ORLANDO GOMES DOS SANTOS NETO

FACTIBILIDADE E REPRODUTIBILIDADE DA RESTRIÇÃO DE DIFUSÃO DA RESSONÂNCIA MAGNÉTICA NO CÉREBRO DO FETO NA SÍNDROME DE TRANSFUSÃO FETO-FETAL

FEASIBILITY AND REPRODUCIBILITY OF DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING OF THE FETAL BRAIN IN TWIN-TWIN TRANSFUSION SYNDROME

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Orientador: Prof. Dr. Cleisson Fábio Andrioli Peralta

FEASIBILITY AND REPRODUCIBILITY OF DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING OF THE FETAL BRAIN IN TWIN-TWIN TRANSFUSION SYNDROME

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do Título de Mestre em Ciências da Saúde, área de concentração em Saúde Materna e Perinatal.

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Dedico este trabalho...

... aos meus pais,
pelo amor e apoio constantes em todos os momentos .

... à minha esposa,
exemplo de amor incondicional, companheira única e exemplar.

... e aos meus filhos,
razão e luz da minha vida...

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E, por fim, a todas as pacientes, pessoas essenciais, sem as quais não seria possível a concretização desse estudo.

Sumário

Símbolos, Siglas e Abreviaturas

AVPL (LAVP) – Ablação dos vasos placentários com laser (*Laser ablation of placental vessels)*

CAISM – Centro de Atenção Integral à Saúde da Mulher – Hospital da Mulher "Prof. Dr. José Aristodemo Pinotti"

CDA (ADC) – Coeficiente de difusão aparente *(Apparent diffusion coefficient*

CI – Intervalo de confiança (*Confidence interval*)

DNPM – Desenvolvimento neuropsicomotor

FCM/UNICAMP – Faculdade de Ciências Médicas/ Universidade Estadual de Campinas

PPT – Parto pré-termo

RD (DW) – Restrição de difusão *(Diffusion-weighted)*

RD-RNM (DW-MRI) – Restrição de difusão da ressonância nuclear magnética *(Diffusion-weighted magnetic resonance imaging)*

RNM (MRI) – Ressonância nuclear magnética *(Magnetic resonance imaging)*

RPPT – Rotura prematura pré-termo

STFF (TTTS) – Síndrome da transfusão feto-fetal *(Twin-twin transfusion syndrome)*

USG – Ultrassonografia *(Ultrasonography)*

Resumo

Introdução: As lesões neurológicas fetais são importante causa de morbimortalidade neonatal. Uma condição relativamente frequente que expõe os fetos a maior risco de lesão cerebral é a síndrome da transfusão feto-fetal grave (STFF). O tratamento de escolha para STFF consiste na ablação dos vasos placentários com laser (AVPL) e mesmo após a sua realização existe possibilidade de lesão neurológica fetal. A ultrassonografia (USG) é ainda o método de escolha para a avaliação de anormalidades encefálicas fetais, e a ressonância magnética (RNM) pode melhorar o diagnóstico em condições específicas. Entretanto, a USG e as imagens ponderadas T1 e T2 da RM não são apropriadas para a detecção de lesões isquêmicas. A Restrição de Difusão da ressonância magnética (RD-RNM) permite a detecção de eventos isquêmicos agudos no cérebro através da avaliação subjetiva e objetiva da difusão microscópica da água. Esta última, pode ser obtida por meio da medida do coeficiente de difusão aparente (CDA) e sua reprodutibilidade no cérebro fetal normal, em gestações únicas, foi recentemente demonstrada.

Objetivo: Testar a factibilidade e a reprodutibilidade da restrição de difusão da ressonância magnética nas avaliações do cérebro fetal em casos de síndrome de transfusão feto-fetal tratados com a ablação dos vasos placentários com laser.

Materiais e Métodos: Este estudo foi realizado no período de maio de 2011 a junho de 2012, após aprovação pelo Comitê de Ética em Pesquisa da FCM/UNICAMP. Pacientes com STFF grave realizaram uma ressonância magnética para a avaliação do cérebro dos fetos antes e depois da AVPL. Os dados foram analisados *off-line* em imagens axiais da restrição de difusão (RD) e em mapas do coeficiente de difusão aparente por dois radiologistas. A avaliação subjetiva foi descrita como a ausência ou a presença de restrição de difusão da água. A avaliação objetiva foi realizada através da colocação de regiões de interesse circulares de 20 mm^2 nas imagens de RD e em mapas de CDA. A concordância subjetiva inter observadores foi avaliada pelo coeficiente de correlação de Kappa. As medidas do CDA realizadas pelo mesmo observador e por observadores diferentes foram comparadas por meio de testes de Bland-Altman proporcionais.

Resultados: As análises foram realizadas em 23 pacientes (46 fetos) com STFF grave, antes e após a AVPL, totalizando noventa e dois exames RD-RNM. Destes, 62 (67%) foram considerados de boa qualidade para avaliação. A concordância entre os radiologistas foi de 100% tanto para a ausência (55/62 = 89%) quanto para a presença (7/62=11%) de restrição de difusão da água. Com relação às concordâncias intra e inter-observadores das medidas do CDA, o teste de Bland-Altman mostrou diferenças percentuais médias de menos de 1,5% e Intervalo de Confiança (IC) de 95% em menos de 18% em todos os locais avaliados.

Conclusões: Nossos dados sugerem que a avaliação RD-RNM do cérebro fetal em STFF é factível e reprodutível. Este método pode representar uma ferramenta útil para o aconselhamento dos pais sobre a evolução neurológica de seus filhos.

Summary

Introduction: The fetal neurological injuries are an important cause of neonatal morbidity and mortality. Severe twin-twin transfusion syndrome (TTTS) is a relatively frequent condition that exposes the fetuses to a higher risk of brain injury. The treatment of choice for TTTS consists in laser ablation of placental vessels (LAPV) and even after its completion there is the possibility of fetal neurologic injury. Ultrasonography (USG) is still the method of choice for evaluation of fetal brain abnormalities, and magnetic resonance imaging (MRI) can improve the diagnostic in specific conditions. However, ultrasonography and the T1 and T2 weighted images of MRI are not suitable for detection of ischemic lesions. Diffusion-weighted (DW) MRI enables the detection of acute hypoxic-ischemic events in the brain through subjective and objective evaluation of the microscopic diffusion of water. An objective evaluation consists of measuring the apparent diffusion coefficient (ADC): the reproducibility of this method in the normal fetal brain in singleton pregnancies was recently demonstrated.

Purpose: To test the feasibility and reproducibility of diffusion-weighted magnetic resonance imaging (DW-MRI) evaluations of fetal brains in cases of twin-twin transfusion syndrome treated with laser ablation of placental vessels.

Materials and Methods: This study was conducted from May 2011 to June 2012, after approval by the Institutional Review Board of FCM/UNICAMP. Patients with severe TTTS received an MRI scan for the evaluation of fetal brain before and after LAPV. Datasets were analyzed offline on axial DW images and apparent diffusion coefficient (ADC) maps by two radiologists. The subjective evaluation was described as the absence or presence of water diffusion restriction. The objective evaluation was performed by the placement of 20-mm^2 circular regions of interest on the DW image and ADC maps. Subjective inter-observer agreement was assessed by the Kappa correlation coefficient. ADC measurements performed by the same observer and by different observers were compared using proportionate Bland-Altman tests.

Results: Analyses were performed in 23 patients (46 fetuses) with severe TTTS before and after LAVP totaling 92 examinations RD-RM. Of these, 62 (67%) were of good quality for evaluation. The agreement between radiologists was 100% in the absent (55/62 = 89%) and in the presence (7/62 = 11%) of restricted diffusion of water. With respect to intra and inter-observer measurements of the ADC, the Bland-Altman plots showed average percentage differences of less than 1.5% and Confidence Interval (CI) of 95% in less than 18% in all regions evaluated.

Conclusions: Our data suggest that DW-MRI evaluation of the fetal brain in TTTS is feasible and reproducible. This method may represent a useful tool for counseling parents about the neurological outcome of their infants.

1. Introdução

As lesões neurológicas fetais são importante causa de morbimortalidade neonatal. Vários fatores maternos, como hemorragias; obstétricos, como prematuridade; e mesmo inerentes ao próprio concepto (malformações, aneuploidias e síndromes gênicas) podem causar danos no encéfalo fetal. As lesões geralmente decorrem de estados hipóxico-isquêmicos, infecções congênitas e malformações. Além disso, as doenças metabólicas hereditárias, especialmente as doenças mitocondriais, também fazem parte deste grupo.[1]

Dentre os principais fatores de risco para lesões hipóxico-isquêmicas encefálicas, as gestações gemelares monocoriônicas diamnióticas complicadas com a síndrome da transfusão feto-fetal (STFF) destacam-se pelo aumento significativo na incidência de danos neurológicos nos fetos por ela acometidos.[2]

A STFF ocorre com uma frequência de 10% a 30% nas gestações gemelares monocoriônicas diamnióticas.[3-6] É caracterizada pela passagem desbalanceada de sangue de um feto (doador) para o outro (receptor), através de anastomoses vasculares placentárias.[9-13]

Em geral, quando os casos de STFF são acompanhados de forma expectante, a taxa de óbito de pelo menos um gêmeo chega a 70%, com danos

neurológicos nos sobreviventes, decorrentes de fenômenos hipóxico-isquêmicos, ocorrendo em 25 a 100% dos casos.[14-17]

Segundo a classificação de Quintero[18,19], os casos de STFF são agrupados em cinco estágios, correlacionando-os com o prognóstico perinatal. No estágio I, há uma discrepância entre os tamanhos das bexigas fetais e entre a quantidade de líquido amniótico nas duas câmaras âmnicas (doador com maior bolsão de líquido amniótico menor do que 2 cm; receptor com maior bolsão de líquido amniótico maior do que 10 cm abaixo de 20 semanas ou maior do que 8 cm acima de 20 semanas de gestação). No estágio II, o feto doador fica com a bexiga permanentemente vazia e em anâmnio (*stuck twin*), enquanto o receptor apresenta bexiga distendida e polidrâmnio. No estágio III, começam as alterações Dopplervelocimétricas em um ou ambos os fetos (aumento de resistência no Doppler da artéria umbilical do doador; aumento no índice de pulsatilidade / ausência ou inversão de fluxo durante a contração atrial no ducto venoso do receptor). No estágio IV, o receptor desenvolve hidropisia. No estágio V, há óbito de um ou ambos os fetos. São considerados casos graves de STFF aqueles em estágios II, III, IV e V. Sem tratamento, as taxas de óbito de pelo menos um dos gêmeos nos estágios II, III e IV variam de 70% a 100%.[14,15,17]

As opções terapêuticas para a STFF são a amniodrenagem seriada, a septostomia e a ablação vascular placentária com laser (AVPL).

A amniodrenagem foi por muito tempo o tratamento de escolha para a doença e ainda vem sendo utilizada em muitos centros especializados em medicina fetal. Tem a vantagem de ser um procedimento tecnicamente fácil e

barato. Proporciona a diminuição do polidrâmnio e permite o prolongamento da gravidez, sem, no entanto, eliminar a causa da STFF. Os estudos mais recentes sobre o uso dessa técnica mostraram sobrevida de 47% a 91% de pelo menos um dos fetos, com a ocorrência de danos neurológicos nos sobreviventes em 22% a 55% dos casos.[20-22]

A septostomia tem sido abandonada pela maioria dos centros em decorrência de suas possíveis complicações, como a banda amniótica e o aprisionamento do cordão umbilical por entre as lâminas de âmnio. Um único estudo randomizado demonstrou não haver diferença significativa na sobrevida entre os casos tratados com septostomia e aqueles tratados com amniodrenagem seriada.[23]

Em 1990, De Lia et al.[24] descreveram uma técnica para oclusão dos vasos placentários com o uso do laser, por visibilização endoscópica dos mesmos na superfície placentária. Desde então este método tem sido estudado e comparado com os demais para o tratamento da STFF.[13,19,24-31]

Os estudos mais recentes sobre os resultados da AVPL demonstram sobrevida de 61 a 83% (pelo menos um neonato/lactente), com sequelas neurológicas clínicas em até 25% dos sobreviventes.[13,19,24-31]

O mais importante estudo randomizado comparando os resultados da amniodrenagem seriada com os da AVPL para tratamento da STFF foi apresentado por Senat et al. em 2004.[28] Neste trabalho, após randomização de 142 pacientes (72 para AVPL e 70 para amniodrenagem), foi demonstrado que os resultados obtidos com o uso do laser foram significativamente melhores do que aqueles observados com a amniodrenagem seriada (Sobrevida de pelo

menos um gêmeo: AVPL – 76% x Amniodrenagem – 56%; Leucomalácia periventricular: AVPL – 7% x Amniodrenagem – 35%; Idade gestacional (IG) ao parto: AVPL – 33 semanas x Amniodrenagem – 29 semanas).

Até o momento, a maioria dos autores concorda que a AVPL para STFF grave oferece risco mínimo às gestantes e que as complicações cirúrgicas graves são raras.[32-42] Em decorrência disso, universalmente, tem sido aceito que os benefícios de tais cirurgias em muito sobrepujam seus riscos. Dentre as intercorrências mais citadas como diretamente relacionadas ao procedimento encontram-se a rotura prematura pré-termo (RPPT) de membranas (9 - 12%), a corioamnionite (2 - 8%), o abortamento ou parto pré-termo (PPT) extremo (2 - 7%) e a perda de líquido amniótico para a cavidade peritoneal materna (2 - 7%).[13,19,24-42]

Como mencionado, o comprometimento no desenvolvimento neuropsicomotor (DNPM) pode ocorrer em até 25% dos neonatos sobreviventes da STFF grave tratada com a AVPL.[13,19,24-31] Os estudos mais importantes sobre o acompanhamento dessas crianças até a fase pré-escolar demonstraram alterações neurológicas graves em 6 a 12 % dos gêmeos.[7,43-46] A maioria dos trabalhos sugere não haver associação entre a gravidade das lesões neurológicas e o estágio da STTF segundo a classificação de Quintero et al.[18,19], nem tampouco diferenças entre fetos doadores e receptores quanto à incidência dessas lesões.[27,42-45]

Alterações no DNPM de crianças que passaram pela STFF podem ser atribuídas aos distúrbios hemodinâmicos e hematológicos pré-natais inerentes à fisiopatologia da doença, ser causadas pela morte de um dos gêmeos, ter

origem nas mudanças hemodinâmicas agudas por ocasião da AVPL e/ou estar associadas à prematuridade e baixo peso ao nascimento.[46-49]

Alguns estudos foram dedicados à avaliação ultrassonográfica transfontanelar de neonatos sobreviventes da STFF tratada por meio da AVPL, tendo como principal objetivo definir se os danos neurológicos tinham origem nos períodos pré (decorrentes da STFF e/ou do seu tratamento) ou pós-natal (decorrentes de fenômenos hipóxico-isquêmicos por causa da prematuridade). Dessa forma, Lopriore et al.[49] avaliaram 48 gêmeos nas primeiras 24 horas de vida, repetiram o exame em mais três ocasiões durante a primeira semana e, depois, uma vez por semana até a alta hospitalar. Relataram incidências de 14% de lesões cerebrais graves e 23% de lesões leves, tendo sido a maioria das graves encontradas logo após o nascimento (provavelmente originadasno período pré-natal).

Tendo como base os achados ecográficos descritos na literatura, as lesões cerebrais do neonato podem ser classificadas como hemorrágicas (infartos hemorrágicos periventriculares; hemorragias intraventriculares) ou isquêmicas da substância branca (leucomalácia periventricular). Outras lesões descritas são o infarto da artéria cerebral média, os cistos porencefálicos, os pseudocistos subependimários, a vasculopatia lentículo-estriada e a ventriculomegalia leve.[19,44,49,50] O exame ecográfico transfontanelar no período neonatal, ainda que de extrema importância, não esclarece em que momento da doença, durante o período pré-natal, o dano cerebral pode ter se instalado.[51]

A ressonância nuclear magnética (RNM) cerebral fetal, além de segura [52,53], tem se mostrado importante ferramenta diagnóstica, podendo

superar a ultrassonografia (USG) na caracterização de lesões parenquimatosas, destrutivas e distúrbios de migração neuronal. Jelin et al.[54] realizaram USG e RNM em 21 gestações monocoriônicas nas quais um dos fetos havia falecido, tendo tido como objetivo a avaliaçãode danos cerebrais no gêmeo sobrevivente. Enquanto a USG não demonstrou alterações, a RNM permitiu o diagnóstico de polimicrogiria, cistos na matriz germinativa, hemorragia intracraniana, ventriculomegalia e atraso no desenvolvimento de giros e sulcos em 33% dos fetos sobreviventes.

Dentre os recursos disponíveis na RNM, a possibilidade de avaliação da restrição de difusão de água nos tecidos tem contribuído sobremaneira para a identificação de processos isquêmicos agudos, principalmente no encéfalo do adulto. A avaliação da restrição de difusão pode ser subjetiva ou objetiva. A primeira contribui para a identificação de áreas suspeitas para isquemia, enquanto a segunda se apóia na realização de medidas que podem ser interpretadas à luz de intervalos de referência, confirmando ou descartando a possibilidade de fenômeno isquêmico. A avaliação objetiva é feita por meio da medida do coeficiente de difusão aparente de água(CDA).

O primeiro estudo sobre a avaliação da restrição de difusão de água e medida do CDA em cérebros de fetos data de 2003.[55] Neste, os autores avaliaram o CDA nas substâncias branca e cinzenta de 15 fetos aparentemente normais, demonstrando ser factível o exame. Posteriormente, dois trabalhos tiveram como objetivo a construção de intervalos de referência do CDA em diferentes regiões de encéfalos normais de fetos com 17 a 37 semanas.[56,57] E, recentemente, sua reprodutibilidade no cérebro fetal normal, em gestações

únicas, foi demonstrada por Boyer et al[58]. Como mencionado anteriormente e demonstrado por alguns autores, tais intervalos de referência tornaram-se particularmente importantes ao permitirem objetividade na avaliação de áreas supostamente isquêmicas no cérebro fetal.[59,60]

Diante da possibilidade de identificação e quantificação das alterações anatômicas e isquêmicas agudas cerebrais fetais por meio da RNM, seu uso em gestações monocoriônicas com STFF grave submetidas à AVPL poderia fornecer informações adicionais sobre as potenciais causas de danos neurológicos nesses fetos. Este conhecimento poderia contribuir para mais adequado aconselhamento dos pais quanto ao prognóstico neurológico dos gêmeos, bem como para possíveis melhorias nas técnicas de tratamento. Antes da execução do mesmo, como passo inicial, seria fundamental a avaliação dafactibilidade e reprodutibilidade do exame de RNM com seus diferentes recursos para este fim. A confirmação de que o método é factível e reprodutível permitiria seu uso no acompanhamento de outras condições associadas ao aumento de risco de danos neurológicos no feto.

2. Objetivo

Testar a factibilidade e a reprodutibilidade da restrição de difusão da ressonância magnética na avaliação do cérebro de fetos com síndrome de transfusão feto-fetal.

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Feasibility and reproducibility of diffusion-weighted magnetic resonance imaging of the fetal brain in twin-twin transfusion syndrome

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Abstract:

Purpose: To test the feasibility and reproducibility of diffusion-weighted magnetic resonance imaging (DW-MRI) evaluations of fetal brain incases of twin-twin transfusion syndrome (TTTS) treated with laser ablation of placental vessels (LAPV).

Materials and Methods: This study was conducted from May 2011 to June 2012, after approval by the Institutional Review Board of UNICAMP. Patients with severe TTTS received an MRI scan for the evaluation of fetal brain before and after LAPV. Datasets were analyzed offline on axial diffusion-weighted (DW) images and apparent diffusion coefficient (ADC) maps by two radiologists. The subjective evaluation was described as the absence or presence of water diffusion restriction. The objective evaluation was performed by the placement of 20-mm^2 circular regions of interest on the DW image and ADC maps. Subjective inter-observer agreement was assessed by the Kappa correlation coefficient. ADC measurements performed by the same observer and by different observers were compared using proportionate Bland-Altman tests.

Results: Ninety-two DW-MRI scans were performed. Sixty-two of them (67%) were considered to be of good quality. Agreement between the radiologists was 100% for the absence (55/62=89%) or presence of diffusion restriction of water. For both intra- and inter-observer agreement of ADC measurements, proportionate Bland-Altman test showed average percentage differences of less than 1.5% and 95% CI of less than 18% for all sites evaluated.

Conclusions: Our data demonstrate that DW-MRI evaluation of the fetal brain in TTTS is feasible and reproducible. This method may represent a useful tool for counseling parents about the neurological outcome of their infants.

Introduction

Ultrasonography (USG) is the method of choice for the assessment of fetal brain abnormalities, and magnetic resonance imaging (MRI) can improve the diagnosis undersome specific conditions (1-5). However, USG and T1- / T2-weighted MRI are not appropriate tools for the detection of acute ischemic fetal brain lesions. These injuries can result from abrupt episodes of hypotensionthat may be related to autoimmune or infectious diseases, cardiovascular abnormalities, monochorionic twinning and fetal interventions (6-10). Diffusion-weighed (DW) MRI allows the detection of acute ischemic events in the brain through subjective and objective evaluation of microscopic diffusion of water (11-13). The latter can be obtained by the measurement of the apparent diffusion coefficient (ADC) and its reproducibility for the normal fetal brain in singleton pregnancies was recently demonstrated (14-16).

Severe twin-twin transfusion syndrome (TTTS) is a relatively frequent condition that exposes the fetuses to a higher risk of brain injury (9,17-22). The hemodynamic instability between the twins, the treatment with the laser ablation of placental vessels (LAPV) and prematurity represent potential threats to the integrity of the fetal brain (23-25). Therefore, we developed a protocol to evaluate the relationship between fetal brain DW-MRI findings before and after LAPV, the results of neonatal transfontanellar ultrasound scans and the

neurological outcome of the infant. However, as a first step, the specific objective of the present study was to test the feasibility and reproducibility of DW-MRI evaluations of normal and hypoxic-ischemic twin brain parenchymas in severe cases of TTTS treated with LAPV.

Materials and Methods

This study was conducted from May 2011 to June 2012 at the State University of Campinas (UNICAMP) and at Vera Cruz Hospital, Campinas, São Paulo, Brazil. The protocol was approved by the Institutional Review Board of FCM/UNICAMP, and all patients who agreed to participate signed an informed consent form prior to enrollment.

Patient selection:

The inclusion criteria for this study were as follows: monochorionic diamniotic twin pregnancies with severe TTTS [stages II to IV, according to the classification by Quintero et al (26,27); no fetal malformations detected by USG prior to LAPV; a cervical length of 15 mm or greater [the 5^{th} percentile according to the methods of To et al. (28) before the procedure; and no maternal contraindications for the MRI study (metallic implants, pacemakers or claustrophobia). The exclusion criteria consisted of the detection of neonatal brain alterations by transfontanellar ultrasound scans performed during the first three days following birth (that were not identified prior to birth) and infant non-attendance at neurological follow-up examinations.

Laser ablation of placental vessels:

All LAPV procedures were performed by the same surgeon (CFAP, seven years of experience in fetoscopic laser surgeries) at Professor José Aristodemo Pinotti Hospital according to the following technique. First, the chorionic plate vessels of the placenta were mapped endoscopically through the amniotic cavity of the recipient fetus. The vascular equator (where most of the arterio-venous anastomoses are expected to be) was identified, and a line of ablation of the chorionic plate was created from one edge of the placenta to the other, including the arterio-venous anastomoses and vessels with unknown courses. Caution was taken to preserve the vessels originating from and returning to the same fetus. At the end of the photocoagulation process, polyhydramnios was drained through the fetoscopy sheath such that the deepest amniotic fluid pocket was less than 8 cm deep.

Magnetic resonance imaging:

All patients received an MRI scan the day before and the day after LAPV. Fetuses were identified during the first MRI examination as the donor or recipient, and the sites of their umbilical cord insertions on the chorionic plate of the placenta were used as landmarks to avoid mislabeling in further scans and at birth.

All MRI examinations were performed at Centro Radiológico Campinas (CRC), Vera Cruz Hospital using a 1.5-Tesla whole-body unit (Signa HDxt - GE Healthcare, Milwaukee - WI) with gradient switching capabilities of 33 mT/m in 276 microseconds (slew rate of 120 T/m/s). Patients fasted for threehoursprior to the scan, and no maternal or fetal sedation was used. The mother was kept in

a supine position, and apnea was only requested in specific situations, especially when at least one of the fetuses was in a breech position. An eight-channel phased-array surface cardiac coil was initially positioned over the lower maternal abdomen. First, T2-weighted sequences (single-shot fast spin-echo; echo time, 180 msec; relaxation time, 3000 msec; slice thickness, 5.0 mm; spacing, 0.0 mm; field of view, 380 × 380 mm; matrix, 320 × 224; partial Fourier factor, 0.5 NEX; and bandwidth, 62.5 Hz per pixel) were acquired for the localization and identification of each fetus as the donor or recipient. Depending on the position of the fetal skulls, the coil was repositioned to optimize the image quality for both twins.

Axial DW sequences of the fetal brains were acquired according to the following protocol: echo time, 95.5; relaxation time: 5100 msec; b-values: 0 and 1000 sec/mm^2; direction: all; echo planar image; NEX 4.00; bandwidth direction: right to left; matrix: 192 x 192; field of view: 380 x 380 mm; slice thickness: 4.0 mm; spacing: 0.0 mm. Fat suppression was achieved using a frequency selective radio frequency pulse. Each DW sequence was acquired over approximately 1 min 10 sec. Apparent diffusion coefficient maps were obtained using b-values of 0 and 1000 sec/mm^2.

All MRI scans were supervised by the same radiologist (GES), who decided which were the best datasets to be stored from each fetal evaluation. Subsequently, two neuroradiologists (MM and RCN, five and six years of experience with fetal neuroradiology, respectively) rated independently each dataset as appropriate or not for the ADC measurements. If more than one

dataset per fetal evaluation was considered to be of good quality by both operators, the first radiologist (GES) randomly selected one of them for further analysis. If none of the datasets available from one fetal evaluation was judged to be appropriate for the ADC measurements by both neuroradiologists, this evaluation was considered unsuccessful. In such way the feasibility of DW-MRI evaluation for the fetal brain was assessed.

The selected datasets were analyzed offline on axial DW images and ADC maps (Advantage Workstation, GE Healthcare, Milwaukee – WI) by MM and RCN, who were blinded to each other's evaluation, their own previous assessment of the same fetus, any information about the mother, the stage of the TTTS and the LAPV (if the dataset was acquired before or after the laser surgery), and the phenotypic particularities of each twin (if the fetus was the donor or recipient; Doppler parameters; vital status of the twin). To guarantee this confidentiality, GES was responsible for labeling each fetal skull and removing all significant information that could enable their identification. Next, the radiologist zoomed in on the images and the field of view was set to allow only the fetal brain to be analyzed in the cine mode. The interval between the evaluations of each fetal brain by both reuroradiologists was at least 15 days, and all images containeddifferent labels than those used to identify them in the previous evaluation. Only the radiologist who prepared the datasets for analysis was aware of each case and the respective evaluations of the two neuroradiologists.

Both subjective and objective assessments of the microscopic diffusion of water were performed. The subjective analysis consisted of the description of absence or presence of water diffusion restriction and its location (Figures 1 and 2). The objective evaluation was performed according to the placement of 20-mm^2 circular regions of interest (ROI) on the ADC maps at the following sites bilaterally: frontal (F), thalamic (T), temporo-parietal (TP), periventricular (PV) and cerebellar (C) (Figure 1).

Prior to the second MRI scan (after the LAPV), all patients underwent USG to assess the vital status of the fetuses. The radiologist who prepared the DW-MRI datasets for offline evaluation was the only person apprised of this information.

After LAPV, all patients were examined by USG every 15 days until delivery. At each evaluation, the fetal brain was scanned for anatomic alterations, which, if found, were described. After birth, surviving neonates underwent transfontanellar ultrasound scans until the first three days of life and every week thereafter until hospital discharge. All surviving infants were followed-up for neurological examination by a neurologist and a physiotherapist at least once until the age of 12 months. The Bayley Scales of Infant and Toddler Development were used to assess the neurodevelopmental status of the infant (29).

Statistical analysis:

Demographic and clinical characteristics of the mothers and neonates were described as medians and ranges for continuous variables and as absolute and relative frequencies for categorical data.

Inter-rater agreement for the subjective evaluation of fetal brain microscopic water diffusion (categorized as normal, abnormal in the whole brain or abnormal in a specific site of the brain) was assessed using Cohen's Kappa coefficients (30). Agreement was considered good if the Kappa value exceeded 0.59.

Due to the small number of fetuses with altered brains in the DW image and ADC maps, repeatability and reproducibility of the measurements were assessed only in fetuses considered to have normal brains. No separate analysis of repeatability and reproducibility was performed for datasets acquired before and after the LAPV, i.e., pre- and post-operative acquisitions were combined as if they were obtained from different fetuses.

Shapiro-Wilk tests were used to assess the distributions of the percentage differences between two measurements [(measurement 1 – measurement 2) / (average of measurements 1 and 2) x 100]. Proportionate Bland and Altman analyses were performed to determine the agreement between measurements performed in each side of the brain (for all sites evaluated: F, T, TP, PV and C) by the same operator, repeatability (variations in repeat measurements made by the same rater, or simply intra-observer variation) and reproducibility (defined in this study as variations in

measurements performed by different observers, or simply inter-observer variation) (31,32). Bias was defined as the average of the percentage differences between two measurements, and the limits of agreement were defined as 1.96 multiplied by the standard deviation of the mean percentage difference. Percentage differences between two measurements were further compared to zero using one-sided t-test. Differences were considered statistically significant if the P-value was less than 0.05.

To assess repeatability, if no discrepancy was found between measurements performed in each side of the brain (for each of the abovementioned sites), left and right ADC values from the first evaluation were combined for comparison with the left and right measurements performed in the second assessment. If a difference between sides was detected, left and right ADC values of the first and second evaluations were compared separately.

For the assessment of reproducibility, the average of the first and second evaluations of both operators was compared.

The data were analyzed using the statistical software SPSS for Macintosh 21.0 (IBM Corp., Chicago, Il, USA) and Excel for Macintosh 2011 (Microsoft Corp., Redmond, WA, USA).

Results

Twenty-three patients (46 twins) met the entry criteria for the study. The median (range) maternal and gestational ages at LAPV and the median (range) gestational age at birth were 29 years (18-39), 22 weeks (18-26) and 32 weeks

(21-37), respectively. Eight patients (35%) were treated at stage II of TTTS, 11 patients (48%) were treated at stage III, and four (17%) were treated at stage IV.

The day following LAPV, 40 of the 46 twins (87%) remained alive. The overall survival rate, survival of at least one twin and survival of both twins at the time of hospital discharge were 57% (26/46), 70% (16/23) and 43% (10/23), respectively.

Ninety-two DW-MRI scans were performed (two evaluations per fetus). From each evaluation, the best dataset was stored for further offline analysis. Sixty-two of the 92 datasets (67%) were considered by both neuroradiologists to be of sufficient quality for the assessment of the microscopic diffusion of water.

Agreement between the radiologists was 100% for the absence (55/62=89%) or presence of diffusion restriction of water and the corresponding sites (7/62=11%: whole brain in six dead fetuses; focal occipital lesion in one fetus); therefore, Cohen's Kappa coefficient for this analysis was 1.0.

In fetuses without diffusion restriction of water (n=55), for both operators, percentage differences between ADC measurements performed in each side of the brain and between the first and second assessments (repeatability) for all sites evaluated (F, T, TP, PV and C) were not significantly different from zero (bias and 95% CI for these comparisons are presented in table 1 and figure 3). Likewise, the mean percentage differences between the averages of the first and second measurements performed by the two operators (reproducibility) for each site of the brain were not significantly different from zero (bias and 95% CI for these comparisons are presented in table 2 and figure 4).

All fetuses with normal ADC measurements demonstrated normal subsequent brain ultrasound evaluations until delivery. All neonates born alive demonstrated normal transfontanellar ultrasound scans until the first three days of life. None of the infants discharged alive from the hospital (n=26) presented with severe neurological compromise, although 12 (46%) were not considered competent in all categories of the Bayley Scales of Infant and Toddler Development.

Discussion

This study demonstrated that DW-MRI evaluation of the fetal brain in cases of severe TTTS treated with LAPV is feasible and reproducible when performed by experienced operators.

Several authors have described the use of DW-MRI for the evaluation of normal and abnormal fetal brains (12-15). More recently, Boyer et al. (16) have demonstrated that the measurement of the ADC was reproducible for the normal fetal brain in singleton pregnancies.

Of the various conditions associated with an increased risk of fetal brain damage, severe TTTS deserves special consideration. Twin-twin transfusion syndrome occurs in approximately 15% of monochorionic twin pregnancies. The pathophysiology itself, which is characterized by an unbalanced blood flow between twins mainly due to arteriovenous anastomosis, predisposes the fetuses to brain injuries (17-20). In addition, LAPV may worsen or be the main cause of brain lesions due to the abrupt hemodynamic changes imposed on the fetuses during the coagulation process or due to accidental bleeding (23-25). A

detailed evaluation of the fetal brain could help physicians counsel parents about the possibilities of neurodevelopmental compromise in their infant. As a first step before performing DW-MRI evaluation of the fetal brain in this disease, the feasibility and reproducibility of the aforementioned method should be tested.

Because the most common presentation of TTTS is the oligo-polyhydramnios sequence, we did not take into consideration cases with the isolated twin anemia-polycytemia sequence. Furthermore, evaluation of the fetal brain by ultrasound and/or by MRI is generally more time-consuming and difficult for a multiple pregnancy than for singleton pregnancies. Without maternal sedation, MRI evaluation of the fetus in a polyhydramnious sac can often be a challenging task. Moreover, because not all twins survive LAPV, this approach enables the assessment of the reproducibility of the evaluation of normal and hypoxic-ischemic fetal brain parenchyma. Despite the efforts made during the acquisition process, some images in the current study were considered inadequate for the evaluation of water diffusion restriction. Most inappropriate datasets were obtained during the evaluation of the recipient twin prior to LAPV. Maternal sedation could have helped to overcome this difficulty, but sedation was not an option in our protocol.

Because most of the twins assessed in the present study were alive after LAPV, we did not have a sufficient number of cases with hypoxic-ischemic brain injuries to enable the evaluation of the intra and inter-observer reliability of the objective evaluation of water diffusion restriction in this condition. Although the agreement between the two observers in identifying poorly perfused tissues was

high, the reproducibility of ADC measurements in hypoxic-ischemic twin brain parenchymas warrants further testing.

The observation that all of our twins with normal prenatal brains (by DW-MRI and USG) who were born alive had normal transfontanellar ultrasound scans reinforces the prenatal USG and MRI findings. It remains unclear why some of the twins who survived the neonatal period and underwent formal neurological evaluation during the first year of life were not considered competent in all categories evaluated of the Bayley scale; this observationmay be partially explained by alterations that might have occurred after the first postnatal transfontanellar scan. This finding suggests that alterations caused by prematurity may have played important roles in determining the neurological outcomes of these children. However, it should be noted that these alterations were not the main focus of our study.

In conclusion, our data demonstrate that DW-MRI evaluation of the fetal brain in TTTS is feasible and reproducible. Therefore, this method may represent a useful tool for counseling parents about the neurological outcome of their infants.

Summary statement: Diffusion-weighted magnetic resonance imaging evaluation of the fetal brain in cases of twin-twin transfusion syndrome treated with laser ablation of placental vessels is feasible and reproducible.

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Table 1. Proportionate Bland and Altman analyses for assessment of the agreement between apparent diffusion coefficient measurements performed in each side of the normal twin brain and between the first and second evaluations (repeatability) performed by two operators.

	Operator 1				Operator 2			
	n = 110		n = 110		n = 110		n = 110	
Site	Left/right Bias (95% CI)	p	Repeatability Bias (95% CI)	P	Left/right Bias (95% CI)	p	Repeatability Bias (95% CI)	p
F	0.450 (-11.7 to 12.6)	0.448	-1.197 (-14.6 to 12.2)	0.069	0.240 (-11.7 to 12.2)	0.807	-0.298 (-12.7 to 12.1)	0.636
T	-0.009 (-9.32 to 9.30)	0.818	-0.794 (-11.5 to 9.89)	0.100	-0.459 (-10.5 to 9.6)	0.416	0.829 (-10.4 to 12.1)	0.173
TP	0.054 (-16.5 to 16.6)	0.947	-1.190 (-18.1 to 15.7)	0.151	-0.352 (-16.0 to 15.4)	0.658	-0.104 (-16.4 to 16.2)	0.896
PV	0.214 (-12.9 to 13.4)	0.739	-0.104 (-17.3 to 17.1)	0.901	-0.141 (-11.7 to 11.4)	0.762	-0.580 (-13.7 to 12.5)	0.375
C	-0.930 (-11.2 to 9.3)	0.065	-0.938 (-11.7 to 9.79)	0.075	0.179 (-13 to 13.4)	0.781	-0.709 (-10.8 to 10.1)	0.179

n: number of compared measurements

Bias: average of percentage differences between two measurements.

95% CI: 95% confidence interval (limits of agreement)

p: p-value obtained by one-sample t-test (percentage differences of measurements compared to zero)

F: frontal; T: thalamic; TP: temporo-parietal; PV: periventricular; C: cerebellar

Table 2. Proportionate Bland and Altman analyses for assessment of the agreement between the averages of the first and second apparent diffusion coefficient measurements of normal twin brains performed by the two operators (reproducibility) for each site.

Site	Operator 1 n = 110 A: Average (SD) of 1st and 2nd measurements	Operator 2 n = 110 B: Average (SD) of 1st and 2nd measurements	C: Average of A and B (SD) n = 110	Reproducibility Bias (95% CI) n = 110	p
F	0.00139 (0.000183)	0.00138 (0.000189)	0.00139(0.000180)	1,240 (-12.7 to 15.2)	0.07
T	0.00128 (0.000195)	0.00127 (0.000171)	0.00128(0.000178)	0.733 (-13.9 to 15.4)	0.31
TP	0.00140 (0.000174)	0.00140 (0.000159)	0.00140(0.00157)	0.900 (-13.8 to 15.6)	0.67
PV	0.00129 (0.000171)	0.00130 (0.000164)	0.00130(0.000157)	-0.814 (-18.8 to 17.1)	0.35
C	0.00140 (0.000170)	0.00140 (0.000181)	0.00140 (0.000170)	-0.045 (-12.0 to 11.9)	0.94

Average (SD): average (standard deviation) of two measurements of apparent diffusion coefficient, expressed in mm^2/s

n: number of compared measurements

Reproducibility: C (Bias) + 1.96 SD of C (95% confidence interval/limits of agreement of C)

p: p-value obtained by one-sample t-test (C compared to zero)

F: frontal; T: thalamic; TP: temporo-parietal; PV: periventricular; C: cerebellar

Figure 1. Diffusion-weighted image (left) and apparent diffusion coefficient map (right) of normal fetal brain and the sites used for the measurements ofthe apparent diffusion coefficient: frontal (F), thalamic (T), temporo-parietal (TP), periventricular (PV) and cerebellar (C).

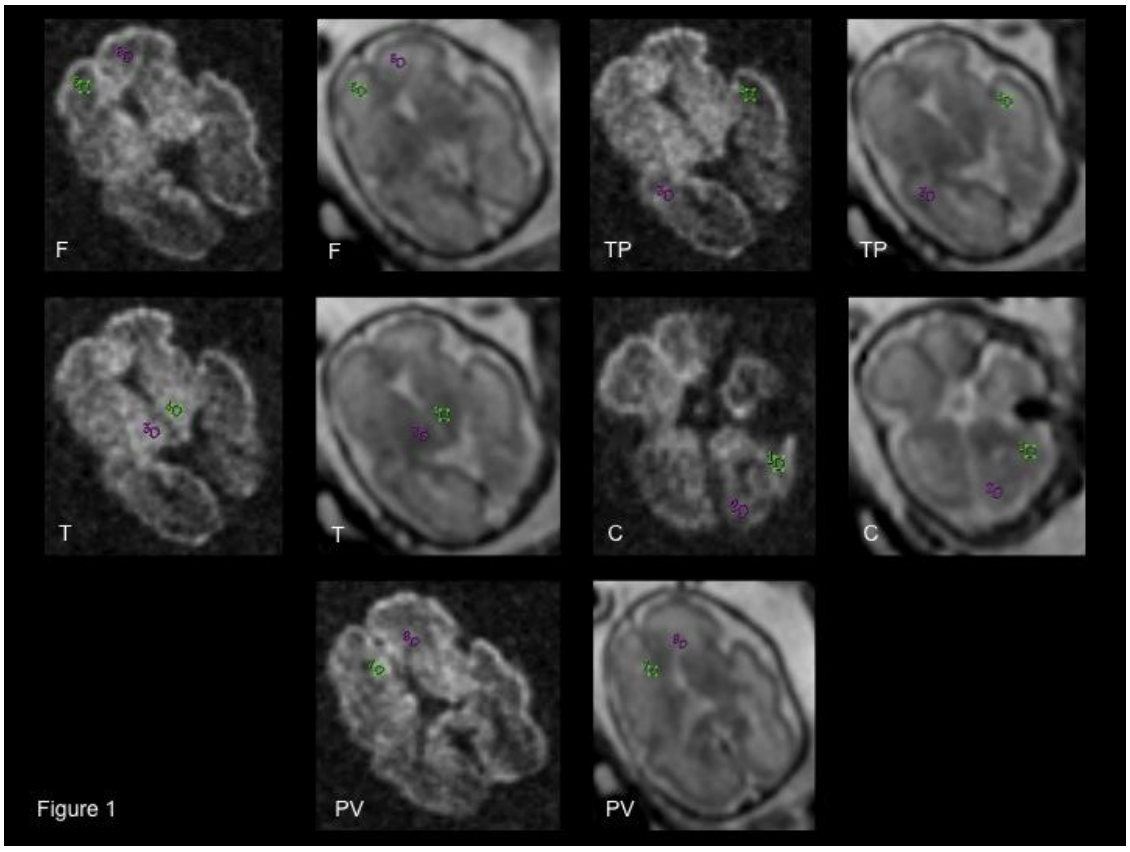

Figure 2. F: Diffusion-weighted image (left) and apparent diffusion coefficient grey and color maps (right) of one fetus with a focal hypoxic-ischemic lesion; W: Diffusion-weighted images (left) and apparent diffusion coefficient maps of a dead fetus after the laser ablation of placental vessels.

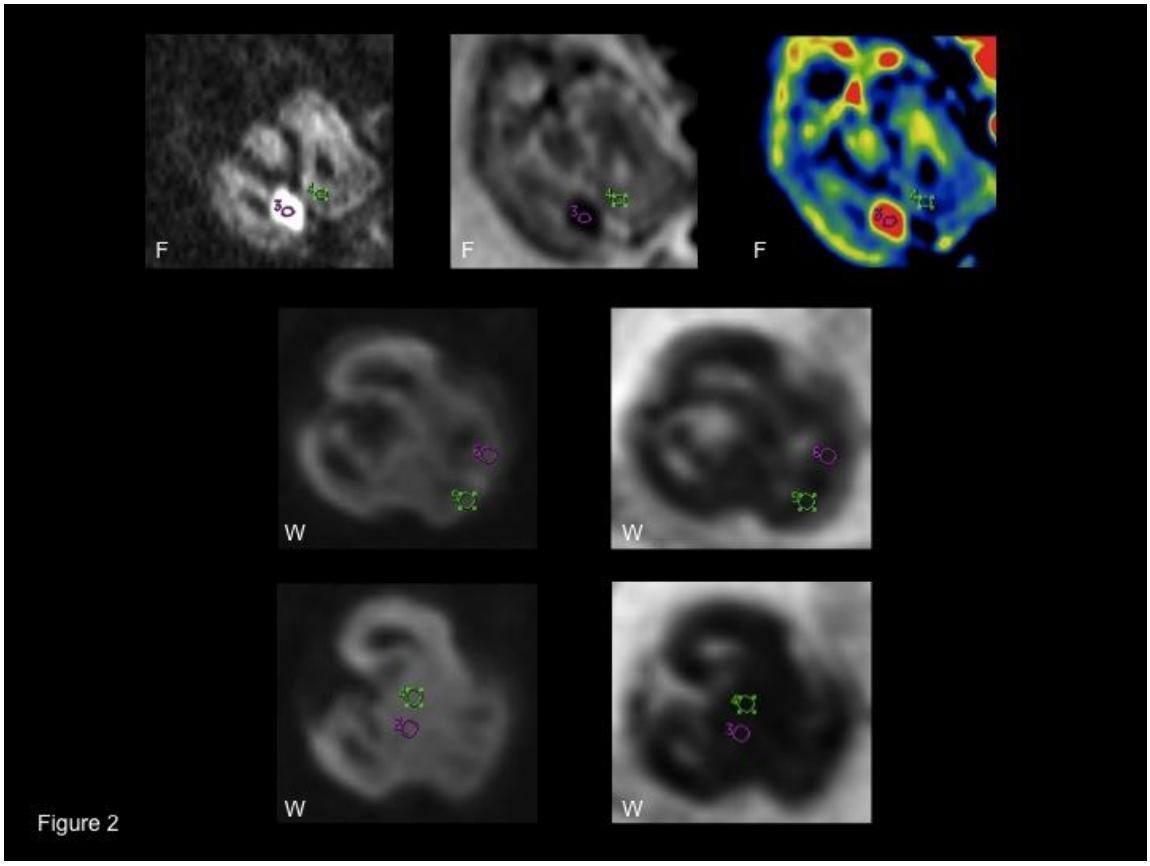

Figure 3. Bland and Altman plots for the analysis of repeatability (intra-observer variation) of apparent diffusion coefficient (ADC) measurements performed by observers one and two in different sites of the normal twin brain. The continuous line represents the bias (average percentage difference) and the dotted lines represent the 95% limits of agreement.

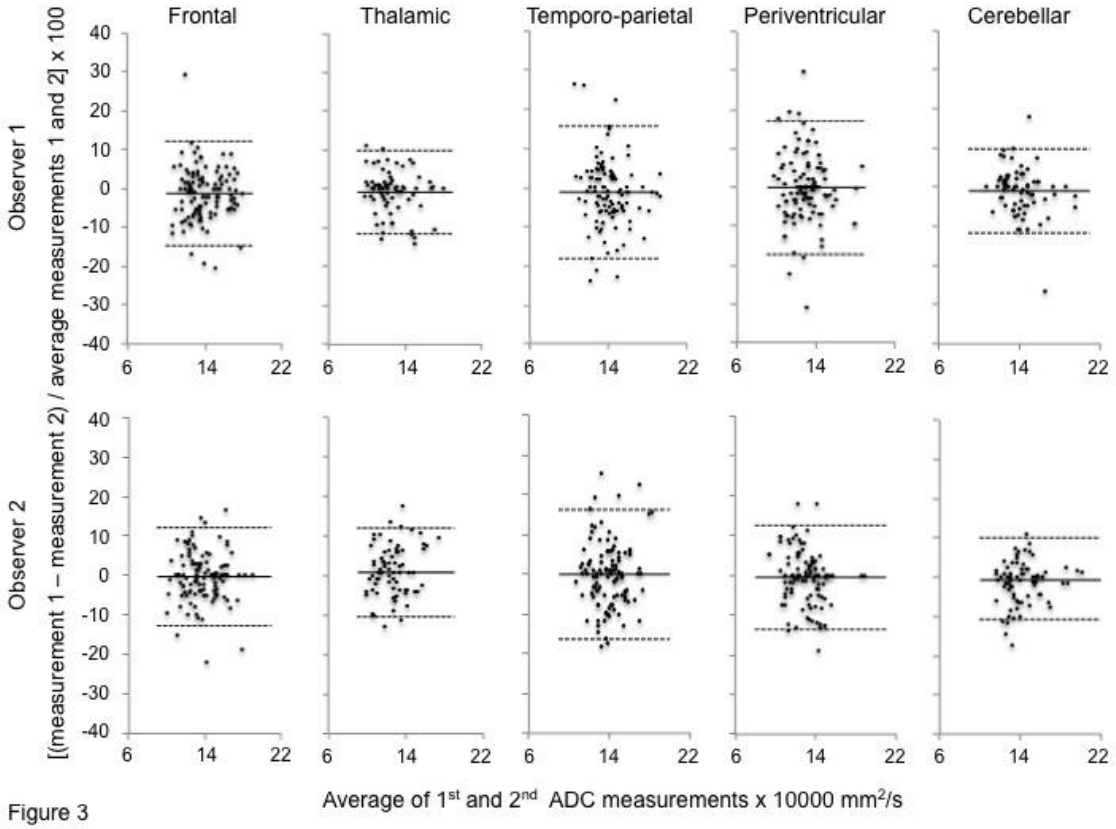

Figure 3

Figure 4. Bland and Altman plots in the analysis of reproducibility (inter-observer variation) of apparent diffusion coefficient (ADC) measurements performed by two observers in the different sites of the normal twin brain. The continuous line represents the bias (average percentage difference) and the dotted lines represent the 95% limits of agreement. Obs 1: average of the first and second ADC measurements performed by observer 1 in the intra-observer analysis; Obs 2: average of the first and second ADC measurements performed by observer 2 in the intra-observer analysis.

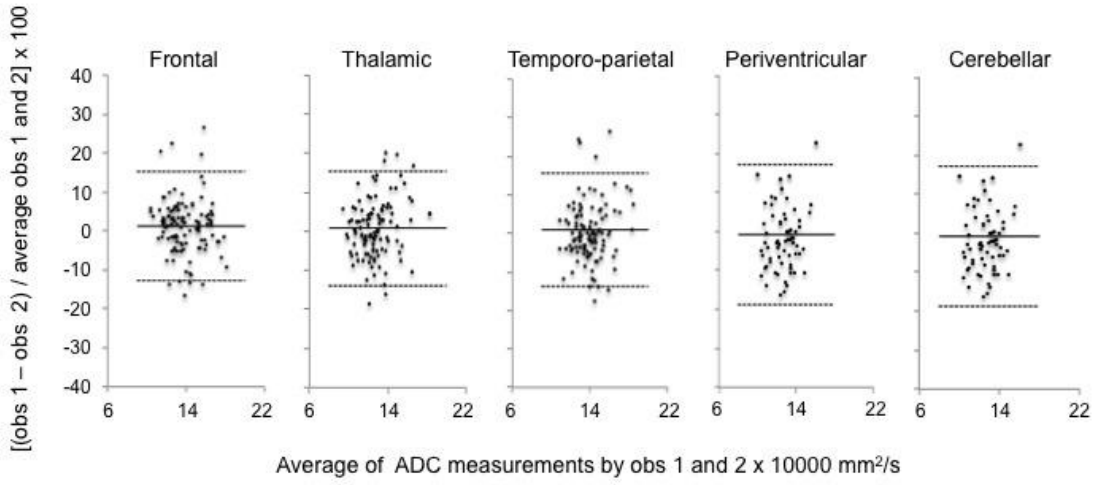

Figure 4

4. Conclusão

A avaliação da restrição de difusão de água cerebral por meio da ressonância magnética é factível em 2/3 dos gêmeos na síndrome de transfusão feto fetal. As medidas do coeficiente de difusão aparente da água em fetos sem lesão neurológica são reprodutíveis pelo mesmo operador e por operadores diferentes. E, observou-se uma alta concordância entre operadores na avaliação subjetiva da restrição de difusão.

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6. Anexo

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TÍTULO DO PROJETO: Avaliação do encéfalo do feto e do neonato em gestações gemelares com transfusão feto-fetal grave submetidas à ablação vascular placentária com laser

MÉDICO RESPONSÁVEL PELO PROJETO: Dr. Cleisson Fábio A. Peralta. CRM:79240; Telefone: (019) 93185008.

DADOS DA PACIENTE:

Nome:_____Sobrenome:_____

Data de nascimento: _____/_____/_____ Idade: _____

RG:_____ Registro no hospital:_____

Endereço:_____

DADOS DO RESPONSÁVEL LEGAL PELA PACIENTE:

Grau de parentesco: _____

Nome:_____Sobrenome:_____

Data de nascimento:_____/_____/_____Idade: _____

RG:_____Endereço:_____

_____

JUSTIFICATIVA E OBJETIVOS DA PESQUISA

A sua gravidez é uma gravidez de alto risco por ser de gêmeos e porque os dois fetos estão ligados em uma única placenta. Além disso, detectamos com o ultrassom que um dos fetos esta roubando sangue do outro através de alguns vasos na placenta que comunicam a circulação sanguínea dos dois bebês. Essa situação é chamada de transfusão feto-fetal. No seu caso, a transfusão feto-fetal está muito grave e, se nada for feito, a chance de que você perca a gravidez (aborto ou trabalho de parto muito prematuro) é muito alta, em torno de 90%. Para casos como o seu, oferecemos um tratamento chamado de ablação dos vasos placentários com laser. É um tratamento que tem o objetivo de fechar os vasos que comunicam as circulações de sangue dos dois fetos. É uma cirurgia pequena, feita com anestesia peridural (a mesma usada para cesareana), que dura mais ou menos 20 minutos, onde nós introduzimos uma minúscula câmera de vídeo no útero, achamos os vasos que estão causando o problema e cauterizamos (bloqueamos) estes vasos com o uso do laser. Sabemos que, com este tratamento, a chance de você levar pelo menos um dos bebês para casa passa para 75%. Mais uma vez reforçamos que, se o tratamento não for realizado, a chance de você levar pelo menos um dos bebês para casa é menor do que 10%. Para este tratamento, a mãe deve ficar internada por 3 dias, tomando medicação para inibir trabalho de parto e também antibiótico para prevenir infecção.

Depois disso, a mãe vai de alta e deverá vir ao hospital para visitas semanais até o nascimento dos bebês.

Neste estudo, que convidamos você a participar, oferecemos avaliações do cérebro dos fetos antes e depois do procedimento de laser, com a ultrassonografia e com a ressonância magnética. Também oferecemos avaliações do cérebro do bebê depois do nascimento, somente com a ultrassonografia, nos casos em que a gestante tiver o parto no CAISM. Os bebês das pacientes que tiverem parto em outros hospitais serão submetidos aos exames de ultrassonografia do cérebro neste outro serviço. A finalidade dessas avaliações é detectar se ocorre alguma alteração na anatomia do cérebro do feto, ou se já existe alguma alteração antes da realização do laser na placenta. Também desejamos saber se ocorre alguma alteração no cérebro do bebê após o nascimento, por causa da prematuridade. Nenhum desses exames (ultrassonografia e ressonância magnética) faz mal ao feto nem ao recém nascido.

Em relação à cirurgia do laser para a placenta, sabemos que é um procedimento seguro, mas que pode apresentar algumas complicações. As principais complicações são o sangramento na placenta, o óbito de um ou ambos os fetos e o aborto ou parto prematuro. Estas complicações podem ocorrem em até 25% dos casos tratados. No entanto, lembramos que se nada for feito no seu caso, a chance de que você perca a gravidez é por volta de 90%. Se a cirurgia de laser for realizada, o risco de perder a gravidez é muito menor que isso.

Sabemos também que, até o momento, nas cirurgias de laser que já foram realizadas, as mães não tiveram complicações graves.

ESCLARECIMENTOS DADOS PELO PESQUISADOR À PACIENTE SOBRE GARANTIAS DA PESQUISA:

1. O pesquisador (Cleisson Fábio A. Peralta) estará sempre disponível, a qualquer momento, para fornecer à paciente ou aos seus responsáveis informações sobre os procedimentos, os riscos e os benefícios relacionados à pesquisa, inclusive para tirar quaisquer dúvidas.

2. A paciente terá liberdade de retirar seu consentimento a qualquer momento e de deixar de participar do estudo, sem que isto traga prejuízo à continuidade da assistência.

3. Esta pesquisa será feita de forma confidencial, e com todo o sigilo e privacidade.

4. A paciente terá a disponibilidade de assistência no CAISM/HC-UNICAMP, por eventuais danos à saúde, decorrentes da pesquisa.

5. A participação nesta pesquisa não garante parto no CAISM. Em princípio, as gestantes que não forem da região para a qual o CAISM é referência deverão ter partos nos serviços de origem (local de onde foram encaminhadas).

CONSENTIMENTO LIVRE E ESCLARECIDO

Declaro que, após convenientemente esclarecido pelo pesquisador e ter entendido o que me foi explicado, consinto em participar do presente Protocolo de Pesquisa.

Campinas, _____de _____de 201___.

_____

Assinatura do sujeito da pesquisa ou responsável legal

_____

Assinatura do pesquisador

_____

Carimbo ou nome legível

Telefones para contato:

Pesquisadores:

Dr. Orlando Gomes dos Santos Neto – (081)99215096

Dr. Cleisson Fabio Andrioli Peralta - (019)93185008.

Comitê de ética em pesquisa: (019)35218936