nos doadores e receptores de transplante de fígado pediátrico e sua associação com a evolução clínica”. Fui informado(a) e orientado sobre do que se trata esse estudo, que é coordenado / orientado pela Dra. Cristina Targa Ferreira.

Afirmo que aceitei participar por minha própria vontade, sem receber qualquer incentivo financeiro ou ter qualquer ônus e com a finalidade exclusiva de colaborar para o sucesso da pesquisa e para ajudar os próximos pacientes. Fui informado (a) dos objetivos estritamente acadêmicos do estudo, que, em linhas gerais, é a avaliação completa do HLA.

Assinatura do(a) paciente:_____

Assinatura da pesquisadora:_____

Porto Alegre, _____ de_____De 20____.

Apêndice B – TERMO DE CONSENTIMENTO ESCLARECIDO - Receptor

Eu_____RG_____
responsável pelo paciente _____receptor de transplante hepático declaro, por meio deste termo, que concordei em participar na pesquisa de campo referente ao projeto de doutorado da Dra. Melina Utz Melere cujo título é: “Avaliação do HLA e do DSA nos doares e receptores de transplante de fígado pediátrico e sua associação com a evolução clínica”. Fui informado(a) e orientado(a) sobre o estudo, seus objetivos e como será realizado. Esse estudo é coordenado / orientado pela Dra. Cristina Targa Ferreira.

Afirmo que aceitei participar por minha própria vontade, sem receber qualquer incentivo financeiro ou ter qualquer ônus e com a finalidade exclusiva de colaborar para o sucesso da pesquisa e para ajudar os próximos pacientes. Fui informado (a) dos objetivos estritamente acadêmicos do estudo, que, em linhas gerais, é a determinação da frequência de anticorpos contra HLA do doador.
Poderei ter acesso aos resultados relacionados à esta pesquisa e os mesmos serão explicados pela Dra. Melina Melere ou por alguém da equipe médica.

Assinatura do(a) paciente ou responsável:_____

Assinatura do(a) pesquisador(a):_____

Porto Alegre, _____ de_____De 20____.

Anexo I. Comprovante CEP

IRMANDADE DA SANTA CASA DE MISERICORDIA DE PORTO ALEGRE - ISCMPA

COMPROVANTE DE ENVIO DO PROJETO

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação do antígeno leucocitário humano e a presença de anticorpo contra o doador no transplante de fígado pediátrico e sua associação com a evolução clínica.

Pesquisador: Melina Utz Melere

Versão: 4

CAAE: 19926219.4.0000.5683

Instituição Proponente: Hospital da Criança Santo Antônio - Santa Casa/RS

DADOS DO COMPROVANTE

Número do Comprovante: 109320/2019

Patrocionador Principal: Financiamento Próprio

Informamos que o projeto Avaliação do antígeno leucocitário humano e a presença de anticorpo contra o doador no transplante de fígado pediátrico e sua associação com a evolução clínica. que tem como pesquisador responsável Melina Utz Melere, foi recebido para análise ética no CEP Irmandade da Santa Casa de Misericordia de Porto Alegre - ISCMPA em 30/08/2019 às 08:57.

Endereço: Avenida Osvaldo Aranha, n° 80, sala 17, Centro Administrativo da Santa Casa, 2° andar.
Bairro: Cidade Baixa **CEP:** 90.035-190
UF: RS **Município:** PORTO ALEGRE
Telefone: (51)3214-8571 **Fax:** (51)3214-8571 **E-mail:** cep@santacasa.tche.br

Anexo II. Normas para publicação – Artigo 1

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Name of Journal: *World Journal of Transplantation*
ManuscriptType: ORIGINAL ARTICLE

Guidelines for Manuscript Preparation and Submission: Prospective Study Core tip: Prospective Study articles are submitted by any author and describe an observational study of a population for a sufficient number of persons over a sufficient number of years to generate incidence and/or mortality rates subsequent to the selection of the study group.

For the initial submission, authors can conveniently make their first upload of their manuscript without restrictions on writing style, file format, or need for accompanying relevant documents. However, it is recommended that the content be written as a high-quality academic article before submitting, according to the following checklist.

1 FIRST SECTION OF MANUSCRIPT WRITING [YES or NO]
1.1 Title [ ]
1.2 Authorship [ ]
1.3 Institution [ ]
1.4 ORCID number [ ]
1.5 Supportive foundations [ ]
1.6 CONSORT 2010 Statement [ ]
1.7 Corresponding author [ ]
1.8 Abstract [ ]
1.9 Key words [ ]
1.10 Core tip [ ]

2 SECOND SECTION OF MANUSCRIPT WRITING [YES or NO]
2.1 Main text [ ]
2.2 Biostatistics [ ]
2.3 Units [ ]
2.4 Illustrations [ ]
2.5 Tables [ ]
2.6 Notes in illustrations and tables [ ]
2.7 Abbreviations [ ]
2.8 Italics [ ]
2.9 Acknowledgements [ ]
2.10 References [ ]
3 ETHICS POLICIES/STATEMENTS [YES or NO]
3.1 Institutional review board [ ]
3.2 Clinical trial registration [ ]
3.3 Informed consent [ ]
3.4 Conflict-of-interest [ ]
4 language editing for manuscripts submitted by non-native Speakers of english

5 steps for submitting manuscripts
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1 FIRST SECTION OF MANUSCRIPT WRITING
All contributions should be written in English; the authors may use either UK or US English language, but the chosen English language usage must be consistent throughout the document. All articles should be prepared with Word-processing software, using 12 pt Book Antiqua font and 1.5 line spacing with ample margins. Required information for each of the manuscript sections is as follows:

1.1 Title. The title should be no more than 18 words. It should summarize the core content of the manuscript, so that the reader may readily understand the key concepts and important findings presented within. This type of succinct and impactful statement will serve to catch readers' attention and stimulate their interest in reading the abstract and/or downloading the full paper. It is also strongly recommended that the title include one or two of the key words associated with the manuscript's topical content, to facilitate the paper being readily found by electronic searches of public databases, such as by Google or in PubMed. Finally, words such as 'exploration', 'research', 'analysis', 'observation', and 'investigation' are to be avoided. The title should not start with 'A', 'An', or 'The' and will not include any Arabic numbers or abbreviations.

1.2 Authorship. Authorship credit should be given in accordance with the standard proposed by the International Committee of Medical Journal Editors (ICMJE) (http://www.icmje.org/). Specifically, authorship is merited by (1) substantial contributions to conception and design of the study, acquisition of data, or analysis and interpretation of data; (2) drafting the article or making critical revisions related to important intellectual content of the manuscript; and (3) provision of final approval of the version of the article to be published. Authors should meet conditions 1, 2 and 3. We consider requests for co-first/co–corresponding authors on a limited basis, making the final decision to allow/deny according to the detailed reasons provided by the authors for justification on a case-by-case basis, with allowance permitting no more than 2 co-first/co–corresponding authors. For the policy of allowing co-first authors and co-corresponding authors who made equal contribution to a manuscript, please visit: /bpg/GerInfo/310. Author names (unabbreviated) should be given as first name, middle name initial (with no period) and family (sur) name, and typed in bold with the first letter capitalized; a hyphen should be included between the syllables of Chinese names. For example, Jason Lamontagne, Laura F Steel, Paul V Harper Jr, Bo Yuan, and Wei-Hong Tang.

1.3 Institution. Author names should be written out first (as first name, middle name initial (with no period) and family (sur)name; with a hyphen included between the syllables of Chinese names) and typed in bold, followed by a comma and the complete name of the affiliated institution, city, province/state, postcode and country typed in non-bold.
For example:
Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China In the case that multiple authors represent a single institution, the authors will be listed together for that institution. For example:
Giuseppe Losurdo, Domenico Piscitelli, Antonio Giangaspero, Mariabeatrice Principi, Francesca Buffelli, Floriana Giorgio, Lucia Montenegro, Claudia Sorrentino, Annacinzia Amoruso, Enzo Ierardi, Alfredo Di Leo, Gastroenterology Section, Department of Emergency and Organ Transplantation, University of Bari, Bari 70124, Italy
In the case that one author represents multiple institutions, the institutions will be listed

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1.4 ORCID number. ORCID provides a persistent digital identifier that distinguishes each researcher from every other researcher globally and, through integration in key research workflows such as manuscript and grant submissions, supports automated linkages between an individual researcher and their own professional activities, thereby ensuring that their work is recognized accurately in a distinctive manner. Please visit the ORCID website at https://orcid.org/ for more information. The corresponding author must provide his/her personal ORCID registration number.

1.5 Supportive foundations. The approved grant application form(s) will be released online, together with the manuscript in order for readers to obtain more information about the study and to increase the likelihood of subsequent citation. Our purpose of publishing the approved grant application form(s) is to promote transparent academic communication, accelerate scientific progress in the related field, and improve effective and productive sharing of research ideas.
Supportive foundation acknowledgment: The complete name(s) of supportive foundation(s) and identification number(s) of grants or other financial support will be provided on the title page of all submitted manuscripts using the following format: Supported by the National Natural Science Foundation of China, No. 30224801.

1.6 CONSORT 2010 Statement. In order to improve the quality of Prospective Study manuscripts, authors should download and complete the 'CONSORT 2010 checklist of information to include when reporting a randomised trial' to ensure that the manuscript meets the requirements of the CONSORT 2010 Statement. Authors must state in the Footnotes section of the manuscript that the guidelines of the CONSORT 2010 Statement have been adopted (see below). Authors must upload the PDF version of the completed checklist to the system.
Sample wording: The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

1.7 Corresponding author. The corresponding author's contact information will be provided in the following format: written out first name, middle name initial (with no period) and family (sur)name (with a hyphen included between the syllables of Chinese names), and followed by the relevant honorifics (such as PhD, MD, Chief of Surgery, Assistant Professor, etc), all typed in bold and ending with a comma. This will be followed immediately by the corresponding author's professional affiliation (non-bold text), written out as complete name of institution, present address, city, province/state and postcode, and country and ending with a period. Immediately following the ending period and a single space will be the corresponding author's E-mail address; this E-mail address must be issued by his/her institution. All the letters in the E-mail address should be typed in lowercase. For example:
Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor, Chief, Department of Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, 5901 E Seventh St, Long Beach, CA 90822, United States. astarnaw@uci.edu

1.8 Abstract. An informative, structured abstract of no more than 350 words should accompany each manuscript. Abbreviations should be avoided, but if used should be spelled out at first mention. The 5 sections of the structured abstract are: Background, Aim, Methods, Results, and Conclusion. Each section should adhere to the word count thresholds (indicated in parentheses) and the content guidelines below: BACKGROUND (no more than 100 words) This section should clearly describe the rationale for the study. It should end with a statement

of the specific study hypothesis. AIM (no more than 20 words) The purpose of the study should be stated clearly, with no or minimal background information, following the format of: “To investigate/study/determine…”

METHODS (no more than 80 words)
This section should describe the materials and methods used for all of the data presented in the proceeding Results section of the abstract. This information should include the following details, as applicable: basic study design (e.g., randomized controlled trial, cross sectional study, cohort study, case series, etc); setting, please specify study location (e.g., primary or tertiary care setting, hospital, general community, etc); number of participants and how they were selected; intervention, the method of administration and the duration; and major statistical methods used.

RESULTS (no more than 120 words)
This section should describe the key findings of the study, including absolute values and risk differences. P values should be presented where appropriate, and not for data that did not reach the threshold of statistical significance. You must provide relevant data to illustrate how the statistical values were obtained (e.g., 6.92 ± 3.86 vs 3.61 ± 1.67, $P < 0.001$).

CONCLUSION (no more than 30 words)
This section should succinctly and cogently present the findings and implications that are within the scope of the data you have presented in the preceding Results section of the abstract. You should state only conclusions that are directly supported by the evidence presented and the implications of the findings presented. This section should be written in the present tense.

1.9 Key words. The ‘Key words’ list will provide 5-10 keywords that reflect the main content of the study. Please do not use abbreviations for the keywords (e.g. Ulcerative colitis, not UC). The first letter of each keyword will be capitalized, and each keyword will be separated by a semicolon. For example: Key words: Non-alcoholic fatty liver disease; Alcoholic liver disease; Non-alcoholic steatohepatitis; Animal models; Insulin resistance; Oxidative stress

1.10 Core tip. Please write a summary of no more than 100 words to present the core content of your manuscript, highlighting the most innovative and important findings and/or arguments. The purpose of the Core Tip is to attract readers’ interest for reading the full version of your article and increasing the impact of your article in your field of study.

2 SECOND SECTION OF MANUSCRIPT WRITING

2.1 Main text. The main text will contain 7 sections, including Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, and References.

2.2 Biostatistics. Any manuscript describing a study (basic research and clinical research) that used biostatistics must include a statement in the Materials and Methods section affirming that the statistical review of the study was performed by a biomedical statistician. Statistical review is performed before the submission or after peer-review. The author(s) will invite an expert in Biomedical Statistics to evaluate the statistical method(s) used in the study, including but not limited to the t-test (group or paired comparisons), chi-square test, ridit, probit, logit and regression (linear, curvilinear, or stepwise) modeling, correlation, analysis of variance, and analysis of covariance. The review by the biomedical statistician is conducted with respect to the following points: (1) Statistical methods are adequately and appropriately described when they are used to verify the results; (2) Statistical techniques are suitable or correct, and compliant with the following Baishideng Publishing Group (Baishideng) directives; (3) Only

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Sample wording: The statistical methods of this study were reviewed by [name(s) of individual(s)] from [name(s) of organization(s)]…
Note: If a biostatistics editor is employed by the authors, the person’s name (first nam and family (sur)name), qualifications, and contact information must be submitted to the editorial office in the form of a letter of confirmation of service. If the biostatistics editing was performed by a commercial service provider, the company’s name and contact information, including URL and E-mail or phone number, must be submitted to the editorial office in the form of a letter of confirmation of service. The letters of confirmation of service must include the corresponding author’s name (first name and family (sur)name) and contact information (E-mail and phone number), and the manuscript title.

2.3 Units. Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h; blood glucose concentration, c (glucose) = 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6-24.5 g/L; CO2 volume fraction, 50 mL/L CO2, not 5% CO2; likewise, for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, etc. Arabic numerals such as 23,243,641 (i.e. 23 million, 243 thousand, and 641) should be written as 23243641, with no commas or spaces. The format for how to accurately write common units and quantities can be found at: https://www.wjgnet.com/bpg/gerinfo/189.

2.4 Illustrations. Figures must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, etc). All figures must have a detailed figure legend that provides a clear and comprehensive description of the information presented in the figure, so that the reader can understand without having to refer back to any other portion of the manuscript. It is necessary to keep all elements compiled in a line-art image. Scale bars (with the length of the bar defined in the legend text rather than on the bar itself) or magnification factors (with textual definition in the legend) can be used. Figure file names should identify the figure and panel. Avoid layering type directly over shaded or textured areas in the figure. Uniform presentation should be used for figures showing the same or similar contents; for example, “Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...”.

2.5 Tables. Tables must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, etc). A brief, one-line title must be provided for each table. Detailed legends should not be included under tables, instead having the information presented in the main text where applicable; the information should complement, but not duplicate the text. Use one horizontal line under the title, a second under the column headings, and a third below the last row of the Table (being above any footnotes). Vertical lines and italics should be omitted.

Please note that tables embedded as Excel files within the manuscript are NOT acceptable. Tables made in Excel that are 2 pages or less should be transformed into a customized Word program table, using the ‘Insert Table’ function. Tables will be located at the very end of your

article document, following the figures. Any tables submitted that are longer/larger than 2 pages will be published as online-only supplementary material.

Tables must be primarily cell-based and fully editable. Do not use the following to organize data or structure the table: (1) Returns (“Enter” key); (2) Tabs; (3) Spaces; (4) Colored text; (5) Cell shading; and (6) Cells within cells. The Software should be Word (preferred; embedded at the end of the manuscript file) or Excel (allowed for longer tables presented as Supplementary Materials). Baishideng does not allow for graphics, boxes or embedded tables to appear in the main body of the manuscript.

2.6 Notes in illustrations and tables. Data with statistical significance in a figure or table should be denoted using superscripted alphabetical lettering, such as aP < 0.05 and bP < 0.01. If there are other series of P values, the alphabetical subscripted denotation format is continued, such as cP < 0.05 vs control, dP < 0.01 vs control, eP < 0.05 vs group A, and fP < 0.01 vs group B. Data that are not statistically significant should not be denoted, i.e. P > 0.05 is not an allowed denotation.

Other notes in tables or under illustrations should be expressed as F1, F2, F3, or sometimes as other superscripted symbols (Arabic numerals); for example, “F: Venn diagram. 1Here, we excluded patients that preintervention was inconsistent with original treatment in our hospital.” In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, etc, in a specified sequence.

2.7 Abbreviations. Standard abbreviations should be defined in the abstract and in the main body of the manuscript upon first mention in the text. In general, terms should not be abbreviated unless they are used two times or more and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.

2.8 Italics. Quantities: t, time or temperature; c, concentration; A, area; l, length; m, mass; V, volume. Genotypes: gyrA, arg 1, c myc, c fos, etc. Restriction enzymes: EcoRI, HindI, BamHI, Kbo I, Kpn I, etc. Biological nomenclature: H. pylori, E. coli, etc. Latin terms: i.e., e.g.,

2.9 Acknowledgements. Brief acknowledgements of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

2.10 References. Prospective Study should be composed of detailed contents, comparisons, and evaluations in relation to other published relevant articles, and an in-depth discussion. Please don't use informal publications. For seminal references, however, the publication date is not strictly limited. You should always cite references that are relevant to your article. Citing more than five references in a single citation, even when separated by a hyphen, should be avoided; for example [1-6],[2-14], and [1,3,4-10,22] are all considered inappropriate reference citations. Moreover, authors should not cite their own unrelated published articles. Citation of references not indexed on PubMed is discouraged, but if a reference that is not indexed by PubMed is necessary, you must provide Baishideng with a printed copy of the first page of the full article. Please update the format of all the references according to the Format for references guidelines. The accuracy of the information of journal citations is very important. We will interlink all references with DOIs in an XML file, so that readers can immediately access the abstracts of cited articles online.

This section includes Coding system, PMID and DOI, Style for journal references, Style for book references, and Format for references (Examples). Specific requirements are as follows:

(1) Coding system
The author should number the references in Arabic numerals according to the citation order in the text. The reference numbers will be superscripted in square brackets at the end of the sentence with the citation content or after the cited author's name, with no spaces. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]." If references are cited directly in the text, they should be included with the direct citation content within the text; for example, "From references[19,22-24], we know that...". Before submitting your manuscript, please ensure that the order of citations inthe text is the same as in the references section, and also ensure the spelling accuracy of the authors' names. Do not list the same citation twice (i.e. with two different numbers).

(2) PMID and DOI
Please provide the PMID number, which is the serial number that roots the abstract for that publication into the PubMed index, and the CrossRef DOI® (Digital Object Identifier) name, which is a unique string created to identify a piece of scholarly content in the online environment for each reference in the References section. The PMID number can be found at http://www.ncbi.nlm.nih.gov/pubmed and the DOI name at http://www.crossref.org/SimpleTextQuery/. The numbers will be used in the electronic (E)-version of the manuscript.

(3) Style for journal references For authors' names, the name of the first author should be typed in bold letters; the family (sur)name of all authors should be typed with the first letter capitalized, followed by their abbreviated first and middle initials. For example, an article by Lian-Sheng Ma and Bo-Rong Pan will be written as Ma LS and Pan BR. The title of the cited article will be written in sentence case. The journal title will be written in its abbreviated form (as shown in PubMed) in italics and followed by the article publication information (not italicized), including the publication date, volume number (in bold numbers), and start page through end page (separated by a hyphen, with no space). The PMID and DOI will follow this information and be written as "[PMID: 11819634 DOI: 10.3748/wjg.13.5396]"

For the authors' names, the name of the first author should be typed in bold letters. The family (sur)name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. The book title will follow the authors' names and not be italicized. The publication information will follow, written as punctuated here: publication number, publication place: publication press, year: start page-end page.
Baishideng uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references. Journal name abbreviations should be those found in the National Center for Biotechnology Information databases.

Anexo III. Carta de Submissão

---------- Forwarded message ---------

De: BPG Submission <submission@wjgnet.com>

Date: qui**., 9 de mai. de 2024 às 17:25**

Subject: World Journal of Gastroenterology - Receipt of Manuscript No.: 96575

To: <flavia.feier@gmail.com>

Cc: <mel_melere@hotmail.com>, <neumann.litx@gmail.com>, <ankalil@terra.com.br>, <luizasnader@gmail.com>, <carolina.soaresdasilva@yahoo.com.br>, <marcofarina2018@gmail.com>, <gcoral@terra.com.br>, <gbobsin@gmail.com>, <j-montagner@hotmail.com>, <targaferreira@gmail.com>

Dear Dr. Feier,

Thank you for submitting your manuscript for consideration of publication in World Journal of Gastroenterology, a peer reviewed, online, and open access journal. Your manuscript has been successfully submitted online. Our editor will verify whether your manuscript meets the requirements for Baishideng's formal receipt of a manuscript, including verification of whether the content of your manuscript falls within the scope of the journal, whether the basic information on the authors is correct (including the names, E-mail addresses, and ORCID information of all authors), and whether your manuscript document and the documents on ethics and academic rules and norms for the column that the manuscript belongs to are correctly provided. If any of the above items do not meet the requirements, your manuscript will be returned to the authors for revision and resubmission. If the manuscript is not resubmitted within 7 d and no reply clearly stating the reason for missing the resubmission deadline is received from the authors, the manuscript will be rejected.

The details of your submission are listed below:
Journal: World Journal of Gastroenterology
Manuscript No.: 96575
Manuscript Source: Invited Manuscript
Title: Human leukocyte antigen compatibility and incidence of donor-specific antibodies in pediatric liver transplant recipients
Column: Observational Study
Authors: Melina Utz-Melere, Flavia Heinz Feier, Jorge Neumann, Antonio Nocchi Kalil, Juliana Montagner, Luiza Nader, Carolina Soares da Silva, Marco Farina, Gabriela Coral, Guilherme Bobsin and Cristina Targa

Received Date: 2024-05-09

Please mention the above manuscript No. 96575 in all future correspondence or when emailing the office for questions.

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First Decision of the Manuscript

If your manuscript meets the requirements, the F6Publishing artificial intelligence system will search the Baishideng database to identify relevant editorial board members and peer reviewers based on the keywords you provided, and then invite 5 of the journal's editorial board members and 15 external peer reviewers to perform a single-blind peer review of the manuscript. After your manuscript is peer reviewed by at least two peer-reviewer(s) and/or editorial board member(s), the first decision will be made, and an evaluation report of the first decision will be provided to all the authors.

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For an invited manuscript, once the "Invited Manuscript ID" is confirmed by our editor, the Article Processing Charge (APC) of the manuscript will be fully waived. For an unsolicited manuscript, there are three levels of APC: (1) Standard APC for general manuscript; (2) 10% discounted APC for manuscripts contributed by young scholar under 45 years-old; and (3) 20% discounted APC for manuscripts whose scientific significance is rated as AA or AB or BB (A: Excellent; B: Good) by peer reviewers. For detailed information on the APC, please visit our website at: https://www.wjgnet.com/bpg/gerinfo/242.

Other Resources

The Reference Citation Analysis (RCA) Scholar Registration is an online tool for fair and objective assessment of your academic influence, based upon your own articles. Please find a useful example here: https://referencecitationanalysis.com/00000002. In addition, the RCA literature database provides the following tools for personal use, free of charge:

Find an Article: https://referencecitationanalysis.com/
Find a Category: https://referencecitationanalysis.com/Categories
Find a Journal: https://referencecitationanalysis.com/SearchJournal
Find a Scholar: https://referencecitationanalysis.com/searchscholar
Find an Academic Assistant: https://referencecitationanalysis.com/searchacademicassistant
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Anexo IV. Normas para publicação – Artigo 2

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Liver Transplantation

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AASLD Abbreviations
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Online Manuscript Submission, Forms, and Licenses

Note: These instructions comply with those formulated by the International Committee of Medical Journal Editors (ICMJE). For further details, authors should consult the following article: International Committee of Medical Journal Editors. "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" *New Engl J Med* 1997, **336**:309–315. The complete document appears at http://www.icmje.org. Manuscripts that do not comply with these Instructions cannot be considered for publication and will be sent back to the authors.

The Editorial Office is pleased to answer any questions you may have about preparing your manuscript in accordance with our guidelines.

Email: livertransplantation@aasld.org

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Prior to submitting, we recommend that you run your manuscript through the PaperPal Preflight service, which instantly checks your manuscript and helps you address the most common errors and omissions.

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SCOPE

Liver Transplantation publishes original clinical and laboratory-based research related to the fields of liver transplantation, liver surgery, and chronic liver disease in both humans and experimental models.

GENERAL INFORMATION

To submit your manuscript electronically and for complete instructions on how to do so, go

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JOURNAL POLICIES

Originality

The Editors require that each manuscript is an original contribution and that it has not been, and will not be, submitted elsewhere while it is under consideration for publication in *Liver Transplantation*. Editors may subject any manuscript submitted for consideration of publication in *Liver Transplantation* to plagiarism-detection software. Manuscripts dealing with material that has appeared or is in press, in brief or preliminary form in other publications will not be considered unless the prior publication is a meeting abstract reporting only summarized information and does not exceed one printed page. The ICMJE has provided details of what is and what is not duplicate or redundant publication. If you are in doubt (particularly in the case of material that you have posted on a web site), we ask you to proceed with your submission but to include a copy of the relevant previously published work or work under consideration by other journals. Authors must draw attention to any published work that concerns the same patients or subjects as the present paper in a covering letter with their article.

Liver Transplantation will consider for review articles previously available as preprints on non-commercial servers such as ArXiv, bioRxiv, etc. Authors may also post the submitted version of their manuscript to non-commercial servers at any time. Authors are requested to update any pre-publication versions with a link to the final published article.

Authorship

Liver Transplantation expects that each person listed as an author has participated sufficiently in the intellectual content, the analysis of data, and/or the writing of the manuscript to take public responsibility for it. Each author must have reviewed the manuscript, believes it represents valid work, and approves it for submission. Moreover, should the Editors request the data upon which the manuscript is based, the authors shall produce it. We ask all authors to confirm that they have met the criteria for authorship as established by the ICMJE, believe that the paper represents honest work, and are able to verify the validity of the results reported.

All persons designated as authors should qualify for authorship and all those who qualify should be listed. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. One or more authors should take

responsibility for the integrity of the work as a whole, from inception to published article. Authorship credit should be based only on 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data for the work; 2) drafting the work or revising it critically for important intellectual content; 3) final approval of the version to be published; and 4) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Conditions 1, 2, 3, and 4 must all be met. Acquisition of funding, the collection of data or general supervision of the research group, by themselves, do not justify authorship. All others who contributed to the work who are not authors should be named in the Acknowledgments section.

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For purposes of the submission process, it is assumed, that the corresponding author speaks for his or her coauthors and certifies that all listed authors participated meaningfully in the study and that they have seen and approved the final manuscript.

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Changes to Authorship

Authors are expected to consider carefully the list and order of authors before submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion, or rearrangement of author names in the authorship list should be made only before the manuscript has been accepted and only if approved by the journal Editor(s). To request such a change, the Editor(s) must receive the following from the corresponding author: (a) the reason for the change in author list and (b) written confirmation (email, letter) from all authors that they agree with the addition, removal, or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

Only in exceptional circumstances will the Editor(s) consider the addition, deletion, or

rearrangement of authors after the manuscript has been accepted. While the Editor(s) considers the request, publication of the manuscript will be suspended. If the manuscript has already been published in an online issue, any requests approved by the Editor(s) will result in a corrigendum.

English Language Assistance

Liver Transplantation publishes only in English. Authors lacking facility with English syntax should seek the appropriate editorial assistance prior to submitting their manuscript. Material requiring major editorial work might be returned without peer- review. To help reduce the possibility of such problems, we encourage such authors to consider using Wolters Kluwer Author Services*.

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Abbreviations, Nomenclature and Symbols

AASLD has compiled a list of abbreviations that are sufficiently common in the field as to not require them to be defined on first use, and the AMA Manual of Style also includes abbreviations that can be used without expansion. For all other abbreviations, do not abbreviate unless a term is used more than three times in the manuscript. In this case, the term should be spelled out the first time it is used in the text, followed by the abbreviation in parentheses, and then the abbreviation should be used for all other instanced in the text. Abbreviations used in figures and tables should be defined in each legend, so that figures and tables can stand alone as much as possible. All abbreviations introduced in the text and the figure and table legends should be included in the list of abbreviations for the title page footnote. Express temperatures as degrees Celsius and other measurements in SI units.

Recently a multi-stakeholder effort developed a consensus on a change in nomenclature and diagnostic criteria for MASLD (formerly NAFLD) and NASH (formerly MASH). Manuscripts submitted to and published in *Liver Transplantation* should employ this revised terminology wherever scientifically appropriate. If the revised terminology is not used, a justification should be given within the paper.

Ethics

All articles dealing with original human or animal data must include a statement on ethics approval within the Methods section, not in the supplementary material.

Research involving human subjects submitted to ***Liver Transplantation*** must abide by the Ethical Principles for Medical Research Involving Human Subjects outlined in the **2013 Declaration of Helsinki**, abide by the **2018 Declaration of Istanbul**, and be approved by the appropriate ethics and/or institutional review committee(s).

A sentence affirming that A) all research was conducted in accordance with both the Declarations of Helsinki and Istanbul, B) all research was approved by the appropriate ethics and/or institutional review committee(s), and C) written consent was given in writing by all subjects must be included within the Methods section of the manuscript. Please include the full name and institution of the review committee, in addition to the approval number, where applicable. If doubt exists whether the research was conducted in accordance with the Declarations of Helsinki and Istanbul, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study. Studies exempt from Institutional Review Board approval must note this exemption in their methods section. All waivers of informed consent must be noted in the methods section. Papers without such explicit statements will be returned without review.

This sentence could read, for example:

Ethical approval for this study (Ethical Committee N° NAC 207) was provided by the Ethical Committee NAC of Geneva University Hospitals, Geneva, Switzerland on 12 February 2015. All research was conducted in accordance with both the Declarations of Helsinki and Istanbul. Written consent was given in writing by all subjects.

For experiments involving animals you must state the care of animal and licensing guidelines under which the study was performed and report these in accordance with the ARRIVE (Animals in Research: Reporting In Vivo Experiments) statement If ethics clearance was not necessary, or if there was any deviation from these standard ethical requests, please state why it was not required. Please note that the Editors may ask you to provide evidence of ethical approval. Please state if you have approval from a National Drug Agency (or similar) and provide details; this can be particularly useful when discussing the use of unlicensed drugs.

Informed Consent and Patient Details

The protection of a patient's right to privacy is essential. Studies on patients or volunteers require ethics committee approval and informed consent, which should be documented in the Methods section of your paper. Appropriate consents, permissions and releases must be obtained where an author wishes to include case details or other personal information or images of patients and any other individuals. Written consents must be retained by the author and copies of the consents or evidence that such consents have been obtained must be

provided to *Liver Transplantation* on request. Unless you have written permission from the patient (or, where applicable, the next of kin), the personal details of any patient included in any part of the article and in any supplementary materials (including all illustrations and videos) must be removed before submission.

Registration of Research

Liver Transplantation endorses the recommendations of the ICMJE regarding the registration of clinical trials. Trials must be registered in a registry that is a primary register of the World Health Organization International Clinical Trials Registry Platform (WHO ICTRP) or in ClinicalTrials.gov, which is a data provider to the WHO ICTRP. The trial registration number must be included on the title page of any manuscript reporting a registered clinical trial. Trials should be registered by the time of first participant consent.

Reporting Guidelines

Compliance with the relevant reporting guideline is mandatory for submission of the following guidelines. You need to: 1. Submit a completed checklist, indicating the page numbers where compliance was achieved 2. Mention in your methods section that the research is being reported in line with the relevant guideline which should be named and cited.

Randomized Controlled Trials
All randomized controlled trials submitted for publication in *Liver Transplantation* should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow chart and ensure that all elements in the CONSORT checklist are covered. A copy of the CONSORT checklist must be uploaded as supplemental material. Refer to the CONSORT statement website at http://www.consort-statement.org for more information. Manuscripts that fail to comply with the CONSORT guidelines or do not include the CONSORT checklist at the time of submission will not be reviewed for publication. All clinical trials should be registered in order to be published in *Liver Transplantation*.

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Systematic reviews should be reported in accordance PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Guidelines (http://www.prisma-statement.org/) and must include the flow diagram as a figure and the checklist as supplemental material (http://www.prisma-statement.org/2.1.2%20-%20PRISMA%202009%20Checklist.pdf).

Cohort, Case-control and Cross-sectional studies
Cohort, Case-control and Cross-sectional studies should all be compliant with the STROBE criteria (Strengthening the reporting of observational studies in epidemiology):http://www.strobe-statement.org/index.php?id=available-checklists - each study type has its own checklist which should be uploaded as a supplemental file.

Diagnostic, Quality Improvement and Qualitative studies
Diagnostic studies should be reported in accordance with the STARD statement criteria (Standards for the Reporting of Diagnostic accuracy studies) flow diagram and checklist please see (http://www.stard-statement.org/). Quality Improvement studies should comply with the Standards for Quality Improvement Reporting Excellence (SQUIRE) criteria: http://squire-statement.org. Qualitative studies require the Consolidated criteria for Reporting Qualitative Research (COREQ) checklist:http://intqhc.oxfordjournals.org/content/19/6/349.long.

Health Economic Evaluation
Health Economic Evaluation studies should conform to the CHEERS statement: http://www.bmj.com/content/346/bmj.f1049.pdf%2Bhtml.

Tumor Marker Prognostic study
Tumor Marker Prognostic studies should be reported in accordance with the REMARK criteria: http://www.equator-network.org/resource-centre/library-of-health-research-reporting/reporting-guidelines/remark.

Before and After studies
Before and After studies measuring particular characteristics of a population or group of individuals at the end of an event or intervention, compares them with those characteristics before the event or intervention, and then gauges the effects of the event or intervention. These studies should conform to the STROBE statement: http://www.strobe-statement.org/index.php?id=available-checklists.

Genetic Association studies
Genetic Association studies must comply with Strengthening the Reporting of Genetic Association Studies (STREGA), an extension of the STROBE statement: http://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1000022.

Experimental Animal studies
Animal studies must be reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments) and must include the checklist as supplemental material. An example of a completed checklist can be found at http://www.nc3rs.org.uk/page.asp?id=1357. (The example checklist is based on an original publication by Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8(6): e1000412. doi:10.1371/journal.pbio.1000412). The institutional protocol number should be included at the end of the abstract of the article.

IDEAL framework
Liver Transplantation welcomes studies reporting in line with the IDEAL framework for interventional therapies: http://www.ideal-collaboration.net.

Qualitative surveys
Qualitative surveys should be reported in accordance with the following criteria: SRQR Guidelines http://www.equator-network.org/reporting-guidelines/srqr/ For synthesis of qualitative research: http://www.equator-network.org/reporting-guidelines/entreq/ Interviews and Focus Groups - COREQ - http://www.equator-network.org/reporting-guidelines/coreq/.

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In addition to completing the financial disclosure form authors must clearly state all relevant sources of funding concerning this article in the Financial support and sponsorship section and all relevant conflicts of interest in the Conflicts of interest section of the submitted manuscript.

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All figures submitted must be owned solely by the author(s). For figures not meeting this requirement, authors must obtain permission for the use of the figure by *Liver Transplantation.* Obtaining this permission is the sole responsibility of the author(s). Credit must be included in the figure legend for all figures being printed with permission.

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Product Information

Medications, materials, and devices must be identified by full non-proprietary name as well as brand name if appropriate. Include the manufacturer's name and location (city and state, or country). Place this information in parentheses in the text, not in a footnote.

Retractions
Wolters Kluwer is a member of the Committee on Publication Ethics (COPE), and also refers to the ICMJE advice on Scientific Misconduct, Expressions of Concern, and Retraction as well as on Overlapping Publications.

ARTICLE TYPES

Original Manuscripts are full-length reports of completed basic, clinical, and experimental research reported in accordance with the Reporting Guidelines stated above. The manuscript must be no more than 5,000 words (including references), include an abstract of no more than 275 words, and have no more than 50 references. Manuscripts should contain the following elements: Abstract, Introduction, Patients and Methods, Results, Discussion, Acknowledgments, and References. Manuscripts that are redundant or contain extraneous material will be returned for shortening, even if otherwise acceptable. Manuscripts should

contain no more than 8 figures and tables, with a maximum of 6 panels per figure. Authors are encouraged to publish additional material (for instance, large tables, figures with forest plots, data from subgroup analyses etc.) as Supplemental Digital Content.

Editorials are invited by the Editor(s) and should be no longer than 1,000 words (including references) and no more than 9 references.

Review Articles and Practice-Based Recommendations are on a variety of topics and may include AASLD practice guidances, in-depth scientific reviews, meeting reports, and comments on social policy. These articles should be no longer than 5,000 words (including references) unless approved by the Editor(s), and the references list should not be exhaustive. While most reviews are invited by the Editor(s), authors interested in contributing reviews are requested to first contact the Editorial Office (livertransplantation@aasld.org) with an outline of the proposed article.

Letters to the Editor raise issues of importance regarding an article recently published in *Liver Transplantation*. If accepted, the letter is sent to the authors of the article who have an opportunity to respond. Letters may be subjected to peer-review and undergo editing for clarity and brevity. They should be no longer than 500 words (including references) and include no more than 5 references and 1 figure. The title should reflect the content of the letter and not be titled the same as the targeted article. Letters to the Editor will be published online only.

Rapid Communications and Brief Reports Rapid Communications and Brief Reports are devoted to short, rapid communication of novel preliminary findings, innovative clinical or surgical techniques, important but limited new data, and rare or new presentations of disease. As the field of hepatology and liver transplantation continues to evolve rapidly, clinicians need to incorporate novel approaches to the complex problems they face daily. Rapid Communications and Brief Reports will provide the opportunity for our community to present early or preliminary results, innovative medical or surgical solutions, important refinement, or additions/changes to pre-existing knowledge, and report technical advances to the community at large. Case reports can be considered but only if they are novel and will potentially change clinical practice. Communications to this section should begin with "To the editor" and an abstract should not be included. The letter may include up to 3 figures and tables total, a limit of 5 references, and no more than 1,000 words. Submissions to this section must adhere to Liver Transplantation's Ethics and Patient's Privacy requirements stated above.

MANUSCRIPT PREPARATION AND FORMATTING INSTRUCTIONS

Initial Submission: To simplify the preparation and submission of manuscripts while minimizing the time spent on formatting requirements, ***Liver Transplantation* will allow initial submissions in a single Word document**. You may submit your text and artwork as one file. Your artwork can be located within the text. **There are no strict formatting requirements, but the manuscript must still include the following essential elements and remain within the word count**: title page, footnote page, abstract, introduction, methods, clinical trials, results, discussion, acknowledgments, references, permissions, abbreviations, and figure legends.

For revised manuscripts, pay careful attention to the formatting guidelines below.

Manuscripts must be written in clear, grammatical English (see English Language Assistance above). Manuscripts not conforming to journal format will be returned to authors for modification.

- Text should be 1.5-spaced.
- Body text size should be no smaller than 10 pt and no larger than 12 pt.
- Do not add line numbers as the system will generate those when the PDF is built.
- Brevity is appreciated. Authors should avoid repeating the same information in the Abstract, Introduction, and Discussion.

Title page, footnotes, abbreviations, and abstract pages must be included in the main body file. Please do not upload separate copies of these documents.

Acceptable document file types for text and tables include .DOC and .DOCX; do not submit a PDF.
Page 1:
Title Page. The following elements are required for every submission:

Title. Include a concise, descriptive, and informative title. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible. The title should not be a sentence. No proprietary or brand names for drugs or agents may be used in article titles. Please, include the study design in the title; for instance, “randomized controlled trial”, or “systematic review”.

Authors. Author names should be listed in the following order: the full first name, middle initials, last name of each author, medical and/or highest academic degrees and the name(s) of the department(s) and institution(s) to which the work should be attributed.

Manuscripts that include a group or consortium in the author list should also list group members whose contributions meet the authorship criteria defined above. To ensure proper indexing of these authors in MEDLINE, please refer to the guidelines from the ICMJE, noting the specific instruction that when a group name is included in the byline, there should be a note “clearly stating that the individual names are elsewhere in the paper and whether those names are authors or collaborators.”

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