UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS DA SAÚDE
Programa de Pós-graduação em Ciências da Saúde

ANDRÉA SIMONE SIQUEIRA DE QUEIRÓS

SHUNT INTRAPULMONAR EM PACIENTES COM ESQUISTOSSOMOSE HEPATOESPLÊNICA: DIAGNÓSTICO ATRAVÉS DE CINTILOGRAFIA COM MMA-Tc 99m

Dissertação de Mestrado

RECIFE, 2013

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Dissertação submetida à Universidade Federal de Pernambuco como parte dos requisitos para a obtenção do grau de Mestre em Ciência da Saúde.

Orientadora: Prof. Dra. Simone Cristina Soares Brandão

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UNIVERSIDADE FEDERAL DE PERNAMBUCO
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RELATÓRIO DA DEFESA DE DISSERTAÇÃO ANDRÉA SIMONE DE QUEIRÓS, ALUNO DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE, TURMA INICIADA EM 2011 (DOIS MIL E ONZE)

Às nove horas do dia dois de agosto de dois mil e treze, no Auditório do Prédio das Pós-Graduações do CCS, tiveram início, pelo Coordenador do Curso, Prof.º Dr. Emanuel Sávio Cavalcanti Sarinho, o trabalho de Defesa da Dissertação, da mestranda **Andréa Simone Siqueira de Queiroz** para obtenção do **Grau de Mestre em Ciências da Saúde** do Centro de Ciências da Saúde da Universidade Federal de Pernambuco. A Comissão Julgadora eleita pelo Colegiado do Curso e homologada pelas Câmaras de Pesquisa e Pós-Graduação foi formada pelos professores: **Dr. Hilton Justino da Silva** na qualidade de Presidente, do Departamento de Fonoaudiologia da UFPE, **Dr. Bruno Severo Gomes**, do Departamento de Medicina Micologia da UFPE e **Dr.ª Cláudia Diniz Lopes Marques**, do Departamento de Medicina Clínica da UFPE. A Dissertação apresentada versou sobre: **"Shunt Intrapulmonar em Pacientes com Esquistossomose Hepatoesplênica: Diagnóstico através de Cintilografia com MMA-Tc 99m"**, tendo como orientador a Prof.ª Simone Cristina Soares Brandão, do Departamento de Medicina Clínica da UFPE. Após a explanação de 30 minutos feita pelo candidato, justificando a escolha do assunto, objetivos da Dissertação, metodologia empregada e resultados obtidos, ilustrados com diapositivos, foram realizadas as arguições pela Banca Examinadora, todos no tempo regulamentar e respondido pela candidata. Ao término das arguições, a Banca avaliou em secreto e proferiu o seguinte resultado: _____. Nada mais havendo a registrar, foram encerrados os trabalhos, do que, para constar, foi elaborado o presente relatório que vai assinado pelo Senhor Presidente e demais membros da Comissão Julgadora. Recife, 2 de agosto de 2013.

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Prof. Sandro Gonçalves de Lima
Profª. Simone Cristina Soares Brandão

Dedico essa dissertação a meus pais, sem muitas palavras, pois elas são incapazes de representar a admiração e o orgulho que eles despertam em mim.

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A Deus, pois N´Ele somos, nos movemos e existimos.

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"As futuras gerações algum dia vão rir da tolice dos filósofos materialistas modernos. Quanto mais estudo a natureza, mais fico maravilhado com os feitos do Criador. Oro enquanto estou trabalhando no laboratório."

Louis Pasteur

RESUMO

Hipertensão portal é responsável pelo surgimento das dilatações vasculares intrapulmonares (DVIP), evento-chave no desenvolvimento da síndrome hepatopulmonar. Esquistossomose hepatoesplênica (EHE) é causa importante de hipertensão portal e não há estudos que avaliem o significado clínico das DVIP nesse grupo. A cintilografia com macroagregados de albumina marcados com tecnécio-99m (MAA-Tc^{99m}) é sensível e específica para o diagnóstico dessa alteração. Os objetivos deste estudo foram: determinar a frequência de DVIP em esquistossomóticos, correlacionando com parâmetros clínicos, laboratoriais, endoscópicos e ultrassonográficos; avaliar grau de reprodutibilidade e valores normais da cintilografia no diagnóstico das DVIP. Cinquenta e um pacientes foram avaliados e divididos de acordo com a presença ou ausência de DVIP. Dos 51 pacientes, 31 apresentaram DVIP. Diferenças estatisticamente significantes entre os grupos foram encontradas no diâmetro de veia esplênica. Os parâmetros de normalidade e grau de reprodutibilidade foram avaliados em nove indivíduos saudáveis e 15 esquistossomóticos, respectivamente. A captação cerebral média foi 7,9 ± 0,01% e a sistêmica 12,4 ± 0,03% nos indivíduos normais. A frequência de DVIP foi elevada, e o menor diâmetro de veia esplênica observado no grupo com DVIP pode significar adaptação à hipertensão portal. A cintilografia mostrou uma excelente reprodutibilidade, com valores de normalidade superiores aos da literatura.

PALAVRAS-CHAVE: Cintilografia. Esquistossomose. Tecnécio.

ABSTRACT

Portal hypertension is responsible for intrapulmonary vascular dilations (IPVD), key event in the development of hepatopulmonary syndrome (HPS). Hepatosplenic schistossomiais (HSS) is an important cause of portal hypertension but there are no studies assessing the IPVD´s clinical significance. ^{99m}Tc-macroaggregated albumin (^{99m}Tc-MAA) scintigraphy has high sensitivity and specificity for IPVD´s diagnostic. The aims of this study were: evaluate the occurrence of IPVD and its association with clinical, laboratory, endoscopic and ultrasound parameters in patients with HSS; determine normal parameters to scintigraphy and evaluate intra and inter observer agreement. Fifty-one patients with HSS was evaluated and divided according to the presence or absence of IPVD. Clinical, laboratory, endoscopic and ultrasound variables were assessed. Of the 51 patients evaluated, it was observed IPVD in 31 patients. Statistically significant differences between groups were found in the diameter of the splenic vein. Normal parameters to scintigraphy and intra and inter observer reproducibility were evaluated in nine healthy patients and 15 patients with HSS, respectively. In the healthy patients, the mean brain uptake of ^{99m} Tc-MAA was 7.9 ± 0.01%, and the mean systemic uptake was 12.4 ± 0.03%. In patients with HSS, the occurrence of IPVD was higher. The lower splenic vein diameter shows that IPVD can be a mechanism of vascular protection against portal hypertension. ^{99m}Tc-MAA scintigraphy is reproducible, mostly when used brain uptake, and normal parameters was a little upper in this study than in other studies.

KEY-WORDS: Scintigraphy. Schistosomiasis. Technetium.

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ALT	Alanina aminotransferase
AST	Aspartato aminotransferase
CEP	Comitê de Ética e Pesquisa
CCS	Centro de Ciências da Saúde
DVIP	Dilatações vasculares intrapulmonares
EDA	Endoscopia digestiva alta
EHE	Esquistossomose hepatoesplênica
FA	Fosfatase alcalina
GGT	Gamaglutiltransferase
HC	Hospital das Clínicas
HDA	Hemorragia digestiva alta
INR	*International Normalized Ratio*
keV	kiloelétron-volts
MAA- Tc^{99m}	Magroagregados de albumina marcados com tecnécio-99m
MBq	Megabequerel
mCi	miliCurie
Nos	Óxido nítrico sintetase
PaO2	Pressão parcial arterial de oxigênio
SHP	Síndrome hepatopulmonar
ROI	Desenho de região de interesse
TCLE	Termo de consentimento livre e esclarecido
TPAE	Tempo de protrombina e atividade enzimática
UFPE	Universidade Federal de Pernambuco
US	Ultrassonografia

SUMÁRIO

1 APRESENTAÇÃO

Alterações hemodinâmicas importantes ocorrem na circulação pulmonar de pacientes com hipertensão portal (SUGA et al, 2009). Nesses pacientes, duas síndromes são reconhecidas: a hipertensão portopulmonar e a síndrome hepatopulmonar (SHP). A hipertensão portopulmonar é uma forma de hipertensão arterial pulmonar, relacionada à hipertensão portal, (GIUSCA et al. 2011) na qual ocorre aumento da resistência vascular pulmonar secundária ao remodelamento originado por lesões do tipo plexiforme (SUSSMAN, 2012).

A SHP é caracterizada por falha na oxigenação arterial, dilatações vasculares intrapulmonares (DVIP) e presença de doença hepática aguda ou crônica (RODRÍGUEZ-ROISIN et al. 2004). A formação das DVIP, também chamadas de *shunts* intrapulmonares, é o evento-chave no desenvolvimento da SHP (RODRÍGUEZ-ROISIN et al. 2004). A patogênese da formação dessas dilatações ainda não está bem estabelecida, mas sabe-se que a hipertensão portal leva ao aumento na produção de substâncias vasodilatadoras, que parecem estar implicadas na formação dos *shunts* intrapulmonares (TUMGOR et al. 2008).

A cirrose hepática é a causa mais estudada associada à formação dos *shunts* intrapulmonares, mas já está bem estabelecido que a causa primária da doença hepática ou o grau de disfunção do fígado não são os fatores mais importantes para a formação das DVIP, e já é documentada sua presença em pacientes não cirróticos com hipertensão portal. (KROWKA 2001, FERREIRA et al. 2009).

O diagnóstico das DVIP pode ser feito através de ecocardiograma transtorácico contrastado ou através de cintilografia com macroagregados de albumina marcados com tecnécio-99m (MAA-Tc^{99m}). O ecocardiograma transtorácico contrastado é o método diagnóstico mais utilizado, mas tem como desvantagens ser examinador-dependente e não quantificar a magnitude do *shunt* intrapulmonar (EL-SHABRAWI et al. 2010). A cintilografia com MAA-Tc^{99m} tem alta sensibilidade na detecção de pequenos *shunts* venoso-arteriais, o que permite diagnosticar precocemente as DVIP (KROWKA et al. 2000), além de quantificar esta alteração.

A motivação para este estudo ocorreu por ser a esquistossomose mansônica a principal causa de hipertensão portal na região Nordeste do Brasil (MACÊDO et al. 2010) e existirem poucos estudos, especialmente usando cintilografia com MAA-Tc^{99m}, que avaliaram a presença e a importância clínica das DVIP nesses pacientes. Este estudo faz parte de um projeto maior, tema de tese de doutorado, intitulada “Prevalência de síndrome

hepatopulmonar em pacientes com cirrose, esquistossomose mansônica na forma hepatoesplênica e doença hepática crônica mista".

As perguntas condutoras foram: qual a freqüência de DVIP em pacientes com esquistossomose hepatoesplênica avaliada pela cintilografia MAA-Tc^{99m}; qual a associação entre a presença de DVIP e os fatores de risco de hipertensão portal; a cintilografia com MAA-Tc^{99m} é um exame de boa reprodutibilidade e o valor de normalidade no diagnóstico de DVIP usado na literatura deve ser o mesmo em nosso meio. O artigo *"Intrapulmonary vascular dilatation evaluated by ^{99m} Tc-MAA scintigraphy and its association with portal hypertension in hepatosplenic schistosomiasis"* foi publicado na revista *PLOS – Neglected Tropical Diseases – QUALIS A1*. O artigo *"Evaluation of normality and reproducibility parameters of ^{99m}Tc-MAA scintigraphy in the diagnosis of intrapulmonary vascular dilatations"* foi publicado na *Annals of Nuclear Medicine – QUALIS A2*.

2 REFERENCIAL TEÓRICO

Esta seção visa fazer uma revisão do referencial teórico, assim como pontuar a justificativa e os objetivos deste estudo.

2.1 Revisão integrativa

Visando proporcionar uma melhor compreensão deste trabalho, esta seção contempla uma revisão dos temas: esquistossomose mansônica, dilatações vasculares intrapulmonares, diagnóstico de dilatações vasculares intrapulmonares, e cintilografia com macroagregados de albumina marcados com tecnécio-99m.

2.1.1 Esquistossomose mansônica

A esquistossomose mansônica é um sério problema de saúde pública, afetando aproximadamente seis milhões de pessoas no Brasil. É mais freqüente na Região Nordeste, no entanto ocorre em todas as regiões do país (SISTEMA DE VIGILÂNCIA EM SAÚDE, 2012). A doença é provocada pelo *Schistosoma mansoni*, parasita intravascular que tem no homem e outros mamíferos seu hospedeiro definitivo. Os ovos são eliminados pelas fezes do hospedeiro infectado. Na água, estes eclodem, liberando larvas ciliadas denominadas miracídios, que infectam o hospedeiro intermediário (caramujo da espécie Biomphalaria). Após quatro a seis semanas, as larvas abandonam o caramujo, na forma de cercárias, que ficam livres nas águas naturais. A transmissão para humanos ocorre através do contato de pele e mucosa com água contaminada (SISTEMA DE VIGILÂNCIA EM SAÚDE, 2012).

O parasita se aloja no sistema venoso portal e mesentérico do ser humano, levando à formação de granulomas periovulares e fibrose em torno desses granulomas (ANDRADE 2009). A infecção pode se manifestar de forma aguda ou evoluir para formas crônicas, sendo a carga parasitária e a resposta imune do hospedeiro os principais fatores determinantes nessa evolução (Da SILVA, 1992).

Na forma aguda, também chamada de febre de Katayama, manifestações clínicas específicas podem estar ausentes, especialmente em indivíduos que vivem em áreas endêmicas (Da SILVA, CHIEFFI & CARRILHO, 2005). A evolução da forma aguda para a forma crônica caracteriza-se por uma marcada diminuição da resposta inflamatória aos ovos do parasita, (COELHO et al. 1996) com formação de fibrose em torno dos granulomas periovulares.

Os ovos do *Schistosoma mansoni* que não ultrapassam o lúmen intestinal são

transportados, seguindo o fluxo sanguíneo do sistema porta, até o fígado, onde se depositam nos vasos pré-sinusoidais e provocam fibrose. Essa fibrose pode acometer a periferia do sistema venoso portal, o que caracteriza as formas intestinal e hepatointestinal, responsáveis por aproximadamente 90% dos casos nas áreas endêmicas, com pouca ou nenhuma manifestação clínica (ANDRADE, 2009).

Em 5 a 10% dos casos, a fibrose se estende até os espaços portais, com invasão da vasculatura periportal e formação da fibrose típica da esquistossomose hepatoesplênica (EHE) (FERREIRA et al. 2009). A patogênese dessa alteração ainda não está bem estabelecida, mas a resposta imune do hospedeiro (MATHEW & BOROS, 1986), mudanças vasculares e uma carga parasitária elevada parecem ser os principais fatores relacionados (ANDRADE, 2009) ao desenvolvimento da forma hepatoesplênica. A fibrose em torno dos granulomas periovulares e a fibrose periportal, também conhecida como fibrose de Symmers, são responsáveis pelo desenvolvimento de hipertensão portal. O aumento da pressão portal é responsável pelo desenvolvimento de esplenomegalia e formação de circulação colateral portossistêmica (ANDRADE, 2009), o que torna a forma hepatoesplênica a principal causa de morbimortalidade entre os pacientes esquistossomóticos (Da SILVA, 1992).

Assim como na circulação portal, também na vasculatura pulmonar dos pacientes com hipertensão portal ocorrem alterações hemodinâmicas, e duas síndromes podem ser identificadas – a hipertensão portopulmonar e a síndrome hepatopulmonar (SHP). A hipertensão portopulmonar é uma forma de hipertensão arterial pulmonar relacionada à hipertensão portal (GIUSCA et al. 2011), caracterizada por aumento da resistência vascular pulmonar secundária ao remodelamento originado por lesões do tipo plexiforme (SUSSMAN, 2012), sendo raros os casos com hipoxemia. Em pacientes cirróticos, a hipertensão portopulmonar associa-se com aumento da mortalidade e é uma contraindicação relativa ao transplante hepático (SUSSMAN, 2012).

A síndrome hepatopulmonar caracteriza-se por um defeito na oxigenação arterial induzida por dilatações vasculares intrapulmonares (DVIP), em associação com doença hepática aguda ou crônica e hipertensão portal (RODRÍGUEZ-ROISIN et al. 2004). A hipoxemia é comum, e pacientes cirróticos com SHP tem pior qualidade de vida e maior mortalidade quando comparado a cirróticos sem essa alteração (FALLON et al. 2008). Em pacientes esquistossomóticos ainda é desconhecida a importância clínica desta alteração.

Apesar da presença de hipertensão portal fazer parte da definição da SHP, há relatos de casos de pacientes que desenvolveram a síndrome de forma secundária a alterações agudas na função hepática, sem aumento da pressão portal (KOCHAR, NEVAH & FALLON 2011). As

alterações hemodinâmicas presentes em indivíduos com SHP incluem diminuição da resistência vascular pulmonar, hipotensão sistêmica, aumento do débito cardíaco e baixa sensibilidade a substâncias vasoconstrictoras (HERVÉ et al. 1998). Não parece haver relação entre a gravidade da doença hepática e o surgimento das DVIP, evento que desencadeia o desenvolvimento da SHP (FERREIRA et al. 2009).

2.1.2 Dilatações vasculares intrapulmonares

Já no final do século XIX, havia suspeitas da relação entre doença hepática crônica e hipoxemia, mas só a partir da segunda metade do século XX surgiram as primeiras descrições das DVIP, em estudos realizados através de cintilografia com MAA-Tc^{99m} e em cirróticos submetidos à necrópsia (HOFFBAUER & RYDELL, 1956; BERTHELOT et al. 1966). Em 1977, foram publicados os primeiros relatos de caso com o uso do termo síndrome hepatopulmonar para descrever as alterações na oxigenação arterial que acontecem em pacientes com doença hepática crônica (KENNEDY & KNUDSON, 1977).

O desenvolvimento das DVIP, também chamadas de *shunts* intrapulmonares, é o evento-chave na patogênese da SHP. Essa anormalidade vascular inclui dilatação difusa ou localizada dos capilares pulmonares e, menos comumente, comunicação arteriovenosa pleural e pulmonar (MACHICAO & FALLON, 2012). Essas alterações podem levar à hipoxemia através de distúrbio no equilíbrio ventilação-perfusão alveolar, limitação da difusão das moléculas de oxigênio ou pela presença de *shunts* arteriovenosos verdadeiros (RODRÍGUEZ-ROISIN et al. 2004).

Existem duas formas de apresentação das DVIP: o tipo I é mais comum, estando presente em 85% dos casos, e caracteriza-se por dilatação vascular pulmonar difusa sem fístulas arteriovenosas, apresentando boa resposta da PaO2 à inalação de oxigênio a 100%; o tipo II é menos comum, afeta aproximadamente 15% dos indivíduos com DVIP, e caracteriza-se pela presença de comunicação arteriovenosa verdadeira e baixa resposta da PaO2 à inalação de oxigênio a 100% (HO, 2008).

A etiopatogenia das DVIP não é plenamente conhecida, mas a hipótese mais aceita sugere que alterações no metabolismo e na síntese de substâncias vasoativas pulmonares, remodelação da microvasculatura pulmonar e predisposição genética estejam implicadas no seu desenvolvimento (MACÊDO & LOPES, 2009). O estímulo que desencadeia essas alterações parece ser a hipertensão portal, que age aumentando a perfusão intestinal e a translocação bacteriana e de endotoxinas, o que estimula a liberação de substâncias como

fator de necrose tumoral alfa e óxido nítrico, e leva a desequilíbrio entre as substâncias vasoconstrictoras e vasodilatadoras (TUMGOR et al. 2008, EL-SHABRAWI et al. 2010).

A presença das DVIP é mais estudada em pacientes com cirrose hepática. No entanto, não parece haver relação entre a etiologia da doença hepática, o grau de disfunção hepática e a presença dos *shunts* intrapulmonares em pacientes adultos (FERREIRA et al. 2009). Em pacientes esquistossomóticos, o aumento da resistência vascular secundário à fibrose portal provoca diminuição do fluxo sanguíneo proveniente do fígado que chega aos pulmões, o que parece ser o fator de estímulo para liberação das substâncias vasoativas responsáveis pela formação das DVIP (KROWKA, 2001; MANDELL, 2007).

Em pacientes cirróticos com SHP, os níveis de óxido nítrico estão aumentados, diminuindo após a realização de transplante hepático, o que sugere sua importância na patogênese das DIVP. (CREMONA et al. 1995, ROLLA et al. 1997, TUMGOR et al. 2008). Em estudos realizados em ratos, a ligação do ducto biliar comum reproduz as alterações na vasculatura pulmonar e nas trocas gasosas que ocorrem em pessoas com DVIP (ZHANG & FALLON, 2012). Nesses animais ocorre aumento dos níveis séricos da enzima óxido nítrico sintetase (NOs) no endotélio pulmonar, demonstrando que os níveis de NOs estão relacionados com o desenvolvimento de DVIP (FALLON et al. 1997).

No entanto, apenas a liberação de substâncias vasodilatadoras não parece ser suficiente para explicar todas as alterações que ocorrem na formação dos *shunts* intrapulmonares, já que estudos com uso de antagonistas de óxido nítrico não mostraram melhora nas alterações da vasculatura pulmonar desses pacientes (El-SHABRAWI et al. 2010).

Estudo realizado em pacientes cirróticos mostrou que cerca de 20% deles tem *shunts* intrapulmonares. Além disso, nos pacientes com DVIP os marcadores ultrassonográficos de hipertensão portal foram mais frequentes que nos pacientes sem DVIP (KALAMBOKIS et al. 2010). Em pacientes esquistossomóticos, não há estudos que avaliem a importância dessas alterações na evolução clínica e no prognóstico deste grupo de pacientes.

2.1.3 Diagnóstico de dilatações vasculares intrapulmonares

O diagnóstico das DVIP pode ser feito através de ecocardiograma transtorácico contrastado, cintilografia com macroagregados de albumina marcados com tecnécio-99m (MAA-Tc^{99m}), arteriografia pulmonar ou tomografia de tórax de alta resolução, sendo os dois primeiros os métodos mais utilizados. (ABRAMS et al. 1995, RODRÍGUEZ-ROISIN et al. 2004, VARGHESE et al. 2007).

O ecocardiograma transtorácico contrastado detecta a presença das DVIP através da injeção de solução salina fisiológica, agitada manualmente, que forma microbolhas. Na presença de *shunt* direita-esquerda, essas microbolhas são rapidamente visualizadas nas câmaras cardíacas esquerdas (KOCHAR, TANIKELLA & FALLON, 2011). É o método diagnóstico mais utilizado, mas tem como desvantagens ser examinador-dependente e não quantificar a magnitude do *shunt* intrapulmonar (RODRÍGUEZ-ROISIN & KROWKA, 2008; KHAN et al. 2011).

A cintilografia com MAA-Tc^{99m} tem boa sensibilidade e especificidade na detecção das DVIP, além de quantificar a magnitude dessa alteração. Suas desvantagens incluem a falta de padronização do procedimento, utilizar substâncias radioativas, expondo o paciente a uma pequena quantidade de radiação, e não diferenciar *shunts* intracardíacos de intrapulmonares (ABRAMS et al. 1998, KROWKA et al. 2000, HOSONO et al. 2002, El-SHABRAWI et al. 2010, KAYMAKOGLU et al. 2003).

2.1.4 Cintilografia com macroagregados de albumina marcados com tecnécio-99m

No final dos anos 70 do século passado surgem os primeiros estudos a utilizar cintilografia com MAA-Tc^{99m} no diagnóstico das DVIP (WOLFE et al. 1977), mas só a partir de 1990 o uso desse método tornou-se mais comum. A cintilografia se baseia na injeção de pequena quantidade de partículas radiomarcadas na circulação sanguínea (ABRAMS et al. 1995, EL-SHABRAWI et al. 2010) e posterior medida da captação nos órgãos de interesse. Para o diagnóstico de DVIP, usa-se macroagregados de albumina como partícula e tecnécio-99m como radioisótopo, o que permite determinar a fração de *shunt* intrapulmonar através do cálculo da proporção do número de partículas que ultrapassa os capilares pulmonares e atinge a circulação sistêmica (El-SHABRAWI et al. 2010).

Os capilares pulmonares normais têm de 8 a 15 μm de diâmetro (RODRÍGUEZ-ROISIN & KROWKA 2008), enquanto as partículas de albumina marcadas com Tc^{99m} têm, em média, mais de 20 μm de diâmetro (KROWKA et al. 2000), com mais de 90% delas ficando retidas no leito vascular pulmonar em indivíduos normais. Na presença de DVIP, como os capilares estão com seu diâmetro aumentado, uma fração dessas partículas sai do leito vascular e é transportada para outros órgãos, o que pode ser quantificado pela relação entre a atividade radioativa intra e extrapulmonar (RODRÍGUEZ-ROISIN, et al. 2004).

Para o diagnóstico, usa-se principalmente a porcentagem de MAA-Tc^{99m} retida no

cérebro, sendo uma captação cerebral relativa aos pulmões maior do que 6% indicativa da presença de DVIP (RODRÍGUEZ-ROISIN et al. 2004). No entanto, alguns trabalhos utilizam a captação sistêmica (ABRAMS et al. 1995, El-SHABRAWI et al. 2010) e outros, a soma da captação cerebral com a captação renal (McADAMS et al. 1996, KAYMAKOGLU et al. 2003) como índices diagnósticos.

A cintilografia com MAA-Tc^{99m} é um exame simples, seguro, pouco invasivo, sensível e específico na detecção de DVIP (El-SHABRAWI et al. 2010). Outra vantagem da cintilografia com MAA-Tc^{99m} é quantificar a DVIP. Essa medida tem relação inversamente proporcional com a oxigenação arterial em ar ambiente. Esses dados podem ser úteis para determinar a gravidade das alterações pulmonares em pacientes com hipertensão portal e doença pulmonar concomitante, determinando a participação do *shunt* intrapulmonar na hipoxemia (ABRAMS et al. 1998, VARGHESE et al. 2007). Além disso, tem importância no prognóstico do paciente, já que captação cerebral acima de 20% é uma contraindicação relativa ao transplante hepático (HENDRICKSE, AZAM & MANDELL 2007).

2.2 Justificativa

A EHE é uma doença endêmica, que afeta aproximadamente seis milhões de brasileiros, sendo considerada a principal causa de doença hepática crônica e hipertensão portal na Região Nordeste do Brasil. Na EHE, na grande maioria dos pacientes, não há alterações importantes na função hepática, e o aumento da pressão portal, principal causa de morbimortalidade, é secundário à fibrose periportal. A hipertensão portal leva a alterações importantes na circulação pulmonar e participa na patogênese de duas síndromes: a hipertensão portopulmonar e a SHP.

A SHP decorre da formação de DVIP, que leva a alterações nas trocas gasosas pulmonares e subsequente hipoxemia. As DVIP podem ser diagnosticadas através de ecocardiograma transtorácico contrastado ou cintilografia com MAA-Tc^{99m}. A patogênese da formação das DVIP ainda não está bem estabelecida, mas sabe-se que a hipertensão portal promove aumento na produção de substâncias vasodilatadoras, levando a formação de *shunts* intrapulmonares. Em pacientes cirróticos, a presença das DVIP é um sinal de mau prognóstico e de piora na qualidade de vida.

Em pacientes esquistossomóticos, não há estudos que avaliem a frequência das DVIP, independente da presença de SHP, nem qual sua importância na evolução clínica e no prognóstico dos pacientes com EHE. No entanto, é provável que nos locais endêmicos para

esquistossomose a presença de DVIP seja elevada, e são necessários estudos que avaliem o significado dessa alteração nesse grupo de pacientes.

Após revisão de literatura, foi realizado estudo transversal no ambulatório de Esquistossomose e no serviço de Medicina Nuclear do Hospital das Clínicas da Universidade Federal de Pernambuco (HC – UFPE), cujos resultados são aqui apresentados. Nesse estudo, avaliou-se a presença de dilatações vasculares intrapulmonares, em pacientes com EHE, através de cintilografia com MAA-Tc^{99m}, e as diferenças demográficas, clínicas, laboratoriais, ultrassonográficas e endoscópicas entre pacientes com e sem essa alteração. Também foi avaliado o grau de reprodutibilidade das medidas que quantificam as DVIP através da cintilografia com MAA-Tc^{99m} e estudado um grupo de indivíduos sem doença hepática para averiguar se os valores de normalidade da cintilografia obtidos da literatura estão de acordo com a nossa realidade.

2.3 Objetivos

Este trabalho tem como objetivo principal avaliar o uso da cintilografia com MAA-Tc^{99m} no diagnóstico de DVIP em pacientes com esquistossomose hepatoesplênica e a associação desta alteração com hipertensão portal. Para isso, a pesquisa tem como objetivos específicos:

i. Avaliar a frequência de DVIP em pacientes com EHE através da cintilografia com MAA-Tc^{99m};
ii. Descrever e comparar as características clínicas, demográficas, laboratoriais, endoscópicas e ultrassonográficas de pacientes com EHE com e sem DVIP;
iii. Avaliar a reprodutibilidade inter e intraobservador das medidas de DVIP pela cintilografia com MAA-Tc^{99m}; e
iv. Quantificar os índices de captação cerebral e sistêmica do MAA-Tc^{99m} em indivíduos normais, a fim de identificar valores de referência para o diagnóstico de DVIP em nosso meio.

3 MÉTODOS

A seguir, estão apresentados o delineamento do estudo, o local de realização, a população do estudo, definição e categorização das variáveis, variáveis dependentes e independentes do estudo, análise estatística e aspectos éticos.

3.1 Delineamento do estudo

Estudo descritivo, transversal, envolvendo comparações intergrupos, no qual foram avaliados consecutivamente 51 pacientes com diagnóstico de EHE acompanhados no ambulatório de esquistossomose do HC-UFPE e nove indivíduos saudáveis, entre novembro de 2010 e dezembro de 2012. O estudo foi aprovado no Comitê de Ética em Pesquisa do Centro de Ciências da Saúde da UFPE e todos os participantes assinaram o termo de consentimento livre e esclarecido.

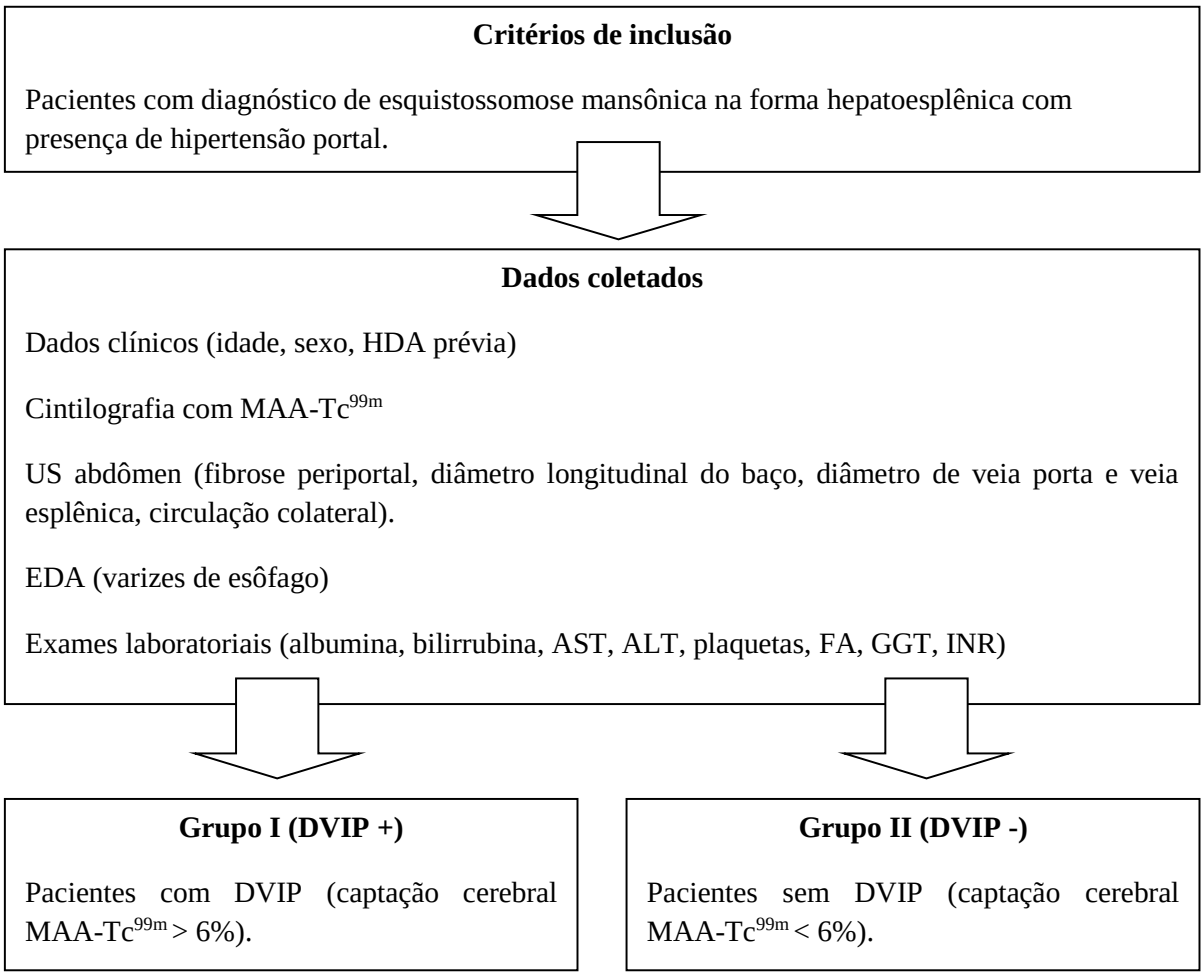
Foram incluídos pacientes de ambos os sexos, maiores de 18 anos, com diagnóstico de EHE. O diagnóstico de EHE foi estabelecido pela história de contato com água de rio em região endêmica e pela presença de fibrose periportal, aumento do lobo esquerdo do fígado e esplenomegalia à ultrassonografia.

Os pacientes esquistossomóticos foram submetidos à avaliação clínica e realizaram exames laboratoriais, endoscopia digestiva alta, ultrassonografia abdominal e cintilografia com MAA-Tc^{99m}. Após realização da cintilografia, os 51 pacientes foram divididos em dois grupos de acordo com a presença ou ausência de DVIP (G1 e G2, respectivamente). Para o diagnóstico de DVIP foi considerado o índice de captação cerebral superior a 6%.

Para avaliação do grau de reprodutibilidade intra e interobservador, foram avaliadas, de forma independente, por dois examinadores, as cintilografias com MAA-Tc^{99m} de 15 pacientes com EHE, encaminhados ao serviço de Medicina Nuclear para pesquisa de DVIP. Cada examinador avaliou em momentos distintos, por duas vezes, cada exame.

Para avaliação dos valores normais, foi realizada cintilografia com MAA-Tc^{99m} em nove indivíduos saudáveis. Antes da realização da cintilografia, todos assinaram o termo de consentimento livre e esclarecido e foram submetidos à avaliação clínica, ultrassonografia de abdômen e ecocardiograma transtorácico, que não mostraram presença de doença hepática ou de *shunt* cardíaco.

Figura 1 - Representação gráfica do delineamento do estudo



Legenda: HDA – hemorragia digestiva; MAA-Tc^{99m} – macroagregados de albumina marcados com tecnécio-99m; US – ultrassonografia; EDA – endoscopia digestiva alta; AST – alanina aminotransferase; ALT – aspartato aminotrasnferase; FA – fosfatase alcalina; GGT – Ɣglutil transferase; INR – *International Normalized Ratio*; DVIP – dilatações vasculares intrapulmonares.

3.2 Local de realização do estudo

O estudo foi realizado no Ambulatório de Esquistossomose e setor de Medicina Nuclear do Hospital das Clínicas – UFPE.

3.3 População do estudo

Pacientes maiores de 18 anos, de ambos os sexos, procedentes do ambulatório de esquistossomose do HC – UPFE. A amostra foi obtida por demanda espontânea no período de novembro de 2010 a dezembro de 2012.

3.3.1 Critérios de inclusão

Pacientes com história prévia de contato com coleção hídrica em área endêmica para esquistossomose, com ultrassonografia (US) de abdômen mostrando fibrose periportal e esplenomegalia e presença de varizes de esôfago à endoscopia digestiva alta (EDA), compatível com o diagnóstico de EHE e hipertensão portal.

Indivíduos com epidemiologia negativa para esquistossomose e US de abdômen normal, sem doenças cardíacas ou pulmonares, para o grupo-controle.

3.3.2 Critérios de exclusão

Pacientes com doença hepática crônica de outras etiologias, com história de doença pulmonar prévia ou presença de *shunts* intracardíacos, pacientes que não realizaram todos os exames solicitados e pacientes esplenectomizados.

3.4 Definição e categorização das variáveis

Esta seção foi subdividida em seis tópicos, visando uma melhor organização do trabalho. Os tópicos abordam: cintilografia com MAA-Tc^{99}m, coleta de dados gerais, exames laboratoriais, ultrassonografia de abdômen, endoscopia digestiva alta e ecocardiograma transtorácico.

3.4.1 Cintilografia com MAA-Tc^{99}m

Para realização da cintilografia com MAA-Tc^{99m}, o paciente foi orientado a ficar na posição ereta por 10 minutos. Após este intervalo, era administrado, por via intravenosa, 0,5 mL de MAA-Tc^{99m} (atividade média de 185 MBq de tecnécio-99m com 300.000 partículas de MAA) em dois minutos. Após 20 minutos da injeção do radiofármaco, as imagens foram realizadas, em gama-câmara de uma cabeça, (modelo STARCAM 3200, General Eletric, Califórnia, USA), utilizando um colimador para baixa energia e todos os propósitos, **f**otopico de 140 kiloelétron-volts (keV) e janela de 20%. Foram obtidas imagens de corpo inteiro, de tórax e crânio. As imagens de corpo inteiro e de tórax foram adquiridas nas projeções anterior e posterior, e as de crânio nas projeções laterais, com o paciente em decúbito dorsal.

Para aquisição das imagens, o detector foi posicionado a aproximadamente dez centímetros do corpo do paciente, utilizando uma matriz de 128x128 por cinco minutos. Posteriormente, as imagens foram analisadas através do desenho de regiões de interesse (ROIs) no crânio e pulmões. As imagens de corpo inteiro foram adquiridas numa velocidade de 7,5 cm/min. Os pacientes tiveram o diagnóstico de DVIP quando a captação cerebral de

MAA-Tc^{99m} foi maior que 6% ou a captação sistêmica maior que 11% (ABRAMS, G. A. et al, 1995).

Para o cálculo da captação cerebral, foi utilizada a relação entre a média geométrica da captação cerebral pela soma entre a média geométrica da captação cerebral e média geométrica da captação pulmonar, corrigido por 0,13, que expressa a fração do fluxo sanguíneo cerebral (Equação 1). Para o cálculo do índice de captação sistêmica, foi utilizada a relação entre a diferença da média geométrica da captação de corpo inteiro e a média geométrica da captação pulmonar pela média geométrica da captação de corpo inteiro (Equação 2).

Equação 1 - Cálculo da captação cerebral

$$\textbf{Captação cerebral}\ (\%) = \frac{\frac{(\textbf{Média geométrica da captação cerebral})}{\mathbf{0,13}}}{\frac{\textbf{média geo. da captação cerebral}}{\mathbf{0,13}} + \textbf{média geo. da captação pulmonar}}$$

Equação 2 - Cálculo da captação sistêmica

$$\textbf{Captação sistêmica}\ (\%) = \frac{(\textbf{Média geo. da captação corpo inteiro} - \textbf{Média geo. da captação pulmonar})}{\textbf{Média geométrica da captação de corpo inteiro}}$$

Foram medidas as captações sistêmica, pulmonar e cerebral. Para diagnóstico de DVIP foram utilizados os índice de captação cerebral (seção 4.1 deste estudo) e sistêmica (seção 4.2 deste estudo). Todos os exames foram avaliados por dois examinadores. Para o estudo de reprodutibilidade, foram utilizados os índices de captação cerebral e sistêmica.

3.4.2 Coleta de dados gerais

A avaliação clínica foi realizada ambulatorialmente, pelo investigador, nos pacientes esquistossomóticos e nos indivíduos normais, sendo composta por anamnese e exame físico. Idade e sexo dos pacientes foram coletados durante consulta médica e registrados em formulário padronizado. Todos os pacientes esquistossomóticos foram submetidos à cintilografia, EDA, US de abdômen, ecocardiograma contrastado e exames laboratoriais. Os indivíduos do grupo-controle não realizaram EDA.

3.4.3 Exames laboratoriais

Exames realizados no laboratório do HC – UFPE. Foram dosados aminotransferases (AST/ALT), fosfatase alcalina (FA), gamaglutamiltransferase (GGT), bilirrubina total, albumina e plaquetas através de espectofotometria automatizada, com uso da Cobas C501, Roche, Diamond Diagnostics, USA. O tempo de protrombina e atividade enzimática (TPAE) foram dosados através do método coagulométrico automatizado e expresso através do *International Normalized Ratio* (INR). As aminotransferases são usadas na avaliação de lesão hepatocelular, sendo o valor de referência da ALT até 31U/mL em mulheres e 41U/mL em homens, enquanto para AST os valores normais são até 31U/mL em mulheres e 37U/mL em homens.

Fosfatase alcalina e gamaglutamiltransferase geralmente indicam acometimento de vias biliares. Em adultos, os valores de referência para a FA variam de 35 – 104U/L em mulheres e 40 – 129U/L em homens. Para GGT, os valores de referência variam de 8 – 41U/L em mulheres e de 12 – 73U/L em homens. Os níveis de plaquetas podem servir como sinal indireto de hipertensão portal, sendo o hiperesplenismo, secundário ao aumento da pressão portal, a causa mais comum de plaquetopenia em pacientes com doença hepática. Seus valores normais variam de 150.000 – 400.000/mm^3.

Bilirrubina, albumina e TPAE são exames usados para avaliação da função hepática. A bilirrubina, principal componente dos pigmentos biliares, é produzida na forma nãoconjugada (bilirrubina indireta) e passa pelo processo de conjugação no fígado (bilirrubina direta). O valor normal da bilirrubina total é de 1,0 mg/dL, da indireta até 0,8mg/dL e da direta até 0,2mg/dL. A albumina é a principal proteína circulante no organismo, e sua produção é feita exclusivamente pelo fígado. Seu valor de referência varia entre 3,5 – 5g/dL.

O fígado tem importante papel na hemostasia, já que os fatores de coagulação são sintetizados em sua maioria no fígado. Na prática clínica, a determinação da atividade de protrombina é o método mais simples e barato para avaliação da coagulação. O valor normal é comparado ao plasma-controle, mas varia entre 11,1 e 13,2 segundos em relação ao controle. Pode ser expresso através do INR, que tem valores normais entre 0,9 – 1,3.

3.4.4 Ultrassonografia de abdômen

A US foi realizada por um único examinador, com o aparelho Acuson X 150 Siemens com transdutor convexo de 3,5 mHz, sendo avaliados e anotados em questionário próprio o diâmetro da veia porta e da veia esplênica, o diâmetro longitudinal do baço e a presença ou ausência da circulação colateral portossistêmica. Também foi avaliada a presença de fibrose

periportal, um dos critérios para inclusão nesse estudo, e o padrão dessa fibrose. A fibrose periportal foi classificada, de acordo com os critérios de Niamey (WHO 2000) em C (fibrose periférica), D (fibrose central), E (fibrose avançada) ou F (fibrose muito avançada).

O valor considerado normal para o diâmetro longitudinal do baço foi de até 12 cm, sendo a esplenomegalia uma característica da forma hepatoesplênica da esquistossomose. Em pacientes com hipertensão portal, são achados comuns o aumento do diâmetro de veia porta e veia esplênica e a presença de circulação colateral. Os valores normais são até 0,9cm para veia esplênica e até 1,2cm para veia porta.

3.4.5 Endoscopia digestiva alta

A EDA foi realizada por examinadores experientes, do serviço de Endoscopia do HC – UFPE, usando o videoendoscópio Olimpus 100, para pesquisa de varizes de esôfago, uma das complicações mais graves da hipertensão portal. A avaliação foi realizada com utilização dos critérios de Beppu, com categorização das varizes em fino, médio e grosso calibre (BEPPU et al. 1981), sendo a presença de varizes um dos critérios diagnósticos de hipertensão portal utilizados para inclusão nesse estudo.

3.4.6 Ecocardiograma transtorácico

O ecocardiograma foi realizado por um único examinador, em todos os pacientes e indivíduos do grupo-controle, utilizando o ecocardiógrafo modelo Vivid I (General Eletric, Califórnia, USA). O objetivo da realização deste exame foi descartar a presença de shunt intracardíaco, um dos critérios de exclusão do estudo.

3.5 Variáveis dependentes e independentes do estudo

Nas tabelas 1 e 2 dos tópicos a seguir estão detalhadas as variáveis deste estudo.

Tabela 1 - Variável dependente

Variável dependente	Definição teórica	Definição operacional	Categorização
Shunt intrapulmonar	Variável categórica definida pela presença de dilatações vasculares intrapulmonares	Captação cerebral de MAA-Tc^{99m} > 6% ou sistêmica > 11%.	Presente Ausente

MAA-Tc^{99m} - macroagregados de albumina marcados com tecnécio99m

Tabela 2 - Variáveis independentes

Variáveis independentes	Definição teórica	Definição operacional	Categorização
Idade	Variável contínua definida pela data de nascimento que consta do Registro Geral fornecido pelo paciente.	Expresso em anos de vida	
Sexo	Variável categórica	Coleta de dados através de anamnese	Masculino Feminino
Hemorragia digestiva alta	Variável categórica definida pela presença de sangramento originado em qualquer ponto do tubo digestivo desde a faringe até o ângulo de Treitz	Coleta de dados através de anamnese	Presente Ausente
Transaminases	Variável contínua usada para detecção de lesão hepatocelular	Dosagem sérica de AST e ALT em U/mL	
Fosfatase alcalina	Variável contínua que indica acometimento de vias biliares	Dosagem sérica de FA em U/L	
gamaglutamiltransferase	Variável contínua que indica acometimento de vias biliares	Dosagem sérica de GGT em U/L	
Plaquetas	Variável contínua que sugere hipertensão portal	Dosagem sérica de plaquetas por mm^3	
Bilirrubinas	Variável contínua utilizada na avaliação	Dosagem sérica de bilirrubinas total em	

	da função hepática	mg/dL	
Albumina	Variável contínua utilizada na avaliação da função hepática	Dosagem sérica da albumina em g/dL	
INR	Variável contínua utilizada na avaliação da função hepática	Dosagem do tempo e atividade enzimática da protrombina, expresso como INR	
Tamanho de veia porta	Variável contínua que sugere presença de hipertensão portal	Avaliação do diâmetro de veia porta por US em centímetros	
Tamanho de veia esplênica	Variável contínua que sugere presença de hipertensão portal	Avaliação do diâmetro de veia esplênica por US em centímetros	
Tamanho do baço	Variável contínua que sugere presença de hipertensão portal	Avaliação do diâmetro longitudinal do baço por US em centímetros	
Presença de circulação colateral	Variável categórica que sugere presença de hipertensão portal	Avaliação ultrassonográfica	Presença Ausência
Padrão de fibrose	Variável categórica que indica a gravidade da fibrose periportal	Avaliação ultrassonográfica pelos critérios de Niamey	C / D / E / F
Varizes de esôfago	Variável categórica usada para determinação indireta da presença de hipertensão portal	Avaliação endoscópica pelos critérios de Beppu	Fino calibre Médio calibre Grosso calibre

3.6 Análise estatística

A amostra de 51 pacientes foi uma amostra de conveniência. Média e desvio-padrão foram utilizados para expressar valores de variáveis quantitativas contínuas. Para comparação dessas variáveis entre dois grupos independentes, usou-se o teste U de Mann – Whitney, a fim de verificar a presença de evidências que valores de um grupo A são superiores aos valores do grupo B. Foi considerado um nível de significância estatística de 5% ($p < 0,05$).

Para expressar valores de variáveis categóricas, utilizou-se frequência. Para comparação dessas variáveis entre dois grupos independentes, usou-se o teste Qui-quadrado de independência para verificar se o grupo ao qual o paciente pertence interfere na medida da variável em questão. Foi considerado um nível de significância estatística de 5% ($p < 0,05$).

Para estimar os valores de referência dos índices de captação cerebral e sistêmica, nos indivíduos do grupo-controle, utilizou-se o método de simulação de Bootsptrap não paramétrico combinado com o método Monte Carlo. O método Bootsprat não-paramêtrico é um método de reamostragem usado quando é necessário estimar para a população uma média derivada de uma pequena amostra, enquanto o método de Monte Carlo permite estimar medidas desconhecidas usando dados amostrais. Esses dois métodos combinados aumentam a precisão da análise (McCRACKEN,1955).

Na avaliação da reprodutibilidade, intra e interobservador, usou-se o índice de concordância kappa e o coeficiente de correlação intraclasse. O índice de concordância kappa é usado quando o resultado é dado de forma categórica (DVIP positivo ou negativo), e o resultado é expresso por valores entre 0 e 1. Se o resultado é maior que 0,80, a concordância é considerada excelente (LANDIS JR & KOCH GG, 1977). O índice de correlação intraclasse é utilizado para avaliar a concordância quando se avaliam medidas quantitativas, e seu resultado também é expresso por valores entre 0 e 1. Um valor maior que 0,75 indica uma concordância satisfatória (SHROUT PE & FLEISS JL, 1979).

3.7 Aspectos éticos

O estudo foi aprovado no Comitê de Ética e Pesquisa (CEP) do Centro de Ciências da Saúde sob o registro CEP/CCS/UFPE 396/2010 (ANEXO A). Todos os pacientes e indivíduos do grupo-controle assinaram o TCLE.

4 SISTEMA ANALÍTICO

A seguir, são apresentados dois artigos originais, sendo o primeiro de acordo com as regras da *Plos neglected tropical diseases* (ANEXO B) e o segundo de acordo com as regras do *Annals of nuclear medicine* (ANEXO C).

4.1 Intrapulmonary vascular dilatation evaluated by 99m Tc-MAA scintigraphy and its association with portal hypertension in schistosomiasis

Abstract: Portal hypertension is responsible for various complications in patients with schistosomiasis, among them intrapulmonary vascular dilations (IPVD). In cirrhotic patients the presence of IPVD is a sign of poor prognosis, but in patients with hepatosplenic schistosomiasis (HSS) there are no studies assessing the significance of this change. The aim of this study was to evaluate the occurrence of IPVD through 99mTc-MAA scintigraphy in patients with HSS and its relationship with clinical, laboratory, endoscopic and ultrasound parameters.

Methods: Cross-sectional study that evaluated 51 patients with HSS. Patients were diagnosed with IPVD when the brain uptake of 99mTc-MAA was higher than 6%. Subsequently, they were divided according to the presence (G1) or absence (G2) of IPVD and the variables were compared between groups.

Results: Overall, 51 patients were assessed, with mean age of 56 ± 12 years. IPVD was observed in 31 patients (60%). There was no statistically significant differences between groups when the clinical, laboratory and endoscopic parameters were compared. As for the ultrasound parameters, the diameter of the splenic vein was lower in G1 (0.9 ± 0.3 cm) than in G2 (1.2 ± 0.4 cm) with p = 0.029.

Conclusion: In patients with HSS, the occurrence of IPVD by 99m Tc-MAA scintigraphy was high and was associated with lower splenic vein diameter, which can be a mechanism of vascular protection against portal hypertension. However, more studies are needed to determine the clinical significance of the early diagnosis and natural evolution of IPVD in this population.

Key-words: Hepatosplenic schistosomiasis, intrapulmonary vascular dilations, 99mTc-MAA scintigraphy, portal hypertension

Introduction: Hepatosplenic schistosomiasis (HSS) is characterized by the presence of liver fibrosis around the intrahepatic branches of the portal vein without damage to the ability of hepatocyte synthesis (ANDRADE 2009). It is estimated that schistosomiasis affects

between 2,5-6 million Brazilians (HEALTH MONITORING SYSTEM, 2012) and about 10% develop the hepatosplenic form, which is the main cause of portal hypertension in Northeastern Brazilian (MACÊDO et al, 2010). Portal hypertension is responsible for a number of complications in patients with schistosomiasis, like esophageal varices, gastrointestinal bleeding or intrapulmonary vascular dilations (IPVD) (FALLON, M. B. et al., 2008, RODRIGUES et al, 2000).

IPVD is the key event in the development of hepatopulmonary syndrome (HPS), when associated with alveolar-arterial difference of O2> 15mmHg and liver disease with portal hypertension (KALAMBOKIS & TSIANOS, 2010). The ethiopageny of IPVD is not yet well established, but portal hypertension appears to be the initial stimulus for vasodilation (MANDELL, 2007). In cirrhotic patients, the occurrence of IPVD is a sign of poor prognosis (FALLON et al, 2008), but in patients with portal hypertension due to HSS, there are no studies assessing the clinical significance and prognosis.

The most commonly used method to diagnose IPVD is transthoracic Doppler echocardiography (RODRÍGUEZ-ROISIN, R. et al, 2004); however, studies have suggested 99mTc-macroaggregated albumin (99m Tc-MAA) scintigraphy as alternative, for being sensitive, specific and because it quantifies the magnitude of IPVD (KALAMBOKIS, G.et al., 2010). In 99m Tc-MAA scintigraphy, radiolabeled particles, which usually have more than 20μm in diameter, are injected and remain retained in the pulmonary vasculature, since capillaries measure 8 to 15μm in diameter (KALAMBOKIS, G.et al., 2010). In the presence of IPVD, as in vasodilation, a fraction of these particles migrate to the systemic circulation, which can be quantified by radioactivity in the intra and extrapulmonary circulation (RODRÍGUEZ-ROISIN, R. et al, 2004).

The aim of this study was to evaluate the occurrence of IPVD through 99m Tc-MAA scintigraphy and its association with clinical, laboratory, endoscopic and ultrasound parameters in patients with HSS.

Material and Methods: This is a descriptive, cross-sectional study involving intergroup comparisons, in which 51 patients with diagnosis of HSS followed as schistosomiasis outpatients at the Clinical Hospital Federal - University of Pernambuco were consecutive evaluated between November 2010 and June in 2012. The study was approved by the Ethics Committee of the Center for Health Sciences - UFPE and all patients signed the informed consent form.

Patients of both sexes, aged over 18 years, with esophageal varices and diagnosis of HSS were included. The diagnosis of HSS was established by history of contact with river

water in endemic regions and the presence of periportal fibrosis, increased left lobe of the liver and ultrasound splenomegaly.

The following exclusion criteria were used: refusal to participate in the study, presence of chronic liver disease of others etiology, previous pulmonary disease and/or intracardiac shunt; technical impossibility to perform the necessary tests and past splenectomy.

Patients were submitted to clinical evaluation and performed laboratory tests, endoscopy, abdominal ultrasound and ^{99m} Tc-MAA scintigraphy. Peripheral blood was assessed for the following: albumin, bilirubin, AST, ALT, platelets, alkaline phosphatase and GGT using automated spectrometry and Cobas C501, Roche Diamond Diagnostics, USA. For measuring the RNI, coagulometric automated method was used.

Endoscopic evaluation was performed using Olympus 100 videoendoscope using the criteria of Beppu, which ranks esophageal varices into fine, medium and large (SHROUT & FLEISS, 1979). Ultrasound was performed by a single examiner with a Siemens Acuson X 150 device using a convex transducer of 3.5 MHz, assessing the following: diameter of the portal vein and splenic vein with normal values of 1.2 and 0.9 cm respectively, longitudinal diameter of the spleen, with normal values of up to 12cm, presence or absence of portal-systemic collateral circulation and pattern of periportal fibrosis, classified as C / D / E / F according to the criteria of Niamey (World Health Organization/TDR/SCH/ULTRASON, 2000).

For scintigraphy with ^{99m} Tc-MAA, the patient was asked to stand in the upright position for 10 minutes and after this interval, 0.5 ml of ^{99m} Tc-MAA was intravenously administered (average activity of 185 MBq of Technetium-99m with 300,000 MAA particles) in two minutes. 20 minutes after tracer injection, the images were obtained in a gamma one-head camera (model STARCAM 3200, General Electric, California, USA), using a low-energy collimator for all purposes, photopeak of 140 Kiloelectronvolt (keV) and 20% window. Static images of chest and skull were obtained. Thorax images were obtained in anterior and posterior projections and skull images were obtained in lateral projections, with the patient in supine position.

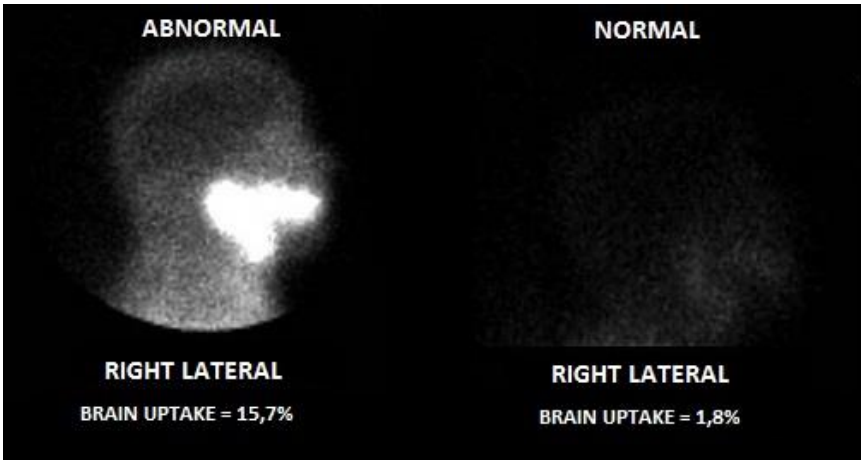
For image acquisition, the detector was positioned approximately 10 cm from the patient's body, using a 128x128 matrix for five minutes. Subsequently, the images were analyzed by drawing regions of interest (ROIs) in the skull and lungs. Patients were diagnosed with IPVD when the brain uptake of ^{99m} Tc-MAA was higher than 6% (Figure 1).

The brain uptake was calculated using the following formula: (geometric mean of brain uptake / 0.13) / (geometric mean of brain uptake / 0.13) + geometric mean of pulmonary

uptake (HOSONO et al, 2002). According to the presence or absence of IPVD, patients were divided into two groups – G1 (positive IPVD) and G2 (negative IPVD) and the clinical (age, sex, history of gastrointestinal bleeding), laboratory, endoscopic and ultrasound features of each group were compared.

To compare quantitative variables, the Mann-Whitney U test was used, which does not need to assume data normality and has power-efficiency around 95%. For qualitative variables, the chi-square test was used to verify if the group to which the patient is interferes in the variable being assessed. Differences between groups were considered statistically significant when p-value was <0.05.

Results: Overall, 51 patients were assessed, 33 female (65%) with mean age of 56 ± 12 years. The presence of IPVD was observed in 31 patients (60%). Table 1 shows the clinical and laboratory parameters and Table 2 shows the endoscopic and ultrasound parameters. The average brain uptake was 9.2 ± 2.6% in the group with IPVD and 3.8 ± 1.2% in patients without IPVD (p <0.005).



(Figura 2) Figure 1: ^{99m}Tc-MAA scintigraphy – abnormal brain uptake in left and normal in right.

When groups were compared, a higher percentage of women in the group with IPVD was observed (74.2% vs 50%), with a tendency to difference (p = .07). There were no statistically significant differences between groups.

When endoscopic parameters were evaluated, there was a greater percentage of varices grades II and III in patients without IPVD. Among ultrasound parameters, there were statistically significant differences between groups when the diameter of the splenic vein measured, with higher values in the group without IPVD (0.9 ± 0.3 in G1 vs 1.2 ± 0.4 cm in G2, p = .029).

Discussion: The occurrence of IPVD ranged from 13 to 47% in cirrhotic patients

(VARGHESE, J. et al.). Ferreira et al, using transthoracic Doppler echocardiography in patients with schistosomiasis already with gas exchange alterations (Da-Ao2> 15mmHg), the IPVD frequency found was 22% (FERREIRA et al, 2011). In this study, using ^{99m}Tc-MAA scintigraphy, IPVD was observed in 60% of the 51 patients evaluated. Shabrawi et al, in a study with children with portal hypertension, found nearly twice the frequency of IPVD when data of scintigraphy with ^{99m}Tc-MAA were compared to those using echocardiography (SHABRAWI et al, 2010).

Studies comparing transthoracic Doppler echocardiography and scintigraphy with ^{99m}Tc-MAA showed conflicting results regarding sensitivity and specificity. This high frequency suggests greater sensitivity of scintigraphy with ^{99m}Tc-MAA, which is able to identify small dilations in the pulmonary vasculature even before the development of HSS (ABOUSSOUAN & STOLLER 2000;). Furthermore, this study was carried out in a General Hospital and all patients had esophageal varices, suggesting that more severe patients are examined, which may be one of the factors for the high incidence of IPVD found. However, there are no studies assessing the importance of early diagnosis of IPVD or the chain that leads to the development of HPS from the emergence of vascular dilatation in HSS.

In cirrhotic patients, it is well established that the presence of IPVD associated with hypoxemia is related to higher MELD score (FERREIRA et al, 2008) and a worse prognosis (EGAWA et al, 1999). Eldridge et al, in a study carried out with patients without abnormal liver function showed the emergence of IPVD during the performance of physical exercises with spontaneous resolution during rest. In this group, the formation of vascular dilatation may be a defense mechanism to increased vascular pressure and blood flow in the pulmonary circulation (ELDRIDGE et al, 2004).

(Tabela 3) Table 1 – Clinical and laboratory parameters of patients with (G1) or without (G2) IPVD

Parameters	Total			
		G1	G2	p-value
Clinical				
Age (years)	55.8 ± 13.2	56.7 ± 13.9	54.6 ± 12.2	0.573
Sex				
woman	33 (64.7%)	23 (74.2%)	10 (50%)	0.078
man	18 (35.3%)	08 (25.8%)	10 (50%)	
Gastrointestinal				

bleeding				
No	30 (58.8%)	20 (63.3%)	10 (50%)	
Yes	21 (41.2%)	11 (36.7%)	10 (50%)	0.35
Laboratory				
Albumin (g/dL)	4.2 ± 0.7	4.2 ± 0.7	4.1 ± 0.7	0.3 ± 8
Bilirrubin (mg/dL)	1.1 ± 0.5	1.1 ± 0.5	1.2 ± 0.5	0.13
INR	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.15
AST (U/L)	38.2 ± 18,5	36.5 ± 17.9	37.1 ± 15.9	0.72
ALP (U/L)	36.2 ± 19.9	33.1 ± 15.8	38.3 ± 20.9	0.19
Platelets (x10^3 p/ mm3)	92 ± 44	100 ± 51	88 ± 36	0.27
Alkaline phosphatase (UI/L)	116 ± 52	107 ± 46.3	126 ± 57.5	0.19
ɣGT (UI/L)	120 ± 82	96 ± 67.6	141 ± 105.2	0.19

RNI – Ratio Normalized International; AST – alanine aminotransferase; ALT – aspartate aminotrasnferase; ɣGT – ɣglutamil transferase

(Tabela 4) Table 2 – Endoscopics and ultrasounds parameters of patients with (G1) or without (G2) IPVD

Parameters	Total	G1	G2	p-valor
Endoscopics				
Esophageal varices				
Grade I	39(70%)	1 – 23 (73,3%)	12 (57,9%)	
Grade II	08 (15%)	2 – 05 (16,7%)	03 (15,8%)	0.31
Grade III	08 (15%)	3 – 03 (10,0%)	05 (26,3%)	
Ultrasound				
Portal vein(cm)	1.3 +/-0.33	1.3 +/- 0.2	1.3 +/- 0.4	0.6
Splenic vein (cm)	1.00 +/-0.3	0.9 +/- 0.3	1.2 +/- 0.4	0.029
Collateral circulation				

No				0.14
Yes	23 (45%)	11 (36%)	12 (60%)	
	28 (55%)	20 (64%)	08 (40%)	
Spleen longitudinal diameter (cm)	15 +/- 9	15.6 +/- 2.5	16.5 +/- 2.2	0.1
Fibrosis				
C	12 (23%)	08 (25.8%)	04 (21.1%)	
D	31 (60%)	19 (61.3%)	12 (57.8%)	0.73
E	08 (17%)	04 (12.9%)	04 (21.1%)	

In patients with schistosomiasis, the clinical relevance of the presence of IPVD and if patients with this alteration have more severe disease is not yet well defined. In this study, no differences in the parameters that evaluated liver function among patients with and without IPVD were observed.

The study by Aller et al showed that in cirrhotic men with IPVD, the serum levels of female sex hormones are higher than in patients without IPVD (ALLER et al, 2001). In patients with schistosomiasis, as there is no significant change in liver function, there should be no difference in the progesterone and estradiol levels in men compared to normal people. The high frequency of IPVD in women found in this study suggests that these hormones may participate in the pathogenesis of the formation of this change, with vasodilatory effect, but further studies are needed to evaluate this association.

In patients with HSS, the obstruction to the hepatic blood flow caused by periportal fibrosis leads to increased pressure in the portal venous system (VEZOZZO et al, 2006). The changes caused by portal hypertension are among the leading causes of morbidity and mortality in patients with schistosomiasis (MUDAWI et al, 2008), and the formation of portal-systemic collateral circulation and increased diameter of the portal vein and splenic vein are the main changes occurring in the portal venous system of patients with schistosomiasis.

This study showed that patients with schistosomiasis and IPVD had smaller diameter of the splenic vein, suggesting that vascular dilation would be mechanisms to reduce the pressure in the portal territory. Moreover, the group with IPVD showed greater proportion of patients with collateral circulation (64% vs 40%), lower average longitudinal diameter of the spleen (15.6 cm x 16.5 cm) and high platelets levels (100x 10^3 vs 88. 10^3) although not

statistically significant. It was also found that the group with IPVD had lower proportion of patients with varices of medium and large diameter, suggesting that this group presents lower portal pressure levels. The number of patients included in this study may not have been sufficient to demonstrate the most significant differences in indirect markers of portal hypertension between groups with and without IPVD.

Conclusion: In patients with HSS, the occurrence of IPVD by 99m Tc-MAA scintigraphy was high and was associated with lower splenic vein diameter, which can be a mechanism of vascular protection against portal hypertension. However, more studies are needed to determine the clinical significance of the early diagnosis and natural evolution of IPVD in this population.

4.2 Evaluation of normality and reproducibility parameters of 99mTc-MAA scintigraphy in the diagnosis of intrapulmonary vascular dilatations

Abstract: The formation of intrapulmonary vascular dilations (IPVD) is the key event for the onset of hepatopulmonary syndrome, vascular changes secondary to portal hypertension that leads to hypoxemia. The diagnosis of IPVD can be made by contrasted transthoracic echocardiography or scintigraphy with technetium-macroaggregated albumin – (^{99m}Tc-MAA) – that quantifies the IPVD magnitude. However, its procedure and diagnostic indices are not yet standardized and well defined in health services. The aims of this study were to define normality values and evaluate the inter- and intra-observer reproducibility degree of diagnostic indexes of IPVD through ^{99m}Tc-MAA scintigraphy.

Methods: Cross-sectional study conducted at the Clinical Hospital - Federal University of Pernambuco (HC-UFPE) between July and December 2012. Fifteen patients with hepatosplenic schistosomiais and nine patients without liver or heart disease (control group) were assessed. After clinical assessment, ultrasound and echocardiography, patients underwent ^{99m}Tc-MAA scintigraphy, and a relative brain uptake value exceeding 6% or systemic uptake value exceeding 11 % was considered diagnostic of IPVD. Each assessment was performed by two independent observers. To analyze the results of the normal group, the nonparametric Bootsptrap method simulation model combined with the Monte Carlo method was used and to analyze inter- and intra-observer reproducibility indexes, the kappa and intra-class correlation coefficient were used.

Results: In normal subjects, the average brain uptake of ^{99m}Tc-MAA was 7.9 % ± 0.01 and systemic uptake was 12.4 ± 0.03 %. The intra-observer agreement was 100 %, with kappa

index of 1.0 ($p < 0.0001$), suggesting a perfect agreement. The inter-observer agreement was also 100 % (kappa = 1.0, $p < 0.0001$) for brain uptake; however, systemic uptake showed kappa = 0.25 ($p = 0.07$), which features tolerable concordance. The intra-class correlation was excellent for both uptake indexes.

Conclusions: The normality values were slightly higher than those reported in studies from other countries. The demographic characteristics of the Brazilian population, the small number of patients or different methodologies can be the causes of such differences. ^{99m}Tc-MAA scintigraphy - showed excellent reproducibility.

Keywords: ^{99m}Tc-MAA scintigraphy, intrapulmonary vascular dilatation, reproducibility

Introduction: The formation of intrapulmonary vascular dilations (IPVD) is the key event for the onset of hepatopulmonary syndrome (HPS), vascular changes secondary to portal hypertension in patients with acute or chronic liver disease associated with hypoxemia (KALAMBOKIS & TSIANOS, 2010). In northeastern Brazil, hepatosplenic schistosomiasis (HSS) is the main cause of portal hypertension, but the presence of IPVD has been little studied in this group of patients (2).

The diagnosis of IPVD, also called intrapulmonary shunt, can be made by contrasted transthoracic echocardiography or scintigraphy with technetium-macroaggregated albumin – (^{99m}Tc-MAA) (RODRÍGUEZ-ROISIN et al, 2004). Transthoracic echocardiogram is the most frequently test used for diagnosis, but it has limitations such as not quantifying the intrapulmonary shunt magnitude and be examiner – dependent (MACEDO & LOPES, 2009).

In ^{99m}Tc-MAA scintigraphy, radiolabeled particles, which typically are more than 20μm in diameter, are injected and retained in the pulmonary vasculature, since capillaries measure 8-15μm in diameter (El-SHABRAWIet al.). In the presence of IPVD, as vasodilation occurs, a fraction of these particles is transferred into the systemic circulation, which can be quantified by the ratio between radioactivity in the intra and extrapulmonary circulation (RODRÍGUEZ-ROISIN, R. et al, 2004).

^{99m}Tc-MAA scintigraphy is a sensitive and specific method and quantifies the IPVD magnitude. However, its procedure is not yet standardized and its normality values are not well defined (SARI, 2012). To diagnose IPVD, cerebral or systemic uptake indexes are mainly used, but other indexes have been suggested (WHYTE et al., 1998). The ideal index would be the most reproducible, easy to perform and the most precise. In addition to this uncertainty, the reference values in normal subjects have been evaluated only in small studies and no Brazilian study has been hitherto conducted with this purpose. The aims of this study were to define the normality parameters and evaluate the inter- and intra-observer

reproducibility of brain and systemic uptake indexes of ^{99m}Tc-MAA scintigraphy in the diagnosis of IPVD.

Materials and Methods: This descriptive study was carried out at the Nuclear Medicine Service, Clinical Hospital, Federal University of Pernambuco (HC - UFPE) between July and December 2012. The study was approved by the Ethics Research Committee of the UFPE Center for Health Sciences and all patients signed an informed consent form.

To assess the intra- and inter-observer reproducibility degree of the diagnostic indexes of ^{99m}Tc-MAA scintigraphy, examinations of 15 patients with chronic liver disease of schistosomotic etiology were analyzed independently by two professionals from the Nuclear Medicine service. Each examiner evaluated scintigraphy results twice at different times.

To evaluate the normality values, ^{99m}Tc-MAA scintigraphy was performed in nine healthy subjects. Before scintigraphy, all these subjects underwent clinical evaluation, abdomen ultrasound and transthoracic echocardiogram, which showed no liver disease or intracardiac shunt.

For scintigraphy, patients were asked to stand in upright position for 10 minutes and after this time interval, 0.5 ml of 99mTc - MAA was intravenously administered (average activity of 185 MBq of technetium - 99m to about 300,000 MAA particles) in two minutes. Twenty minutes after radiotracer injection, still images of skull, chest and whole body were obtained. The chest and whole body images were acquired in anterior and posterior projections and those of skull in lateral projections, with patient in supine position.

Images were obtained on a one-head gamma camera model STARCAM 3200, General Electric, California, USA, using a low-energy collimator for all purposes, photo peak of 140 kiloelectron-volt (keV) and 20% window. For image acquisition, the detector was positioned at approximately 10 cm from the patient's body. For chest and skull images, a 128x128 matrix was used, with time of five minutes for the acquisition of each incidence. Whole body images were acquired at a rate of 7.5 cm / min.

The images were analyzed by drawing regions of interest (ROIs) in each incidence. The brain uptake index was calculated from the ratio between the geometric mean of counts of right and left skull incidences and the geometric mean of counts of anterior and posterior chest incidences corrected by 0.13, which expresses the brain blood flow fraction (Formula & CORTESE, 1994).

(Equação 3) Formule 1 - Brain uptake calculation

$$Brain\ uptake\ (\%) = \frac{\frac{Geometric\ mean\ of\ brain\ uptake}{0.13}}{\frac{Geometric\ mean\ of\ brain\ uptake}{0.13} + Geometric\ mean\ of\ pulmanary\ uptake}$$

The systemic uptake index was the relative percentage between the difference of the geometric mean of counts of anterior and posterior whole body incidences and geometric mean of anterior and posterior chest incidences by the geometric mean of counts of anterior and posterior whole body incidences (Formula 2), with value equal to or greater than 11 % as indicative of IPVD.

(Equação 4) Formula 2 - Systemic uptake calculation

$$Systemic\ uptake\ (\%) = \frac{(Geometric\ mean\ of\ whole\ body\ uptake - Geometric\ mean\ of\ pulmonary\ uptake)}{Geometric\ mean\ of\ pulmanary\ uptake}$$

In assessing the intra- and inter-observer reproducibility, the kappa concordance index and the intra-class correlation coefficient were used. The kappa concordance index is used when the result is given categorically (positive or negative IPVD), and the result is expressed with values between 0 and 1. If the result is greater than 0.80, the agreement is considered excellent (LANDIS & KOCH, 1977). The intra-class correlation coefficient is used to assess agreement when quantitative measures are assessed, and the result is also expressed by values between 0 and 1. Value greater than 0.75 indicates satisfactory agreement (SHROUT & FLEISS, 1979).

In normal individuals, brain and systemic uptake indexes were measured. To estimate the reference values for brain uptake and systemic uptake in patients in the control group, the nonparametric Bootsptrap simulation model combined with the Monte Carlo method was used. The nonparametric Bootsptrap is a re-sampling model used when it is necessary to estimate an average from a small sample for a given population, while the Monte Carlo method allows estimating unknown measures using sample data. These two methods when combined enhance the analysis accuracy (MCCRACKEN,1955).

Results: The mean value in the control group was 12.4 % ± 0:03 for systemic uptake (Figure 1) and 7.9 ± 0.01% for brain uptake (Figure 2), with low dispersion rate for both measures. Figure 3 shows an abnormal scintigraphy and figure 4 shows a normal scintigraphy.

The intra-observer agreement rate was 100 %, with a kappa index of 1.0 and p-value = 0.0001, suggesting perfect agreement. The same result was obtained using both brain and systemic uptake. The intra-class correlation coefficient was equal to 0.995 for brain uptake and 0.997 for systemic uptake, which is considered excellent agreement (Tables 1 and 2).

When the measures between two different observers are analyzed, there is also a 100% agreement, with kappa index of 1.0 and p - value = 0.0001 for brain uptake, suggesting perfect agreement (Table 3). When the systemic uptake is analyzed, however, a kappa value of 0.25 was found, which features tolerable agreement (Table 4). The intra-class correlation was excellent for both systemic uptake and brain uptake.

Discussion: IPVD is the key event in the development of hepatopulmonary syndrome and your natural history has not been sufficiently understood. In cirrhotic patients, the presence of IPVD is evidence of poor prognosis, especially when associated to HPS. In HSS patients, there are no studies assessing the clinical significance and prognosis. (RODRÍGUEZ-ROISIN & KROWKA 2008).

Pulmonary arteriography is the gold standard test for diagnosing intrapulmonary shunt (RODRÍGUEZ-ROISIN & GÓMEZ, 2008), but because of its invasiveness it is not widely used. Transthoracic echocardiography is considered by some authors as the best test for diagnosing IPVD (KALAMBOKIS & TSIANOS, 2010, PALIKHE et al., 2013), but the main disadvantages is the examiner dependence and not quantifying the shunt magnitude .

This study was the first to evaluate the results of ^{99m}Tc-MAA scintigraphy in a Brazilian cohort of normal individuals as well as the inter- and intra-observer reproducibility degree of the main diagnostic indexes of IPVD provided by this test. The results of this study showed that the diagnostic values are slightly higher than those provided by studies from other countries and that ^{99m}Tc-MAA scintigraphy provides diagnostic indexes with excellent reproducibility degree, especially considering the brain uptake index.

^{99m}Tc-MAA scintigraphy is a simple and safe test, with sensitivity and specificity similar to those Doppler echocardiography (MACÊDO & LOPES, 2009). Moreover, it has the advantage of quantifying the shunt magnitude, which seems to be important in assessing the prognosis of these patients (ABRAMS et al, 1998).

The extra pulmonary ^{99m}Tc-MAA uptake is inversely proportional to arterial oxygenation in ambient air, which may be useful to determine the severity of lung disorders in patients with portal hypertension and pulmonary disease.

Although brain uptake of ^{99m}Tc - MAA less than 6% is considered normal, studies carried out with normal patients are few, since although minimally invasive, it exposes

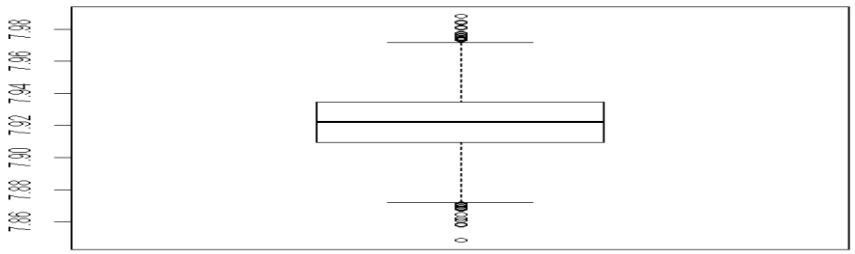
individuals to a small radiation dose. The first study to assess normal patients was published in 1977. Eight patients without chronic liver disease underwent scintigraphy with ^{99m}Tc - MAA and all had mean brain uptake less than or equal to 6 % (NAEIJE, 2003). Another study published in 2003 with seven healthy subjects showed values ranging from 2 to 7 % of brain uptake index. Thus, patients with brain uptake above 7% were considered positive for the presence of IPVD (KAYMAKOGLU, S, 2003). The study that assessed the largest group of normal patients had 12 individuals, and showed mean brain uptake of 2 ± 1 % (KROWKA, M. J. et al., 2000).

This study evaluated patients without chronic liver disease and without cardiac shunt to determine the reference value for ^{99m}Tc-MAA scintigraphy in this sample. The normality values found were slightly higher than value considered standard in literature. The demographic characteristics of the Brazilian population, the small number of patients in each study or the use of different methodologies in the performance of tests for the analysis of control groups can be the causes of such differences. In this study, the type of statistical analysis used and the low standard deviation values observed point to the veracity of results; however, further studies should be carried out to confirm these results.

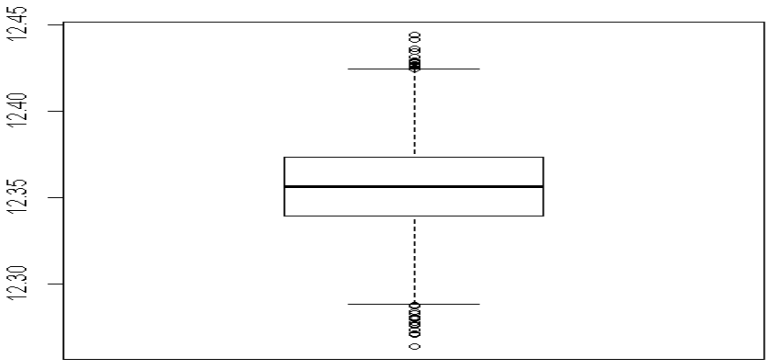
The excellent reproducibility degree of this study shows that ^{99m}Tc-MAA scintigraphy is an examiner independent test. The brain uptake index was more appropriate than the systemic uptake index for both categorical results, with specific cutoff value, as for quantitative results.

Conclusion: The normality values of 99mTc-MAA scintigraphy were slightly higher than those reported in studies from other countries. Moreover, 99mTc-MAA scintigraphy showed excellent reproducibility, particularly when brain uptake is used as index for the diagnosis of IPVD.

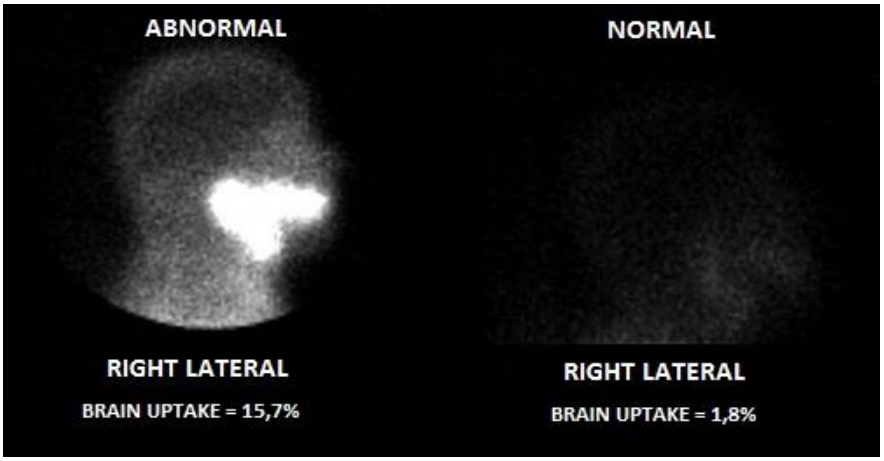
(Figura 3) Figure 1 - Mean and dispersion, in percentage, of systemic uptake of 99mTc - MAA in normal individuals



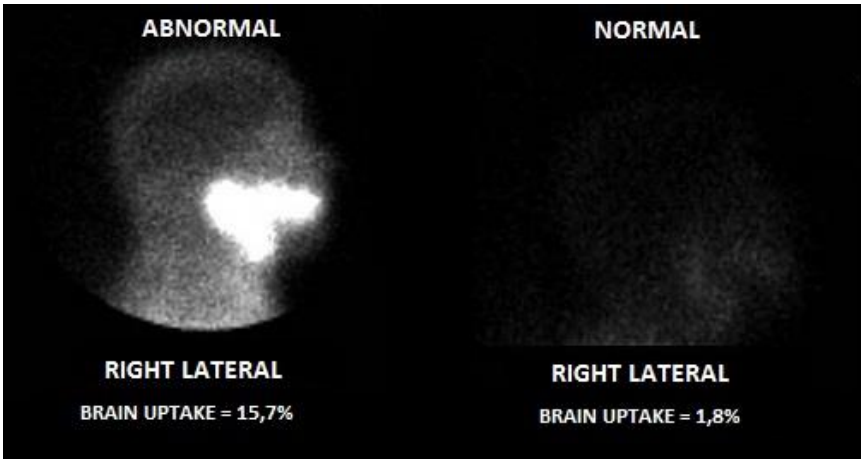
(Figura 4) Figure 2 - Mean and dispersion, in percentage, of brain uptake of 99mTc - MAA in abnormal individuals



(Figura 5) Figure 3 – Abnormal scintigraphy



(Figura 6) Figure 4 – Normal scintigraphy



(Tabela 5) Table 1. Intra-observer agreement analysis of scintigraphy results in relation to the diagnosis of pulmonary vascular dilatations - brain uptake

Brain uptake	Observer 1 (measure 2)		Agreement index	Kappa index (CI(95%))	Intra-class correlation
	Positive	Negative			
Observer 1 (measure 1)					
Positive	04	00	100.0%	1.0 (0.49; 1.00)	0.995 (0.980; 1.001)
Negative	00	11		p-value = 0.0001	

(Tabela 6) Table 2. Intra-observer agreement analysis of scintigraphy results in relation to the diagnosis of pulmonary vascular dilatations – systemic uptake.

Systemic uptake	Observer 1 (measure 2)		Agreemet index	Kappa index (CI(95%))	Intra-class correlation
	Positive	Negative			
Observer 1 (measure 1)					
Positive	01	00	100.0%	1.0 (0.49; 1.00)	0.997 (0.993 – 1.002)
Negative	00	14		p-value =	0.0001

(Tabela 7) Table 3. Inter-observer agreement analysis of scintigraphy results in relation to the diagnosis of intrapulmonary vascular dilatations – brain uptake.

Brain uptake	Observer 2		Agreement index	Kappa index (CI(95%))	Intra-class correlation
	Positive	Negative			
Observer 1 (measure 1)					
Positive	04	00	100.0%	1.0 (0.49; 1.00)	0.986 (0.946; 1.025)
Negative	00	11		p-value = 0.0001	
Observer 1 (measure 2)					
Positive	04	00	100.0%	1.0 (0.49; 1.00)	0.996 (0.987; 1.006)
Negative	00	11		p-value = 0.0001	

(Tabela 8) Table 4. Inter-observer agreement analysis of scintigraphy results in relation to the diagnosis of intrapulmonary vascular dilatations – systemic uptake.

Systemic uptake	Observer 2		Agreement index	Kappa index (CI(95%))	Intra-class correlation
	Positive	Negative			
Observer 1 (measure 1)					
Positive	01	00	73.3%	0.25 (0.01; 0.58)	0.988 (0.963 – 1.013)
Negative	04	10		p-value = 0.07	
Observer 1 (measure 2)					
Positive	01	00	73.3%	0.25 (0.01; 0.58)	0.973 (0.915; 1.030)
Negative	04	10		p-value = 0.07	

5 CONCLUSÕES E CONSIDERAÇÕES FINAIS

Através deste estudo, concluiu-se que:

- Em pacientes com EHE, a ocorrência de DVIP pela cintilografia com MAA-Tc^{99m} foi elevada
- A presença de DVIP esteve associada à menor diâmetro da veia esplênica, o que pode significar um mecanismo de proteção vascular contra o regime de hipertensão portal.
- A cintilografia com MAA-Tc^{99m} apresentou uma excelente reprodutibilidade, principalmente quando utilizada a captação relativa cerebral como índice diagnóstico de DVIP.
- Os valores de normalidade da cintilografia com MAA-Tc^{99m} foram ligeiramente superiores aos descritos na literatura. As características demográficas da população brasileira, o pequeno número de pacientes ou as diferentes metodologias entre os estudos podem ser as causas dessas diferenças.

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APÊNDICE A – Ficha para avaliação de pacientes

Identificação do Paciente

Nome: _____

Nº do prontuário:_____

Nº da identidade: _____Idade:_____Sexo:_____

Endereço:_____

_____

Dados da doença

Diagnóstico:_____

Tempo de diagnóstico:_____

Presença de hemorragia: ( ) S ( ) N _____

Medicações em uso:_____

Presença de ascite: ( ) S ( ) N

Encefalopatia: ( ) S ( ) N

Presença de doença pulmonar: ( ) S ( ) N

Fumantes: ( ) S ( ) N

Resultados de exames – Data (___/___/___)

Albumina:_____TPAE / INR:_____

Bilirrubina:_____Plaquetas:_____

TGO:_____TGP:_____

Fosfatase:_____ gama-GT:_____

Endoscopia – Data (___/___/___)

VVEE: ( ) S ( ) N Calibre: _____

GHP: ( ) S ( ) N

USG abdômen – Data (___/___/___)

FPP: ( ) S ( ) N Grau: _____

Tamanho do baço: ( ) < 12 cm ( ) > 12 cm _____

VP: _____ VE:_____

Exame cintilográfico – Data (___/___/___)

01	Capitação cérebro A1	Shunts A1	Pulmões A1	PD A1	PE A1	Capitação cérebro A2	Shunts A2	Pulmões A2	PD A2	PE A2
02	Capitação cérebro B1	Shunts B1	Pulmões B1	PD B1	PE B1	Capitação cérebro B2	Shunts B2	Pulmões B2	PD B2	PE B2

APÊNDICE B – Captação relativa do MAA-Tc^{99m} no grupo-controle

	Captação sistêmica	Captação cerebral
1	8,9	4,7
2	19,9	14,1
3	8,4	7,2
4	16,1	9,2
5	12,2	7,9
6	12	6
7	11	6,7
8	12,4	8,7
9	10,3	6,8

ANEXO A – Aprovação do Comitê de Ética em pesquisa do Centro de Ciências da Saúde – UFPE

SERVIÇO PÚBLICO FEDERAL
UNIVERSIDADE FEDERAL DE PERNAMBUCO
Comitê de Ética em Pesquisa

Of. Nº. 354/2010 - CEP/CCS

Recife, 16 de dezembro de 2010

Registro do SISNEP FR – 378924
CAAE – 0393.0.172.000-10
Registro CEP/CCS/UFPE Nº 396/10
Título: **Prevalência de Síndrome Hepatopulmonar em Pacientes com Cirrose, Esquistossomose Mansônica na Forma Hepatoesplênica e Doença Hepática Crônica Mista.**
Pesquisador Responsável: Liana Gonçalves de Macêdo

Senhor(a) Pesquisador(a):

Informamos que o Comitê de Ética em Pesquisa Envolvendo Seres Humanos do Centro de Ciências da Saúde da Universidade Federal de Pernambuco (CEP/CCS/UFPE) registrou e analisou de acordo com a Resolução N.º 196/96 do Conselho Nacional de Saúde, o protocolo de pesquisa em epígrafe, liberando-o para início da coleta de dados em 16 de dezembro 2010.

Ressaltamos que a aprovação definitiva do projeto será dada após a entrega do relatório final, conforme as seguintes orientações:

a) Projetos com, no máximo, 06 (seis) meses para conclusão: o pesquisador deverá enviar apenas um relatório final;
b) Projetos com períodos maiores de 06 (seis) meses: o pesquisador deverá enviar relatórios semestrais.

Dessa forma, o ofício de aprovação somente será entregue após a análise do relatório final.

Atenciosamente

Prof. Geraldo Bosco Lindoso Couto
Coordenador do CEP/ CCS / UFPE

A
Doutoranda Liana Gonçalves de Macêdo
Programa de Pós-Graduação em Medicina Tropical - CCS/UFPE

Av. Prof. Moraes Rego s/n, 1º Andar, Cid. Universitária, 50670-901, Recife - PE, Tel/fax: 81 2126 8588; cepccs@ufpe.br

ANEXO B – Instruções para publicação na *PLOS Neglected Tropical Diseases*

Submission Guidelines

PLOS Neglected Tropical Diseases publishes original research articles of importance to the NTDs community and the wider health community. We will consider manuscripts of any length; we encourage the submission of both substantial full-length bodies of work and shorter manuscripts that report novel findings that might be based on a more limited range of experiments.

The writing style should be concise and accessible, avoiding jargon so that the paper is understandable for readers outside a specialty or those whose first language is not English. Editors will make suggestions for how to achieve this, as well as suggestions for cuts or additions that could be made to the article to strengthen the argument. Our aim is to make the editorial process rigorous and consistent, but not intrusive or overbearing. Authors are encouraged to use their own voice and to decide how best to present their ideas, results, and conclusions.

PLOS Neglected Tropical Diseases is committed to the highest ethical standards in medical research. Accordingly, we ask authors to provide specific information regarding ethical treatment of research participants, patient consent, patient privacy, protocols, authorship, and competing interests. We also ask that reports of certain specific types of studies adhere to generally accepted standards. Our requirements are based on the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, issued by the International Committee for Medical Journal Editors.

Manuscript Organization

Most manuscripts should be organized as follows. Instructions for each element appear below.

- Title
- Authors and Affiliations
- Abstract
- Author Summary
- Introduction
- Methods
- Results
- Discussion
- Acknowledgments
- References
- Supporting Information Captions
- Uniformity in format facilitates the experience of readers and users of the journal. To provide flexibility, however, the Results and Discussion can be combined into one Results/Discussion section.
- Other elements
- Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately.
- Tables are inserted immediately after the first paragraph in which they are cited.
- Supporting information files are uploaded separately.

- Please refer to our downloadable sample files to make sure that your submission meets our formatting requirements:
- Download sample title, author list, and affiliations page (PDF)

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Titles should be written in title case (all words capitalized except articles, prepositions, and conjunctions). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

All attributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments hors must meet the criteria for authorship as outlined in the authorship policy.

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

Corresponding author

One corresponding author should be designated in the submission system as well as on the title page.

One corresponding author should be designated in the submission system. However, this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication.

Include an email address for each corresponding author listed on the title page of the manuscript.

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include the consortium or group name in the author list, and include the full list of members in the Acknowledgments or in a Supporting Information file.

Cover letter

Upload a cover letter as a separate file in the online system.

The cover letter should address the following questions:

- Why is this manuscript suitable for publication in *PLOS Neglected Tropical Diseases*?
- Why will your study inspire the NTDs community, and how will it drive the understanding of NTD pathobiology, epidemiology, prevention, treatment, control, or policy?

If your study addresses an infection that is outside our detailed scope, you must first send a pre-submission inquiry indicating why you consider the infection to be a neglected tropical disease.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract succinctly introduces the paper. It should not exceed 250–300 words. It should mention the techniques used without going into methodological detail and summarize the most important results with important numerical results given.

The Abstract is conceptually divided into the following three sections with these headings: Background, Methodology/Principal Findings, and Conclusions/Significance.

Do not include any citations in the Abstract. Avoid specialist abbreviations.

Author Summary

We ask that all authors of research articles include a 150- to 200-word non-technical summary of the work, immediately following the Abstract. Subject to editorial review and author revision, this short text is published with all research articles as a highlighted text box.

Distinct from the scientific abstract, the Author Summary should highlight where the work fits in a broader context of life science knowledge and why these findings are important to an audience that includes both scientists and non-scientists. Ideally aimed to a level of understanding of an undergraduate student, the significance of the work should be presented simply, objectively, and without exaggeration.

Authors should avoid the use of acronyms and complex scientific terms and write the author summary using the first-person voice. Authors may benefit from consulting with a science writer or press officer to ensure that they effectively communicate their findings to a general audience.

Introduction

The Introduction should put the focus of the manuscript into a broader context. As you compose the Introduction, think of readers who are not experts in this field. Include a brief review of the key literature. If there are relevant controversies or disagreements in the field, they should be mentioned so that a non-expert reader can delve into these issues further. The Introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

Methods

This section should provide enough detail for reproduction of the findings. Protocols for new methods should be included, but well-established protocols may simply be referenced. Detailed methodology or supporting information relevant to the methodology can be published on our web site.
This section should also include a section with descriptions of any statistical methods employed. These should conform to the criteria outlined by the Uniform Requirements, as follows:

Results
The Results section should include all relevant positive and negative findings. The section may be divided into subsections, each with a concise subheading. Large datasets, including raw data, should be submitted as supporting files; these are published online alongside the accepted article. The Results section should be written in past tense.

Discussion
The Discussion should be concise and tightly argued. It should start with a brief summary of the main findings. It should include paragraphs on the generalizability, clinical relevance, strengths, and limitations of your study.
You may wish to discuss the following points also:

- How do the conclusions affect the existing knowledge in the field?
- How can future research build on these observations and what are the key experiments that must be done?

Copyediting manuscripts
Please note that accepted manuscripts are not subject to detailed copyediting. Therefore, please carefully review your manuscript, paying special attention to spelling, punctuation, and grammar, as well as scientific content.

Acknowledgments
Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.
Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.
Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the online submission system.

References
Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on pre-print servers, if the manuscript is submitted to a journal and also publicly available as a pre-print

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”). Instead, include those data as supplementary material or deposit the data in a publicly available database.

- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts or author summaries.

Make sure the parts of the manuscript are in the correct order before ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the"Vancouver" style.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Supporting Information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All Supporting Information will be subject to peer review. All file types can be submitted, but files must be smaller than 10 MB in size.

Authors may use almost any description as the item name for a Supporting Information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting files should be publication-ready, as they are not copyedited.

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List Supporting Information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations we recommend that you cite Supporting Information in the manuscript text, but this is
not requirement. If you cite Supporting Information in the text, citations do not need to be in numerical
order.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order upon first appearance in the manuscript file.
Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.
At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

Tables
Cite tables in ascending numeric order upon first appearance in the manuscript file.
Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.
Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Data reporting
All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.
Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.
To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors.
Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

Accession numbers
All appropriate datasets, images, and information should be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). Accession numbers should be provided in parentheses after the entity on first use.

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Funding statement
This section should describe sources of funding that have supported the work. Please include relevant grant numbers and the URL of any funder's web site. Please also include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript." If this statement is not correct, you must describe the role of any sponsors or funders, and amend the aforementioned sentence as needed.

Competing interests
The corresponding author is asked at submission to declare, on behalf of all authors, whether there are any financial, personal, or professional interests that could be construed to have influenced the work.
Any relevant competing interests of authors must be available to editors and reviewers during the review process and will be stated in published articles.

Prior publication
When submitting a manuscript, all authors are asked to indicate that they have not submitted a similar manuscript for publication elsewhere. If related work has been submitted elsewhere, then a copy must be included with the manuscript submitted to PLOS. Reviewers will be asked to comment on the overlap between related submissions.

Guidelines for Specific Study Types
Human and animal research
All research involving humans and animals must have been approved by the authors' institutional review board or equivalent committee(s), and that board must be named by the authors in the manuscript. For research involving human participants, informed consent must have been obtained (or the reason for lack of consent explained, e.g. the data were analyzed

anonymously) and all clinical investigation must have been conducted according to the principles expressed in the Declaration of Helsinki. It must be stated in the Methods section of the paper whether informed consent was written or oral. If informed consent was oral, it must be stated in the paper: (a) why written consent could not be obtained, (b) that the IRB approved the use of oral consent, and (c) how oral consent was documented.
Authors should be able to submit, upon request, a statement from the research ethics committee or institutional review board indicating approval of the research. We also encourage authors to submit a sample of a patient consent form, and may require submission on particular occasions.
All animal work must have been conducted according to relevant national and international guidelines. In accordance with the recommendations of the Weatherall report, we specifically require authors to include details of animal welfare and steps taken to ameliorate suffering in all work involving non-human primates. The institution that approved the study must be named, and it must be stated in the paper that the study was conducted adhering to the institution's guidelines for animal husbandry.

Patient privacy and informed consent for publication

Our human participant policy conforms to the Uniform Requirements of the International Committee of Medical Journal Editors:

Patients have a right to privacy that should not be infringed without informed consent. Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. Complete anonymity is difficult to achieve, and informed consent for publication should be obtained if there is any doubt. If data are changed to protect anonymity, authors should provide assurance that alterations of the data do not distort scientific meaning. When informed consent has been obtained it should be indicated in the published article.

For papers that include identifying information, or potentially identifying information, authors must download the *Consent Form for Publication in a PLOS Journal* from our web site, which the patient, parent, or guardian must sign once they have read the paper and been informed about the terms of the PLOS content license.

Once authors have obtained the signed consent form, it should be filed securely in the patient's case notes and the manuscript submitted to PLOS should include this statement indicating that specific consent for publication was obtained: “The patients in this manuscript have given written informed consent (as outlined in the PLOS consent form) to publication of their case details.”

Clinical trials
We follow the World Health Organization’s (WHO) definition of a clinical trial:
A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health

outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

PLOS Neglected Tropical Diseases requires that all trials be registered and, as of August 13, 2013, supports the position of the that trials that are registered after the trial commences or retrospectively will be considered. For all trials, authors are asked to provide the trial registration information and to register their trial in an approved registry. For trials that were registered after the trial began or retrospectively, authors are asked to provide the following information:

- The trial registration information (or indicate that registration is in process)
- The reason for late registration, explained within the Methods section
- A statement in which all authors affirm that any trials on the same or a related drug or intervention they're involved in are registered, and provide (either as part of the statement or in the supplementary information) links to the published versions of the trials or the registration numbers. This statement will be published in the Methods section.

The editors reserve the right to inform authors' institutions or ethics committees about unregistered trials that have been carried out. Authors will also be asked to submit an accurate summary of the trial's results to the relevant registry (if there is such a mechanism) within a year of study completion or at the time of publication, whichever is the earliest.

Authors of trials must adhere to the CONSORT reporting guidelines appropriate to their trial design. Before the paper can undergo peer review, authors must: 1) provide in the manuscript the trial registry, trial registration number, and IRB, and 2) provide a copy of the trial protocol (or a link to an open access version of the protocol) and a completed CONSORT checklist as supporting files (these documents will also be published alongside the paper, if accepted). The CONSORT flow diagram must be included as Figure 1. Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and PLOS reserves the right to request a copy of the patient consent form. Information on statistical methods or participants beyond what is indicated in the CONSORT statement should be reported in the Methods section.

PLOS supports the public disclosure of all clinical trial results, as mandated, for example, by the FDA Amendments Act, 2007. For trials in registries that permit posting of trial results, *PLOS Neglected Tropical Diseases* requires that an accurate summary of the trial's results be submitted to the relevant registry (if there is such a mechanism) within a year of study completion or at the time of publication, whichever is the earliest.

Systematic reviews and meta-analyses

Reports of systematic reviews and meta-analyses must adhere to the PRISMA Statement or alternative guidelines appropriate to the study design, and include the completed checklist and flow diagram to accompany the main text. Authors must complete the appropriate reporting checklist not only with page references, but also with sufficient text excerpted from the manuscript to explain how they accomplished all applicable items.

Abstracts should follow PRISMA for Abstracts, using the PLOS abstract format. Authors must also state within the Methods section of their paper whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information.

The journal supports the prospective registration of systematic reviews. Authors whose systematic review was prospectively registered (e.g., in a registry such as PROSPERO) should provide the registry number in their abstract. Registry details and protocols will be made available to editors and reviewers, and included with the paper if the report is ultimately published.

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Reports of studies of diagnostic accuracy must adhere to the STARD requirements or alternative guidelines appropriate to the study design (see the EQUATOR web site) and include a completed checklist as supporting information. Authors must complete the appropriate reporting checklist not only with page references, but also with sufficient text excerpted from the manuscript to explain how they addressed all applicable items.

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If a prospective analysis plan (from the study's funding proposal, IRB or other ethics committee submission, study protocol, or other planning document written before analyzing the data) was used in designing an observational study, authors must include the relevant prospectively written document with the manuscript submission for access by editors and reviewers and eventual publication alongside the accepted paper. If no prospectively written document exists, authors should explain how and when they determined the analyses being reported.

Microarray experiments

Reports of microarray experiments must conform to the MIAME guidelines, and the data from the experiments must be deposited in a publicly accessible database.

Other Article Types

If you are submitting content other than a research article, read the guidelines for other article types.

ANEXO C – Instruções para publicação na *Annals of Nuclear Medicine*

Manuscripts should be organized as follows: title page, main document, acknowledgments, figures and tables (each figure and table should be on a separate page).

Title Page
The title page should include:

- The first name(s) (spelled out in full), middle initial(s) (if any), and surname(s) of all author(s)
- A concise and informative title
- A short title of up to 30 characters
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- Footnotes referring to the title (marked with an asterisk)
- Sources of funding for the article, if any

Main Document
The main document should be organized as follows: abstract, manuscript text, references, figure legends, and tables (if you want to insert the tables into the main document).
Because this journal follows a double-blind review policy, author information should not be included in the main document.

Abstract
Except for case reports and review articles, the abstract should contain a maximum of 350 words and include four clearly identifiable elements of content: objective, methods, results, and conclusions. These sections should be preceded by headings (i.e., Objective, Methods, Results, and Conclusions). For case reports and review articles, an unstructured abstract consisting of one complete paragraph in no more than 200 words is required. All abstracts should be submitted with three to five key words.

Manuscript Text Formatting
The manuscript text (except for case reports and review articles) should be arranged under the following headings: Introduction, Materials and Methods, Results, and Discussion. For case reports, the manuscript text should be arranged under the following headings: Introduction, Case report, and Discussion. While there are no specific length limitations for short communications, authors are encouraged to keep them concise.

- Use a normal, plain font (e.g., 12-point Times Roman) for the manuscript text.
- Every page must be typewritten double-spaced, leaving a 3-cm margin on all sides.
- Use italics for emphasis.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Number pages consecutively, beginning with the Abstract. Type the page number in the upper right-hand corner of each page.
- Type the short title of up to 30 characters in the header.
- Paragraphs should begin with an indentation of at least five-spaces length.

- Reference numbers in the text should be in parentheses.
- Footnotes on the text should be numbered consecutively.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations. Note: If you use Word 2007, do not create the equations with the default equation editor but use MathType instead.

Abbreviations and Units

Abbreviations in the manuscript text should be defined the first time they are mentioned, unless they are common, easily recognizable ones such as cm, ml, g, min, s, Bq, Gy, Sv, R, etc. and should be used consistently thereafter. Nomenclature, units and abbreviations should conform to IUPAC recommendations and Système Internationale (SI). Chemical formulae should be in keeping with the guidelines of the American Chemical Society.

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The list of references should only include works that are cited in the manuscript text and in any tables and figures that have been published or accepted for publication. Personal communications, unpublished data, manuscripts in preparation, or manuscripts submitted for publication are not acceptable in the references but may be cited parenthetically in the manuscript text.

References must be typed double-spaced and numbered consecutively in order of appearance. Do not use footnotes or endnotes as a substitute for a reference list. Where there are six or fewer authors, all authors should be listed. Where there are seven or more authors, only the first six should be listed, followed by “et al.” The journal uses the Vancouver style for references. Journal names are abbreviated according to the list of journals indexed in Index Medicus. References should be cited using numbers in square brackets on the line, e.g., Ames et al. [1] reported…

Figure Legends

Figure legends should be included in the manuscript text and not in the figure file.

Acknowledgments

Acknowledgments of people, grants, funds, etc. who contributed substantially to the work should be saved separately from the main document. The names of funding organizations should be written in full. Because this journal follows a double-blind review policy, authors should submit the main document and the acknowledgments separately.

A conflict-of-interest statement should always be inserted in the Acknowledgments section, even when no such conflict exists.

Figures

- All figures including photographs, graphs, diagrams, etc. are to be cited in the manuscript text and numbered consecutively using Arabic numerals, and given titles.
- Figure parts should be denoted by lowercase roman letters (a, b, etc.). If figures are supplied with uppercase labeling, lowercase letters will still be used in the figure legends and citations.
- A figure legend should be supplied for each figure. All elements found in the figure must be identified in the legend. Any previously published material must be identified by citing the original source in the form of a reference at the end of the legend. The figure legends should be included in the manuscript text and not in the figure file.
- The number of the figures must be kept to the minimum required for clarity of the manuscript text.

- Color figures will always be published in color in the online version. In print, however, they will appear in color only if the author agrees to make a contribution (€ 950 per article) to printing costs.

Tables

- All tables are to be numbered using Arabic numerals and given titles.
- Tables should always be cited in the text in consecutive numerical order.
- For each table, a table title should be supplied. The table title should explain clearly and concisely the components of the table.
- Any previously published material must be identified by citing the original source in the form of a reference at the end of the table title.
- Footnotes to tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data) and included beneath the table body.
- Horizontal lines should be drawn below the title, below column headings, and at the end of the table. Do not use vertical lines.