JOÃO RENATO BENNINI JUNIOR

GASTROSQUISE: ULTRASSONOGRAFIA NA ESTIMATIVA DO PESO FETAL E PREDIÇÃO DE DESFECHOS PERINATAIS

GASTROSCHISIS: ULTRASONOGRAPHY FOR FETAL WEIGHT ESTIMATION AND PREDICTION OF PERINATAL OUTCOMES

CAMPINAS
2014

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Ciências Médicas

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GASTROSCHISIS: ULTRASONOGRAPHY FOR FETAL WEIGHT ESTIMATION AND PREDICTION OF PERINATAL OUTCOMES

Tese de Doutorado apresentada à Pós-Graduação em Tocoginecologia, da Faculdade de Ciências Médicas da Universidade Estadual de Campinas – UNICAMP para obtenção do Título de Doutor em Ciências da Saúde, área de concentração em Saúde Materna e Perinatal.

Thesis presented to the Programme of Obstetrics and Gynecology, Faculty of Medical Sciences, University of Campinas - UNICAMP for obtain the title Ph.D grade in concentration area of Maternal and Perinatal Health

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO JOÃO RENATO BENNINI JUNIOR E ORIENTADA PELO Prof. Dr. CLEISSON FÁBIO ANDRIOLI PERALTA

Assinatura do Orientador

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Campinas, 2014

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

B439g

Bennini Junior, João Renato, 1978-
Gastrosquise : ultrassonografia na estimativa do peso fetal e predição de desfechos perinatais / João Renato Bennini Junior. -- Campinas, SP : [s.n.], 2014.

Orientador : Cleisson Fábio Andrioli Peralta.
Coorientador : Ricardo Barini
Tese (Doutorado) - Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Gastrosquise. 2. Ultrassonografia. 3. Peso fetal. I. Peralta, Cleisson Fábio Andrioli. II. Barini, Ricardo, 1955-. III. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. IV. Título.

Informações para Biblioteca Digital

Título em outro idioma: Gastroschisis : ultrasonography for fetal weight estimation and prediction of perinatal outcomes
Palavras-chave em inglês:
Gastroschisis
Ultrasonography
Fetal weight
Área de concentração: Saúde Materna e Perinatal
Titulação: Doutor em Ciências da Saúde
Banca examinadora:
Cleisson Fábio Andrioli Peralta [Orientador]
Maria de Lourdes Brizot
Adolfo Wenjaw Liao
José Guilherme Cecatti
Maria Laura Costa do Nascimento
Data de defesa: 25-02-2014
Programa de Pós-Graduação: Tocoginecologia

BANCA EXAMINADORA DA TESE DE DOUTORADO

Aluno: JOÃO RENATO BENNINI JUNIOR

Orientador: Prof. Dr. CLEISSON FABIO ANDRIOLI PERALTA

Coorientador: Prof. Dr. RICARDO BARINI

Membros:

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Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

Data: 25/02/2014

Dedico este trabalho...

Aos meus filhos, Maria Cecília e João Felipe, como um incentivo aos estudos.

A todos que fazem do ensino e da pesquisa objetivos de vida.

Agradecimentos

À Carolina Maria...

A todos que contribuíram para a idealização, realização e finalização deste trabalho.

Sumário

Símbolos, Siglas e Abreviaturas

2DUS – *Two-dimensional ultrasound*

3DUS – *Three-dimensional ultrasound*

AC – *Abdominal circumference*

AFI – *Amniotic fluid index*

BPD – *Biparietal diameter*

CAISM – Centro de Atenção integral à Saúde da Mulher

CI – *Confidence interval*

DAI – Dilatação de alças intestinais intra-abdominais

DLD – *Delta luminal diameter of extra-abdominal intestinal loops*

DP – Desvio padrão

EFW – *Estimated fetal weight*

EPN – *Exclusive parenteral nutrition*

FDL – *Femur diaphysis length*

FGR – *Fetal growth restriction*

g – *gram*

GA – *Gestational age*

HC – *Head circumference*

IBD – *Intra-abdominal bowel dilation*

k – *Number of paired comparisons*

kg – *kilogram*

MSPE – *Mean sign percentage error*

NICU – *Neonatal intensive care unit*

OFD – *Occipito-frontal diameter*

p – Nível de significância estatística

RCF – Restrição de crescimento fetal

SD – *Standard deviation*

ThiV – *Fetal thigh volume*

UNICAMP – *State University of Campinas*

UNICAMP – Universidade Estadual de Campinas

US2D – Ultrassonografia bidimensional

US3D – Ultrassonografia tridimensional

VOCAL™ – *Virtual Organ Computer-aided Analysis*

Resumo

Introdução: A literatura é controversa sobre o papel da ultrassonografia pré-natal na predição de desfechos perinatais nos casos de gastrosquise. O peso ao nascimento é descrito como um importante fator prognóstico em neonatos com este tipo de malformação e estudos relatam que fórmulas ultrassonográficas criadas especificamente para esses casos apresentam melhor desempenho na estimativa do peso fetal, mas não há consenso sobre qual o melhor modelo de predição de peso a ser utilizado. **Objetivos:** Avaliar o papel de parâmetros ultrassonográficos pré-natais na predição de desfechos perinatais em casos de gastrosquise. Criar uma nova fórmula ultrassonográfica para estimativa de peso fetal que não utilize medidas abdominais e compará-la à outras fórmulas com parâmetros ultrassonográficos bidimensionais (US2D) e tridimensionais (US3D) quando aplicadas em fetos com gastrosquise. **Métodos:** Para avaliar o desempenho de parâmetros ultrassonográficos pré-natais na predição de desfechos perinatais foi realizado um estudo de coorte retrospectiva envolvendo fetos com o diagnóstico de gastrosquise isolada. Para criar e validar a nova fórmula US2D foram utilizados dados referentes à gestantes e fetos normais coletados em um estudo prévio publicado pelo nosso grupo. Foi realizado um estudo retrospectivo transversal envolvendo fetos com

gastrosquise, para comparar a nova fórmula US2D com diferentes fórmulas US2D e US3D já publicadas. Os sujeitos foram selecionados entre aqueles acompanhados na Divisão de Obstetrícia do CAISM / UNICAMP. O tamanho da amostra foi estimado em 56 pacientes para avaliar o desempenho de parâmetros ultrassonográficos pré-natais na predição de desfechos perinatais e 27 pacientes para comparar as fórmulas de estimativa de peso fetal. Os dados maternos, gestacionais e pós-natais foram descritos como freqüências relativas e absolutas, média e desvio padrão (DP), mediana e limites. A normalidade dos dados contínuos foi testada utilizando-se o teste de Kolmogorov–Smirnov. Testes *t* de amostras independentes e testes de qui-quadrado foram utilizados na comparação de dados contínuos e categóricos, respectivamente. Análises de regressão polinominal até o terceiro grau foram consideradas para criar a nova fórmula US2D de estimativa do peso fetal sem medidas abdominais. Cálculo do erro percentual médio ± DP, testes *t* unilaterais, testes *t* de amostras pareadas com correção de Bonferroni e testes de variância para amostras pareadas foram usados para avaliar e comparar a acurácia e precisão das fórmulas. A associação entre dados contínuos foi testada utilizando-se os coeficientes de correlação de Pearson ou Spearman e regressão logística univariada, conforme indicado. Valores de $p < 0,05$ foram considerados significativos. **Resultados:** Foram incluídos 44 casos de fetos com gastrosquise para avaliar a predição de desfechos perinatais por meio de parâmetros ultrassonográficos pré-natais. A presença de dilatação de alças intestinais intra-abdominais (DAI) fetais aumentou o risco de complicacões intestinais pós-natais e a presença de restrição de crescimento fetal (RCF) diminuiu o risco

deste mesmo desfecho. Nenhum outro parâmetro ultrassonográfico pré-natal pode significativamente predizer os desfechos perinatais avaliados. Foram usados os dados referentes aos mesmos grupos de fetos normais do estudo prévio para respectivamente criar (150 fetos) e validar (60 fetos) a nova fórmula US2D, que foi a seguinte: peso fetal estimado = 623.324 + 0.165 x DBP x CC x CF^2 (DP: 12,25%). Na comparação entre as fórmulas US2D e entre as fórmulas US2D e US3D, foram utilizados 44 e 28 fetos com gastrosquise isolada, respectivamente. Os melhores desempenhos na estimativa de peso dos fetos com gastrosquise foram obtidos com o modelo US2D proposto por Siemer e colaboradores e com os modelos US2D e US3D criados no estudo prévio desenvolvido pelo nosso grupo, não havendo diferença estatísiticamente significativa entre os mesmos. **Conclusões:** Em fetos com gastrosquise o achado de múltipla DAI associa-se a complicações intestinais pós-natais e a presença de RCF possui um efeito protetor para este mesmo desfecho. A nova fórmula US2D sem medidas abdominais não melhorou a estimativa do peso ao nascimento dos fetos com gastrosquise da nossa população em relação às outras fórmulas US2D e US3D avaliadas. Na nossa amostra de pacientes com gastrosquise o modelo US2D de Siemer e colaboradores e os modelos US2D e US3D criados no estudo prévio desenvolvido pelo nosso grupo apresentaram os melhores desempenhos na estimativa do peso fetal.

Palavras-chave: gastrosquise, ultrassonografia, peso fetal.

Summary

Background: The role of prenatal ultrasonographic parameters for the predicition of perinatal outcomes in fetuses with gastroschisis is still controversial. Birthweight is described as a prognostic factor and some studies report that ultrasonographic formulas especifically created for these cases have a better performance for fetal weight estimation, but there is no consensus about which is the best one. **Objectives:** To evaluate prenatal ultrasonographic parameters as predictors of adverse perinatal outcomes in fetuses with gastroschisis. To create a new birthweight predicting ultrasonographic model without abdominal measurements and compare this new formula with other two-dimensional (2DUS) and three-dimensional (3DUS) fetalweight predicting models already published when aplied to fetuses with gastroschisis. **Methods:** To evaluate the performance of prenatal ultrasonographic parameters as predictors of perinatal outcomes in fetuses with gastroschisis a retrospective cohort study was conducted. To create and validate the new 2DUS formula the same data from normal fetuses colected in a previous study of our group was used. A retrospective cross-sectional study encompassing fetuses with gastroschisis was carried out to compare the new 2DUS formula with other 2DUS and 3DUS formulas already published. The patients were selected among

those followed at the Division of Obstetrics of the Center for Integral Assistance to Women's Health of the State University of Campinas. The sample size was estimated in 56 patients to evaluate prenatal ultrasonographic predictors and perinatal outcomes and 27 patients to compare the fetal weigth estimating formulas. Maternal, pregnancy and postnatal data were described as absolute and percentual frequencies, mean and standard deviation (SD), median and range. Continuous data were tested for their normal distribution using the Kolmogorov–Smirnov test. Independent samples *t* tests and chi-square tests were used in the assessment of continuous and categorical variables, when appropriate. Polynomial stepwise regression analyses up to the third order were considered to generate a new 2DUS weight-predicting model without abdominal measurements. Calculation of the mean percentage error ± SD, one-sample *t* tests, paired samples *t*-tests with Bonferroni adjustment and correlated variance tests for paired samples were used to compare the performances of the formulas. The potential association between continuous data was tested by means of Pearson or Spearman's Correlation Coefficient and univariate logistic regression, as indicated. A two-tailed p-value of less than 0.05 was considered statistically significant. **Results:** Forty-four fetuses were included to evaluate the ultrasonographic prental parameters as predictors of perinatal outcomes. The presence of fetal multiple intra-abdominal bowel dilation (IBD) was associated with increased incidence of intestinal complications and the presence of fetal growth restriction (FGR) had a protective effect over this outcome. No other prenatal ultrasographic parameter could significantly predict the perinatal outcomes evaluated. It was used the same data from our previous study on 150

normal fetuses and 60 normal fetuses to respectively generate and validate the new 2DUS formula, which was: estimated fetal weight = 623.324 + 0.165 x BPD x HC x FDL^2 (SD: 12.25). In the comparison between the 2DUS formulas and between the 2DUS and 3DUS formulas it was included 44 and 28 fetuses, respectively. The best performance for weight prediction in fetuses with gastroschisis was achieved using the 2DUS model created by Siemer *et al.* and the 2DUS and 3DUS models previously published by our group. **Conclusions:** In fetuses with gastroschisis the findings of multiple IBD increases the risk of postnatal bowel complications and the presence of FGR decreases the risk of this outcome. The new 2DUS formula without abdominal measurements did not improve fetal weight estimation in fetuses with gastroschisis of our population when compared to other 2DUS and 3DUS formulas evaluated. The 2DUS weight estimating model of Siemer *et al.* and the 2DUS and 3DUS models previously published by our group had the best performance for this purpose.

Key words: gastroschisis, ultrasonography, fetal weight.

1. Introdução

Gastrosquise é o nome dado a uma falha de continuidade que acomete toda a espessura da parede abdominal fetal anterior. Geralmente é um defeito de pequeno diâmetro (2 a 3 cm), localizado 95% das vezes à direita do cordão umbilical (Sadler, 2010). Através deste orifício ocorre herniação de órgãos abdominais para a cavidade amniótica, sem a cobertura do peritônio ou do âmnio. A estrutura mais freqüentemente herniada é o intestino, mas herniação de outros órgãos, como estômago, bexiga e fígado, também pode ocorrer (Garcia *et al.*, 2010).

A etiologia da gastrosquise é motivo de debate. Uma hipótese bastante difundida é que sua patogênese envolve um acidente vascular e dentro desta linha duas teorias foram desenvolvidas: uma sugere que a involução da veia umbilical direita causa uma necrose na parede abdominal, levando a um defeito paraumbilical (deVries, 1980), e a outra postula que a artéria onfalomesentérica involue prematuramente, causando um enfraquecimento da parede abdominal, através do qual o conteúdo intestinal subsequentemente hernia (Hoyme *et al.*, 1981). Estas duas teorias são suportadas pela observação de que a gastrosquise associa-se à atresia intestinal, uma condição que também acredita-se estar associada com alterações isquêmicas (Louw e Barnard, 1955).

Além disso, dados retrospectivos sugerem um maior risco de gastrosquise e atresia intestinal em pacientes usuárias de drogas vasoconstritoras como efedrina, pseudoefedrina ou cocaína, assim como em pacientes tabagistas (Werler *et al.*, 2003). Entretanto, estudos epidemiológicos mais recentes sugerem que estas explicações podem ser insuficientes. Feldkamp *et al.* (2007) observam que as veias umbilicais não nutrem o mesênquima na parede abdominal, o que não explicaria um enfraquecimento desta região por uma alteração no desenvolvimento da veia umbiical direita. Além disso, o suprimento vascular da parede abdominal deriva de uma rica rede de vasos que se originam da aorta dorsal e que não é dependente e nem se anastomosa com os vasos umbilicais ou vitelínicos. Um grande estudo epidemiológico avaliando as associações entre exposição materna a agentes vasoativos e defeitos congênitos, observou que em mulheres jovens estes agentes possuem um papel menor, se é que possuem algum, na etiologia da gastrosquise, mas podem ter um papel maior em mulheres com mais de 25 anos (Werler *et al.*, 2009). Explicações para a origem da gastrosquise não relacionadas a problemas vasculares incluem falha da incorporação do ducto vitelínico ao cordão umbilical (Stevenson *et al.*, 2009), falha da fusão mediana dos folhetos laterais da parade abdominal (Feldkamp *et al.*, 2007) ou um defeito na diferenciacão do mesênquima embrionário responsável pela formação da parede abdominal (Duhamel, 1963). A ruptura intrauterina de uma onfalocele também foi proposta como um mecanismo de formação da gastrosquise (Shaw, 1975).

A gastrosquise ocorre em cerca de 1 a cada 4000 nascidos vivos. Dados recentes mostram um aumento de 10 a 20 vezes na incidência desta malformação em todos os grupos etários nas últimas duas décadas, embora ainda não haja uma explicação clara para isso (Baird e MacDonald, 1981; Alvarez e Burd, 2007; Loane *et al*., 2007; Castilla *et al*., 2008).

Estima-se que apenas 1% dos casos de gastrosquise apresente algum tipo de aneuploidia e casos com alteração de um único gene são ainda mais raros (Sadler, 2010), mas a observação do aumento da incidência de gastrosquise em algumas familias sugere que fatores genéticos desempenham um papel relevante na causa desta doença. Fatores de risco não-genéticos também são importantes, evidenciados, por exemplo, pela maior ocorrência de gastrosquise em mulheres jovens, pelo aumento da incidência geral de gastrosquise nos últimos anos e pela ocorrência de casos agrupados (Rasmussen e Frias, 2008).

Entretanto, apesar do reconhecimento da importância dos fatores de risco não-genéticos na causa da gastrosquise, apenas a baixa idade materna é bem estabelecida como um destes fatores, limitando o desenvolvimento de estratégias de prevenção (Rasmussen e Frias, 2008). Mais recentemente, outros fatores de risco não-genéticos como o tabagismo (Hackshaw *et al*., 2011) e a ingesta de álcool (Richardson *et al*., 2011) foram descritos em estudos envolvendo um grande número de pacientes.

Antes da ultrassonografia ser utilizada rotineiramente no pré-natal, um aumento na concentração sérica de alfa-feto proteína costumava ser o único indicador pré-natal de gastrosquise. Com o uso rotineiro da ecografia no primeiro trimestre para datação e no segundo trimestre para rastreamento de

malformações, quase todos os casos de gastrosquise passaram a ser diagnosticados antes do nascimento, com um estudo reportando uma taxa de detecção de 90% na Europa (Garne *et al.*, 2005).

O aspecto ecográfico habitual da gastrosquise é a visualização de múltiplas alças intestinais flutuando livremente na cavidade amniótica. Tipicamente, o defeito na parede abdominal ocorre à direita da inserção do cordão umbilical, que está normalmente inserido na parede abdominal. Este diagnóstico pode ser feito a partir de 11-12 semanas de gestação, quando a herniação fisiológica intestinal já deve ter retornado para a cavidade peritoneal (Cullen *et al.*, 1990). O principal diagnóstico diferencial é com a onfalocele, na qual as alças herniadas são cobertas pelo peritônio e o cordão umbilical se insere no ápice do saco herniário. Outros diagnósticos diferenciais incluem lesões císticas do cordão umbilical, cistos de úraco, extrofia de bexiga/cloaca e a Pentalogia de Cantrell. Excluir a possibilidade de uma onfalocele é de fundamental importância, visto que esta condição, diferentemente da gastrosquise, freqüentemente associa-se com malformações em outros órgãos e aneuploidias (David *et al.*, 2008).

Exceto por uma maior incidência de alterações intestinais, crianças com gastrosquise geralmente não possuem outras anomalias anatômicas associadas e tendem a apresentar crescimento e desenvolvimento neurológico normais durante a infância. A atresia intestinal é a alteração anatômica mais comumente associada. Estudos recentes relatam a presença de atresia intestinal em 7% a 28% dos casos (Arnold *et al.*, 2007; Kronfli *et al.*, 2010).

O prognóstico dos casos de gastrosquise é determinado principalmente pelo grau de lesão intestinal que ocorre durante o período fetal. Esta lesão resulta provavelmente da combinação entre o contato da alça com o líquido amniótico e a constrição intestinal no orífico do defeito abdominal, sendo que a maior parte da injúria parece ocorrer no final da gestação (Langer *et al.*, 1989; Langer *et al.*, 1990). A lesão nas alças intestinais pode causar disfunção na motilidade e na absorção, o que prolonga a necessidade de nutrição parenteral e, em alguns casos, pode causar perda irreversível da função intestinal (Wales e Christison-Lagay, 2010). O diagnóstico pré-natal provê a possibilidade de controlar o modo, local e época do parto, com o objetivo de minimizar estas complicações.

A melhor via de parto para os fetos com gastosquise é motivo de debate. Alguns autores argumentam que o parto vaginal pode provocar lesões nas alças intestinais e defendem o uso rotineiro da cesárea. Entretanto esta hipótese não encontra respaldo na literatura, que não demonstra diferença no prognóstico dos casos nascidos por cesariana quando comparados aos casos nascidos por parto normal. Desta forma, a conduta mais adequada em relação à via de parto talvez dependa de uma discussão entre o obstetra e gestante (Segel *et al.*, 2001; Salihu *et al*, 2004).

O melhor momento para realizar o parto dos casos de gastrosquise também é um tópico controverso. Alguns centros recomendam a realização do parto no limite do termo, por volta de 36-37 semanas, com o objetivo de diminuir a exposição das alças intestinais ao líquido amniótico e assim reduzir a intensidade da reação inflamatória na superfície das mesmas. Há evidências de

que mediadores inflamatórios e citocinas (interleucinas 6 e 8) presentes no líquido amniótico possuem um papel no desenvolvimento de danos no plexo nervoso mioentérico e nas células intersticiais de Cajal nos casos de gastrosquise. Como acredita-se que estes efeitos inflamatórios sobre as alças intestinais aumentam com o passar da gestação, alguns autores acham que a antecipação do parto poderia diminuí-los (Srinathan *et al*., 1995; Luton *et al*., 1999; Salihu *et al*, 2004; Guibourdenche *et al*., 2006; Vargun *et al*., 2007). Parto pré-termo é mais frequente em gestações de fetos com gastrosquise (incidência de 28% contra 6% nos casos de gestações de fetos normais), desta forma o parto pode ser induzido com sucesso em grande parte dos casos com 36-37 semanas de gestação, provavelmente por causa desta tendência natural (Lausman *et al*., 2007). A literatura, entretanto, é controversa sobre os benefícios da antecipação do parto. O argumento contra a antecipação do parto é que o baixo peso ao nascimento tem um efeito negativo sobre o prognóstico destas crianças, com neonatos com pesos inferiores a dois quilos demorando mais tempo para atingirem nutricão enteral completa e ficando mais tempo sob ventilação assistida (Charlesworth *et al*., 2007). Alguns estudos propõem a antecipação do parto de maneira seletiva nos casos que apresentem sinais de dilatação e espessamento da parede das alças intestinais na avaliação ecográfica, pois a presença destes sinais correlaciona-se com mau prognóstico, incluindo sofrimento e óbito fetal, em algumas, mas não em todas as séries de casos (Langer *et al*., 1993; Piper e Jaksic, 2006). Entretanto, esses dados não são muito claros por não haver padronização na maneira de se medir as alças

intestinais e nem consenso sobre a definição de dilatação intestinal de acordo com a idade gestacional.

A maioria dos autores recomenda que o parto seja realizado em centros terciários, de maneira a permitir acesso imediato a cuidados neonatais e cirúrgicos especializados. Um estudo avaliando resultados pós-natais observa que o nascimento de casos de gastrosquise em centros com acesso imediato a atendimento de neonatologia e cirurgia pediátrica terciários associa-se com diminuição do risco de complicações pós-natais, quando comparado com casos nascidos em hospitais sem acesso imediato a estes tipos de atendimentos (Christison-Lagay *et al.*, 2011; Nasr e Langer, 2011). Entretanto, esta recomendação não é um consenso. Um trabalho recente com 118 casos de gastrosquise observa que o tempo para realização da primeira cirurgia não foi preditivo de nenhum desfecho clínico desfavorável relevante. Estes dados sugerem que os potenciais benefícios da regionalização do acompanhamento dos casos de gastrosquise não seriam suportados por um menor tempo para a realização da intervenção cirúrgica (Bucher *et al.*, 2012).

A gastrosquise leva a uma perda hídrica significativa por evaporação pelas alças expostas. Após o parto, deve ser iniciada repoisção de líquido por via endovenosa. Descompressão gástrica, por meio de sonda, também deve ser realizada com o objetivo de prevenir ou diminuir distensão intestinal. As alças herniadas devem ser envolvidas em compressas embebidas em solução salina fisiológica, colocadas em posição central na parede abdominal com o recém-nascido em decúbito lateral direito para prevenir acotovelamento do mesentério e cobertas com silo plástico para diminuir as perdas hídricas evaporatovas e a

instabilidade térmica. Um exame detalhado do neonato deve ser feito para excluir a coexistência de outras anomalias, com atenção especial à busca de sinais de atresia, necrose ou perfuração intestinal (Christison-Lagay *et al.*, 2011).

A conduta cirúrgica nos casos de gastrosquise varia de centro para centro e e modificou-se nas últimas décadas. O objetivo da cirurgia é devolver as vísceras herniadas para a cavidade abdominal com o menor risco de lesão, seja esta por trauma direto ou por aumento da pressão intra-abdominal. As principais opções de conduta cirúrgica são: (a) redução primária com fechamento da fascia; (b) colocação de silo, reduções seriadas e fechamento tardio da fascia; (c) redução primária ou seriada, sem o fechamento da fascia. O intervalo de tempo após o nascimento e o local onde deve ser realizada a intevenção cirúrgica são motivos de debate, podendo ser um reparo imediato na sala de parto, redução e fechamento na unidade de terapia intensiva neonatal ou fechamento tardio no centro cirúrgico (Coughlin *et al.*, 1993; Bianchi *et al.*, 2002). Em todos os casos, inspeção intestinal à procura de sinais de bandas obstrutivas, perfuração ou atresia deve ser realizada cuidadosamente. Bandas atravesando as alças devem ser divididas antes da colocação do silo ou fechamento abdominal primário para evitar obstrução intestinal subsequente. Estabelecimento precoce de um acesso venoso central deve ser considerado, pois hipomotilidade intestinal está invariavelmente presente (Christison-Lagay *et al.*, 2011).

A taxa geral de sobrevivência do casos de gastrosquise é muito boa, variando de 90% a 97% (Molik *et al.*, 2001; Aina-Mumuney *et al.*, 2004). Já complicações gastrointestinais, tais como perfuração ou atresia, ocorrem em

10% a 20% dos casos (Brantberg *et al.*, 2004). A presença destas complicações associa-se com taxas de mortalidade de até 28% e maior tempo de hospitalização e nutrição paraenteral, com seus riscos associados de infecção, distúrbios de crescimento, disfunções metabólicas e doenças hepáticas (Langer *et al.*, 1993; Wilson e Johnson, 2004; Nick *et al.*, 2006; Badillo *et al.*, 2008). Desta forma, a identificação pré-natal de fatores associados com o prognóstico perinatal ajudaria na triagem de casos que se beneficiariam de um acompanhamento mais próximo das condições fetais e de algum tipo de intervenção, como a antecipação do parto ou nascimento em hospitais especializados, além de ajudar no aconselhamento dos pais (Huh *et al.*, 2010).

A dilatação de alças intestinais extra-abdominais tem sido um dos fatores prognósticos pré-natais mais estudado, mas seu papel na predição da evolução pós-natal dos casos de gastrosquise ainda é motivo de debate. Alguns estudos demonstram correlação entre a presença de dilatação de alças extra-abdominais e complicações intestinais e outros não. Diferenças na definição de dilatação e amostras pequenas dos estudos limitam o estabelecimento de um consenso sobre quais medidas são consistentemente preditoras de mau prognóstico (Alsulyman *et al.*, 1996; Japaraj *et al.*, 2003; Nick *et al.*, 2006; Badillo *et al.*, 2008). Além disso, alguns autores sugerem que a dilatação das alças intestinais exteriorizadas pode ser um fenômeno fisiológico em muitos fetos com gastrosquise no terceiro trimestre, que apresentam ótima evolução pós-natal (Huh *et al.*, 2010).

A avaliação quanto a presença de dilatação intra-abdominal das alças intestinais também tem sido estudada como um fator prognóstico pré-natal.

Este achado é menos comum nos fetos com gastrosquise, com uma incidência estimada em 8% a 17% (Brantberg *et al.*, 2004; Nick *et al.*, 2006), e estudos recentes demonstram que a presença deste sinal correlaciona-se melhor com a ocorrência de complicações pós-natais do que a presença de dilatação das alças extra-abdominais. Entretanto, diferenças na definição de dilatação intra-abdominal e amostras pequenas dos estudos também limitam o estabelecimento de um consenso sobre o papel deste achado (Nick *et al.*, 2006; Huh *et al.*, 2010; Kuleva *et al.*, 2012).

Avaliando 117 pacientes com gastrosquise, um trabalho relata que herniação hepática junto com o intestino estava presente em 6% dos casos e este achado se correlacionou com uma taxa de sobrevivência significativamente menor (43% x 97%), considerando-se a idade gestacional e o peso ao nascimento. Estes autores observam que a presença de herniação hepática demonstra ser um fator de mau prognóstico pós-natal e deve ser avaliada nos exames de ecografia pré-natais (McClellan *et al.*, 2011). A literatura, entretanto, ainda é escassa na avaliação deste fator prognóstico.

Um outro fator prognóstico que tem sido avaliado é a dilatação do estômago, também com resultados diversos. Avaliando 34 casos, 13 (38%) dos quais apresentavam dilatação estomacal, um estudo observa que este achado se correlacionou com maior incidência de cardiotocografia não reativa, vólvulo intestinal e morte neonatal e maior tempo para alimentação por via oral e de hospitalização (Aina-Mumuney *et al.*, 2004). Já um outro trabalho, avaliando 89 fetos com gastrosquise, 32 (33%) dos quais com dilatação do estômago, encontrou resultados diferentes. Este estudo observa que a presença de

dilatação estomacal correlacionou-se apenas com maior ocorrência de líquido meconial ao nascimento, mas não se correlacionou com outros resultados adversos perinatais (Alfaraj *et al.*, 2011).

Parâmetros Dopplervelocimétricos também já foram avaliados na predição de complicações intestinais de fetos com gastrosquise. Um estudo prospectivo com 17 fetos com gastrosquise avalia a relação entre o índice de pulsatilidade da artéria mesentérica superior fetal e má evolução pós-natal, definida como necessidade de ressecção intestinal ou tempo de hospitalização maior que 50 dias. Neste estudo, o índice de pulsatilidade da artéria mesentérica superior não foi capaz de predizer má evolução pós-natal (Abuhamad *et al.*, 1997).

A presença de herniação vesical junto com as alças intestinais parece se correlacionar com maior risco de sofrimento fetal nos casos de gastrosquise, mas não com maior risco de complicações intestinais. Um estudo recente, observa que 6 de 105 (6%) dos casos de gastrosquise acompanhados em um centro apresentavam herniação vesical. Um destes casos evoluiu com óbito fetal e outros quatro tiveram parto cesárea por sinais de sofrimento fetal. Os cinco sobreviventes evoluiram bem após o tratamento pós-natal, mas os autores observam que a presença de herniação da bexiga nos casos de gastrosquise pode ser um sinal para um acompanhamento mais rigoroso do bem-estar fetal (Mousty *et al.*, 2012).

Recentemente, um estudo caso-controle com 106 casos de gastrosquise, avalia a correlação de múltiplos fatores ultrassonográficos pré-natais com o desenvolvimento de complicações intestinais neonatais. Os seguintes fatores ecográficos pré-natais foram avaliados: feto pequeno para a idade gestacional,

presença de dilatação intra ou extra-abdominal de alças intestinais, espessamento de parede de alça intestinal e dilatação estomacal. Estes fatores foram utilizados para tentar predizer a presença de atresia, perfuração, necrose ou vólvulo ao nascimento, sendo que 14% dos casos apresentaram alguma destas complicações. Este trabalho observa que nenhum dos marcadores ecográficos foi preditivo de morte fetal ou neonatal e apenas a presença de dilatação de alças intra-abdominais se correlacionou com a ocorrência de complicações intestinais pós-natais (Kuleva *et al.*, 2012).

Muitas publicações relatam que o peso ao nascimento é também um importante fator na evolução pós-natal dos casos de gastrosquise. Estes autores observam que recém-nascidos com gastrosquise pequenos para a idade gestacional apresentam maiores taxas de morbidade e mortalidade (Charlesworth *et al.*, 2007; Netta *et al.*, 2007; Nicholas *et al.*, 2009). Desta forma, a predição mais precisa possível do peso fetal é de extrema importância para tomada de decisões nesses casos. A estimativa do peso fetal nos fetos com gastrosquise tem sido feita essencialmente por fórmulas geradas a partir de fetos normais que utilizam medidas ultrassonográficas bidimensionais (US2D) do pólo cefálico, membros e abdome. Entretanto, alguns estudos relatam que fórmulas criadas especificamente para fetos com defeitos de parede abdominal, que não levam em consideração as medidas do abdome, possuem uma maior acurácia na estimativa do peso desses fetos (Siemer *et al.*, 2008; Chaudhury *et al.*, 2010; Nicholas *et al.*, 2010; Adams *et al.*, 2012).

Em um estudo publicado em 2010, Bennini *et al.* criaram, a partir de fetos normais, um modelo US2D de estimativa de peso que utiliza medidas do pólo

cefálico, abdome e fêmur, e outros dois modelos que utilizam como único parâmetro o volume da coxa fetal medido por meio da ultrassonografia tridimensional (US3D). Na comparação das fórmulas, os desempenhos dos novos modelos US2D e US3D não apresentaram diferença estatística entre si, mas foram significativamente melhores do que os dos modelos US2D e US3D testados que haviam sido gerados em outras populações. Apesar dessas observações, nunca foram criadas fórmulas US2D de estimativa de peso específicas para fetos com gastrosquise geradas a partir de uma amostra da nossa população. Da mesma maneira, fórmulas que utilizam parâmetros ultrassonográficos tridimensionais (US3D), como o volume da coxa fetal, para a estimativa de peso nunca foram avaliadas em fetos com gastrosquise.

Portanto, avaliando as publicações mais recentes, observamos que, apesar do reconhececimento da importância da identificação de fatores pré-natais que possam predizer o risco de complicações perinatais nos casos de gastrosquise, a literatura é controversa sobre quais seriam estes fatores e sobre o desempenho dos mesmos. Além disso, considerando-se a importância do peso ao nascimento no prognóstico dos casos de gastrosquise, ainda são necessários mais estudos para avaliar qual a melhor maneira de estimar peso desses fetos.

2. Objetivos

2.1. Objetivo geral

Avaliar o papel da ultrassonografia na estimativa do peso e predição de desfechos perinatais desfavoráveis em fetos com gastrosquise isolada.

2.2. Objetivos específicos

- Criar uma nova fórmula US2D para estimativa de peso que não utilize medidas abdominais gerada a partir de fetos normais da nossa população e comparar o seu desempenho com os de outras fórmulas US2D e US3D quando aplicadas em fetos com gastrosquise isolada.

- Avaliar o papel de parâmetros ultrassonográficos na predição de desfechos perinatais em fetos com gastrosquise isolada.

3. Publicações

Artigo 1 – Bennini JR, Marussi EF, Barini R, Peralta CFA. Birth weight prediction for fetuses with gastroschisis using two- and three-dimensional ultrasound.

Artigo 2 – Bennini JR, Marussi EF, Barini R, Peralta CFA. Gastroschisis: fetal longitudinal follow-up and perinatal outcomes

3.1. Artigo 1

Manuscript number: UOG-2014-0093

Dear Dr Bennini

We are pleased to receive your manuscript entitled Birth weight prediction for fetuses with gastroschisis using two- and three-dimensional ultrasound by Bennini, Joao; Marussi, Emilio; Barini, Ricardo; Peralta, Cleisson Fábio. We will shortly be assigning it to one of the Journal's Editors who will handle the peer review of the paper.

To track the progress of your manuscript through the editorial process using our web-based system, simply point your browser to:

http://mc.manuscriptcentral.com/uog

Please remember in any future correspondence regarding this article to always include its manuscript ID number UOG-2014-0093.

Many thanks for submitting your manuscript

Yours sincerely

Sarah Hatcher
Managing Editor

Birth weight prediction for fetuses with gastroschisis using two- and three-dimensional ultrasound

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Abstract

Objectives: To create a new two-dimensional ultrasound (2DUS) birthweight predicting model without abdominal measurements and compare this new formula with other 2DUS and three-dimensional ultrasound (3DUS) models when aplied to fetuses with gastroschisis.

Methods: To create and validate the new 2DUS formula it was used the same group of normal fetuses enrolled in a previous study published by our group. To compare the 2DUS and 3DUS formulas it was realized a retrospective cross-sectional study using fetuses with gastroschisis. Polynomial stepwise regression analyses was used to generate the new weight-predicting model. Calculation of the mean percentage error ± standard deviation, one-sample *t* tests, paired samples *t*-tests and correlated variance tests for paired samples were used to compare the performances of the formulas.

Results: A total of 44 and 28 fetuses with gastroschisis were used to compare the 2DUS and 3DUS formulas, respectively. The weight predicting models with highest accuracies and precisions were the 2DUS formula of Siemer *et al.* and ours previously published 2DUS and 3DUS models, with no statistic difference among their performances.

Conclusions: The new 2DUS formula did not improve fetal weight estimation in fetuses with gastroschisis when compared to other 2DUS and 3DUS formulas evaluated.

Key words: gastroschisis, prenatal ultrasonography, fetal weight, three-dimensional ultrasonography, fetal thigh volume.

Introduction

Accurate estimation of weigh in fetuses with gastroschisis is important because abnormal patterns of growth may influence this disease's baseline risks of perinatal mortality and morbidity.[1-5] The assessment of fetal weight in such cases has been essentially based on traditional two-dimensional ultrasound (2DUS) formulas, which were generated from anatomically normal fetuses and take into account the measurements of the head, limbs and abdomen. Nevertheless, some studies report that equations elaborated specifically for fetuses with abdominal wall defects, which do not include the abdominal measurements, produce better results when applied to fetuses with gastroschisis.[6-9]

Another option to estimate fetal weight is to use three-dimensional ultrasound (3DUS) birth-weight predicting models. Some of these formulas rely on the use of single predictors of weight, such as the thigh volume, which reflects not only the length of the limb but also the amount of soft tissue that surrounds it.[10] The use of 3DUS formulas to estimate the weight in fetuses with gastroschisis has not been reported yet.

The aim of this study was to create a new 2DUS formula without abdominal parameters and to compare, in fetuses with isolated gastroschisis, the performances of 2DUS and 3DUS birth-weight predicting models generated in our population, with those of previously published 2DUS formulas elaborated specifically for fetuses with abdominal wall defects.

Methods

This was a retrospective cross-sectional two-stage study carried out at Professor José Aristodemo Pinotti Hospital, Center for Integral Assistance to Women's Health of the State University of Campinas (UNICAMP), including normal fetuses evaluated between July 2007 and January 2009 and fetuses with gastroschisis evaluated between February 2007 and November 2013. The ethics committee of the State University of Campinas Medical School approved this protocol. Since this was a retrospective study and all the data were obtained from routine assessments of normal and pathological cases, we were exempt from the application of informed consent forms.

Common eligibility criteria for the two stages of the study consisted of the following: 1. No maternal diseases associated with alterations in fetal growth, such as pre-eclampsia or diabetes. 2. Singleton pregnancy. 3. Well-defined gestational age (GA) based on the known date of the last menstrual period and/or the measurement of embryonic/fetal crown-rump length during the first trimester, interpreted based on the reference intervals reported by Robinson and Fleming.[11]

First stage of the study: Elaboration and validation of a new 2DUS birth-weight predicting model without abdominal measurements

Two groups of normal fetuses were evaluated to create and validate a new specific 2DUS birth-weight predicting model without abdominal measurements. These patients were the same used in a previous study by our group to create 2DUS and 3DUS formulas for the estimation of fetal weight in our population.[10]

Second stage of the study: Comparison of the accuracies of the new 2DUS birth-weight predicting model with those of previously published 2DUS and 3DUS equations in fetuses with gastroschisis

This stage of the study involved the evaluation of fetuses with isolated gastroschisis between February 2007 and November 2013 with the purpose of comparing the 2DUS birth-weight predicting model created in this study with other 2DUS and 3DUS formulas previously published. The specific eligibility criteria for this part of the study were delivery less than 14 days after the last ultrasound scan and the presence of isolated fetal gastroschisis diagnosed during obstetric scans in our institution.

Demographic and clinical characteristics of the mother, including age, GA at delivery, the mode of delivery and the clinical characteristics of the newborn (weight and Apgar scores) were collected from the mother's hospital files. At our institution, neonates are categorized as small, normal or large for gestational age if their weights fall below the 10^{th}, between the 10^{th} and the 90^{th} or above the 90^{th} percentile of the reference intervals defined by Alexander et al.[12], respectively. All neonates were weighed immediately after birth in the delivery

room on the same precision electronic Filizola Baby scale (Filizola SA, Weighting and Automation, Campo Grande, MS, Brazil), which has a precision of 5 g and is calibrated every two weeks. The date and time of each scan and birth were recorded to allow calculation of the time from scan to birth.

Conventional 2DUS measurements and fetal thigh volumetry using the VOCAL™ (Kretztechnik, Austria) technique were performed as previously described by our group.[10]

All ultrasound examinations were performed transabdominally, with a Voluson 730 Expert scanner, equipped with a RAB 4-8L probe (GE Medical Systems, Milwaukee, WI, USA) by one of two physicians (JRB; CFAP).

Statistical analysis

Maternal demographic characteristics and pregnancy and postnatal data were described as absolute and relative frequencies, the average and standard deviation (SD) or the median and range.

Creation and validation of a new 2DUS birth-weight predicting model without abdominal measurements:

Using data from the first stage of the study and weight of the neonate as the dependent variable, polynomial stepwise regression analyses up to the third

order were considered to generate a new weight-predicting model with the following conventional 2DUS measurements as predictors: biparietal diameter (BPD), head circumference (HC) and femur diaphysis length (FDL). To construct the new 2DUS formula, Eigenvalue, tolerance, variance inflation factor, condition index and variance proportion were calculated to check for multicollinearity among independent variables.[13] The criteria for multicollinearity were: Eigenvalue less than 0.1; tolerance value less than 1 - r^2; variance inflation factor greater than 1 / (1 - r^2); condition index greater than 0.30; and variance proportion greater than 0.8. For the best-fit equation, Kolmogorov-Smirnov test was performed to check for normality of the standardized residuals.

Comparison of previously published 2DUS and 3DUS equations with the new 2DUS model applied to fetuses with gastroschisis:

For this part of the study, a sample size of 27 patients with gastroschisis has a power of 80% to detect a difference in means of -6.24 (the difference between a Group 1 mean, μ_1, of -0.27 and a Group 2 mean, μ_2, of 5.97) assuming that the common standard deviation is 8.00 using a two group t-test with a 0.05 two-sided significance level.

The EFW calculated using each birth-weight predicting model was adjusted for the interval between the last sonographic examination and the date of delivery according to the individual trend of growth of each fetus.[14]

The original 2DUS formulas described by Siemer *et al.*[6] and Honarvar *et al.*[15], as well as the 2DUS and 3DUS equations reported by our group[10] were applied to data obtained from the fetuses with gastroschisis, in order to compare their performances with that of the new 2DUS model generated in the present study. The criteria for the selection of these formulas were: 1. The absence of abdominal parameters in the formulas generated by Siemer *et al.*[6] and Honarvar *et al.*[15], as well as in the 3DUS model created by our group.[10] 2. The demonstration that our previous 2DUS equation including the abdominal circumference (AC) had better performance than those of other formulas when applied to normal fetuses in our population.[10]

The technique used for the measurement of total fetal thigh volume in fetuses with gastroschisis was the same used in the original article to generate the 3DUS equation.[10] The formulas used for comparison with our new 2DUS model are described in Chart 1.

The performances of each of these equations were analyzed by the calculation of systematic and random errors. The systematic error, or accuracy, was evaluated by calculating the mean sign percentage error (MSPE): [(estimated fetal weight – actual birth weight) / actual birth weight x 100], with 95% confidence intervals (CI). To determine the presence and extent of bias, the MSPE were compared to zero using one-sample *t*-tests. Paired samples *t*-tests with Bonferroni adjustments were used to detect significant differences between the accuracies of these formulas. The adjusted *p*-value (*p*'), which was

calculated according to the Bonferroni method, was obtained by the formula *p*' = k x *p*-value, where k was the number of paired comparisons and the p-value was obtained from each paired samples t-test.[16,17] In this manner, for the comparison of our new 2DUS model with the original 2DUS functions of Siemer *et al.*[6], Bennini *et al.*[10] and Honarvar *et al.*[15] (three paired comparisons), each *p*' was obtained by the formula *p*' = 3 x the *p*-value of the paired samples *t*-test. This method has the restriction that the *p*' cannot exceed 1.0. The random error, or precision, was evaluated by calculating the standard deviation (SD) of the MSPE. In order to compare the random errors of two equations, correlated variance tests for paired samples were used.[18] For each paired comparison, the variances were considered to be significantly different if the *p* value obtained from the r (Pearson's correlation coefficient) distribution table was less than 0.05. The r value was calculated by the formula $r = (F - 1) / \sqrt{(F + 1)^2 - 4 \times r^2 \times F}$, where F is the ratio of the variances of the groups being compared. All *p* values exceeding 0.20 were referred to as $p > 0.20$.

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

Results

In order to generate and validate the new 2DUS formula, 210 patients (150 in the formula-generating group; 60 in the formula-validation group) were evaluated.

The best-fit 2DUS formula without AC measurement for the estimation of weight in normal fetuses was: EFW = 623.324 + 0.165 x BPD x HC x FDL^2 (SD of predicted values: 12.25; r = 0.774; r^2 = 0.599; p < 0.001). The results of the Kolmogorov-Smirnov tests revealed normal distributions of the standardized residuals of the equation.

For both the formula-generating and the formula-validation groups, no statistically significant differences were noted between the accuracies ($p < 0.01$) and precisions ($p < 0.01$) of the new 2DUS model in the prediction of birth weight.

In total, 61 fetuses with gastroschisis were evaluated throughout the study period. Among them, 44 (72%) met the entry criteria and 17 (28%) were not included in the final analysis due to the following reasons (Chart 2): delivery more than 14 days after the last ultrasound scan (5 cases); loss of follow-up (7 cases); incomplete 2DUS measurements in the last scan before birth (1 case); spontaneous fetal demise (3 cases); ongoing pregnancy (1 case).

Twenty-eight (64%) of the gastroschisis fetuses included had stored 3DUS datasets of their thighs. Therefore, the comparison of 2DUS formulas was performed using 44 patients (2DUS gastroschisis group) and the comparison of 2DUS and 3DUS models was carried out with 28 cases (2DUS/3DUS gastroschisis group). Demographic and clinical characteristics of the 44 cases of gastroschisis are presented in Table 1.

2DUS gastroschisis group:

When applied to the 2DUS gastroschisis group, the formulas of Siemer *et al.*[6] and Bennini *et al.*[10] showed the lowest MSPE and SD (0.60 ± 12.76 and -1.54 ± 11.39, respectively), with no significant difference of the MSPE from zero (p = 0.37 and 0.76, respectively). The new 2DUS formula and the Honarvar *et al.*[15] equation significantly overestimated the neonatal weights ($p < 0.01$ for both models) with MSPE and SD = 10.39 ± 12.26 and 11.84 ± 16.69, respectively (Table 2).

When comparing the accuracies of these equations, the performance of the new 2DUS formula was not different from that of Honarvar's formula, but it was significantly worse than those of Siemer's and Bennini's equations (Table 2).

The precision of the new 2DUS model was not significantly different from those of Bennini's and Honarvar's equations, but it was significantly worse than that produced by Siemer's formula (Table 2).

When comparing the formulas of Siemer *et al.*[6] and Bennini *et al.*[10] no statistic difference between the accuracies and precisions were observed (Table 2).

2DUS/3DUS gastrosqhisis group results:

When applied to the 2DUS/3DUS gastroschisis group, the formula of Siemer *et al.*[6] and the 3DUS model of Bennini *et al.*[10] showed the lowest MSPE and SD (0.13 ± 9.36 and -0.33 ± 9.98, respectely), with no significant difference of the MSPE from zero (p = 0.94 and 0.86, respectely). The 2DUS formula of Bennini *et al.*[10] produced a MSPE and SD = -3.14 ± 9.62, also with no significant difference (p = 0.10) of the MSPE from zero (Table 3).

When comparing the three formulas applied to the 2DUS/3DUS gastroschisis group, no statistic difference between the accuracies and precisions were observed (Table 3).

Discussion

The main question raised in this study was whether 2DUS or 3DUS birth-weight predicting models that do not include fetal abdominal measurements and are generated in our population allow better estimations of fetal weights in cases of gastroschisis than do other particular 2DUS equations (generated in our

population and including the AC, or elaborated elsewhere specifically for the estimation of weight in fetuses with gastroschisis).

In a previous study we have demonstrated that 2DUS and 3DUS birth-weight predicting models created from a representative sample of our population have better performances to estimate fetal weights in our patients. Therefore, we elaborated a new 2DUS model without fetal abdominal measurements and compared the performance of this new model with those of our previous 2DUS and 3DUS formulas and those of 2DUS equations created specifically for the estimation of weight in fetuses with gastroschisis.

With regard to the 2DUS models, our results demonstrated that, in terms of accuracy and random error, the two best formulas were those proposed by Siemer *et al.*[6] and Bennini *et al.*[10] None of this models produced a systematic bias in weight estimation and the differences between their MSPE and SD were not statistically significant.

The new 2DUS formula created in this study had worse performance than those of our previous 2DUS equation and of Siemer*'s* formula. This was not expected, if we consider that the new 2DUS model did not incorporate fetal abdominal measurements and was generated from fetuses of the same population of the cases of gastroschisis. The reasons for these findings are not clear. One could hypothesize that, because fetuses with gastroschisis tend to be smaller than anatomically normal fetuses, this should be followed by smaller phenotypic

variations. If we consider that Siemer's equation was elaborated using preterm neonates, it seems reasonable that the precision of their equation would be better even when applied to different populations.

The main weakness of this study was the small sample size to allow the evaluation of the impact of other variables, such as the content herniated through the abdominal wall defect, on the accuracies of each of these formulas. For the same reason, it was not possible to evaluate properly the performance of each model in the prediction of fetal growth restriction.

In conclusion, the 2DUS model without abdominal measurements generated in our population did not improve birth-weight estimation in relation to previously published formulas. The 2DUS model proposed by Siemer *et al.*[6] and the 2DUS and 3DUS models created by Bennini *et al.*[10] were the best to predict birth-weight in our fetuses with gastroschisis.

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Chart 1. Original 2DUS and 3DUS birth-weight prediction models used for comparison with the new 2DUS formula created in this study

Author	Method	Equation
Siemer *et al.*[6]	2DUS	EFW (g) = -145.577 + (23.724 x FDL^2) + (1.255 x BPD^3) + (0.001 x e^{OFD}) - (0.0000406 x 10^{FDL}) + (1.03 x e^{FDL})
Hornavar *et al.*[15]	2DUS	EFW (kg) = (0.042x FDL^2) + (0.32 x FDL) - 1.36
Bennini *et al.*[10]	2DUS	EFW (g) = -562.824 + (11.962 x AC x FDL) + (0.009 x BPD^2 x AC^2)
Bennini *et al.*[10]	3DUS	EFW (g) = 1025.383 + 12.775 x ThiV

2DUS: Two-dimensional ultrasound

3DUS: Three-dimensional ultrasound

EFW: Estimated fetal weight

BPD: Biparietal diameter (cm)

OFD: Occipito-frontal diameter (cm)

AC: Abdominal circumference (cm)

FDL: Femur diaphysis length (cm)

ThiV: Fetal thigh volume measured by the VOCAL™ technique (cm^3)

Chart 2. Gastroschisis population flow chart

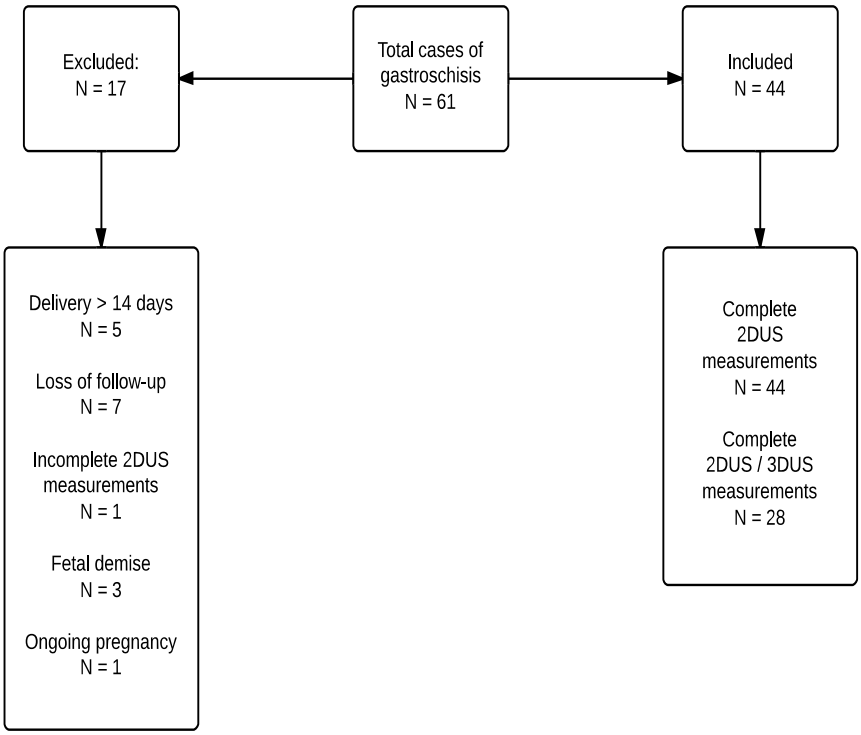

Table 1. Characteristics of the group of fetuses with gastroschisis

Mother and pregnancy	**n = 44**
Age (years)*	21.1 ± 5.2 (13.3 – 43.9)
GA at delivery (weeks)*	36.1 ± 1.7 (31.1 – 38.9)
Parity	
Nuliparous	32 (73%)
Multiparous	12 (27%)
Delivery mode	
Vaginal	6 (14%)
Caesarean section	38 (86%)
Interval scan - birth (hours)*	112 ± 100 (2 – 336)
Neonate	**n = 42**
Weight (grams)*	2287 ± 518 (1430 – 3575)
< 2000	13 (30%)
2000 – 2500	17 (39%)
> 2500 - 3000	11 (25%)
> 3000	3 (6%)
Apgar	
1º minute**	8 (1 – 10)
5º minute**	9 (7 – 10)
Size	
Small	21 (48%)
Adequate	23 (52%)

GA: gestational age

*Mean ± standard deviation (minimum – maximum)

**Median (minimum – maximum)

Table 2. Comparison between the accuracies and precisions of the 2DUS birth-weight predicting models in fetuses with gastroschisis

2DUS gastrochisis group (n = 44)				
	New 2DUS model	Honarvar *et al.*[15]	Siemer *et al.*[6]	2DUS Bennini *et al.*[10]
MSPE (95%CI)	10.39 (5.99 - 14.79)	11.84 (6.76 - 16.90)	0.60 (-3.28 - 4.48)	-1.54 (-5.00 - 1.92)
p*	< 0.01	< 0.01	0.76	0.37
± SD	± 14.47	± 16.69	± 12.76	± 11.39
	p' / *p***	*p*' / *p***	*p*' / *p***	
2DUS Bennini *et al.*[10]	< 0.01 / 0.05	< 0.01 / < 0.01	0.41 / > 0.20	
Siemer *et al.*[6]	< 0.01 / < 0.01	< 0.01 / < 0.01	-	
Honarvar *et al.*[15]	0.24 / 0.05	-	-	

MSPE: Mean sign percentage error (accuracy)
95%CI: 95% confidence interval of MSPE
SD: Standard deviation of MSPE (precision)
*p**: *p*-value for one-sample t-test for comparison of MSPE to zero
p': p-value for paired samples t-tests with Bonferroni adjustment for comparison of MSPE
*p***: *p*-value for correlated variance in paired samples for comparison of SD

Table 3. Comparison between the accuracies and precisions of the 2DUS and 3DUS birth-weight predicting models in fetuses with gastroschisis

3DUS gastroschisis group (N = 28)			
	3DUS Bennini *et al.*[10]	Siemer *et al.*[6]	2DUS Bennini *et al.*[10]
MSPE (95%CI)	-0.33 (-4.20 - 3.53)	0.13 (-3.50 - 3.75)	-3.14 (-6.87 - 0.59)
*p**	0.86	0.94	0.10
± SD	± 9.98	± 9.36	± 9.62
	p' / *p***	*p'* / *p***	
2DUS Bennini *et al.*[10]	0.50 / >0.20	0.15 / > 0.20	
Siemer *et al.*[6]	0.61 / > 0.20	-	

MSPE: Mean sign percentage error (accuracy)
95%CI: 95% confidence interval of MSPE
SD: Standard deviation of MSPE (precision)
*p**: *p*-value for one-sample t-test for comparison of MSPE to zero
p': p-value for paired samples t-tests with Bonferroni adjustment for comparison of MSPE
*p***: *p*-value for correlated variance in paired samples for comparison of SD

3.2. Artigo 2

Manuscript number: UOG-2014-0092

Dear Dr Bennini

We are pleased to receive your manuscript entitled Gastroschisis: fetal longitudinal follow-up and perinatal outcomes by Bennini, Joao; Marussi, Emilio; Barini, Ricardo; Peralta, Cleisson Fábio. We will shortly be assigning it to one of the Journal's Editors who will handle the peer review of the paper.

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Many thanks for submitting your manuscript

Yours sincerely

Sarah Hatcher
Managing Editor

Gastroschisis: fetal longitudinal follow-up and perinatal outcomes

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Abstract

Objectives: To evaluate longitudinal prenatal ultrasonographic parameters as predictors of adverse outcomes in fetuses with gastroschisis.

Patients and methods: Retrospective cohort study of fetuses with isolated gastroschisis. The prenatal predictors of outcome evaluated in this study were: fetal growth, amniotic fluid volume, changes in the extra-abdominal bowel luminal diameter and the occurrence of simple or multiple intra-abdominal bowel dilation. The outcome variables evaluated were: fetal or neonatal death, neonatal intestinal complications, length of stay of the neonate in the neonatal intensive care unit, duration of exclusive parenteral nutrition and the time from birth to hospital discharge. The relationship between continuous data was tested by means of Pearson's or Spearman's correlation coefficients and univariate logistic regression.

Results: Forty-four fetuses were included. The presence of fetal multiple intra-abdominal bowel dilation was associated with an increased incidence of intestinal complications and the presence of fetal growth restriction was less frequent in patients with this outcome. There was no significant relation between the other longitudinal prenatal ultrasonographic predictors and the postnatal outcomes evaluated.

Conclusions: In fetuses with gastroschisis, evidence of multiple intra-abdominal intestinal dilations and normal fetal growth increases the risk of postnatal bowel complications.

Key words: gastroschisis, prenatal ultrasonography, pregnancy outcome.

Introduction

Gastroschisis is a congenital abdominal wall defect that occurs in 1-5 per 10,000 births.[1] The exact pathophysiology of this disease remains unknown, while many theories have been postulated.[2,3] Although the postnatal survival rates are high (90-95%), the concurrence of gastrointestinal alterations such as atresia, stenosis, perforation, necrosis or volvulus increases the lengths of neonatal hospitalization and parenteral nutrition, as well as the mortality rates.[4-9]

It is established that identifying prenatal factors associated with worse postnatal outcomes improves counselling and facilitates the management of these cases.[5,8] Case-control studies have demonstrated the importance of fetal ultrasound parameters, particularly bowel dilation, as predictors of a poorer prognosis.[5,10-16] However, there are no data regarding the influence of fetal changes throughout pregnancy on postnatal results. Therefore, the aim of this study was to evaluate the relation between prenatal ultrasonographic parameters evaluated longitudinally and postnatal outcomes in cases of gastroschisis.

Methods

This investigation was a retrospective cohort study conducted at Professor José Aristodemo Pinotti Hospital, Center for Integral Assistance to Women's Health of the State University of Campinas (UNICAMP), including fetuses with gastroschisis evaluated between February 2007 and November 2013. The ethics committee of the State University of Campinas Medical School approved this protocol.

The sample size for this study was calculated considering a proportion of intestinal complications of 7% in the group of fetuses with gastroschisis without intra-abdominal bowel dilation (IBD) and 38% in the group with IBD[10] (equivalent to an odds ratio of 8.14). A chi-square test with a bilateral significance level of 5% has a power of 80% to diagnose a significant difference between the groups when the sample size is 28 patients in each group.[17]

The patients were selected among those followed-up in the Division of Obstetrics at our institution. The inclusion criteria were: 1. Singleton pregnancy. 2. Well-defined gestational age (GA) based on the known date of the last menstrual period and/or measurement of the embryonic/fetal crown-rump length during the first trimester, interpreted based on the reference intervals reported by Robinson and Fleming.[18] 3.

The presence of isolated fetal gastroschisis diagnosed during obstetric scans in our institution and confirmed by postnatal clinical examination. 4. The absence of any other fetal anatomical alteration detected during pregnancy or after birth. 5. At least three fetal ultrasound examinations, with delivery and neonatal follow-up in our hospital.

Demographic and clinical characteristics of the mother, including age, parity, GA at delivery and mode of delivery were collected from the mother's hospital records. The following neonatal parameters were obtained from the neonate's hospital files: birth-weight, Apgar scores, length of stay in the hospital and in the neonatal intensive care unit (NICU), the presence of intestinal complications (atresia, stenosis, perforation, necrosis or volvulus), duration of exclusive parenteral nutrition (EPN) and discharge of a live neonate from the hospital.

In our institution, fetuses with gastroschisis are followed-up according to a protocol that includes ultrasound examinations every two weeks from 24 to 34 weeks of gestation and on a weekly basis thereafter. The ultrasound scans include evaluations of the fetal growth, the amount of amniotic fluid, umbilical artery Doppler indices, the content herniated through the abdominal wall defect and the presence of extra and/or intra-abdominal intestinal dilations. The maximum extra-abdominal intestinal luminal diameter (inner to inner wall) is always measured. Intra-

abdominal intestinal dilations are described as absent or present if the lumen of an intestinal segment is ≤ 6 mm or > 6 mm, respectively. Intra-abdominal intestinal dilations are subsequently classified as simple (1 segment) or multiple (> 1 segment). All ultrasound examinations are performed or supervised by JRB (eight years of experience in fetal medicine).

The following longitudinal prenatal ultrasound parameters (predictor variables) were analyzed in this study: 1. Fetal growth: the estimated fetal weights (EFWs) were calculated using Bennini's formula and interpreted according to local conditional reference intervals of weight.[19] Fetal growth restriction (FGR) was defined as a progressive and evident change in the pattern of growth towards the inferior limits of these intervals, even if not reaching values below the 10th centile. 2. Amniotic fluid index (AFI): this parameter was measured and interpreted according to the reference intervals produced by Phelan *et al.*[20] Oligo and polyhydramnios were defined as AFIs that persistently stayed below or above, or crossed the limits of the 10th and 90th centiles of these reference intervals, respectively. 3. Changes in the extra-abdominal luminal diameter (delta luminal diameter - DLD): this parameter was defined as the difference between the last and the first maximum luminal diameter of extra-abdominal bowel loops divided by the number of weeks between the measurements. 4. IBD: defined as the presence and/or the

development of one or more dilated segments of intra-abdominal intestinal loops. 5. Multiple IBD: defined as the presence and/or the development of at least two dilated segments of intra-abdominal intestinal loops during the follow-up period.

The outcome variables evaluated in this study were: 1. Fetal or neonatal death. 2. Neonatal intestinal complications: atresia, stenosis, perforation or necrosis. 3. Length of stay of the neonate in the NICU. 4. Duration of EPN. 5. Time from birth to hospital discharge.

Maternal, pregnancy and postnatal data were described as absolute and relative frequencies, the average and standard deviation (SD) or the median and range. Continuous data were tested for normal distribution using the Kolmogorov–Smirnov test. Independent-sample t-tests or Mann–Whitney U tests and chi-square tests were used to assess continuous and categorical variables, when appropriate. The relationships between continuous variables were tested using Pearson's or Spearman's correlation coefficients and univariate logistic regression analysis was performed when appropriate. Differences were considered statistically significant if the two-tailed p-value was less than 0.05. The data were analyzed using the statistical software packages SPSS 20.0 (Chicago, Il, USA) and Excel for Mac 2011 (Microsoft Corp., Redmond, WA, USA).

Results

In total, 61 fetuses with gastroschisis were evaluated throughout the study period. Among them, 44 (72%) met the entry criteria (study group) and 17 (28%) were not included in the final analysis for the following reasons (Figure 1): ongoing pregnancies (4 cases); loss of follow-up (7 cases); less than three ultrasound fetal evaluations (5 cases); amnioexchange procedure (1 case). Maternal and perinatal data are shown in Table 1.

With regard to the serial prenatal ultrasound parameters, 33 (75%) fetuses evolved with FGR and 11 (25%) exhibited normal growth; 25 (57%) had a normal AFI, nine (20%) had oligohydramnios and 10 (23%) developed polyhydramnios; 28 (63%) fetuses did not show any sign of IBD, 13 (30%) had simple IBD and three (7%) developed multiple IBD; the median (range) extra-abdominal DLD was 1.10 (-7.40 - 4.00) mm per week. The median (range) time from birth to hospital discharge, stay in the NICU and duration of EPN were 35 (3 - 90) days, 28 (3 - 69) days and 23 (0 - 55) days, respectively. After the exclusion of one case of fetal demise and one case of neonatal death, fourteen (14/42 = 33%) neonates had intestinal complications, which included bowel atresia (7 cases = 17%), bowel necrosis (4 cases = 9%), bowel perforations (2

cases = 5%) and bowel stenosis (1 case = 2%). Except for the case of bowel stenosis, the other 13 (93%) neonates with intestinal complications underwent bowel resection. Data on the ultrasound predictors and postnatal outcomes are shown in Table 2.

None of the longitudinal prenatal ultrasound findings significantly influenced the occurrence of fetal or neonatal death, the time from birth to hospital discharge, the length of stay of the neonate in the NICU or the duration of EPN. FGR correlated negatively and the presence of multiple IBD correlated positively with the postnatal identification of intestinal complications (Table 3). All three fetuses with multiple IBD had postnatal intestinal complications, specifically, two cases of atresia and one case of stenosis. None of these fetuses had multiple IBD in the beginning of the follow-up. Multiple IBD in the fetus yielded a sensitivity of 21% and a 100% specificity in the prediction of neonatal bowel complications.

Discussion

This study demonstrated that the identification of multiple IBD in fetuses with gastroschisis during serial ultrasound examinations is associated with the detection of intestinal complications in the neonate. In addition, our data showed that FGR was less frequent among cases with postnatal intestinal alterations.

The positive relation between fetal IBD and neonatal bowel complications in cases of gastroschisis is well known.[5,10,13,21] Our results reinforce this knowledge and are similar to the findings published by Huh *et al*.[10] In their original work involving 43 cases of gastroschisis, these researchers reported neonatal bowel complications in all cases that presented fetal multiple IBD. None of their cases with simple IBD had neonatal intestinal alterations. In our series, two of 13 patients with single IBD had postnatal bowel complications whereas all three cases with multiple IBD presented this outcome. Our finding that all cases with multiple IBD were detected in the third trimester of pregnancy reinforces the importance of a serial prenatal follow-ups in these patients.

The association between FGR with a lower rate of neonatal intestinal complications has not been reported in the literature. In fact, many studies demonstrate that a low birthweight in cases of gastroschisis is related to higher neonatal morbidity and mortality rates.[22-24] The outcomes evaluated in these studies are mainly clinical, with no special attention to the occurrence of intestinal complications. The reasons for our findings are not clear, however it may be speculated that fetuses with gastroschisis and lower estimated weights have smaller abdominal circumferences due to greater exteriorization of abdominal contents through larger defects. This condition might somehow lower the pressure

on the bowel and minimize the risk of intrauterine ischemia. Further studies are necessary to better clarify our findings.

The main limitation of our study is most likely the small number of patients. No other prenatal predictor evaluated in our series significantly influenced the postnatal outcomes, however, there was a trend towards a significantly higher incidence of adverse postnatal outcomes when these markers were present. This finding warrants further analyses in a larger sample.

In conclusion, our study reinforces the association of multiple IBD with neonatal intestinal complications and adds the importance of a longitudinal prenatal follow-up for detecting this marker. In addition, we raise the question of a possible association between the pattern of fetal growth and the occurrence of adverse postnatal outcomes, which must be confirmed by further studies.

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Chart 1. Gastroschisis population flow chart

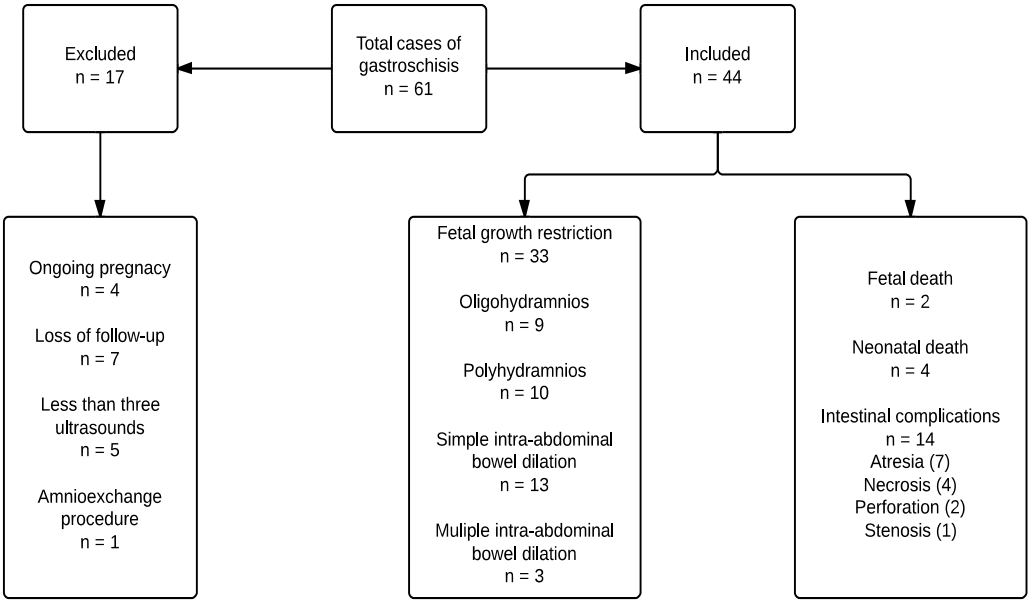

Table 1. Maternal, pregnancy and perinatal data of the study population

Mother and pregnancy	**n = 44**
Maternal age (years)*	20.3 ± 3.7 (13.3 – 33.0)
GA at delivery (weeks)*	36.4 ± 1.4 (32.0 – 38.9)
Parity	
Nuliparous	33 (75%)
Multiparous	11 (25%)
Delivery mode	
Vaginal	7 (16%)
Caesarean section	37 (84%)
Fetal death	2 (5%)
Number of scans**	7 (3 – 12)
Neonate	**n = 42**
Weight (grams)*	2332 ± 483 (1490 – 3575)
< 2000	9 (21%)
2000 – 2500	19 (45%)
> 2500 - 3000	12 (29%)
> 3000	2 (5%)
Apgar	
1º minute**	8 (1 – 10)
5º minute**	9 (7 – 10)
Size	
Small	19 (45%)
Adequate	23 (55%)

GA: gestational age

*Mean ± standard deviation (minimum – maximum)

**Median (minimum – maximum)

Table 2. Ultrasound predictors and outcomes evaluated

Ultrasound predictor	
Fetal growth	
FGR	33 (75%)
Normal growth	11 (25%)
AFI	
Normal	25 (57%)
Oligohydramnios	9 (20%)
Polyhydramnios	10 (23%)
IBD	
Absent	28 (63%)
Simple (1 segment)	13 (30%)
Multiple (≥ 2 segments)	3 (7%)
Extra-abdominal DLD* (mm/week)	1.10 (-7.40 - 4.00)
Outcomes	
Death	6 / 44 (14%)
Fetal	2 (5%)
Neonatal	4 (9%)
Intestinal complication	14 / 42 (33%)
Atresia	7 (17%)
Necrosis	4 (9%)
Perforation	2 (5%)
Stenosis	1 (2%)
Days of hospital stay*	35 (3 - 90)
Days in the NICU*	28 (3 - 69)
Days of EPN*	23 (0 - 55)

FGR: fetal growth restriction; AFI: amniotic fluid index; IBD: intra-abdominal bowel dilation; DLD: delta luminal diameter; NICU: neonatal inensive care unit; TPN: total parenteral nutrition
*Median (minimum – maximum)

Table 3. Comparisons between ultrasound predictors and outcome variables

		Fetal / Neonatal death			Intestinal complication			Days of		Days of		Days of	
		+	-	p	+	-	p	Hospital	p	NICU	p	EPN	p
FGR	+	4 (9%)	29 (66%)	§ 0.63	7 (17%)	24 (57%)	**§ 0.02**	36 (3 - 90)*	# 0.32	30 (3 - 67)*	# 0.57	23 (2 - 55)*	# 0.39
	-	2 (5%)	9 (20%)		7 (17%)	4 (9%)		26 (9 - 76)*		23 (9- 69)*		20 (0 - 37)*	
Oligohydramnios	+	2 (6%)	7 (20%)	§ 0.28	2 (6%)	6 (19%)	§ 1.00	38 (26 - 90)*	# 0.19	33 (11 - 67)*	# 0.07	25 (9 - 41)*	# 0.41
	-	2 (6%)	23 (68%)		7 (22%)	17 (53%)		32 (9 - 61)*		23 (9 - 51)*		20 (0 -42)*	
Polyhydramnios	+	2 (6%)	8 (22%)	§ 0.56	5 (15%)	5 (15%)	§ 0.27	40 (3 - 76)*	# 0.49	38 (3 - 69)*	# 0.13	26 (2 - 26)*	# 0.51
	-	2 (6%)	23 (66%)		7 (20%)	17 (50%)		32 (9 - 61)*		23 (9 - 51)*		20 (0 -42)*	
Any IBD	+	1 (2%)	15 (34%)	§ 0.39	5 (12%)	10 (24%)	§ 1.00	40 (15 - 76)*	# 0.49	32 (11 - 69)*	# 0.61	27 (5 - 55)*	# 0.54
	-	5 (12%)	23 (52%)		9 (21%)	18 (43%)		34 (3 - 90)*		26 (3 - 67)*		21 (0 - 55)*	
Multiple IBD	+	0 (0%)	3 (7%)	§ 1.00	3 (7%)	0 (0%)	**§ 0.03**	53 (18 - 76)*	# 0.43	48 (18 - 69)*	# 0.25	26 (5 - 27)*	# 0.92
	-	6 (14%)	35 (79%)		11 (26%)	28 (67%)		35 (3 - 90)*		28 (3 - 67)*		22 (0 - 55)*	
Extra-abdominal DLD		0.92* (-7.40 - 1.09)	1.37* (-0.13 - 4.00)	# 0.08	1.05* (-7.40 - 3.70)	1.37* (-0.13 - 4.00)	# 0.27	‡ 0.09	† 0.58	‡ 0.03	† 0.87	‡ 0.12	† 0.49

FGR: fetal growth restriction; IBD: intra-abdominal bowel dilation; DLD: delta luminal diameter;
NICU: neonatal intensive care unit; EPN: exclusive parenteral nutrition
* Median (minimum – maximum)
‡ Spearman's correlation coefficient
§ Fisher's exact test
# Independent samples Mann-Whitney U test
† Logistic univariate regression

4. Discussão geral

Nosso estudo apresentou resultados variados em relação aos descritos na literatura médica. Para algumas questões nossos achados confirmaram os relatos de publicações prévias, para outras as conclusões foram opostas ao esperado e foram levantadas novas hipóteses para perguntas que já pareciam ter respostas definidas.

No que diz respeito ao papel da ultrassonografia na predição de desfechos perinatais em casos de gastrosquise, a nossa observação de que a presença de múltipla DAI no feto associa-se com maior risco de complicações intestinais pós-natais confirma os resultados de estudos recentes. Esta confirmação é importante, pois se em fetos sem defeito de fechamento da parede abdominal a presença de múltipla DAI é considerada fortemente sugestiva de obstrução intestinal, nos casos de gastroquise este achado poderia ser apenas uma consequência da hipomotilidade intestinal que com freqüência é observada nesses pacientes. Nossos resultados confirmam que a presença de múltipla DAI não é uma achado que pode ser considerado normal em fetos com gastrosquise e, apesar de pouco sensível, possuiu alta especificidade para a presença de complicações intestinais no período pós-natal.

Um outro fator avaliado que, embora menos específico, também pode estar associado à obstrução intestinal em fetos sem defeitos da parede abdominal é o polidrâmnio. Na nossa amostra de fetos com gastrosquise esse sinal não se correlacionou estatisticamente com nenhum dos desfechos avaliados, mas foi mais freqüente nos casos de óbito fetal/neonatal e com complicações intestinais pós-natais. Além disso, os casos de gastrosquise com polidrâmnio tiveram uma tendência a maior tempo de internação hospitalar, internação em unidade de terapia intensiva neonatal e nutrição parenteral exclusiva. Desta forma, achamos que este é um sinal que merece ser mais bem avaliado em estudos futuros.

Um achado totalmente novo do nosso trabalho foi o efeito protetor que a RCF apresentou para a ocorrência de complicações intestinais pós-natais. Como vários estudos associam o baixo peso ao nascimento a maiores taxas de morbidade e mortalidade pós-natais, o esperado seria que a RCF se associasse a desfechos perinatais mais desfavoráveis. Conforme discutido em um dos artigos gerados a partir deste estudo, uma possível explicação seria que a RCF obervada nesses casos fosse uma conseqüência de uma menor medida da circunferência abdominal fetal devido a uma maior herniação do conteúdo intra-abdominal para a cavidade amniótica, já que a fórmula US2D usada para a estimativa de peso no estudo inclui medidas do abdome fetal. Na nossa opinião esta hipótese, embora precise ser confirmada em estudos futuros, faz sentido por dois motivos. Primeiro, as complicações intestinais estudadas (necrose, atresia, estenose e perfuração) podem ser secundárias a fenômenos isquêmicos e uma maior herniação do conteúdo intra-abdominal faria com que

as alças intestinais sofressem uma menor pressão e assim ficassem menos sujeitas à isquemia (vale lembrar que a dilatação de alças intestinais é um fenômeno freqüente nesses casos). Segundo, nossa taxa de RCF foi de 75% e nossa taxa de baixo peso ao nascimento de 45%, o que reforça a possibilidade de uma subestimativa do peso devido a uma menor medida da circunferência abdominal fetal. De qualquer forma, como já mencionado, estas hipóteses precisam ser mais bem avaliadas em trabalhos futuros.

Um resultado inesperado do trabalho foi o fato do nosso novo modelo US2D de estimativa do peso fetal ter apresentado um desempenho pior do que o do modelo US2D sem medidas abdominais proposto por Siemer *et al.* (2008) e do que os modelos US2D com medidas abdominais e US3D com o volume da coxa fetal propostos por Bennini *et al.* (2010). Esses resultados não eram esperados, pois dos modelos US2D avaliados a nossa nova fórmula é a única que incorpora dois fatores que de acordo com a literatura deveriam melhorar o desempenho na estimativa de peso dos fetos com gastrosquise em relação aos demais modelos testados: não utiliza medidas abdominais e foi gerada a partir de pacientes da mesma população dos fetos com gastrosquise. Vale lembrar que o modelo US3D previamente publicado pelo nosso grupo também foi gerado a partir de fetos da nossa população e não utiliza medidas abdominais fetais, mas como não encontramos relato do uso deste tipo de parâmetro (volume da coxa fetal medido por US3D) na estimativa de peso dos casos de gastrosquise, o seu desempenho era uma incógnita. Desta forma, a expectativa era de que o nossa nova formula US2D apresentasse um desempenho no mínimo semelhante às demais fórmulas avaliadas.

As razões para tais achados não nos são claras. Uma hipótese seria que, como os fetos com gastosquise tendem a ser menores, o impacto de variações fenotípicas interpopulacionais na predição do peso destes fetos também seria menor. Além disso, o modelo US2D proposto por Siemer *et al.* (2008) foi gerado a partir de uma amostra maior de pacientes e envolvendo somente fetos prematuros (idade gestacional inferior a 37 semanas), isto também pode ter contribuído para o melhor desempenho apresentado por esta fórmula quando comparado à nossa nova fórmula US2D. Uma opção futura seria conseguir um número de casos suficientes para gerar uma fórmula sem medidas abdominais geradas a partir de fetos prematuros da nossa população e novamente fazer esta comparação entre os modelos.

Achamos interessante ressaltar que os nossos modelos US2D e US3D prévios apresentam bom desempenho tanto em fetos normais como em fetos com gastrosquise e, portanto, podem ser utilizados em ambas as situações. Isto facilita a prática diária, pois elimina a necessidade de usar uma fórmula de estimativa de peso para cada situação.

Apesar de termos atingido o tamanho amostral esperado para a avaliação da estimativa do peso fetal por meio da ultrassonografia, o número de pacientes incluídos não permitiu a avaliação do impacto de outras variáveis, tais como tipo de estruturas herniadas pelo defeito abdominal ou posição do fígado em relação ao mesmo, nas acurácias das fórmulas estudadas. Pelo mesmo motivo, não foi possível avaliar adequadamente a sensibilidade e especificidade de cada modelo no diagnóstico de restrição de crescimento fetal. Este pequeno número

de casos se deve ao fato da gastrosquise ser uma doença rara e havermos utilizado pacientes atendidas em um único centro.

O pequeno tamanho amostral, associado à falta de padronização da avaliação ultrassonográfica pré-natal do casos de gastrosquise, também limitou muitas das conclusões em relação à avaliação dos preditores ultrassonográficos pré-natais e desfechos perinatais.

Desta forma, o estabelecimento de uma colaboração multicêntrica, preferencialmente com a utlização de protocolos prospectivos com parâmetros ultrassonográficos claramente definidos a serem avaliados, seria uma solução para conseguirmos casuísticas maiores e com avaliações padronizadas e assim esclarecer muitas das dúvidas que ainda pairam sobre o papel da ultrassonografia pré-natal nos casos de gastrosquise.

5. Conclusão geral

- A nova fórmula US2D sem medidas abdominais gerada a partir de nossos pacientes não melhorou a estimativa do peso ao nascimento de fetos com gastrosquise da nossa população em relação às outras fórmulas US2D e US3D já publicadas avaliadas. O modelo US2D de Siemer *et al.* (2008) e as fórmulas US2D e US3D de Bennini *et al.* (2010) apresentaram os melhores desempenhos na estimativa de peso dos fetos com gastrosquise da nossa população, não havendo diferença estatisticamente significativa entre os mesmos.

- Em fetos com gastrosquise o achado de DAI múltipla no período pré-natal aumenta o risco de complicações intestinais (atresia, estenose, perfuração e necrose) pós-natais e a presença de RCF possui um efeito protetor para este mesmo desfecho. Não houve relação entre os demais parâmetros ecográficos pré-natais e os desfechos perinatais avaliados.

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7. Anexos

7.1. Anexo 1 - Parecer da Comissão de Pesquisa do DTG/CAISM

UNICAMP

Comissão de Pesquisa do DTG / CAISM

Campinas, 9 de outubro de 2012.

Protocolo nº: 052/2012

O protocolo de pesquisa *"Gastrosquise: relação entre parâmetros ultrassonográficos pré-natais e a evolução pós-natal"* do pesquisador João Renato Bennini Júnior, orientado pelo Prof. Dr. Cleisson Fábio A. Peralta, foi aprovado pela Comissão de Pesquisa do DTG/CAISM em 09/10/2012.

Atenciosamente,

PROF. DR. JOSÉ GUILHERME CECATTI
Presidente da Comissão de Pesquisa do DTG/CAISM

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7.2. Anexo 2 - Aprovação do projeto no CEP

FACULDADE DE CIENCIAS MEDICAS - UNICAMP (CAMPUS CAMPINAS)

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Gastrosquise: relação entre parâmetros ultrassonográficos pré-natais e a evolução pós-natal

Pesquisador: Joao Bennini

Área Temática:

Versão: 3

CAAE: 10261212.7.0000.5404

Instituição Proponente: Hospital da Mulher Prof. Dr. José Aristodemo Pinotti - CAISM

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 234.076

Data da Relatoria: 07/03/2013

Apresentação do Projeto:

O pesquisador esclarece que dados recentes mostram um aumento de 10 a 20 vezes na incidência da gastrosquise em todos os grupos etários nas últimas duas décadas, embora ainda não haja uma explicação clara para isso. Associa-se a altas taxas de complicações intestinais, como perfuração, atresia e necrose, com significativa morbidade. Apesar de reconhecer a importância da identificação de fatores pré-natais que possam predizer o risco de complicações nos casos de gastrosquise,a literatura é controversa sobre quais seriam estes fatores e sobre o desempenho dos mesmos.

Método:

Estudo observacional analítico retrospectivo do tipo caso-controle, no qual os casos serão os fetos com gastrosquise que apresentaram complicações intestinaisv e os controles aqueles que não apresentaram. Os sujeitos serão selecionados entre aqueles acompanhados na Área de Obstetrícia do CAISM / UNICAMP. O tamanho da amostra foi estimado em 56 pacientes. As características demográficas maternas, gestacionais e pós-natais serão descritas utilizando-se freqüências absolutas e relativas, média ± desvio padrão e mediana e percentis. Análise de regressão logística simples será realizada para o cálculo das razões de chances quando as variáveis independentes categóricas forem estudadas em relação à ocorrência ou não de complicações intestinais neonatais. A normalidade da distribuição dos dados contínuos será avaliada com o teste de Kolmogorov-Smirnoff e as comparações serão feitas com o teste t de amostras independentes ou

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com o teste de Mann-Whitney. Valores de p menores que 0,05 serão considerados estatisticamente significativos.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar as associações entre parâmetros ultrassonográficos pré-natais e complicações intestinais pós-natais em casos de gastrosquise.

Objetivo Secundário:

Avaliar as relações entre a presença de complicações intestinais pós-natais, (definidas como perfuração, obstrução ou necrose) com os seguintes parâmetros ultrassonográficos pré-natais: oligoâmnio, dilatação de alças intestinais extra-abdominais,dilatação de alças intestinais intra-abdominais, índice de pulsatilidade da artéria mesentérica superior fetal,dilatação do estômago fetal, herniação hepática e herniação vesical.

Avaliação dos Riscos e Benefícios:

Trata-se de um estudo observacional analítico retrospectivo do tipo caso-controle, portanto sem riscos previsíveis aos sujeitos de pesquisa.

Não haverá benefícios diretos aos sujeitos de pesquisa, sendo que a definição de fatores prognósticos pré-natais ajudaria na identificação dos fetos que se beneficiariam de acompanhamento mais rigoroso ou mesmo de parto e intervenção precoce, além de trazer mais informações para os cirurgiões e possibilitar melhor aconselhamento dos pais, resultando em potenciais benefícios sociais.

Comentários e Considerações sobre a Pesquisa:

Projeto bem qualificado, com desenho adequado para a baixa prevalência da doença e para os objetivos propostos. Apresentou cálculo de tamanho amostral, baseado em pressupostos estatísticos corretos. Poderá trazer subsídios importantes para o grupo de cirugiões e neonatologistas que cuidam destes pacientes, além de fornecer importantes dados ao grupos de medicina fetal que fornecem informações prognósticas aos familiares.

Considerações sobre os Termos de apresentação obrigatória:

Os investigadores apresentaram aprovação da Comissão de Pesquisa do Departamento de Tocoginecologia do CAISM/UNICMAP e Folha de rosto devidamente preenchida. Projeto gerado pela Plataforma Brasil, com adequação da área temática. Há solicitação de dispensa do TCLE, por se tratar de estudo retrospectivo.

Recomendações:

Pendências atendidas.

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Conclusões ou Pendências e Lista de Inadequações:

Aprovado com dispensa do TCLE.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Em estudos retrospectivos, caso o pesquisador encontre dados que possam modificar o prognóstico ou tratamento dos sujeitos de pesquisa, recomenda-se que tais informações sejam transmitidas aos participantes e/ou anexadas ao prontuário para conhecimento da equipe clínica.

CAMPINAS, 02 de Abril de 2013

Assinador por:
Fátima Aparecida Bottcher Luiz
(Coordenador)

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7.3. Anexo 3 - Ficha de coleta de dados

Projeto de pesquisa: "Gastrosquise: relação entre parâmetros ultrassonográficos pré-natais e a evolução pós-natal"

Pesquisador responsável: Dr. João Renato Bennini Júnior

Caso nº:_____ HC nº: _____ Data da coleta dos dados: _____/_____/_____

DADOS DA GESTANTE

Nome: _____ Sobrenome: _____

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

G: _____ PN: ____ C: ____ A: _____

DADOS DA ULTRASSONOGRAFIA

Data:_____/_____/_____ Hora: _____:_____

DBP:____ OF:____ CC:_____ DAT:_____ DAAP:_____ CA:_____ CF:_____

Volume da coxa:_____

Peso fetal estimado: _____ ILA (cm): _____ Oligo/Polidrâmnio: _____

Maior calibre de alça: extra-abdominal: _____ intra-abdominal: _____

DADOS DO PARTO

Data: ____/____/____ Hora: _____:_____ Tipo de parto: PN: ☐ C: ☐ F: ☐

Peso do RN: _____ AIG: ☐ PIG: ☐ GIG: ☐

Obs: _____

DADOS PÓS-NATAIS

Complicações intestinais: _____

Tipo da complicação: _____

Tempo UTI neonatal:_____

Tempo nutrição enteral total:_____

Tempo de internação:_____

7.4. Anexo 4 - Artigo: "Birth-weight prediction by two- and three-dimensional ultrasound imaging"

Ultrasound Obstet Gynecol 2010; 35: 426–433
Published online 9 December 2009 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.7518

Birth-weight prediction by two- and three-dimensional ultrasound imaging

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KEYWORDS: birth-weight estimation; fetal growth; fetal thigh volume; three-dimensional ultrasound; two-dimensional ultrasound

ABSTRACT

Objectives *To compare the accuracies of birth-weight predicting models derived from two-dimensional (2D) ultrasound parameters and from total fetal thigh volumes measured by three-dimensional (3D) ultrasound imaging; and to compare the performances of these formulae with those of previously published equations.*

Methods *A total of 210 patients were evaluated to create a formula-generating group (n = 150) and a prospective-validation group (n = 60). Polynomial regression analysis was performed on the first group to generate one equation based on 2D ultrasound measurements, one based on fetal thigh volume measured by the multiplanar technique (ThiM) and one based on fetal thigh volume obtained by the Virtual Organ Computer-aided AnaLysis (VOCAL™) method (ThiV). Paired-samples t-tests with Bonferroni adjustments were used to compare the performances of these equations in the formula-finding and the prospective-validation groups. The same approach was used to compare the accuracies of the new 2D and 3D formulae with those of both original and modified 2D equations from previous publications, as well as the 3D model reported by Chang* et al.

Results *The formulae with the best fit for the prediction of birth weight were: estimated fetal weight (EFW) = −562.824 + 11.962 × AC × FDL + 0.009 × BPD² × AC² (where AC is abdominal circumference, FDL is femur diaphysis length and BPD is biparietal diameter), EFW = 1033.286 + 12.733 × ThiM, and EFW = 1025.383 + 12.775 × ThiV. For both the formula-generating and the prospective-validation groups, there were no significant differences between the accuracies of the new 2D and 3D models in the prediction of birth weight. When applied to our population, the performances of the modified and original versions of the previously published 2D equations and the performance of the original 3D formula reported by Chang* et al. *were all significantly worse than our models.*

Conclusions *We believe that the greatest sources of discrepancy in estimation of birth weight are the phenotypic differences among patients used to create each of the formulae mentioned in this study. Our data reinforce the need for customized birth-weight prediction formulae, regardless of whether 2D or 3D measurements are employed. Copyright © 2009 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Accurate estimation of fetal weight is a major concern in perinatal care, because abnormal intrauterine growth is associated with increased neonatal morbidity and mortality[1–4]. For the past 30 years, the assessment of fetal size and growth has essentially been based on predictive models derived from two-dimensional (2D) ultrasound measurements. Although widely used in routine clinical practice, these formulae provide weight estimates with errors of up to 20% when compared with actual birth weights[5–11].

The advent of three-dimensional (3D) ultrasound imaging has allowed the accurate and reliable calculation of fetal organ volumes[12–15]. Some authors have demonstrated that the prediction of birth weight using fetal limb volumetry is more precise than that obtained using conventional 2D ultrasound parameters[15–20]. These studies have compared the accuracy of their new 3D

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Accepted: 7 September 2009

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volume-derived equations with that of the traditional 2D measurement models when both are applied to their 3D formula-generating and validation groups. An issue that remains unclear is whether these findings reflect a true improvement offered by 3D ultrasound imaging or simply result from phenotypic differences between the patients used to create each formula.

The aim of this study was to compare the accuracies of 2D and 3D birth-weight predicting models generated from the same sample of patients. In addition, we compared the performances of these formulae with those of previously published 2D and 3D equations when applied to our patients.

METHODS

This was a two-stage prospective cross-sectional study carried out at the Center for Integral Assistance to Women's Health over a 19-month period (between July 2007 and January 2009). The ethics committee of the State University of Campinas Medical School approved this protocol. All patients who agreed to participate signed an informed consent form.

The first phase of the study was carried out over the initial 15 months in order to compose a formula-finding group. The second phase was undertaken during the last 4 months to gather patients for validation of our new birth-weight predicting models. Data from the first subset of women were also used in another report aimed specifically at the comparison between the multiplanar and Virtual Organ Computer-aided AnaLysis (VOCAL™, GE Medical Systems, Zipf, Austria) techniques for the assessment of fetal thigh volume[21].

The eligibility criteria for the entire study were: patients admitted to the hospital for delivery or because of a high probability of the spontaneous onset of labor within the next few days; no maternal diseases or conditions associated with alterations in fetal growth, such as pre-eclampsia, diabetes or tobacco use; singleton pregnancy; well defined gestational age based on the known date of the last menstrual period and/or the measurement of embryonic/fetal crown–rump length during the first trimester, interpreted based on the reference intervals reported by Robinson and Fleming[22]; normal fetal anatomy during obstetric scans and confirmed by postnatal clinical examination; delivery < 49 complete hours after the 2D measurements; and 3D volume acquisition performed at our institution. Exclusion criteria were: multiple pregnancies; the presence of maternal diseases or conditions associated with alterations in fetal growth; uncertain gestational age; fetal anomaly detected by ultrasound imaging or after birth; delivery > 49 complete hours after the 2D and 3D ultrasound evaluations; and delivery in other hospitals. The amount of amniotic fluid was not used as a selection criterion. The patients were non-consecutive because ultrasound examination depended on the weekly schedules of the two physicians. Nonetheless, the inclusion process respected the chronological sequence in which the women were admitted to the hospital.

Demographic characteristics of the mother, including age, gestational age and parity, were recorded at the time of the scan. The subjects included in this study were mostly of mixed race and came from lower socioeconomic backgrounds. These characteristics are representative of the great majority of patients seeking assistance from the public health system of our country. Data on the gestational age at birth, mode of delivery and clinical characteristics of the newborn (weight and Apgar scores) were collected from the mother's hospital records. At our institution, neonates are categorized as small, normal or large for their gestational age if their weights fall below the 10th, between the 10th and the 90th, or above the 90th percentile of the reference intervals defined by Alexander *et al.*[23], respectively. All neonates were weighed immediately after birth in the delivery room on the same precision electronic Filizola Baby scale (Filizola SA, Weighting and Automation, Campo Grande, MS, Brazil), which has a precision of 5 g and is calibrated every 2 weeks. The date and time of each scan and birth were recorded to allow calculation of the time from scan to birth.

All ultrasound examinations were performed transabdominally, with a Voluson 730 Expert scanner, equipped with a RAB 4-8L probe (GE Medical Systems, Milwaukee, WI, USA) by either one or both of two previously selected physicians (J.R.B. and C.F.A.P., with 3 and 10 years' experience with 3D ultrasound imaging, respectively). Examination of the same patient by both operators was limited to cases used for analysis of intraobserver and interobserver variations, which are discussed in another manuscript[21]. Patients who were not included in the assessment of the repeatability and reproducibility of measurements were examined by one physician only. When a woman was evaluated by both physicians, only the first assessment by the first operator (J.R.B.) was used in this analysis.

Two-dimensional ultrasound measurements

Conventional 2D ultrasound fetal biometry was performed as follows. Head measurements were obtained in the axial view at the level of the cavum septi pellucidi, where both thalami could be seen symmetrically, and the anterior and posterior aspects of the cerebral falx were equidistant to the parietal bones. Biparietal diameter (BPD) was measured from the outer edge of the proximal parietal bone to the inner edge of the distal skull table, in a line perpendicular to the orientation of the cerebral falx. The head circumference (HC) was calculated using the scanner's automatically generated ellipse including the outer margins of the fetal skull. Abdominal circumference (AC) was measured in a transverse circular view of the abdomen at the level of the stomach and the portoumbilical vein complex. Anteroposterior (APD) and laterolateral (LLD) diameters across the center of the abdominal image were obtained. The calculation of AC was obtained from the equation $(APD + LLD) \times \pi/2$. Femur diaphysis length

(FDL) was measured in a plane in which the full femoral diaphysis was almost parallel to the transducer surface. The measurement was taken from one end of the diaphysis to the other.

Three-dimensional ultrasound measurements

The acquisition and storage of 3D datasets were performed as follows. Initially, the transducer was held over the longitudinal aspect of the fetal femur. Several thigh volumes were acquired using automatic sweeps, but two to four of them were stored for further analysis. The 3D volume box was adjusted for the size of the thigh, and the sweep angle was set at between 30 and 70° depending on the gestational age. The slowest sweep velocity (four frames per s) was chosen in order to guarantee the best resolution of the image, and the acquisition process was repeated if there was any maternal or fetal movement. Volumes affected by motion artifacts were not stored.

Thigh volumetry using both the multiplanar and VOCAL techniques was performed offline with 4D view software version 5.3 (GE Medical Systems) on datasets retrieved from the scanner by the same operator who examined the patient. For this purpose, the physician selected only one dataset among all those stored for a specific patient, on the basis of image quality, and measured thigh volumes using both methods (multiplanar and VOCAL).

Total fetal thigh volumetry by the multiplanar technique was performed as previously described by Chang *et al.*[16] and by ourselves[21]. Briefly, the surface of the thigh was drawn manually in every axial view 3 mm apart from each other, from one end of the femur diaphysis to the other. At the end of this process, the total thigh volume (ThiM) was automatically calculated by the built-in scanner software.

Thigh volumetry by the VOCAL technique was performed as previously described by our group[21]. The dataset containing the fetal thigh was displayed on the screen in three orthogonal planes. The sagittal view of the femur was exposed in one of these planes, and this image was rotated so that the thigh and whole diaphysis were identified in a perpendicular position. Two demarcating arrows were positioned at each end of the diaphysis to define the limits of the thigh to be included in the volume calculation. Volume estimates (ThiV) were computed utilizing the VOCAL program with a manual trace at 30° of rotation.

Statistical analysis

Comparison of the demographic characteristics of the formula-generating and prospective-validation groups

Patients in the formula-generating group were compared with those from the prospective-validation group with regard to age, gestational age at the time of the scan, parity, mode of delivery, time from scan to birth, weight of the neonate, Apgar scores and neonatal weight category (small-, normal- or large-for-gestational age). Independent-samples *t*-tests and Chi-square tests were used for the assessment of continuous and categorical variables, respectively. The Mann–Whitney *U*-test was used for comparison of Apgar scores between the two groups. Two-tailed $P < 0.05$ was considered statistically significant.

Creation of new two- and three-dimensional ultrasound models

Using data from the first phase of the study and weight of the neonate as the dependent variable, polynomial stepwise regression analyses up to the third order were considered to generate three birth-weight predicting models with the following predictors: conventional 2D ultrasound measurements, total fetal thigh volume measured using the multiplanar method (ThiM) and total fetal thigh volume measured by the VOCAL technique (ThiV). The formulae including thigh volumetry were also used in another report of the comparison of these methods[21]. To construct the 2D formula, eigenvalue, tolerance, variance inflation factor, condition index and variance proportion were calculated to check for multicollinearity among independent variables[24]. The criteria for multicollinearity were: eigenvalue < 0.1, tolerance value $< 1 - r^2$, variance inflation factor $> 1/(1 - r^2)$, condition index > 0.30 and variance proportion > 0.8. These tests were not performed when the 3D measurement models were derived, as in such cases only the first, second and third powers of thigh volumes were evaluated as independent variables. For all best-fit equations, Kolmogorov–Smirnov tests were performed to check for normality of the standardized residuals.

Comparison of previously published equations with the models created in this study

The original and modified 2D ultrasound formulae described by Hadlock *et al.*[8,9], Woo *et al.*[10] and Hsieh *et al.*[11], as well as the original 3D ultrasound equation reported by Chang *et al.*[16], were applied to our data in order to compare their performances with those of our new models. The criterion for the selection of these specific 2D equations was the presence of the same parameters (BPD, AC and FDL) used in our new 2D formula. The reason for choosing Chang's 3D model was that we used the same multiplanar technique for the measurement of total fetal thigh volume. The modified 2D ultrasound models (obtained from the original formulae published by Hadlock *et al.*[8,9], Woo *et al.*[10] and Hsieh *et al.*[11]) were generated by the calculation of new coefficients derived from multiple regression analysis using their equations' terms and our data. By using this approach, we sought to minimize potential biases in comparing the prediction errors of functions generated from two different study populations. The original and modified formulae used for comparison with our new models are described in Table 1.

Table 1 Original and modified two-dimensional (2DUS) and three-dimensional (3DUS) ultrasound imaging birth-weight prediction models used for comparison with the new formulae created in this study

Formula	*Parameter*	*Equation*
Hadlock *et al.*[8]	2DUS	$\text{Log}_{10}\ \text{EFW} = 1.3598 + 0.051 \times \text{AC} + 0.1844 \times \text{FDL} - 0.0037 \times \text{AC} \times \text{BPD}$
Modified Hadlock *et al.*[8]	2DUS	$\text{Log}_{10}\ \text{EFW} = 2.368 + 0.011 \times \text{AC} + 0.068 \times \text{FDL} + 0.001 \times \text{AC} \times \text{BPD}$
Hadlock *et al.*[9]	2DUS	$\text{Log}_{10}\ \text{EFW} = 1.335 - 0.0034 \times \text{AC} \times \text{FDL} + 0.0316 \times \text{BPD} + 0.0457 \times \text{AC} + 0.1623 \times \text{FDL}$
Modified Hadlock *et al.*[9]	2DUS	$\text{Log}_{10}\ \text{EFW} = 1.252 - 0.004 \times \text{AC} \times \text{FDL} + 0.027 \times \text{BPD} + 0.048 \times \text{AC} + 0.192 \times \text{FDL}$
Woo *et al.*[10]	2DUS	$\text{Log}_{10}\ \text{EFW} = 1.54 + 0.15 \times \text{BPD} + 0.00111 \times \text{AC}^2 - 0.0000764 \times \text{BPD} \times \text{AC}^2 + 0.05 \times \text{FDL} - 0.000992 \times \text{FDL} \times \text{AC}$
Modified Woo *et al.*[10]	2DUS	$\text{Log}_{10}\ \text{EFW} = 1.794 + 0.080 \times \text{BPD} + 0.001 \times \text{AC}^2 - 0.000046 \times \text{BPD} \times \text{AC}^2 + 0.126 \times \text{FDL} - 0.002 \times \text{FDL} \times \text{AC}$
Hsieh *et al.*[11]	2DUS	$\text{Log}_{10}\ \text{EFW} = 2.7193 + 1.745 \times 0.001 \times \text{BPD}^2 \times \text{FDL} - 7.6742 \times 0.0001 \times \text{AC} \times \text{BPD}^2 - 0.1432 \times \text{FDL} + 9.4962 \times 0.001 \times \text{AC} \times \text{BPD}$
Modified Hsieh *et al.*[11]	2DUS	$\text{Log}_{10}\ \text{EFW} = 2.369 - 0.00012 \times \text{BPD}^2 \times \text{FDL} - 0.000096 \times \text{AC} \times \text{BPD}^2 + 0.077 \times \text{FDL} + 0.003 \times \text{AC} \times \text{BPD}$
Chang *et al.*[16]	3DUS	$\text{EFW} = 1080.8735 + 22.44701 \times \text{ThiM}$

AC, abdominal circumference (in cm); BPD, biparietal diameter (in cm); EFW, estimated fetal weight (in g); FDL, femur diaphysis length (in cm); ThiM, fetal thigh volume measured by the multiplanar technique (in cm^3).

In both the formula-generating and the prospective-validation groups, the performances of each of these equations were analyzed by the calculation of systematic and random errors. The systematic error, or accuracy, was evaluated by calculating the mean sign percentage error ((estimated fetal weight − actual birth weight)/actual birth weight × 100). The random error, or precision, was evaluated by calculating the SD of the mean sign percentage error. Paired-samples *t*-tests with Bonferroni adjustments were used to detect significant differences between the accuracies of these formulae. The adjusted *P* value (*P′*), which was calculated according to the Bonferroni method, was obtained from the formula $P' = k \times P$, where k was the number of paired comparisons and the *P* value was obtained from each paired-samples *t*-test[25,26]. In this manner, for the comparison of the accuracies of our new 2D and 3D formulae, the *P′* for each paired comparison was obtained by the formula $3 \times P$ value. Similarly, for the comparison of our 2D model with the original and modified functions of Hadlock *et al.*[8,9], Woo *et al.*[10] and Hsieh *et al.*[11] (eight paired comparisons), each *P′* was obtained by the formula $P' = 8 \times P$ value of the paired-samples *t*-test. This method has the restriction that the *P′* cannot exceed 1.0. In order to compare the random errors of two equations, correlated variance tests for paired samples were used[27]. For each paired comparison, the variances were considered to be significantly different if the *P* value obtained from the *r* (Pearson's correlation coefficient) distribution table was less than 0.05. The *r* value was calculated using the formula: $r = (F - 1)/\sqrt{((F+1)^2 - 4 \times r^2 \times F)}$, where *F* is the ratio of the variances of the groups being compared. All *P* values exceeding 0.200 were referred to as $P > 0.200$.

The data were analyzed using the statistical software packages SPSS 16.0 (SPSS, Chicago, IL, USA) and Excel for Windows 2007 (Microsoft Corp., Redmond, WA, USA).

RESULTS

A total of 254 patients were evaluated throughout the whole study period. The first 182 women were examined during the initial 15 months (Phase 1) with the purpose of composing a formula-generating group. Of these, 153 met the entry criteria. A total of 29 cases were excluded because their deliveries occurred more than 49 h after the scan. During the last 4 months of the study (Phase 2), another 72 patients were evaluated in order to create a prospective-validation group. Ten of these were excluded because their deliveries occurred more than 49 h after the scan.

Of the 153 patients from the formula-generating group, three more were withdrawn from final analysis because fetal thigh volumetry was not possible owing to poor image quality in the 3D dataset. For the same reason, two of the 62 cases in the prospective-validation group were eliminated. Demographic and clinical data for the remaining 150 patients from the first group and 60 patients from the second group are presented in Table 2. No statistically significant differences were noted between these groups with regard to maternal age, gestational age at the time of the scan, parity, mode of delivery, time from scan to birth, weight of the neonate, Apgar scores or neonatal weight category.

The best-fit formulae for the estimation of fetal weight using 2D ultrasound parameters and 3D fetal thigh volumes were: estimated fetal weight (EFW) = $-562.824 + 11.962 \times \text{AC} \times \text{FDL} + 0.009 \times \text{BPD}^2 \times \text{AC}^2$ (SD of predicted values, 554.261; $r = 0.899$; $r^2 = 0.808$; $P < 0.001$), EFW = $1033.286 + 12.733 \times \text{ThiM}$ (SD of predicted values, 566.521; $r = 0.919$; $r^2 = 0.845$; $P < 0.001$) and EFW = $1025.383 + 12.775 \times \text{ThiV}$ (SD of predicted values, 570.6299; $r = 0.926$; $r^2 = 0.857$; $P < 0.001$). The results of the Kolmogorov–Smirnov tests revealed normal distributions of the standardized residuals for all equations.

For both the formula-generating and the prospective-validation groups, no statistically significant differences

Table 2 Demographic characteristics of the formula-generating and prospective-validation groups

Characteristics	*Formula-generating group (n = 150)*	*Prospective-validation group (n = 60)*	P
Mother			
Age (years)	26.4 ± 6.3 (15–43)	27.1 ± 6.1 (16–43)	0.535*
Gestational age (weeks)	38.4 ± 2.3 (29.7–41.7)	38.5 ± 5.8 (29.4–42.4)	0.970*
Parity			0.457†
Nulliparous	47	22	
Parous	103	38	
Mode of delivery			0.629†
Vaginal	68 (45.3)	25 (41.7)	
Cesarean section	82 (54.7)	35 (58.3)	
Time between scan and birth (h)	18.1 ± 14.7 (0.7–48.6)	19.1 ± 16.0 (0.7–48)	0.676*
Neonate			
Weight (g)	3124.7 ± 616.5 (1445–4500)	3247.4 ± 698.3 (1475–4750)	0.211*
< 2500 g	21 (14)	7 (11.7)	0.567†
2500–4000 g	118 (78.7)	46 (76.7)	
> 4000 g	11 (7.3)	7 (11.7)	
Apgar score			
1 min	9 (2–10)	9 (2–10)	0.983‡
5 min	10 (6–10)	10 (7–10)	0.718‡
Size			0.909†
Small	16 (10.7)	7 (11.7)	
Adequate	119 (79.3)	46 (76.7)	
Large	15 (10.0)	7 (11.7)	

Values are mean ± SD (range), *n* (%) or median (range). *Independent-samples *t*-test. †Chi-square test. ‡Mann–Whitney *U*-test.

Table 3 Accuracy and precision of birth-weight prediction of the two-dimensional (2D) and three-dimensional (3D) equations created in this study in the formula-generating and prospective-validation groups

		Versus 2D formula		*Versus 3D formula (ThiM)*	
Group/Formula	*MSPE ± SD (%)*	*P' MSPE*	*P SD*	*P' MSPE*	*P SD*
Formula-generating group					
New 2D formula	−0.27 ± 8.35				
New 3D formula (ThiM)	0.69 ± 7.64	0.594	> 0.200		
New 3D formula (ThiV)	0.73 ± 7.99	0.627	> 0.200	1.000	0.100
Prospective-validation group					
New 2D formula	1.27 ± 8.78				
New 3D formula (ThiM)	−0.82 ± 8.24	0.402	> 0.200		
New 3D formula (ThiV)	0.06 ± 8.08	1.000	> 0.200	0.087	> 0.200

MSPE ± SD, mean sign percentage error (accuracy) ± SD (precision) of MSPE; P' MSPE, paired-samples *t*-tests with Bonferroni adjustment for comparison of MSPE; *P* SD, correlated variance in paired samples for comparison of SD; ThiM, thigh volume measured using multiplanar technique; ThiV, thigh volume measured using VOCAL technique.

were noted between the accuracies and precisions of our 2D and 3D models in the prediction of birth weight (Table 3).

The performances of previously published formulae, when applied to our population, were significantly worse than those of our new models (Tables 4 and 5). Although our 2D function allowed the estimation of birth weight with a mean ± SD sign percentage error of −0.27 ± 8.35% in the formula-finding group, the result obtained from the original model reported by Hsieh *et al.*[11] was 5.97 ± 15.34%. The performances of all other original and modified 2D models were even worse than the model described by Hsieh *et al.*[11] (Table 4). In the prospective-validation group, our 2D equation had an accuracy of 1.27%, which was not significantly different from those obtained using the original and modified formulae of Hsieh *et al.*[11] and the original models reported by Woo *et al.*[10] and Hadlock *et al.*[9] (Table 4). However, the random errors produced by all formulae tested in this study were significantly worse than those resulting from our new 2D model, in both the formula-finding and the prospective-validation groups. Similarly, the performance of our new 3D function that used ThiM was significantly better than that of Chang's equation, in both the formula-finding and the prospective-validation groups (Table 5).

Table 4 Accuracy and precision of birth-weight prediction of the two-dimensional (2D) equation created in this study and of the original and modified previously published 2D models

Parameter	*New 2D formula*	*Hsieh* et al.[11]		*Woo* et al.[10]		*Hadlock* et al.[8]		*Hadlock* et al.[9]	
		Original	*Modified*	*Original*	*Modified*	*Original*	*Modified*	*Original*	*Modified*
Formula-generating group									
MSPE ± SD (%)	−0.27 ± 8.35	5.97 ± 15.34	7.94 ± 16.07	6.06 ± 15.19	18.72 ± 16.04	−12.43 ± 15.35	11.25 ± 16.18	7.64 ± 15.68	10.13 ± 15.96
P MSPE		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P SD		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Prospective-validation group									
MSPE ± SD (%)	1.27 ± 8.78	5.71 ± 17.72	7.23 ± 17.61	5.73 ± 17.47	18.41 ± 17.51	−13.76 ± 14.81	10.65 ± 17.64	6.71 ± 16.69	8.99 ± 17.25
P MSPE		0.336	0.056	0.376	< 0.001	< 0.001	< 0.001	0.064	< 0.001
P SD		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

MSPE ± SD, mean sign percentage error (accuracy) ± SD (precision) of MSPE; *P* MSPE, paired-samples *t*-tests with Bonferroni adjustment for comparison of MSPE; *P* SD, correlated variance in paired samples for comparison of SD.

Table 5 Comparison of the accuracy and precision of a previously published three-dimensional (3D) formula with those of our new 3D equation using thigh volumetry as determined by the multiplanar method

Formula	*Formula-generating group*	*Prospective-validation group*
New 3D formula (ThiM) (MSPE ± SD) (%)	0.73 ± 8.00	−0.82 ± 8.24
Chang *et al.*[11] formula (MSPE ± SD) (%)	52.75 ± 12.27	50.64 ± 12.40
P MSPE	< 0.001	< 0.001
P SD	< 0.001	< 0.001

MSPE, mean sign percentage error (accuracy) ± SD (precision) of MSPE; *P* MSPE, paired-samples *t*-tests for comparison of MSPE; *P* SD, correlated variance in paired samples for comparison of SD; ThiM, thigh volume measured using multiplanar technique.

DISCUSSION

This study shows that there is no statistically significant difference in the prediction of birth weight by 2D and 3D formulae that are generated from the same set of patients. Conversely, it demonstrates that 2D and 3D equations that were created using different populations have significantly lower performances in the prediction of birth weight in our patients than our models.

The main question raised in this study was whether the superior accuracy of 3D formulae over previously published 2D equations reflects a true advantage of 3D ultrasound imaging, or whether it is simply a result of phenotypic differences between the patients used to create each of these formulae. Chang *et al.*[16] concluded that 3D ultrasound assessment of fetal thigh volume has better accuracy in predicting birth weight than 2D ultrasound formulae previously produced by Warsof *et al.*[5], Thurnau *et al.*[7] and Hadlock *et al.*[9]. The 3D equation yielded a mean value of error of 0.0 g, percentage error of 0.7%, absolute error of 176.1 g and absolute percentage error of 5.8%, whereas the results for the same parameters using the 2D formulae were −206.2 g, −6.2%, 249.0 g and 7.5% (Warsof *et al.*[5]), −708.4 g, −20.8%, 708.4 g and 20.8% (Thurnau *et al.*[7]), and −224.9 g, −6.7%, 260.1 g and 7.8% (Hadlock *et al.*[9]). These values were all significantly different from those obtained with Chang's equation. Similarly, Lee *et al.*[20] demonstrated that a different way of measuring fetal thigh volume, the fractional limb volume, can also be used to predict birth weight with better accuracy than the formulae reported by Shepard *et al.*[6] and Hadlock *et al.*[9] Other authors have used fetal upper arm volumes[17] and abdominal organ volumes[18] to estimate birth weight, and have also demonstrated an improved performance of these formulae compared with those of traditional 2D equations. Our results, at least in part, contradict these findings. We observed that, if 2D and 3D formulae are generated from the same set of patients, they have similar accuracies for the prediction of birth weight. More recently, in 176

prolonged pregnancies, Lindell and Marsal demonstrated that fetal weight could be estimated using 2D sonography with the same accuracy as 3D sonography[28].

In this study, we compared the accuracies of the most commonly used 2D formulae and of the 3D equation reported by Chang *et al.*[16] with those of our new 2D and 3D models. We did not compare our 3D equation with the formulae generated by Song *et al.*[19] and Lee *et al.*[20] because the techniques used to calculate fetal thigh volume were different. Comparisons with the equations created by Schild *et al.*[18] were not possible either, because they used fetal abdominal organ volumes in association with thigh volumes to predict weight. The selection of conventional 2D formulae for comparison with our models was based on the predictor variables included in these functions. We chose only the formulae that used BPD, FDL and AC, which were the parameters used in our equation. In addition, we took care to generate modified 2D ultrasound models from the original formulae published by Hadlock *et al.*[8,9], Woo *et al.*[10] and Hsieh *et al.*[11] for comparison with our 2D equation. As mentioned previously in this article, by using this approach, we sought to minimize potential biases in comparing the prediction errors of formulae generated from two different study populations.

Our results support the claim that birth-weight predicting formulae should be customized to each specific population. We noticed striking differences in the prediction of birth weight between our formulae and others. When we applied the original equations reported by Hsieh *et al.*[11], Woo *et al.*[10] and Hadlock *et al.*[8,9] to the patients in our formula-finding group, we obtained corresponding mean ± SD sign percentage errors of $5.97 \pm 15.34\%$, $6.06 \pm 15.19\%$, $-12.43 \pm 15.35\%$ and $7.64 \pm 15.68\%$. These results were all significantly different from those obtained using our new 2D equation (P' MSPE < 0.001; P SD < 0.001). The performances of the modified models were even worse when applied to the formula-generating group. Regarding the prospective-validation group, there were no differences observed in the accuracies of the original and modified formulae of Hsieh *et al.*[11], the original model of Woo *et al.*[10] or the original model of Hadlock *et al.*[9] and the accuracy of our 2D equation. However, in this group, these formulae yielded significantly higher random errors than those generated by our model. Moreover, it is important to consider that we used paired-samples *t*-tests with Bonferroni adjustment for the comparison of the accuracies of different equations, in both the formula-finding and the prospective-validation groups. This statistic was chosen instead of ANOVA because our intention was to compare specific pairs of observations rather than all possible combinations of groups of measurements. This approach is known to be highly conservative in controlling the overall Type I error when multiple comparisons are made. Therefore, we were extremely rigid in considering all possible original and modified formulae (we used a total of eight different equations) for comparison with our 2D model, as the corrected P values obtained for each paired comparison resulted from the multiplication of each P value derived from the paired samples *t*-test by eight. In their article regarding fractional limb volume, Lee *et al.*[20] also applied a modified formula generated by Hadlock *et al.*[9] to their patients and noticed a better performance than that obtained with the original equation. In our evaluation, the modified models of all previously published 2D equations did worse than the original models at predicting birth weight in our population. When we compared our 3D equation to that of Chang *et al.*[16], the differences were even greater than those observed in the comparison of our 2D equation with the other 2D models.

One may argue that our findings are the result of inaccurately performed 2D and 3D measurements. We believe this hypothesis to be extremely unlikely because the 2D ultrasound measurements of BPD, HC, AC and FDL have been employed universally for years, and the physicians who evaluated the patients in this study have significant experience in performing obstetric ultrasound examinations. The greater discrepancy found in the comparison of the 3D formulae may have resulted from a systematic difference in the method of fetal thigh volumetry because the training process to become experienced with 3D ultrasound imaging and fetal organ volumetry is more complex and time consuming. All of the steps described by Chang *et al.*[16], however, were followed in our patients. Moreover, before data acquisition for this analysis, both sonographers involved in fetal thigh volumetry underwent a training process and each evaluated 50 datasets. In addition, in a separate analysis, we demonstrated that both the repeatability and reproducibility of total fetal thigh volume measurements using the multiplanar technique are acceptable, and are comparable with the results obtained by other authors[20,21].

In conclusion, we believe that the greatest sources of discrepancy in estimation of birth weight are the phenotypic differences among the patients used to create each of the formulae mentioned in this study. We suppose that lower errors would result from the use of simpler processes, which could give 2D measurements an advantage. In addition, we believe that our data reinforce the need for customization of birth-weight prediction formulae, regardless of whether 2D or 3D measurements are employed.

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