Yuri Costa Sarno Neves

**Avaliação ultrassonográfica de pacientes com febre amarela grave
na fase aguda e na convalescença**

Tese apresentada à Faculdade de Medicina
da Universidade de São Paulo para obtenção
do título de Doutor em Ciências

Programa de Radiologia

Orientadora: Profa. Dra. Maria Cristina
Chammas

Coorientadora: Dra. Ho Yeh Li

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LISTA DE ABREVIATURAS

AST: aspartato aminotransferase ou transaminase glutâmico-oxalacética

FA: febre amarela

IMC: índice de massa corpórea

RT-PCR: reação em cadeia da polimerase

UTI: unidade de terapia intensiva

US: ultrasonography

ICU: intensive care unit

INR: international normalized ratio

TS: transient elastography

ARFI: acoustic radiation force impulse,

SWE: shear-wave elastography

LS: liver stiffness

kPa: kilopascals

YF: yellow fever

LISTA DE SIGLAS

HCFMUSP	Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo
UMIN	Unidade de Tétano e de Terapia Intensiva
UTI-DMP	Unidade de Terapia Intensiva – Departamento de Moléstias Infecto-Parasitárias
SES-SP	Secretaria de Saúde do Estado de São Paulo
InRad	Instituto de Radiologia
PACS	Picture Archiving and Communication Systems
PAMB	Prédio dos Ambulatórios

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RESUMO

Neves YCS. *Avaliação ultrassonográfica de pacientes com febre amarela grave na fase aguda e na convalescença* [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2021.

Introdução: A febre amarela (FA) é uma doença hemorrágica causada por um arbovírus endêmico na América do Sul, com surtos recentes nos últimos anos. No Brasil, a epidemia recente entre dezembro de 2017 e abril de 2018 resultou em muitos óbitos, principalmente por febre hemorrágica grave, com hepatite aguda, insuficiência renal aguda, coagulopatia e choque circulatório. Não há estudos sobre os achados de imagem nesta doença e seus efeitos tardios no parênquima hepático. **Objetivos:** Descrever os achados de ultrassonografia (US) abdominal de pacientes com FA aguda grave, relacionando-os com dados clínicos e laboratoriais, e determinar a frequência e o grau de fibrose hepática dos sobreviventes na convalescença com base na US com avaliação por elastografia baseada na onda de cisalhamento (SWE). **Métodos:** Este é um estudo combinado com dois braços - primeiro, uma coorte retrospectiva entre janeiro e abril de 2018 com pacientes internados em unidade de terapia intensiva (UTI) com FA grave confirmada por reação em cadeia da polimerase. US abdominal à beira do leito foi realizada 48 horas após a admissão. As imagens foram analisadas independentemente por dois radiologistas. Os exames laboratoriais foram coletados em até 12 horas após a aquisição das imagens. Em um segundo momento, uma investigação de coorte de acompanhamento foi realizada com pacientes sobreviventes por meio de varreduras de SWE realizadas 6 meses após o início dos sintomas. A regressão logística multivariada foi realizada para identificar preditores de óbito em 30 dias. Todos os testes foram bicaudais e os valores finais de p abaixo de 0,05 foram considerados estatisticamente significativos. **Resultados:** Publicação 1: 46 pacientes foram avaliados com US à beira do leito na UTI. Os exames laboratoriais mostraram níveis séricos elevados de aspartato aminotransferase, bilirrubina total e creatinina (5319 U/L, 6,2 mg/dL e 4,3 mg/dL, respectivamente). 26/46 (56,5%) pacientes morreram dentro de 30 dias da admissão (mediana de 5 dias; intervalo interquartil: 2-9 dias). A mediana de tempo entre o início dos sintomas e a US foi de 7 dias. Os achados ultrassonográficos mais frequentes foram: espessamento da parede da vesícula biliar (80,4%), aumento da ecogenicidade do córtex renal (71,7%), aumento da ecogenicidade do parênquima hepático (65,2%), líquido perirrenal (52,2%), ascite (30,4%). O aumento da ecogenicidade renal foi associado ao óbito em 30 dias (84,6% versus 55,0%; $p = 0,046$) e mostrou-se um

preditor independente deste desfecho após a análise multivariada (OR: 10,89; $p = 0,048$). Publicação 2: 18 pacientes sobreviventes foram avaliados com SWE 6 meses após o início dos sintomas (mediana de tempo de 185 dias; intervalo interquartil: 180-191 dias). A mediana de rigidez hepática (do inglês *liver stiffness*, LS) foi de 5,3 (4,6 - 6,4) kPa. 2/18 (11,1%) foram classificados como Metavir F2, 1/18 (8,3%) como F3 e 1/18 (8,3%) como F4; esses dois últimos pacientes apresentavam características de congestão hepática cardiogênica na análise Doppler. Nenhum paciente tinha história de doença hepática ou alterações morfológicas de cirrose no modo de escala de cinza. A insuficiência cardíaca foi associada à LS aumentada ($p = 0,024$) e fibrose significativa ($\geq F2$; $p = 0,039$). Hipertensão arterial sistêmica e diabetes mostraram tendência de associação com maiores valores de LS ($p = 0,063$ e $p = 0,066$, respectivamente); SAPS-3 na admissão à UTI apresentou tendência semelhante com fibrose significativa ($\geq F2$; medianas de 71 versus 47; $p = 0,053$). LS foi significativamente correlacionada com idade ($p = 0,497$, $p = 0,036$) e alanina aminotransferase na admissão ($p = -0,507$, $p = 0,032$). Nenhum paciente dos 7 que receberam sofosbuvir apresentou fibrose hepática 6 meses após o início dos sintomas *versus* 4/11 (36,4%) indivíduos $\geq F2$ no grupo que não recebeu o medicamento ($p = 0,119$).

Discussão e Conclusão: Este é o primeiro estudo a descrever os achados de imagem em pacientes com FA. Encontramos achados reprodutíveis de US abdominal na FA aguda grave, em órgãos envolvidos na fisiopatologia da doença (efeitos diretos da infecção viral), que podem se relacionar com a gravidade e o prognóstico de pacientes de UTI. O aumento da ecogenicidade renal foi independentemente associado a óbito em 30 dias. Também encontramos baixa frequência de fibrose hepática durante o acompanhamento. A idade e a insuficiência cardíaca foram associadas ao aumento do LS na convalescença; SAPS-3 elevado também pode estar relacionado à fibrose hepática neste período. Assim, a US é uma ferramenta valiosa durante a fase aguda da FA grave e também pode ter um papel no acompanhamento de pacientes com idade e / ou comorbidades após a alta hospitalar. Em conclusão, este trabalho contribui para o nosso conhecimento sobre a história natural e as alterações e imagem desta doença rara.

Descritores: Febre amarela; Ultrassonografia; Técnicas de imagem por elasticidade; Abdome; Fígado; Injúria renal aguda.

ABSTRACT

Neves YCS. *Ultrasound evaluation of patients with severe yellow fever in the acute fase and convalescence* [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2021.

Introduction: Yellow fever (YF) is a hemorrhagic disease caused by an arbovirus endemic in South America, with recent outbreaks in the last years. In Brazil, the recent epidemic between December 2017 and April 2018 resulted in many deaths, mainly due to severe hemorrhagic fever, with acute hepatitis, acute renal failure, coagulopathy and circulatory shock. There are no studies regarding imaging findings in this disease and its late-term effects on liver parenchyma. **Objectives:** Describe the abdominal ultrasonography (US) findings of patients with severe acute YF, relating them to clinical and laboratory data, and to determine the frequency and grade of liver fibrosis in convalescent survivors based on US with shear-wave elastography (SWE) evaluation. **Methods:** This is a combined study with two arms – first, a retrospective cohort between January and April 2018 with patients admitted to intensive care unit (ICU) with polymerase chain reaction-confirmed severe YF. Bedside abdominal US was performed within 48 h of admission. Images were independently analyzed by two radiologists. Laboratory tests were collected within 12 h of image acquisition. Second, a follow-up cohort investigation was carried out with surviving patients using SWE scans performed 6 months after onset of symptoms. Multivariable logistic regression was performed to identify 30-day mortality predictors. All tests were two-tailed and final p values under 0.05 were considered statistically significant. **Results:** Publication 1: 46 patients were evaluated with bedside US at ICU. Laboratory tests showed high serum levels of aspartate aminotransferase, total bilirubin and creatinine (5319 U/L, 6.2 mg/dL and 4.3 mg/dL, respectively). 26/46 (56.5%) patients died within 30 days of admission (median time: 5 days; interquartile range: 2-9 days). Median interval between onset of symptoms and US was 7 days. Most frequent US findings were: gallbladder wall thickening (80.4%), increased renal cortex echogenicity (71.7%), increased liver parenchyma echogenicity (65.2%), perirenal fluid (52.2%), ascites (30.4%). Increased renal echogenicity was associated with 30-day mortality (84.6% versus 55.0%; $p = 0.046$) and an independent predictor of this outcome after multivariate analysis (OR: 10.89; $p = 0.048$). Publication 2: 18 surviving patients were evaluated with SWE 6 months after onset of symptoms (median time: 185 days; interquartile range: 180-191 days). Median liver stiffness (LS) was 5.3 (4.6 – 6.4) kPa. 2/18 (11.1%) were classified as Metavir F2, 1/18 (8.3%) as F3 and 1/18 (8.3%) as F4; these two last patients had features of cardiogenic liver congestion on Doppler analysis. No patient had history of liver disease or showed morphologic changes of cirrhosis on grayscale mode. Cardiac failure was associated with increased LS ($p = 0.024$) and significant fibrosis ($\geq F2$; $p = 0.039$). Systemic arterial hypertension and diabetes showed a tendency of association with higher LS values ($p = 0.063$ and $p = 0.066$, respectively); SAPS-3 at ICU admission showed a similar tendency with significant fibrosis ($\geq F2$; medians of 71 versus 47; $p = 0.053$). LS was significantly correlated with age ($p = 0.497$, $p = 0.036$) and alanine aminotransferase at admission ($p = -0.507$, $p = 0.032$). No patient who received sofosbuvir showed liver fibrosis at 6 months after

onset of symptoms) *versus* 4/11 (36.4%) $\geq$ F2 individuals in the group that did not receive the drug ($p = 0.119$). **Discussion and conclusion:** This is the first study to describe imaging findings in patients with YF. We found reproducible abdominal US findings in severe acute YF, in organs involved in the pathophysiology of the disease (direct effects of viral infection), that may relate to severity and prognosis of ICU patients. Increased renal echogenicity was independently associated with 30-day mortality. We also found a low frequency of liver fibrosis during the follow-up. Age and cardiac failure were associated with increased LS in convalescence. Increased SAPS-3 might be related to liver fibrosis after survival. Thus, US is a valuable tool during the acute phase of severe YF and can also have a role in the follow up of patients of age and / or with comorbidities after hospital discharge.

Descriptors: Yellow fever; Ultrasonography; Elasticity imaging techniques; Abdomen; Liver; Acute kidney injury.

1. INTRODUÇÃO

1.0 INTRODUÇÃO

Febre Amarela

A febre amarela (FA) é uma doença íctero-hemorrágica causada por um arbovírus do gênero *Flavivirus* e transmitida por mosquitos dos gêneros *Haemagogus*, *Sabethes* e *Aedes*, endêmica em regiões tropicais de países da América do Sul e da África Subsaariana, onde representa grave problema de saúde pública¹⁻³, com surtos recentes nos últimos anos⁴⁻⁷. No Brasil, a transmissão urbana foi interrompida na década de 40 graças a campanhas de vacinação em massa e programas de controle de vetores⁸. No entanto, a enzootose continua presente e a infecção humana se manteve dentro de um ciclo silvático⁹⁻¹¹. No final de 2017 e início de 2018, surgiu uma epidemia com os primeiros casos relatados em Minas Gerais¹² e posterior expansão progressiva para a Região Metropolitana de São Paulo (notadamente no município de Mairiporã), com letalidade geral próxima de 35%¹³. Entre os pacientes internados na unidade de terapia intensiva (UTI), no entanto, um estudo recente observou letalidade intra-hospitalar de 67,0%¹⁴.

O espectro clínico da FA compreende desde infecção subclínica (casos habitualmente não diagnosticados), doença febril leve sem icterícia e febre hemorrágica grave¹⁵. Neste último grupo, os pacientes cursam com insuficiência renal aguda (frequentemente exigindo terapia de substituição renal), hepatite fulminante, pancreatite, sangramento gástrico, coagulopatia, acidose metabólica grave rapidamente progressiva e choque circulatório, apresentando elevada letalidade^{14,16-18}. O acometimento hepático parece ser o grande responsável pela morte dos pacientes, tendo sido descrita associação entre os níveis de transaminases e de

bilirrubina direta e prognóstico^{3,19}. Ribeiro e colaboradores demonstraram a associação entre aspartato-aminotransferase (AST) e creatinina elevada e mortalidade por FA grave¹⁶. Casadio e cols., em outra publicação recente, chamam atenção para elevação da lipase e consumo de fator V para o desfecho fatal, e destacam que carga viral não foi um fator preditor independente de óbito entre os pacientes internados na UTI²⁰. No entanto, em artigo recente com inclusão e pacientes hospitalizados na UTI e em enfermaria, Kallas e colegas reinserem carga viral como fator prognóstico importante e atribuem relevância para AST, contagem de neutrófilos elevada e idade avançada²¹. Diante dos dados mais atuais, é nítida a busca por fatores prognósticos fidedignos e que possam guiar a terapêutica.

O tratamento da FA consiste em medidas de suporte clínico intensivo; não há terapia antiviral específica com eficácia comprovada até o momento²². Ensaios clínicos estão em andamento com sofosbuvir (medicação aprovada para tratamento de hepatite C, que demonstrou ação *in vitro* e *in vivo* contra o vírus da febre amarela²³), ainda sem resultados definitivos²⁴. Ainda assim, houve avanços significativos em face da última epidemia na melhora do manejo clínico, com uso de anticonvulsivantes se sintomas de encefalopatia ou níveis arteriais de amônia elevados, terapia de troca plasmática precoce e administração de doses terapêuticas de inibidor de bomba de prótons^{14,25}. Alguns casos foram submetidos a transplante hepático, com resultados variados^{19,26,27}.

A vacina com vírus vivo atenuado, desenvolvida em 1936, continua sendo medida primordial na prevenção da doença²⁸, porém possui contra-indicações bem definidas, devido ao risco de efeitos adversos graves²⁹. Ressalta-se ainda o efeito protetor somente a partir de 10 dias após a vacinação, como evidenciado na coorte recente em que três pacientes com FA grave haviam sido vacinados nos 10 dias

anteriores à internação ¹⁴. Nesses casos, a doença vacinal foi excluída em função de todos os vírus detectados por reação em cadeia da polimerase (RT-PCR) terem sido selvagens. Tal constatação reforça a recomendação de saúde pública de apenas viajar para áreas endêmicas após transcorrido o período de 10 dias da aplicação da vacina.

As principais experiências prévias da literatura em relação à FA no Brasil e no mundo foram adquiridas até década de quarenta do século XX, após o que, com controle dos casos urbanos secundário à vacinação em massa, os casos tornaram-se escassos. Assim, muitos recursos e tecnologias da medicina desenvolvidos nas últimas décadas não foram estudados no contexto desta doença. Além disso, os surtos haviam ocorrido e ocorrem principalmente em regiões com escassez de recursos de saúde, que muitas vezes carecem desses recursos específicos. Apesar de se saber sobre a fisiopatologia da doença e sua predileção (tropismo) e lesão direta de órgãos vitais como fígado e rins^{17,18,30,31}, não há estudos de imagem até o momento avaliando as alterações nestes sítios. Pouco se sabe também acerca dos efeitos tardios da doença sobre o parênquima hepático após o insulto agudo e a convalescência, pois poucos pacientes foram adequadamente acompanhados. Alguns trabalhos sugerem a recuperação total do fígado, como ocorre em outras hepatites, sem sinais ou sintomas de hepatopatia crônica ou cirrose ³²⁻³⁴. No entanto, uma série de casos recente demonstrou recaída tardia de hepatite em alguns pacientes sobreviventes de FA grave, ou seja, uma nova elevação de transaminase dentro de 6 meses da recuperação da função hepática, uma entidade completamente nova e não estudada³⁵. Portanto, o acompanhamento tardio de pacientes com FA não é um tópico bem estabelecido na literatura.

Ultrassonografia abdominal

A ultrassonografia (US) abdominal é um método de diagnóstico por imagem versátil, portátil, não emissor de radiação ionizante e de baixo custo relativo³⁶. Fundamentalmente, os equipamentos de US médica utilizam *softwares* específicos (modos) que permitem a avaliação, em tempo real, da morfologia das estruturas ecorrefringentes em escalas de cinza (modo B), da perviedade e padrão de fluxo sanguíneo nos leitos vasculares (modos Doppler colorido e espectral/pulsado), e das propriedades viscoelásticas (elasticidade) dos tecidos ensonados (modo Elastografia).

A papel da US na avaliação de alguns órgãos intra-abdominais merece destaque em certas doenças agudas (infecciosas ou não). A análise renal pela US mostrou validade na avaliação de doença renal aguda em pacientes críticos. Apesar de a princípio considerado inespecífico, o achado de aumento da ecogenicidade do parênquima renal evidenciou especificidade e sensibilidade altas em alguns estudos (de até 96% e 67%, respectivamente)^{37,38}. Além disso, o método permite exclusão de outras causas de disfunção renal, como insuficiência pós-renal (com dilatação do sistema coletor) e doença parenquimatosa crônica (através da avaliação do tamanho dos rins, espessura do parênquima e diferenciação córtico-medular)^{39,40}. A US detecta espessamentos da parede da vesícula biliar, com amplo diagnóstico diferencial, não restrito às colecistites^{41,42}. Quanto à avaliação vesicular nas doenças febris hemorrágicas agudas, temos a descrição de espessamento parietal difuso nas afecções agudas do parênquima hepático adjacente, principalmente na hepatite A e na dengue, sendo que nesta última existe uma relação entre o grau de espessamento e a gravidade do quadro clínico^{43,44}. A pesquisa de coleções líquidas e líquido livre

abdominal é outra aplicabilidade do exame, importante na cirrose e em quadros inflamatórios abdominais⁴⁵⁻⁴⁷.

O uso da US na avaliação hepática é amplo e bem estabelecido na literatura⁴⁸. A US é considerada ferramenta de escolha na caracterização de inicial de cirrose (sinais de redistribuição volumétrica, contornos irregulares, bordos rombos e alargamento das fissuras), na avaliação da perviedade das estruturas vasculares e no *screening* de carcinoma hepatocelular nos pacientes portadores de hepatopatia crônica e hipertensão portal^{49,50}. O método é ainda fundamental como na avaliação inicial no pré-transplante hepático⁵¹⁻⁵⁴ e no rastreamento das complicações pós-operatórias do transplante⁵⁵⁻⁵⁸, com a vantagem de permitir avaliações seriadas.

Elastografia hepática por SWE

A avaliação não invasiva do *status* do parênquima hepático para estimativa do grau de fibrose apresenta algumas modalidades disponíveis. Entre elas, a Elastografia por US mostrou-se um método seguro, com performances comparáveis às da biópsia convencional^{59,60}. Além disso, é importante destacar que a biópsia hepática, apesar de historicamente considerada o método padrão-ouro, apresenta considerável variabilidade intra e interobservador e erros potenciais de amostragem^{61,62}, além das desvantagens do procedimento invasivo, com riscos intrínsecos não desprezíveis⁶³. Portanto, a US com elastografia impõe-se como método importante na avaliação longitudinal de pacientes com hepatopatias⁶⁴.

O estudo do fígado por meio das ondas sonoras iniciou-se com a elastografia transitória (do inglês *transient elastography*, *TS*), baseada no princípio físico de compressibilidade dos meios e transmissão da onda mecânica por meio de

movimentos do transdutor; esta tecnologia popularizou-se com o nome comercial de FibroScan®⁶⁵. Atualmente, existem outros métodos comparáveis disponíveis no mercado, como a *Acoustic Radiation Force Impulse* (ARFI), que, embora ainda menos amplamente testada que a consagrada e mais bem estabelecida TS (que teve mais tempo e mais estudos acumulados de comparação com biópsia hepática ao longo dos anos), vem mostrando desempenhos praticamente equivalentes (quando não superiores) à TS na aferição e estadiamento da fibrose hepática^{59,66}. Esta última técnica (ARFI), também conhecida como elastografia por onda de cisalhamento (do inglês *shear-wave elastography*, SWE), baseia-se na aferição da velocidade da onda de cisalhamento (uma onda transversal oriunda do ponto de interesse para o estudo, no órgão-alvo), gerada a partir da emissão de pulsos sonoros pelo transdutor, sem necessidade de compressões pelo examinador⁶⁷. A partir da velocidade (em m/s) desta onda, estipula-se a medida de dureza do parênquima hepático (do inglês *liver stiffness*, LS, em kilopascals), que por sua vez é uma estimativa do grau de fibrose. Apresenta algumas vantagens em relação ao método anterior, como o fato de estar sempre acoplada ao aparelho de ultrassonografia convencional, com geração de imagens simultâneas em escalas de cinza (modo B). Desse modo, há a possibilidade de seleção da área do órgão a ser estudada, e, portanto, menor erro de aferição na profundidade em casos de ascite acentuada ou obesidade com grande panículo adiposo, por exemplo⁶⁶. Vale ressaltar que o FibroScan® também fornecer uma medida de padrão de qualidade ou traçado da onda obtida.

Dentre as técnicas de ARFI comercialmente disponíveis, temos dois principais tipos: a SWE bidimensional (2D-SWE) e a SWE de ponto (p-SWE), ambas com resultados semelhantes⁶⁸⁻⁷⁷. Recentemente foi validada uma nova técnica de p-SWE, a S-SWE, desenvolvida no equipamento RS80A® US system (Samsung Medison Co.,

Ltd.), demonstrando boa reprodutibilidade e confiabilidade para a avaliação de fibrose hepática, comparável àquela dos métodos antecessores⁷⁸⁻⁸⁰.

Existem fatores intrínsecos do paciente que podem afetar a performance da SWE e devem ser considerados antes do exame. A própria obesidade, reconhecidamente um fator de dificuldade técnica na realização da US, reduz a acurácia do método; o IMC (índice de massa corpórea) dos pacientes é habitualmente registrado em estudos com elastografia⁸¹. Edema e congestão hepática cardiogênica, em pacientes com insuficiência cardíaca, por exemplo, aumenta a dureza do fígado e superestima o grau de fibrose⁸². Inflamação e atividade necroinflamatória nas hepatites agudas ou reagudizadas também leva à superestimativa do grau de fibrose, por aumentar a LS^{33,83,84}.

Os valores de corte para estadiamento ou graduação da fibrose hepática dentro da escala Metavir apresentam grande variabilidade e sobreposição de faixas a depender do fabricante da máquina de US com SWE e conforme a doença sendo estudada (hepatites C ou B, por exemplo), principalmente para valores de fibrose leve ou moderada, motivo pelo qual um consenso recente propôs uma classificação baseada na variação (delta) da LS (e não no valor absoluto ou na velocidade da onda de cisalhamento) e utilizando a “regra de 4” para técnicas de ARFI para estimar o risco de complicações relacionadas à hipertensão portal e à insuficiência hepática (ascite, hemorragia varicosa e encefalopatia)⁶⁰. Este mesmo consenso advoga o uso de *cutoffs* mais altos para afirmar com maior valor preditivo positivo a presença de doença hepática crônica (acima de 13 kPa). Valores abaixo de 9 kPa na ausência de sinais clínicos praticamente excluíam doença hepática crônica (maior valor preditivo negativo).

Motivação do trabalho

Até o presente momento, não há estudos descritivos de imagem médica em pacientes com FA. Em face da busca de fatores prognósticos que se relacionem a piores desfechos da doença, o uso da US à beira do leito no contexto da UTI (onde são internados a maioria dos seus casos graves) pode ser uma ferramenta valiosa, especialmente na relação entre os seus achados e os de exames laboratoriais. O exame pode vir a ser decisivo para tomada de condutas imediatas, principalmente nas regiões com recursos laboratoriais parcos, além da sua reconhecida praticidade e aplicabilidade para avaliação pré-transplante (terapia proposta para alguns pacientes com hepatite fulminante), com checagem de fluxos vasculares hepáticos⁵³. Utilizando as palavras-chave “*Yellow fever*” e “*Ultrasonography*” com o conectivo “*AND*”, não foi encontrado nenhum estudo indexado, nas plataformas / bases de dados MeSH / PubMed, da NCBI, Biblioteca Virtual de Saúde (BVS, do Ministério da Saúde), Cochrane Library, Scopus, Web of Science e Google Scholar.

Não são conhecidos os efeitos tardios secundários à infecção viral por FA no parênquima hepático. Como mencionado, foi descrita a recuperação completa dos casos sobreviventes, sem sinais ou sintomas relacionados à hepatopatia crônica³⁴. Outras hepatites agudas cursam com recuperação total do parênquima, com normalização da LS^{32,33}. No entanto, até a data da elaboração deste projeto, através de revisão abrangente da literatura, não foram encontradas publicações acerca das alterações morfotexturais ou elastográficas hepáticas a longo prazo em pacientes com febre amarela. Frente a estas observações, a avaliação do fígado com SWE em pacientes convalescidos poderia contribuir para a melhor compreensão das alterações hepáticas após insulto agudo e, possivelmente, elucidar a causa de astenia

crônica e de elevações tardias de transaminases³⁵, predizendo ou associando-se à eventual recaída tardia e direcionando para acompanhamento específico os casos que porventura desenvolvam hepatopatia crônica.

2. OBJETIVOS

2.0 OBJETIVOS

- Avaliar os achados ultrassonográficos abdominais dos pacientes na fase aguda da febre amarela grave e relacioná-los com dados clínicos e laboratoriais
- Determinar a frequência e estimar o grau de fibrose hepática dos pacientes sobreviventes na fase de convalescência da febre amarela por meio da Elastografia com técnica SWE e avaliar possíveis preditores clínicos e laboratoriais de fibrose

3. DESENVOLVIMENTO E RESULTADOS

3.0 DESENVOLVIMENTO E RESULTADOS

3.1 ORGANIZAÇÃO DO TEXTO DE DESENVOLVIMENTO

As publicações do trabalho foram agrupadas de acordo com os objetivos da tese:

Avaliar os achados ultrassonográficos abdominais dos pacientes na fase aguda da febre amarela grave e relacioná-los com dados clínicos e laboratoriais

- Publicação 1: *Ultrasound findings and laboratory predictors of early mortality in patients with severe yellow fever (Neves et al 2021 – publicado em periódico internacional indexado)*

Determinar a frequência e estimar o grau de fibrose hepática dos pacientes sobreviventes na fase de convalescência da febre amarela por meio da Elastografia com técnica SWE e avaliar possíveis preditores clínicos e laboratoriais de fibrose

- Publicação 2: *Staging liver fibrosis after severe yellow fever with ultrasound elastography in Brazil: A six-month follow-up study (Neves et al 2021 – publicado em periódico internacional indexado)*

3.2 TEXTO SISTEMATIZADO SOBRE A PUBLICAÇÃO 1

Durante o recente surto de casos de FA oriundos dos arredores de São Paulo, no final de 2017 e início de 2018, o Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) começou a receber casos graves da doença, que rapidamente tornou-se uma epidemia, regulados pela Secretaria de Saúde do Estado de São Paulo (SES-SP). O Hospital então rapidamente tornou-se uma referência na condução e tratamento destes pacientes. Os casos encaminhados, em sua maioria graves, eram internados na Unidade de Tétano e de Terapia Intensiva (UMIN) do Serviço de Moléstias Infecto-Parasitárias (UTI-DMP). Novas descobertas e conhecimentos sobre o manejo da doença no contexto da terapia intensiva eram necessários e estavam sendo desenvolvidos em curto espaço de tempo.

Nesse contexto, a busca pelo melhor entendimento da fisiopatologia da doença e fatores prognósticos relevantes era imperativa. Logo percebeu-se que não havia estudos de imagem acerca das alterações inflamatórias provocadas pelo vírus. Impulsionado pela necessidade de afastar trombose do sistema venoso portal que pudesse vir a dificultar ou privar o paciente de um possível transplante hepático (que se configurava como uma nova alternativa possivelmente válida para os casos de hepatite fulminante por FA), o Instituto de Radiologia (InRad) do HCFMUSP foi ativado para a realização de US abdominal com Doppler hepático. Dessa forma, foram observadas alterações repetidas e consistentes nos pacientes internados na UTI, em órgãos diretamente implicados na fisiopatologia da infecção viral, de maneira que uma avaliação sistemática dos pacientes com FA foi rapidamente implementada. A descrição inicial destes achados inéditos e o seu possível uso como fatores prognósticos e que viessem a trazer nova luz aos mecanismos de patogenicidade da

arbovirose foram vistos com bastante entusiasmo científico pelas equipes de Infectologia e Radiologia, que entraram em colaboração.

Assim nasceu o primeiro trabalho sobre imagem abdominal e FA. Os dados coletados da avaliação ultrassonográfica à beira do leito de UTI, conjuntamente com informações clínicas e laboratoriais da admissão, foram acessados então retrospectivamente a partir dos sistemas de dados do hospital, em face do desfecho fatal (portanto uma coorte retrospectiva). Todos os pacientes eram testados e confirmados à chegada com o teste da reação em cadeia da polimerase em tempo real (RT-PCR). Não houve outros critérios de exclusão. O aparelho de ultrassonografia utilizado encontrava-se disponível para o uso e era deslocado do terceiro andar do InRad – HCFMUSP para a UMIN. As imagens foram arquivadas digitalmente via *sistema Picture Archiving and Communication System (PACS)* no sistema de imagens do HCFMUSP (iSite – PHILIPS). Os exames laboratoriais coletados já faziam parte da rotina assistencial dos pacientes incluídos no estudo. Dessa maneira, não houve custos adicionais para os departamentos ou investigadores.

Outras especificações metodológicas:

- Apenas 1 (um) radiologista com experiência em ultrassonografia (pesquisador responsável) ficou encarregado por fazer os exames à beira-leito;
- Todas as imagens foram revisadas retrospectivamente por pelo menos 2 (dois) radiologistas (sendo um deles o mesmo que subiu ao leito do paciente e realizou o exame); um terceiro radiologista foi requisitado em alguns casos de dúvida para fornecer a opinião final quanto às variáveis ultrassonográficas.

- O exame ultrassonográfico do abdome superior (da mesma forma como os de laboratório) já fazia parte da rotina assistencial dos pacientes na UTI, e era solicitado para avaliar principalmente vascularização hepática; não obstante, cabe salientar que, em face de uma doença “reemergente” e ainda pouco conhecida, o protocolo de exame foi intencional e sistematicamente estendido para estudar outros órgãos e estruturas, em todos os pacientes.

Nas próximas páginas está o artigo produzido e publicado na revista de alto impacto em radiologia *American Journal of Roentgenology*, com o ineditismo da descrição inicial de uma casuística relevante de pacientes com essa doença rara na fase aguda e uma importante descoberta acerca do valor prognóstico de um dos achados de imagem.

3.3 ARTIGO 1

ULTRASOUND FINDINGS AND LABORATORY PREDICTORS OF EARLY MORTALITY IN PATIENTS WITH SEVERE YELLOW FEVER

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Ultrasound Findings and Laboratory Predictors of Early Mortality in Patients With Severe Yellow Fever

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OBJECTIVE. Yellow fever is a hemorrhagic disease caused by an arbovirus endemic in South America; outbreaks have occurred in recent years. The purpose of this study was to describe abdominal ultrasound findings in patients with severe yellow fever and correlate them with clinical and laboratory data.

MATERIALS AND METHODS. A retrospective cohort study was performed between January and April 2018. The subjects were patients admitted to an ICU with polymerase chain reaction–confirmed yellow fever. Bedside sonography was performed within 48 hours of admission. Images were independently analyzed by two board-certified radiologists. Laboratory test samples were collected within 12 hours of image acquisition. Multivariable logistic regression analysis was performed to identify 30-day mortality predictors; $p < .05$ was considered statistically significant.

RESULTS. Forty-six patients (40 [87%] men, six [13%] women; mean age, 47.5 ± 15.2 years) were evaluated with bedside sonography. Laboratory tests showed high serum levels of aspartate aminotransferase (5319 U/L), total bilirubin (6.2 mg/dL), and creatinine (4.3 mg/dL). Twenty-six (56.5%) patients died within 30 days of admission (median time to death, 5 days [interquartile range, 2–9 days]). The most frequent ultrasound findings were gallbladder wall thickening (80.4%), increased renal cortex echogenicity (71.7%), increased liver parenchyma echogenicity (65.2%), perirenal fluid (52.2%), and ascites (30.4%). Increased renal echogenicity was associated with 30-day mortality (84.6% versus 55.0%; $p = .046$) and was an independent predictor of this outcome after multivariate analysis (odds ratio, 10.89; $p = .048$).

CONCLUSION. Reproducible abdominal ultrasound findings in patients with severe yellow fever may be associated with severity of disease and prognosis among patients treated in the ICU.

Yellow fever (YF) is a mosquito-borne hemorrhagic disease caused by an arbovirus of genus *Flavivirus* that occurs in tropical regions of South America and Africa [1]. In Brazil, the urban transmission cycle was interrupted in the 1940s by a massive vaccination campaign and vector control programs [2]. However, the enzootic remains present, and human infection continues to occur in a sylvatic cycle [3]. At the beginning of 2018, a YF outbreak occurred on the outskirts of São Paulo; 419 cases were confirmed between December 2017 and March 2018, and the mortality rate was 36%. In one recent study [4] investigators described the clinical characteristics of patients with YF admitted to the ICU. The in-hospital mortality rate was 67.0%, and there were high frequencies of acute renal failure (often requiring renal replacement therapy), fulminant hepatitis, pancreatitis, gastric bleeding, and rapidly progressive severe metabolic acidosis.

Because severe YF is associated with a rapidly progressive decline in clinical status, quick clinical decisions should be based on fast, accessible bedside evaluations. Abdominal ultrasound (US) is a low-cost and radiation-free imaging method suited to evaluation of the liver and kidneys, allowing assessment of vascular status and parenchymal changes [5–7]. US also depicts fluid collections and intraperitoneal free fluid in inflammatory and congestive states [8, 9].

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Little is known about the role of imaging in the management of YF, particularly regarding organs directly affected by the viral infection, such as liver and kidneys. To our knowledge, there have been no studies of the US findings in YF. The aims of this study were to describe the abdominal US changes in patients with severe YF and to correlate these findings with clinical and laboratory data.

Materials and Methods

A retrospective cohort study was conducted to assess abdominal US findings in patients with YF admitted to the infectious diseases ICU of a tertiary-care teaching hospital in São Paulo, Brazil (Hospital das Clínicas, University of São Paulo), from January 15 to April 12, 2018. All cases were confirmed by serum polymerase chain reaction for YF virus RNA. ICU admission criteria included one or more of the following: serum aspartate or alanine aminotransferase level greater than 3000 IU/L; international normalized ratio (INR) greater than 1.5; platelet count less than 90,000/mm³; acute kidney injury; hemorrhage; encephalopathy; hemodynamic instability. Patients who met any of these criteria were transferred directly from the health center or emergency department to the ICU.

Bedside US examinations were performed within 48 hours of patient admission by a board-certified radiologist (4 years of experience) using a Logiq P6 system (2008 model, GE Healthcare) with a convex transducer (mean frequency, 3.5 MHz) and standard abdominal preset (gain, 50 dB; dynamic range, 75 dB). Patients were in supine position, frequently with an elevated headboard. No patient was receiving parenteral nutrition. The Doppler analysis parameters were pulse repetition frequency, 15 cm/s; gain, 28 dB; frequency, 2 MHz; sample volume, 3 mm; minimal wall filter. The scanning protocol included gray-scale and color and spectral Doppler modes to assess the following: liver size (maximum left lobe length, measured in the parasagittal plane), morphology (contours, borders, and fissure width), echogenicity, and vasculature (portal vein patency and flow velocity, hepatic artery patency and spectral pattern, and hepatic vein patency and spectral pattern); gallbladder wall thickness (considered normal if ≤ 0.3 cm [10]) and content; kidney size, parenchymal thickness and echogenicity, and perirenal fluid; pancreatic size, echogenicity, and peripancreatic fluid collections; spleen size and echogenicity; and presence and amount of intraabdominal free fluid.

Ascites was subjectively categorized into minimal if there was only a thin laminar anechoic fluid collection in the pelvis, mild if fluid was encountered in other peritoneal spaces (i.e., perihepatic and perisplenic), moderate if a substantial accumulation of fluid was observed, and large if fluid displaced organs [9]. Liver echogenicity was considered increased if equal to or greater than that of the spleen. Kidney echogenicity was considered increased if equal to or greater than that of the spleen or higher than that of the liver [5, 7, 11].

Data acquired were archived in the PACS (iSite, version 4.1.110.0, Philips Healthcare). Each patient had at least 30 images acquired; no videos were available. All images were independently analyzed by two board-certified radiologists (6 and 4 years of experience in US). A third radiologist (10 years of experience) was requested in cases of disagreement and provided the final opinion.

Clinical and laboratory data were retrieved retrospectively from medical records. Demographic data, Simplified Acute Physiology Score 3, and clinical outcome (death within 30 days) were

evaluated. Laboratory samples were collected within 12 hours of the US examination. Because of the retrospective nature of the study and the anonymity of participants, the requirement for informed consent was waived. The research protocol was approved by the research ethics committee of the Hospital das Clínicas, Faculdade de Medicina, University of São Paulo. We followed the Strengthening the Reporting of Observational Studies in Epidemiology statement checklist for observational studies [12].

For descriptive purposes, categorical variables were presented through relative and absolute frequencies and compared by chi-square or Fisher exact test, as appropriate. Distributions of continuous variables were assessed for normality by skewness and kurtosis and by graphic methods. Variables with normal distribution were presented as mean and SD and compared by independent-samples *t* test. Otherwise, they were presented as median and interquartile range (IQR) and compared by Mann-Whitney nonparametric test. Independent predictors of the primary outcome 30-day mortality were identified through multivariable logistic regression analysis by the backward stepwise approach. A stepwise approach was preferred over a full-model strategy because of the limited sample size and events-per-variable number. Variables significant at the .10 level in univariate analysis were included in the initial models and maintained according to Wald statistics. All tests were two-sided, and final *p* < .05 was considered statistically significant. All analyses were conducted with SPSS software (SPSS Statistics for Microsoft Windows, version 24.0, IBM).

Results

Ninety-nine patients with severe hemorrhagic fever suspicious for YF were admitted to the ICU during the study period. Forty-nine patients were evaluated with bedside US, 46 of whom (40 men [87%], six women [13%]; mean age, 47.5 $\pm$ 15.2 (SD) years) had positive test results for YF. Figure 1 shows the patient enrollment and exclusion process, and Table 1 shows the clinical and demographic data on subjects with YF who underwent evaluation. Laboratory tests showed high serum levels of aspartate aminotransferase (5319 U/L), total bilirubin (6.2 mg/dL), and creatinine (4.3 mg/dL). All patients were residents of or had traveled to areas at risk of YF days before their symptoms started.

The median interval between onset of symptoms and US examination was 7 days (IQR, 5–8 days). Six patients (13.0%) had been vaccinated for YF less than 10 days before admission. None was considered to have vaccinal disease, because only wild virus was detected with polymerase chain reaction. Laboratory tests (Table 1) revealed high serum levels of alanine aminotransferase, aspartate aminotransferase, and total bilirubin. Mean serum creatinine and urea serum concentrations were also elevated. Coagulation disorders were frequent, with high median INR and low median platelet count.

Two (4.3%) patients underwent liver transplant. One died of complications and recurrent onset of hepatitis 9 days after admission and 7 days after transplant. The other survived and was discharged 180 days after admission. Twenty-five (56.8%) of the 44 patients who did not undergo transplant died within 30 days of admission, mainly of acute hepatitis, coagulopathy, and renal dysfunction (total of 26 deaths [26/46 patients, 56.5%]). The median time between admission and death was 5 days (IQR, 2–9 days). Eighteen (40.9%) patients were discharged from the hospi-

Ultrasound of Yellow Fever

tal (median length of hospital stay, 11 days [IQR, 7–23 days]). One patient died after 94 days of hospitalization with hospital-acquired pneumonia.

Regarding abdominal US findings, we observed that 30 (65.2%) patients had increased liver parenchyma echogenicity (Fig. 2). One (2.2%) patient had morphologic changes of mild chronic hepatitis (coarse liver borders and minimal widening of fissures), and another patient had established cirrhosis of unknown cause with nodular liver contours and enlargement of the left lobe. Except for one patient with previous atrial fibrillation who had heart failure and thus a pattern of cardiogenic congestion on Doppler analysis, all patients had normal portal vein flow (median velocity, 20 cm/s), pervious and pulsatile hepatic veins, and normal biphasic hepatic artery flow. All patients had patent splenic and superior mesenteric veins and inferior vena cava, as visualized with color Doppler ultrasound.

Gallbladder wall thickening was found in 37 (80.4%) patients, and multiple patterns of delamination were noted (Figs. 3A–3D): eight patients had homogeneous and regular thickening; 18 patients had thickening and perivesicular laminar fluid collection; and 11 patients had gallbladder wall delamination with marked thickening. Heterogeneous material was found in the gallbladder lumen in four (8.7%) patients (Figs. 3E and 3F). Gallstones were found in three (6.5%) patients, two of whom also had slight gallbladder wall thickening. No patient had gallbladder distention.

In all patients the kidneys were of normal size and parenchymal thickness. Increased cortical echogenicity was found in 33 (71.7%) patients, and perirenal fluid was found in 24 (52.2%) (Fig. 4). Fourteen (30.4%) patients had intraabdominal free fluid: seven (15.2%), a minimal amount; six (13.0%), mild ascites; and one (2.2%), moderate ascites. Four (8.7%) patients had imaging findings suggestive of pancreatitis (peripancreatic fluid collections or increase in size of any portion of the pancreas) (Table 1). Absolute interobserver agreement on all five most prominent US findings (increased liver and renal echogenicity, gallbladder wall thickening, perirenal fluid, free fluid) was always greater than 93.5% (mean $\kappa = 0.90$).

Comparison between individuals with and without the five main US findings showed that time between onset of symptoms and performance of bedside US did not differ significantly (median, 7 days versus 6 days for all comparisons; $p = .11$ for liver echogenicity; $p = .52$ for renal echogenicity; and $p = .75$ for gallbladder thickening). Patients with increased liver echogenicity had lower fibrinogen levels (119 versus 167 mg/dL; $p = .006$). Higher serum creatinine and urea levels were found in patients with increased renal echogenicity (mean creatinine concentration, 4.78 versus 3.11 mg/dL, $p = .13$; mean urea level, 107 versus 72 mg/dL, $p = .16$); however, these differences were not statistically significant (Table 2). Gallbladder wall thickening was associated with an elevated INR (median, 1.64 versus 1.33; $p = .03$). Patients with findings suggestive of pancreatitis at US also had higher serum levels of lipase (1076 U/L [IQR, 718–4495 U/L] versus 208 U/L [IQR, 136–672 U/L]; $p = .04$). There were no other statistically significant associations between US findings and clinical or laboratory variables.

Our univariate analysis of early mortality (Table 2) showed that patients who died within 30 days of admission were more likely to have increased renal echogenicity (84.6% versus 55.0%; $p = .046$). This group had higher serum levels of aspartate aminotransferase

TABLE 1: Clinical, Laboratory, and Sonographic Findings of 46 Patients With Severe Yellow Fever Admitted to the Infectious Diseases ICU (Descriptive Statistics)

Variable	Value
Age (y)	
Mean $\pm$ SD	47.5 $\pm$ 15.2
Median and interquartile range	44.8 (34.9–62.0)
Range	19.7–75.4
Sex	
Male	40 (87)
Female	6 (13)
Diabetes	7 (17.9)
Hypertension	10 (25.6)
Dyslipidemia	1 (2.6)
Cardiac disease	3 (7.7)
COPD or asthma	1 (2.6)
Alcohol abuse	
Current	21 (46.7)
Previous	5 (12.8)
SAPS 3 score ^a	59 $\pm$ 13
Death within 30 d (no transplant)	25 (56.8)
Time between admission and death (d) ^b	5 (2–9)
Discharge (no transplant)	18 (40.9)
Length of hospital stay (d) ^b	11 (7–23)
Liver transplant	2 (4.3)
Death	1
Days after admission	9
AST (U/L) ^b	5319 (3247–7000)
ALT (U/L) ^b	2686 (1790–3380)
Total bilirubin (mg/dL) ^b	6.2 (4.5–7.7)
Alkaline phosphatase (U/L) ^a	146 $\pm$ 51
GGT (U/L) ^a	308 $\pm$ 182
Urea (mg/dL) ^a	98 $\pm$ 70
Creatinine (mg/dL) ^a	4.3 $\pm$ 3.4
Fibrinogen (mg/dL) ^a	136 $\pm$ 58
Factor V (%) ^a	73 $\pm$ 48
INR ^b	1.50 (1.27–2.26)
Platelets (1000 U/mm ³) ^b	57 (45–83)
Lipase (U/L) ^b	236 (138–710)
Time between onset of symptoms and sonography (d) ^b	7 (5–8)
Increased liver echogenicity	30 (65.2)
Increased renal cortex echogenicity	33 (71.7)

(Table 1 continues on next page)

TABLE 1: Clinical, Laboratory, and Sonographic Findings of 46 Patients With Severe Yellow Fever Admitted to the Infectious Diseases ICU (Descriptive Statistics) (continued)

Variable	Value
Perirenal fluid	24 (52.2)
Intraabdominal free fluid	14 (30.4)
Gallbladder wall thickening	37 (80.4)
Homogeneous thickening	8 (17.4)
Perivesicular laminar fluid	18 (39.1)
Delamination	11 (23.9)
Heterogeneous gallbladder content	4 (8.7)
Peripancreatic fluid collections or increase in pancreatic size	4 (8.7)
Ultrasound features of chronic liver disease	2 (4.3)
Left hepatic lobe length (cm) ^b	8.3 (7.0–9.4)
Portal vein flow velocity (cm/s) ^b	20 (15–25)

Note—Values are number with percentage in parentheses unless otherwise indicated. COPD = chronic obstructive pulmonary disease, SAPS 3 = Simplified Acute Physiology Score 3, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = γ -glutamyl transferase, INR = international normalized ratio.

^aMean $\pm$ SD.

^bMedian and interquartile range.

(median, 7000 versus 3723 U/L; $p = .002$), creatinine (mean, 5.6 versus 2.6 mg/dL; $p = .002$), urea (mean, 117 versus 73 mg/dL; $p = .03$), and lipase (median, 501 versus 187 U/L; $p = .01$) and also presented with higher INRs (mean, 1.99 versus 1.27; $p < .001$). Lower platelet count (median, 47 versus 67 1000/mm³; $p = .04$) and factor V serum concentration (mean, 51% versus 101% of normal; $p < .001$) were also found among patients who died.

The initial multivariable model included renal hyperechogenicity; aspartate transaminase, γ -glutamyl transferase, lipase, creatinine, and fibrinogen levels; platelet count; and INR. Urea and factor V were not included because of high correlation with creatinine concentration (correlation coefficient, 0.851; $p < .001$) and INR (correlation coefficient, 0.879; $p < .001$). After multivariable analysis, renal hyperechogenicity (odds ratio, 10.89; $p = .048$), platelet count (odds ratio, 1.03 per unit; $p = .03$), creatinine concentration (odds ratio, 1.48 per unit; $p = .02$) and INR (odds ratio, 4.44 per unit; $p = .02$) were predictive of 30-day mortality in the final model (Table 3).

Discussion

To our knowledge, this study is the first to assess abdominal US findings in patients with severe YF. US is a valuable tool in the ICU, where most of patients with severe YF are admitted, owing to the advantages of being readily accessible and portable. US was not available during previous outbreaks of YF, either because the technology had not yet been developed or because of lack of resources in underdeveloped countries in which the outbreaks occurred. Duarte-Neto et al. [13] published an observational study showing the efficacy of US-guided postmortem liver biop-

sy during the YF epidemic, finding it a reliable diagnostic alternative to conventional autopsy. We used US at admission and aimed to compare the findings with clinical and laboratory data—with interesting results.

We found reproducible liver and renal US findings in this group of patients with severe YF that may correlate with acute direct damage to these organs. This observation is supported by viral tropism to the liver and renal parenchyma, hallmarks of the pathophysiology of YF [14–20]. In our study, as in previous reports from our institution [4, 21, 22], patients presented to the ICU with high levels of hepatic transaminases, bilirubin, and creatinine, markers of tissue injury. Increased liver parenchymal echogenicity, although not entirely specific, has been associated with fatty deposition [23–25]. It is also known that hepatitis C virus is a causative agent of hepatic steatosis [26, 27]; this suggests that a similar mechanism or pattern of liver injury may be involved in YF.

Also well documented is the relation between steatosis and metabolic disorders in the pathogenesis of nonalcoholic steatosis and steatohepatitis [28]. In our sample, some patients did report having disorders such as diabetes, hypertension, and dyslipidemia. Some patients also had a history of alcohol abuse, a condition also associated with hepatic fat deposition. However, the prevalence of increased liver echogenicity in our study (65.2%) was greater than that of alcoholic and nonalcoholic fatty liver diseases combined in the general population (estimated to be $\approx 4\%$ and 10–35%) [29–31]. Moreover, we found no association between increased liver echogenicity and diabetes, hypertension, dyslipidemia, or alcohol abuse, indicating viral infection as the most likely causative agent.

Increased renal cortical echogenicity has been associated with acute kidney injury (in the absence of US features of chronic kidney disease). Although this finding is generally thought to be nonspecific, it has been reported to have 96% specificity (and 67% PPV) for the presence of parenchymal kidney disease [7, 11]. Accordingly, we found higher values of serum creatinine in patients with increased renal echogenicity; more important, this imaging finding was found to be an independent predictor (OR, 10.89; $p = .048$) of early death in the ICU, suggesting that US could have a role in predicting worse prognosis in this setting.

Gallbladder wall thickening in the absence of intrinsic gallbladder disease is a rather nonspecific finding and a common manifestation after other acute forms of viral hepatitis, such as hepatitis A, and dengue [10, 32]. In contrast to renal hyperechogenicity, however, there was no correlation between this finding and disease severity. The heterogeneous vesicular content found in four patients had features of hemorrhagic material and may represent another site of bleeding considering that most of these patients had severe coagulopathy, although the importance of this finding is still to be determined.

Limitations

Limitations of this study must be acknowledged. Liver and renal parenchyma echogenicity is a somewhat subjective (operator dependent) finding. Thus, we used an objective criterion by comparing those organs with the spleen and added two-step analysis by two radiologists. Echogenicity also varies according to equipment and technical adjustments (presets), for which we established a standard protocol. The sensitivity of US for detect-

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TABLE 2: Characteristics of Sample According to Primary Outcome (Death in 30 Days)

Variable	Death		p
	No (n = 20)	Yes (n = 26)	
Age			.41
Mean ± SD	45.4 ± 15.5	49.1 ± 15.0	
Median and interquartile range	43.6 (33.4–59.5)	45.8 (35.2–64.1)	
Range	19.7–69.4	26.5–75.4	
Male sex	16 (80.0)	24 (92.3)	> .99
Liver hyperechogenicity	13 (65.0)	17 (65.4)	> .99
Gallbladder thickening	15 (75.0)	22 (84.6)	.47
Homogeneous	3 (20.0)	5 (22.7)	
Perivesicular laminar fluid collection	6 (40.0)	12 (54.5)	.52
Delamination	6 (40.0)	5 (22.7)	
Renal hyperechogenicity	11 (55.0)	22 (84.6)	.046
Perirenal fluid	8 (40.0)	16 (61.5)	.23
Free fluid	5 (25.0)	9 (34.6)	.54
US features of pancreatitis	1 (5.0)	3 (11.5)	.62
Blood in gallbladder	1 (5.0)	3 (11.5)	.62
Heterogeneous gallbladder content	1 (5.0)	2 (8.7)	> .99
US features of chronic liver disease	0 (0)	2 (8.7)	.50
Diabetes	1 (7.7)	6 (23.1)	.39
Hypertension	2 (15.4)	8 (30.8)	.45
Dyslipidemia	0 (0)	1 (3.8)	> .99
Alcohol abuse (current)	9 (47.4)	12 (46.2)	> .99
SAPS 3 ^a	58 ± 12	59 ± 13	.73
AST (U/L) ^b	3723 (1569–5140)	7000 (5070–8307)	.002
ALT (U/L) ^b	2033 (1485–2912)	2760 (1980–5504)	.12
Total bilirubin (mg/dL) ^b	6.0 (3.2–8.0)	6.2 (5.3–7.7)	.38
Alkaline phosphatase (U/L) ^a	130 ± 37	158 ± 58	.06
GGT (U/L) ^a	364 ± 202	265 ± 156	.07
Urea (mg/dL) ^a	73 ± 54	117 ± 75	.03
Creatinine (mg/dL) ^a	2.6 ± 2.5	5.6 ± 3.4	.002
Fibrinogen (mg/dL) ^a	156 ± 72	122 ± 40	.05
Factor V (%) ^a	101 ± 46	51 ± 37	< .001
INR ^b	1.27 (1.10–1.53)	1.99 (1.46–2.83)	< .001
Platelet count (1000/mm ³) ^b	47 (37–70)	67 (54–83)	.04
Lipase (U/L) ^b	187 (84–305)	501 (184–1561)	.01

Note—Values are number with percentage in parentheses unless otherwise specified. US = ultrasound, SAPS 3 = Simplified Acute Physiology Score 3, AST = aspartate aminotransferase, ALT = alanine aminotransferase, GGT = γ-glutamyl transferase, INR = international normalized ratio.

^aMean ± SD.

^bMedian and interquartile range.

ing mild degrees of steatosis is low, ranging from 55% to 90% [25]. We had technical difficulties in performing examinations in tight ICU rooms with multiple medical devices. We therefore chose to follow a fixed protocol to save time, aiming at the most relevant points in an abdominal scan that could be used as pos-

sible preoperative guidance for liver transplant. We emphasize that this study included only patients admitted to the ICU; it is not clear whether subjects with milder forms of YF have the same findings and at the same frequency. Last, this study lacked a control group.

TABLE 3: Multivariate Analysis With Predictors of 30-Day Mortality Among Patients With Severe Yellow Fever Admitted to the ICU

Variable	Odds Ratio	95% CI	p
Renal hyperechogenicity	10.89	1.03–115.49	.048
Platelet count (1000/mm ³) (each unit)	1.03	1.00–1.06	.03
Creatinine concentration (mg/dL) (each unit)	1.48	1.04–2.12	.03
INR (each unit)	4.44	1.24–15.82	.02

Note—Model adjusted for aspartate transaminase, γ-glutamyl transferase, lipase, and fibrinogen levels in addition to the significant variables displayed in the table.
INR = international normalized ratio.

Conclusion

This unprecedented investigation of YF improved our knowledge of this reemerging disease through the use of a diagnostic tool not available in past epidemics. Abdominal US proved to be a practical and feasible method that may guide patient care in the ICU by facilitating estimation of the severity of disease, especially regarding renal failure. Severe YF is a life-threatening condition that leads to hepatic failure and renal dysfunction. In this scenario, patients usually need life support in the ICU, and US can be a useful bedside tool. We found reproducible abdominal US findings in severe YF, and increased renal echogenicity was especially associated with higher risk of death.

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(Figures start on next page)

Ultrasound of Yellow Fever

Fig. 1—Flow diagram shows patient enrollment. HBV = hepatitis B virus infection, HAV = hepatitis A virus infection, PCR = polymerase chain reaction.

Fig. 2—32-year-old man with 10 days of fever, right upper quadrant pain, and jaundice. Serum polymerase chain reaction result was positive for yellow fever virus. This case is example of right upper quadrant ultrasound findings of yellow fever. **A** and **B**, Abdominal ultrasound (US) images show increased liver echogenicity and intraabdominal free fluid (arrow, **B**). **C**, US image shows normal portal, hepatic artery, and hepatic vein flow and pleural effusion (arrow).

Fig. 3—Gallbladder findings in patients with yellow fever. **A**, 32-year-old man with 10 days of fever, right upper quadrant pain, and jaundice (same patient as in Fig. 2). Abdominal ultrasound (US) image shows diffuse and homogeneous gallbladder wall thickening (arrow). **B**, 22-year-old man with 8 days of fever, malaise, and hemorrhagic vomiting. Abdominal US image shows gallbladder wall thickening and perivesicular laminar fluid collection (arrow). (Fig. 3 continues on next page)

Fig. 3 (continued)—Gallbladder findings in patients with yellow fever.

C and D, 37-year-old man with 4 days of fever, headache, and myalgia. Long-axis (**C**) and short-axis (**D**) US images show gallbladder wall delamination with marked thickening (arrow).

E and F, 26-year-old man with 4 days of fever, headache, and myalgia. Long-axis (**E**) and short-axis (**F**) US images show heterogeneous material (arrow) in gallbladder lumen corresponding to autopsy evidence of biliary tree hemorrhage.

Fig. 4—Renal findings in patients with yellow fever. Both patients died despite intensive care.

A and B, 26-year-old man with 4 days of fever, headache, and myalgia (same patient as in Figs. 3E and 3F). Abdominal ultrasound (US) images show increased renal echogenicity and perirenal fluid collection (arrow).

C and D, 30-year-old man with 8 days of fever and confusion. Serum polymerase chain reaction result was positive for yellow fever. US images show increased renal cortex echogenicity (arrow) compared with liver (L, **C**) and spleen (S, **D**).

SUPPLEMENTAL DATA

eTable 1. Comparison between imaging findings and demographic, clinical and laboratorial characteristics of patients with severe YF.

Variable	Liver echogenicity		p value	Renal echogenicity		p value	Gallbladder thickening		p value
	Increased (30)	Normal (16)		Increased (33)	Normal (13)		Thickened (37)	Normal (9)	
Age (mean $\pm$ SD)	49.0 $\pm$ 13.7	44.6 $\pm$ 17.7	0.351	48.2 $\pm$ 15.3	45.7 $\pm$ 15.2	0.634	46.0 $\pm$ 14.8	53.7 $\pm$ 15.9	0.213
Median and quartiles	44.8 (35.2 – 62.9)	45.2 (27.6 – 61.6)		45.4 (35.2 – 63.9)	43.0 (34.0 – 61.1)		43.9 (34.9 – 57.9)	61.1 (43.0 – 63.9)	
Range	30.2 – 75.4	19.7 – 73.5		20.5 – 75.4	19.7 – 67.2		19.7 – 75.4	26.5 – 73.5	
Male gender	25 (83.3)	15 (93.8)	0.649	29 (87.9)	11 (84.6)	1.000	32 (86.5)	8 (88.9)	1.000
Time between symptoms and US (Median and quartiles)	7 (6 – 9)	6 (4 – 8)	0.114	7 (5 – 8)	6 (5-9)	0.525	7 (5 - 8)	6 (5 - 9)	0.748
Diabetes	7 (25.9)	0 (0)	0.077	6 (20.0)	1 (11.1)	1.000	6 (18.8)	1 (14.3)	1.000
Hypertension	8 (29.6)	2 (16.7)	0.693	8 (26.7)	2 (22.2)	1.000	6 (18.8)	4 (57.1)	0.057
Dyslipidemia	1 (3.7)	0 (0)	1.000	1 (3.3)	0 (0)	1.000	1 (3.1)	0 (0)	1.000
Alcohol abuse (current)	17 (56.7)	4 (26.7)	0.068	16 (48.5)	5 (41.7)	0.746	18 (50.0)	3 (33.3)	0.469
AST (U/L) (Median and quartiles)	5306 (3648 – 7000)	5406 (1854 – 7000)	0.611	5714 (3648 – 7573)	4124 (3724 – 7000)	0.179	5714 (3648 – 7000)	3789 (1976 – 7000)	0.169
ALT (U/L) (Median and quartiles)	2609 (1580 – 3380)	2746 (1911 – 4379)	0.636	2634 (1790 – 3067)	2787 (1842 – 5504)	0.714	2676 (1802 – 3932)	2705 (1078 – 3144)	0.533
Total bilirubin (mg/dL) (Median and quartiles)	6.4 (5.0 – 7.4)	5.7 (3.1 – 8.3)	0.533	6.3 (5.1 – 7.4)	6.1 (2.6 – 8.6)	0.724	6.1 (4.6 – 7.4)	6.3 (2.3 – 7.7)	0.489
Alkaline phosphatase (U/L) (mean $\pm$ SD)	143 $\pm$ 50	149 $\pm$ 55	0.729	1445 $\pm$ 54	148 $\pm$ 47	0.868	146 $\pm$ 53	144 $\pm$ 49	0.924
GGT (U/L) (mean $\pm$ SD)	308 $\pm$ 166	307 $\pm$ 215	0.986	286 $\pm$ 165	364 $\pm$ 218	0.197	294 $\pm$ 159	365 $\pm$ 260	0.295
Urea (mg/dL) (mean $\pm$ SD)	107 $\pm$ 77.0	81 $\pm$ 51	0.165	107 $\pm$ 75	75 $\pm$ 60	0.163	100 $\pm$ 73	89 $\pm$ 59	0.644
Creatinine (mg/dL) (mean $\pm$ SD)	4.8 $\pm$ 3.5	3.4 $\pm$ 3.0	0.205	4.8 $\pm$ 3.3	3.1 $\pm$ 3.5	0.133	4.6 $\pm$ 3.3	3.3 $\pm$ 3.9	0.394
Fibrinogen (mg/dL) (mean $\pm$ SD)	119 $\pm$ 44	167 $\pm$ 67	0.006	127 $\pm$ 49	159 $\pm$ 73	0.095	129 $\pm$ 52	167 $\pm$ 70	0.152
Factor V (%) (mean $\pm$ SD)	76 $\pm$ 49	66 $\pm$ 46	0.507	69 $\pm$ 47	82 $\pm$ 51	0.422	67 $\pm$ 47	96 $\pm$ 47	0.121
INR (Median and quartiles)	1.63 (1.28 – 2.56)	1.43 (1.17 – 2.09)	0.406	1.62 (1.30 – 2.26)	1.36 (1.20 – 1.92)	0.262	1.64 (1.30 – 2.56)	1.33 (1.00 – 1.46)	0.029
Platelets (1000 units/mm ³) (Median and quartiles)	59 (44 – 83)	57 (47 – 69)	0.712	56 (47 – 77)	62 (43 – 115)	0.542	57 (47 – 81)	56 (43 – 113)	0.923

Note.—Data presented as n (%), except if specified otherwise. SD = Standard deviation. YF = yellow fever. US = ultrasonography. AST = Aspartate aminotransferase. ALT = Alanine aminotransferase. GGT = Gamma-glutamyl transferase. INR = International normalized ratio.

3.4 TEXTO SISTEMATIZADO SOBRE A PUBLICAÇÃO 2

Com o aumento crescente no número de casos de FA graves direcionados para a UMIN (UTI-DMP) e a melhora do padrão de atendimento e suporte intensivo, alguns pacientes começaram a ter alta hospitalar, com boa recuperação clínica. Apesar da elevada letalidade intra-hospitalar, alguns indivíduos, cujas características ainda não eram bem definidas, sobreviveram e entraram, portanto, em convalescência. A partir daí, passariam a ser acompanhados ambulatorialmente (a princípio semanalmente e, a posteriori, gradativamente de forma mais espaçada) na unidade de Infectologia do Prédio dos Ambulatórios (PAMB) do HCFMUSP.

Pouco se sabia acerca da evolução clínico-laboratorial e imaginológica neste período, após o insulto hepático e renal agudo. As poucas experiências da literatura, com poucos relatos de casos, sugeriam uma recuperação plena do parênquima do fígado após a infecção aguda, sem sinais ou sintomas de hepatopatia crônica nos sobreviventes, à semelhança do que ocorre com outras hepatites virais. Infelizmente, a biópsia hepática, clássico padrão-ouro para o diagnóstico de fibrose, com suas limitações e potencial morbidade do procedimento (riscos inerentes), não era factível ou justificável para estes pacientes, uma vez que já se encontravam inclusive assintomáticos. Apresentou-se então uma oportunidade de acompanhar evolutivamente os mesmos para entender as mudanças no fígado através de uma ferramenta não invasiva outrora indisponível em surtos anteriores – a elastografia hepática, com resultados na literatura comparáveis aos da biópsia convencional. Este método, de fácil acessibilidade e disponível nos equipamentos de US mais modernos da instituição, poderia ser aplicado aos pacientes em sobreviventes da FA grave.

Foi então criado o seguinte fluxo: os pacientes que retornassem ao Ambulatório de Infectologia para a consulta clínica de 6 meses após o início dos

sintomas seriam direcionados desta para a coleta de exames laboratoriais (de sangue) no próprio PAMB e em seguida no mesmo dia ao Serviço de Ultrassonografia, no terceiro andar do InRad – HFCMUSP, para a realização ambulatorial de US com SWE, após assinatura do Termo de Compromisso Livre e Esclarecido (TCLE).

Outras especificações metodológicas:

- Este intervalo de tempo (6 meses após o início dos sintomas) foi escolhido pensando-se que, com o período de convalescência, já poderiam ser atestados sinais de cronicidade possivelmente relacionados ao insulto inicial (e não alterações inflamatórias residuais / em resolução, o que desejar-se-ia evitar, pois denotariam falsos-positivos).
- Todos os exames foram adquiridos pelo mesmo radiologista (pesquisador responsável), após treinamentos intensivos na técnica elastográfica (promovidos por pesquisador co-autor do trabalho, com ampla experiência e referência nacional na área), com obtenção de múltiplas medidas objetivas (quantitativas) do parênquima hepático;
- Optamos, pela ausência de consenso quanto a pontos de corte para as faixas de fibrose em FA, por utilizar preferencialmente os valores brutos de elasticidade (em kPa), como vem sendo mais recomendado nas mais novas referências científicas;
- Foi efetuada a correção de Bonferroni para múltiplas comparações, na análise estatística.

Com isso pudemos estudar o parênquima hepático de pacientes que haviam sido infectados pelo vírus da FA e sobreviveram após um quadro grave, no longo prazo. A possibilidade de encontrar uma maior frequência de fibrose hepática nestes indivíduos não poderia ser descartada. Em face das novidades da literatura acerca de hepatite recorrente de início tardio em alguns destes pacientes, o trabalho tornou-se ainda mais relevante, e a avaliação longitudinal com técnicas modernas de imagem poderia trazer benefícios inclusive na detecção de pacientes que necessitassem de acompanhamento por hepatopatia. A despeito das perdas (seja por informações de contato errôneas ou incompletas ou por falta às consultas), conseguimos recrutar uma relevante casuística de pacientes nunca antes estudada na ciência médica. Foi possível também associar e correlacionar as variáveis elastográficas com dados clínicos e laboratoriais da internação e desta forma avaliar possíveis preditores de fibrose.

A seguir, portanto, o segundo trabalho desta tese, publicado em periódico de alto impacto em doenças infecciosas e parasitárias, a *PLOS Neglected Tropical Diseases*.

3.5 ARTIGO 2

STAGING LIVER FIBROSIS AFTER SEVERE YELLOW FEVER WITH ULTRASOUND ELASTOGRAPHY IN BRAZIL: A SIX-MONTH FOLLOW-UP STUDY

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RESEARCH ARTICLE

Staging liver fibrosis after severe yellow fever with ultrasound elastography in Brazil: A six-month follow-up study

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Data Availability Statement: All relevant data are within the manuscript and its [Supporting Information](#) files.

Abstract

Background

Yellow fever (YF) is a hemorrhagic disease caused by an arbovirus endemic in South America, with recent outbreaks in the last years. Severe cases exhibit fulminant hepatitis, but there are no studies regarding its late-term effects on liver parenchyma. Thus, the aim of this study was to determine the frequency and grade of liver fibrosis in patients who recovered from severe YF and to point out potential predictors of this outcome.

Methodology/Principal findings

We followed-up 18 patients who survived severe YF during a recent outbreak (January–April 2018) in Brazil using ultrasound (US) with shear-wave elastography (SWE) at 6 months after symptoms onset. No patient had previous history of liver disease.

Median liver stiffness (LS) was 5.3 (4.6–6.4) kPa. 2 (11.1%) patients were classified as Metavir F2, 1 (8.3%) as F3 and 1 (8.3%) as F4; these two last patients had features of cardiogenic liver congestion on Doppler analysis. Age and cardiac failure were associated with increased LS ($p = 0.036$ and $p = 0.024$, respectively). SAPS-3 at ICU admission showed a tendency of association with significant fibrosis ($\geq F2$; $p = 0.053$). 7 patients used sofosbuvir in a research protocol, of which none showed liver fibrosis ($p = 0.119$).

Conclusions/Significance

We found a low frequency of liver fibrosis in severe YF survivors. US with SWE may have a role in the follow up of patients of age and / or with comorbidities after hospital discharge in severe YF, a rare but reemergent disease.

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Abbreviations: ICU, intensive care unit; kPa, kilopascals; LS, liver stiffness; SWE, shear-wave elastography; US, ultrasound; YF, yellow fever.

Author summary

Yellow fever (YF) is a viral disease transmitted by mosquitoes and represents an important health problem in countries of South America and Africa, with recent outbreaks in the past few years. Severe cases lead to fulminant hepatitis and death; it has also been reported that surviving patients tend to recover / “regenerate” the liver after the acute phase of the disease. However, there are no prior investigations on this matter. Thus, we followed up a group of previously healthy patients who had severe YF 6 months after the initial symptoms, using a non-invasive ultrasound technique of estimating the liver fibrosis (scar tissue) grade, called elastography. In our findings, we report a low frequency of liver fibrosis. Thus, we concluded that patients who had severe YF are not likely to have late-term liver fibrosis or cirrhosis. Nevertheless, there are some specific individuals (older or with chronic diseases) that might need further evaluation.

Introduction

Yellow fever (YF) is a mosquito-borne hemorrhagic disease caused by an arbovirus of genus *Flavivirus* that occurs in tropical regions of South America and Africa [1]. In Brazil, urban transmission cycle was interrupted in 1940's due to massive vaccination campaign and vector control programs [2]. However, the enzootic remains present and human infection continues to occur in a sylvatic cycle [3]. In the beginning of 2018, a YF outbreak occurred on the outskirts of Sao Paulo; 538 cases were confirmed between January and December 2018, with a lethality rate of 34.6% [4]. The pathophysiology of the disease involves viral tropism and acute direct damage to vital organs such as liver and kidneys [5–7], in severe cases leading to fulminant hepatitis and acute renal failure (often requiring replacement therapies) [8–11], with high in-hospital case fatality rates (of up to 67%, according to recent studies [12–14]).

It is not clear whether liver fibrosis arises after initial insult in severe form surviving patients. Some reports showed that patients recover completely from other acute hepatitis, with no signs or symptoms of chronic hepatitis or cirrhosis [15,16]. However, specific longitudinal data in YF patients after recovery is lacking. Due to the rarity of the disease and the absence of specific technologies in prior outbreaks, few patients were adequately followed up in order to clarify this matter. Additionally, a recent series demonstrated a late-onset relapsing hepatitis, a newly discovered entity, in patients who recovered from the acute phase of the disease [17].

In this context, non-invasive assessment of liver fibrosis with ultrasound (US) using new shear-wave elastography (SWE) techniques can be an important and accessible tool to investigate liver parenchyma status after acute hepatitis [18]. US-based elastography techniques are low-cost and radiation-free imaging methods [19], with performances comparable to those of biopsy [20]. It is also worth noting that liver biopsy with histological examination, although historically considered the reference standard for staging fibrosis, has considerable interobserver variability [21] and the disadvantage of being an invasive procedure, with intrinsic risks, and thus not suitable for longitudinal monitoring. Hence, the aim of this study was to determine whether patients who recovered from severe yellow fever developed liver fibrosis based on SWE evaluation and to point out potential predictors (clinical and laboratory) of cirrhosis during the follow up time.

Methods

Ethics statement

All enrolled patients signed an Informed Consent Form. Research protocol was approved by the Ethics Committee of Hospital das Clínicas da Faculdade de Medicina da Universidade da

Universidade de São Paulo—HCFMUSP—CAPPesq (n° 2.833.376). We followed the STROBE Statement checklist for observational studies [22].

Study design

This is a cohort study of patients who were admitted to an infectious diseases intensive care unit (ICU) of a teaching tertiary-care hospital in São Paulo (Hospital das Clínicas, University of São Paulo) with the diagnosis of severe YF, confirmed by serum polymerase chain reaction (PCR). Criteria for severe YF and to be admitted to the ICU were pre-specified in a prior study [23]. Individuals discharged between January and April 2018 were contacted by phone, invited to participate in the study and to attend to the Hospital's Ultrasonography Department to perform a non-enhanced abdominal US with SWE. Scans were carried at 6 months after symptoms onset. There were no exclusion criteria.

SWE evaluations were performed by a board-certified radiologist (5 years of training in US) on an RS80A US system (Samsung Medison Co., Ltd.) equipped with S-SWE (Samsung shear-wave elastography, a type of point shear wave technique) using a convex probe (CA1-7A). All patients fasted for at least 4 hours prior to the examination. Liver stiffness (LS) measurements, expressed in kilopascals (kPa), were done using an intercostal approach, with the patient placed in a supine position with the right arm maximally abducted above the head and performing expiratory breath-holds. Regions of interest (ROIs) were placed in the right lobe of the liver at a depth of approximately 2–4 cm from its capsule, avoiding large vessels. At least 5 valid measurements were obtained, and the median LS value was taken as a representative value if, after consecutive measurements, an interquartile range (IQR)/median ratio $\leq 30\%$ was achieved. S-SWE technique also automatically calculated a reliability measurement index (RMI), which demonstrated the reliability of each measurement. The RMI ranged from 0 to 1.0, with an RMI ≥ 0.4 being considered acceptable, according to the manufacturer's instruction. We used the cutoffs for staging fibrosis (correlating to Metavir scoring system) of 7 kPa for $\geq$ F3 and 9.7 kPa for F4 [24–26]. Scanning protocol also included grayscale (B-mode), color and spectral Doppler modes. Data acquired were archived in PHILIPS iSite Picture Archiving and Communication Systems (PACS) version 4.1.110.0. Each patient had at least 30 images acquired; there were no videos available.

Clinical, demographic and anthropometric data were collected during a short interview and also acquired from medical records (including the Simplified Acute Physiology Score 3—SAPS-3). Laboratory tests were performed in the same day of US elastography examination (and also retrieved from medical records from previous ICU admission) and included liver enzymes, bilirubin levels, serum creatinine and urea, platelets, prothrombin time and international normalized ratio (INR).

Statistical analyses

For descriptive purposes, categorical variables were presented through relative and absolute frequencies and compared by means of the chi-squared or Fisher exact test, as appropriate. Continuous variables distributions were assessed for normality by skewness and kurtosis and graphical methods. Those with normal distribution were presented as mean and standard deviations and compared by the independent samples Student T test. Otherwise, they were presented as medians and quartiles and compared by the Mann-Whitney non-parametric test. In order to address the association between liver stiffness and demographic and laboratory variables, correlation analysis was performed through the Spearman method, since US SWE parameters were non-normally distributed.

All tests were two-tailed and final *p* values under 0.05 were considered statistically significant. All analyses were conducted with the SPSS software (IBM Corp. SPSS Statistics for Windows, version 24.0. Armonk, NY).

Results

Eighteen individuals were included in the study and performed an evaluation with elastography at a median time of 185 (180–191) days after onset of symptoms. **Fig 1** shows patient enrolment. Losses were due to incorrect or incomplete contact information or no attendance after 2 attempts of recruitment. Fifteen (83.3%) patients were male; median age was 47 (41–57) years and median body mass index was 23.6 (21.6–24.8) kg/m². No patient had prior history of liver disease or was submitted to liver transplantation. Serologies for hepatitis A, B and C were negative for all patients. Two patients (11.1%) had been vaccinated for YF less than 10 days before admission. None was considered to have vaccinal disease, because only wild virus was detected with PCR. Median length of hospital stay was 11 days (8–23) and median SAPS-3 at admission was 49 (45–60). At admission to the UCI, laboratory tests revealed high serum levels of aspartate aminotransferase, alanine aminotransferase and total bilirubin (medians of 3479 U/L, 2531 U/L and 4.37 mg/dL, respectively), median serum creatinine of 1.43 mg/dL (1.06–4.59 mg/dL) and median serum urea concentration of 62 mg/dL (26–124 mg/dL). Median INR was 1.23 (1.10–1.40), and median platelet count was 55 units/mm³ (40,000–72,000 units/mm³). Lab results at 6 months after symptoms onset were within normal ranges. Seven (38.9%) patients used sofosbuvir for 10 days in a research protocol during the hospitalization [17,27]. **Table 1** shows clinical, demographic, laboratory and elastography data.

Regarding US findings with elastography evaluation (SWE) (**Fig 2**), median shear-wave velocity and LS were 1.33 m/s (1.24–1.46) and 5.3 (4.6–6.4) kPa, respectively. Two (11.1%) patients had mild fibrosis (F2), one (8.3%) had moderate fibrosis (F3) and one (8.3%) had cirrhosis (F4); these two last patients had signs of congestive heart failure, with features of

Fig 1. Flow diagram of patient enrolment. Of the 43 patients who survived severe yellow fever and were discharged, we managed to evaluate 18 patients with ultrasound elastography after 6 months of symptoms onset. Reasons for the losses of follow-up are specified on the right. PCR = Polymerase chain reaction. YF = Yellow fever. ICU = Intensive care unit. US = Ultrasound. SWE = Shear-wave elastography.

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Table 1. Clinical, demographic, laboratory and elastography data of 18 patients who had severe yellow fever and were followed up to 6 months after onset of symptoms (descriptive statistics), Brazil, 2018*.

Variable	N = 18
Age (years) (mean ± SD)	45.9 ± 14.8
Median and quartiles	47 (41–57)
Range	19–69
Male gender	15 (83.3)
Body mass index (median and quartiles)	23.6 (21.6–24.8)
Hypertension	4 (22.2)
Diabetes	3 (16.7)
Cardiac failure	2 (11.1)
Alcohol use (current)	9 (50.0)
SAPS-3 at admission (median and quartiles)	49 (45–60)
Length of hospital stay (days) (median and quartiles)	11 (8–23)
Sofosbuvir use	7 (38.9)
Laboratory results at admission	
AST (U/L) (Median and quartiles)	3479 (2260–4124)
ALT (U/L) (Median and quartiles)	2531 (1790–2815)
Total bilirubin (mg/dL) (median and quartiles)	4.37 (1.99–8.06)
Alkaline phosphatase (U/L) (median and quartiles)	106 (100–132)
GGT (U/L) (median and quartiles)	279 (211–533)
Urea (mg/dL) (median and quartiles)	62 (26–124)
Creatinine (mg/dL) (median and quartiles)	1.43 (1.06–4.59)
INR (median and quartiles)	1.23 (1.10–1.40)
Platelets (1000 units/mm ³) (median and quartiles)	55 (40–72)
Laboratory results at 6 months after onset of symptoms	
AST (U/L) (median and quartiles)	24 (21–26)
ALT (U/L) (median and quartiles)	27 (19–37)
Total bilirubin (mg/dL) (median and quartiles)	0.45 (0.30–0.61)
Alkaline phosphatase (U/L) (median and quartiles)	71 (61–77)
GGT (U/L) (median and quartiles)	31 (23–51)
Urea (mg/dL) (median and quartiles)	32 (26–37)
Creatinine (mg/dL) (median and quartiles)	0.87 (0.83–1.11)
INR (median and quartiles)	0.99 (0.96–1.03)
Platelets (1000 units/mm ³) (median and quartiles)	207 (175–246)
Time since onset of symptoms and elastography (days) (median and quartiles)	185 (180–191)
Shear wave velocity (m/s) (median and quartiles)	1.33 (1.24–1.46)
Liver stiffness (kPa) (median and quartiles)	5.3 (4.6–6.4)
Liver fibrosis categories (METAVIR)	
F0/F1	14 (77.8)
F2	2 (11.1)
F3	1 (5.6)
F4	1 (5.6)

* Data presented as no. (%), except if specified otherwise. SD, Standard deviation. SAPS-3, Simplified Acute Physiology Score 3. AST, aspartate aminotransferase. ALT, alanine aminotransferase. GGT, gamma-glutamyl transferase. INR, international normalized ratio.

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Fig 2. Right upper quadrant ultrasound of two patients using point shear-wave elastography technique. (A) A 48-year-old male patient, and (B), a 38-year-old male patient, both asymptomatic, 6 months after onset of severe yellow fever. Images depict placement of regions of interest (ROIs) on the right lobe of the liver at expiratory breath-hold. Shear-wave velocities (in m/s) were generated, with corresponding liver stiffness values after multiple measurements of 5.3 kPa and 4.6 kPa, respectively. Both subjects were then classified as F0/F1 on the Metavir scoring system for staging of fibrosis. RMI = Reliability measurement index.

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cardiogenic liver congestion on Doppler analysis. All remaining patients had Doppler evaluation of superior abdomen (portal vein, hepatic veins and hepatic artery) within normal ranges. No patient showed morphologic changes of cirrhosis on grayscale mode (Table 1).

Table 2 compares clinical and demographic variables and laboratory results at admission with LS values obtained 6 months after onset of symptoms. Heart failure was associated with increased LS ($p = 0.024$); systemic arterial hypertension and diabetes showed a tendency towards this association ($p = 0.063$ and $p = 0.066$, respectively). After exclusion of the outlier patient (LS = 22.4 kPa, with cardiogenic liver congestion), there was a tendency towards statistical significance in the correlations between LS and age ($p = 0.416$, $p = 0.096$) and between LS and alanine aminotransferase at admission ($p = -0.479$, $p = 0.052$) (Fig 3). We found no

Table 2. Differences in fibrosis estimations of 18 patients who had severe yellow fever 6 months after onset of symptoms according to clinical and demographic characteristics, Brazil, 2018.

Variable		Liver stiffness (kPa) (median and quartiles)	p value
Gender	Female	6.40 (5.90–22.40)	0.075
	Male	5.10 (4.40–9.30)	
Hypertension	Yes	7.60 (5.55–22.40)	0.063
	No	4.95 (4.40–7.00)	
Diabetes	Yes	9.30 (5.20–22.40)	0.066
	No	5.10 (4.40–7.00)	
Cardiac failure	Yes	15.85 (9.30–22.40)	0.024†
	No	5.15 (4.50–7.00)	
Alcohol use (current)	Yes	5.90 (5.20–9.30)	0.102
	No	4.80 (4.40–22.40)	
Sofosbuvir use	Yes	4.60 (4.40–6.40)	0.070
	No	5.90 (4.80–22.40)	

†Statistical significance.

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Fig 3. Correlations between liver stiffness and clinical and laboratorial variables. Age (A) and alanine aminotransferase (B) and its correlations with liver stiffness, using the Spearman method (levels of significance of $p = 0.096$ and $p = 0.052$, respectively). kPa = Kilopascals.

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significant association between LS and gender, BMI, alcohol consumption, hospitalization time or other laboratorial results.

Considering the cutoff for significant fibrosis ($\geq F2$), differences in clinical and laboratory data during hospitalization are shown in [Table 3](#). Cardiac failure was associated with a Metavir

Table 3. Clinical and laboratory data at hospitalization according to liver fibrosis staging (with or without significant fibrosis) of 18 patients at 6 months after onset of symptoms of severe yellow fever, Brazil, 2018.

Variable	Fibrosis stage		
	F0/1 (n = 14)	$\geq F2$ (n = 4)	p value
Age (years) (median and quartiles)	46.5 (38.0–48.0)	57 (47–61.5)	0.242
Male gender	12 (85.7)	3 (75.0)	1.000
Body mass index (median and quartiles)	22.7 (21.3–24.3)	27.8 (23.2–31.8)	0.151
Hypertension	2 (14.3)	2 (50.0)	0.197
Diabetes	1 (7.1)	2 (50.0)	0.108
Cardiac failure	0	2 (50.0)	0.039†
Alcohol use (current)	6 (42.9)	3 (75.0)	0.576
SAPS-3 at admission (median and quartiles)	47 (42–60)	71 (54–73)	0.053
Length of hospital stay (days) (median and quartiles)	10 (8–25)	18 (12–21)	0.709
Sofosbuvir use	7 (50.0)	0	0.119
AST (U/L) (median and quartiles)	3238 (2260–4124)	3518 (2327–4102)	0.915
ALT (U/L) (median and quartiles)	2631 (1790–3067)	1937 (1475–2410)	0.243
Total bilirubin (mg/dL) (median and quartiles)	3.2 (2.0–8.1)	6.1 (3.3–14.9)	0.524
Alkaline phosphatase (U/L) (median and quartiles)	105 (100–129)	140 (89–184)	0.524
GGT (U/L) (median and quartiles)	261 (211–533)	371 (174–566)	0.791
INR (median and quartiles)	1.2 (1.1–1.4)	1.3 (1.2–1.7)	0.396
Fibrinogen (mg/dL)	133 (121–146)	148 (124–215)	0.594
Factor V (%)	92 (65–133)	118 (67–132)	0.873

*Data presented as no. (%), except if specified otherwise.

†Statistical significance. SAPS-3, Simplified Acute Physiology Score 3. AST, aspartate aminotransferase. ALT, alanine aminotransferase. GGT, gamma-glutamyl transferase. INR, international normalized ratio.

<https://doi.org/10.1371/journal.pntd.0009594.t003>

score $\geq$ F2 (50% versus 0; $p = 0.039$). There was a tendency towards an association between higher SAPS-3 values at hospital admission and significant fibrosis at 6 months after onset of symptoms (medians of 71 versus 47; $p = 0.053$). There were no statistically significant differences in the frequencies or median values of other variables between these two groups of participants.

No patient who received sofosbuvir showed liver fibrosis at 6 months after onset of symptoms, compared with 4 (36.4%) individuals who had fibrosis ($\geq$ F2) in the group that did not receive the drug. This difference did not reach statistical significance.

Discussion

This is a unprecedented investigation on this rare but reemergent and severe disease. Our study managed to determine the frequency of liver fibrosis in severe YF surviving patients, utilizing a modern US SWE technique, with good reproducibility and correlation to liver biopsy[18]. High grades of LS were found in a small percentage of patients, similar to the prevalence in the general population[28–30], indicating that the liver recovers at least in great portion after the initial viral insult (analogous to what was suggested in prior reports for other viral hepatitis [15,16]). Thus, US with SWE showed to be a valid and noninvasive method to evaluate these patients, without the disadvantages of conventional biopsy[31].

Nevertheless, some clinical characteristic seemed to be associated with increased LS. Older age was predictably correlated to higher LS values, as expected; chronic diseases such as diabetes and hypertension also demonstrated a tendency towards this association. We found a tendency to a negative correlation between alanine aminotransferase at admission and LS; it is more likely that this association be spurious than a real and protective factor. Higher SAPS-3 showed a tendency of an association with significant fibrosis and could potentially represent a predictor of long-term fibrosis. Cardiac failure, a condition that determines hepatic congestion, was present in two of 18 patients at 6 months after onset of symptoms, both of which had increased LS; however, this result was most likely a “false-positive”, since edema of liver tissue leads to increased stiffness not related to fibrous tissue[32]. For a similar reason, acquiring a “baseline” SWE at hospitalization would not be valid (nor feasible) due to inflammation and edema of the hepatic parenchyma during the acute phase, also associated to increased LS [16,33,34]. No other clinical or laboratory variable showed to be a predictor of fibrosis.

Sofosbuvir administration during the hospitalization period, as a part of a randomized clinical trial for treatment of acute YF occurring concomitantly in our institution[27], might have altered the proportions of fibrosis during the follow up period. Corroborating with this is the fact that no patient that used sofosbuvir was categorized as having significant fibrosis at the time of completion of 6 months after onset of symptoms (although that difference did not reach statistical significance). There was also a report of high incidence of late-onset relapsing hepatitis in these patients (that is, a new elevation in aminotransferase levels within 6 months after an improvement in or normalization of liver function, occurring in up to 45%, according to a recent study[17]), but this have not seemed to alter our results.

No patient in our sample was adequately immunized for YF or had vaccinal disease. It is important to note that, by the time of this outbreak, the city of São Paulo and its outskirts were not yet included in the areas of universal (i.e. permanent) YF vaccination, presenting a low vaccination coverage [35,36].

Some limitations of this work must be acknowledged. The small sample size, inherent to the epidemiology of the disease, must be taken into account. Although not sufficient to ensure adequate power to the statistical analysis and to confirm the hypothesis, it retains great relevance and represents a unique window of opportunity in the context of a rare disease, never

yet studied in this scale. The observational nature of the investigation brings a series of usual bias, and thus we tried to avoid them by following strict rules and protocols. There was no control group whatsoever; we then needed to compare our findings to populational statistics, not always comparable (e.g., with the same characteristics) to our group of individuals. The lack of a baseline evaluation, in turn, can be justified by the aforementioned argument—it would not be possible to evaluate patients at the acute phase of yellow fever with SWE since acute inflammation changes (increases) LS. It is also of note that no patient had prior history of liver disease and hepatitis viruses were ruled out. There were many losses of follow up due to logistic problems, lack of contact information or no attendances, and this might have affected the results. Furthermore, survival bias is likely to have occurred, since patients that died at hospital could have had a higher probability of developing liver fibrosis if fatal outcome have not happened. Also, there are still no trustworthy cutoffs for fibrosis using acoustic radiation force impulse (SWE techniques) by different fabricators and to different diseases[18,37,38], hence we preferably used the absolute values of LS (continuous variable) to express our results.

In conclusion, we believe our work increases the knowledge of the natural history of this rare disease. US can be a tool to evaluate the liver in patients during the acute phase of severe YF, as it was demonstrated in our previous study [23], but can also be of value and have a role in the follow up of patients of age and / or with comorbidities after hospital discharge. Increased SAPS-3 might be associated with liver fibrosis after survival, but this result needs to be confirmed by further studies.

Supporting information

S1 STROBE checklist.

(DOCX)

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4. DISCUSSÃO

4.0 DISCUSSÃO

Esse é o primeiro estudo que aborda os achados de imagem na FA. Através deste trabalho inédito, encontramos alterações de imagem médica à US consistentes com trabalhos prévios de outras afecções e facilmente reproduzíveis na fase aguda dessa doença grave, uma descrição inicial que amplia o nosso conhecimento acerca da sua fisiopatologia. A amostra obtida foi similar, em termos sócio-demográficos, àquelas dos outros estudos recentemente publicados, com maioria masculina, um dado não completamente elucidado e sem fator causal evidente. Um dos achados da US (hiperecogenicidade renal) mostrou-se fator prognóstico *independente* de óbito precoce na UTI, um dado relevante no contexto da terapia intensiva. Em outras palavras, mesmo com o resultado da creatinina sérica mantendo sua importância no contexto da UTI, para este achado de US, existe valor prognóstico adicional com o resultado de aumento ou não da ecogenicidade dos rins. Diferentemente, hiperecogenicidade hepática e espessamento parietal vesicular, a despeito da alta frequência, não apresentaram a mesma associação. Portanto, a US, um método acessível, portátil e não emissor de radiação ionizante, previamente não disponível em surtos anteriores de FA, pode ser uma ferramenta importante na avaliação destes pacientes, inclusive na modalidade *point-of-care*, para a tomada de decisões rápidas à beira do leito.

Graças ao acompanhamento longitudinal dos pacientes, foi possível avaliar se em 6 meses havia hepatopatia crônica nos sobreviventes. A nossa amostra não apresentou taxas altas de fibrose hepática na elastografia SWE, um resultado "negativo" que felizmente se mostrou alinhado com estudos prévios que relatavam esta melhora do parênquima hepático e com importante validade científica. Os poucos

casos com fibrose avançada podem ter sido falsos-positivos em função de congestão hepática coexistente. Um estudo por biópsia destes pacientes, no futuro, poderia mostrar se de fato se tratava desta hipótese. Conforme comentado na Publicação 2, uma avaliação *baseline* com SWE também não seria factível, em função não só das dificuldades logísticas de um aparelho moderno (e volumoso) de US com a tecnologia na UTI, como pelo fato de a avaliação na fase aguda da hepatite viral trazer uma superestimativa dos valores de LS.

A despeito da amostra limitada de indivíduos estudados, ressalta-se que a casuística é relevante, levando-se em conta o fato de ser uma doença considerada rara, de alta letalidade e sem outras amostras previamente relatadas. Na seleção dos pacientes, incluímos apenas formas graves da FA, além de terem sido feitas avaliações por radiologistas treinados e com equipamento relativamente moderno, o que limita o escopo de generalização (validade externa) do estudo, porém os resultados aplicam-se com grande validade interna a outros pacientes que porventura venham a ser hospitalizados em instituições terciárias e quaternárias e admitidos em UTIs e unidades de alta complexidade. O viés de sobrevivência de fato pode ter ocorrido durante o acompanhamento dos pacientes, pois os óbitos poderiam ser casos de maior fibrose caso houvessem sobrevivido, mas este é um fator esperado para este tipo de estudo de coorte. As perdas de seguimento ocorridas no segundo trabalho, decorrentes de inúmeras dificuldades e problemas logísticos, também configuram um potencial fator de afastamento dos resultados da realidade, uma vez que os dados obtidos poderiam ter sido algo diferentes caso todos os sobreviventes originais tivessem sido avaliados por elastografia. Por outro lado, o fato de a própria elastografia hepática por US fornecer múltiplas medidas e informações objetivas tende a mitigar esta limitação e compensar de certa forma o próprio número reduzido

de indivíduos amostrados. Por fim, o estudo carece de um grupo controle; no entanto, por ser uma descrição inicial de uma doença com acompanhamento dos casos, a pesquisa retém grande significado científico.

Portanto, este estudo traz grandes avanços na pesquisa médica sobre FA, contribuindo para o maior entendimento dos seus mecanismos fisiopatológicos e de patogenicidade, no curto e longo prazo. Os resultados do nosso trabalho poderão ser utilizados para embasar novas pesquisas em FA no futuro.

5. CONCLUSÕES

5.0 CONCLUSÕES

- 1. A FA grave produz achados de imagem ultrassonográfica abdominais reprodutíveis e relacionados à lesão de órgãos-alvo envolvidos na fisiopatologia da doença**

- 1.1 Hiperecogenicidade renal é um fator preditor independente de morte em 30 dias (pior prognóstico) no contexto da UTI

- 2. Os pacientes que sobrevivem a um quadro de FA grave não parecem desenvolver graus significativos de fibrose hepática, estimada pela US com SWE, no acompanhamento a longo prazo após a convalescência**

- 2.1 Idade avançada, diabetes, hipertensão e SAPS-3 à admissão na UTI são potenciais preditores de maior rigidez hepática (LS) no acompanhamento de pacientes convalescidos

- 2.2 Insuficiência cardíaca pode representar uma causa de falsos-positivos, por aumento da dureza hepática não relacionada à fibrose

6. ANEXOS

6.0 ANEXOS

6.1. TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE
SÃO PAULO - HCFMUSP

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DADOS DA PESQUISA

Título da pesquisa: Avaliação Ultrassonográfica de Pacientes com Febre Amarela Aguda e na Convalescença

Pesquisador principal: Yuri Costa Sarno Neves

Departamento/Instituto: Departamento de Radiologia e Oncologia/Instituto de Radiologia (InRad)

Prezado(a),

Convidamos o Sr(a). a participar desta pesquisa, que tem o intuito de esclarecer algumas questões a respeito da febre amarela, infecção viral que causa febre hemorrágica grave em muitos casos. No momento, vivemos em uma situação de epidemia da doença no Brasil, com grave problema de saúde pública. Vários aspectos da doença ainda não são compreendidos pois não foram estudados a fundo com os atuais recursos médicos de que dispomos hoje (novas modalidades de terapia intensiva, novos métodos de imagem).

Os objetivos deste projeto são avaliar as alterações no exame de ultrassonografia do abdome na fase aguda da febre amarela e determinar se ocorre sequela no fígado após a melhora (convalescença), ou seja, se há o desenvolvimento de doença hepática crônica, doença que exige acompanhamento médico. Para tanto, será utilizada no projeto a ultrassonografia realizada à admissão na Unidade de Tétano e de Terapia Intensiva do HCFMUSP, exame que já faz parte da rotina habitual de exames de todos os pacientes que admitidos com a doença, com a finalidade de determinar se existe doença hepática prévia e para avaliar a vascularização do fígado, caso haja a necessidade de transplante hepático. Após a melhora e antes da alta, realizaremos outra avaliação com ultrassom abdominal, desta vez com a técnica de Elastografia, que permite a medida do grau de fibrose (dureza) do fígado, sem nenhum tipo de radiação ionizante ou contraste endovenoso. O exame será feito de forma adicional, no terceiro andar do Hospital (Instituto de Radiologia), no momento da alta e em mais duas ocasiões, dois e seis meses após a sua alta hospitalar.

Essas informações estão sendo fornecidas para sua participação voluntária neste estudo, que visa contribuir para a nossa compreensão sobre a doença febre amarela e, eventualmente, ajudar os próximos pacientes. Concordando em participar, o(a) Sr(a). receberá, como benefício, acompanhamento ultrassonográfico após a internação durante o período o estudo, podendo diagnosticar alterações no fígado, sem nenhum custo ou risco adicional. O único desconforto que o(a)

Sr(a). poderá apresentar é o gel de contato utilizado no exame, que pode estar um pouco frio. Não haverá qualquer modificação no acompanhamento normal que o (a) Sr(a). receberia caso não estivesse participando do projeto, tampouco haverá qualquer prejuízo no atendimento em caso da negativa na participação. Com o término do estudo, o(a) Sr(a). continuará o seu acompanhamento habitual no HCFMUSP. É garantida a liberdade da retirada de consentimento em qualquer fase da pesquisa, a qualquer momento e deixar de participar do estudo sem penalização alguma, seja à continuidade de seu tratamento na Instituição, ao sigilo ou à privacidade.

O(a) Sr(a). receberá uma via deste Termo de Consentimento assinada pelo pesquisador responsável.

A sua participação voluntária é isenta de despesa. O Sr(a). terá a garantia do acesso a tratamento hospitalar se necessário. É também garantida indenização por eventuais danos decorrentes da pesquisa.

As informações obtidas serão analisadas em conjunto com outros pacientes, não sendo divulgada a identificação de nenhum paciente. Os dados e o material coletado serão utilizados somente para pesquisa científica. Novo consentimento deverá ser obtido em caso de uso futuro dos dados desta pesquisa; toda nova pesquisa só será realizada com aprovação do sistema CEP-CONEP.

Em qualquer etapa do estudo ou após o término do mesmo, o(a) Sr(a). terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de dúvidas. O principal investigador é o Dr. Yuri Costa Sarno Neves, que pode ser encontrado no endereço: Rua Dr. Ovídio Pires de Campos, 75 - Cerqueira César, São Paulo - SP, 05403-010. Telefone: 2661-6000 (InRad Portaria 1). E-mail: ycsneves@gmail.com. Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Ovídio Pires de Campos, 225 – 5º andar – tel: (11) 2661-7585, (11) 2661-1548, (11) 2661-1549; e-mail: cappesq.adm@hc.fm.usp.br.

Fui suficientemente informado a respeito do estudo “Avaliação Ultrassonográfica de Pacientes com Febre Amarela Aguda e na Convalescença”. Eu discuti as informações acima com o Pesquisador Responsável Dr. Yuri Costa Sarno Neves sobre a minha decisão em participar nesse estudo. Ficaram claros para mim os objetivos, os procedimentos, os potenciais desconfortos e riscos e as garantias. Concordo voluntariamente em participar deste estudo, assino este termo de consentimento e recebo um via rubricada pelo pesquisador.

Data: ____/____/____

Assinatura do participante/representante legal

Data: ____/____/____

Assinatura do responsável pelo estudo

DADOS DE IDENTIFICAÇÃO DO PARTICIPANTE DA PESQUISA OU RESPONSÁVEL LEGAL

1. NOME:.....

DOCUMENTO DE IDENTIDADE Nº: SEXO M ☐ F ☐

DATA NASCIMENTO:/...../.....

ENDEREÇO: Nº

APTO.....

BAIRRO: CIDADE:

CEP: TELEFONE: DDD (.....)

2. RESPONSÁVEL LEGAL:.....

NATUREZA (grau de parentesco, tutor, curador etc.):

DOCUMENTO DE IDENTIDADE Nº: SEXO M ☐ F ☐

DATA NASCIMENTO:/...../.....

ENDEREÇO: Nº

APTO.....

BAIRRO: CIDADE:

CEP: TELEFONE: DDD (.....)

6.2 PARECER CEP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO ULTRASSONOGRÁFICA DE PACIENTES COM FEBRE AMARELA AGUDA E NA CONVALESCENÇA

Pesquisador: Maria Cristina Chammas

Área Temática:

Versão: 1

CAAE: 92651718.7.0000.0068

Instituição Proponente: Hospital das Clínicas da Faculdade de Medicina da USP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.833.376

Apresentação do Projeto:

PROJETO NOVO - AVALIAÇÃO ULTRASSONOGRÁFICA DE PACIENTES COM FEBRE AMARELA AGUDA E NA CONVALESCENÇA.

Objetivo da Pesquisa:

Este será um estudo misto composto de corte transversal inicial e avaliação prospectiva. Serão incluídos no estudo todos pacientes com diagnóstico

de febre amarela no ano de 2018, confirmado por PCR (polymerase chain reaction) viral sérico, encaminhados à Unidade de Tétano e de Terapia Intensiva (UMIN) do Serviço de Moléstias Infecto-Parasitárias do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP). Critério de exclusão: hipertensão portal ou outras alterações do contorno e ecotextura do fígado. Será incluídos pacientes que apresentem PCR positivo para febre amarela.

Avaliação dos Riscos e Benefícios:

Avaliar os achados ultrassonográficos na fase aguda e convalescença de pacientes co febre amarela. Objetivos secundários: será realizado elastografia hepática na convalescença e com seguimento aos 2 meses e aos 6 meses após a doença.

Comentários e Considerações sobre a Pesquisa:

O objetivo fundamental refere-se ao estudo dos achados ultrassonográficos na doença aguda, seu acompanhamento, verificar alterações na elevação tardia das transaminases no intuito de predizes

Endereço: Rua Ovídio Pires de Campos, 225 5º andar
Bairro: Cerqueira Cesar **CEP:** 05.403-010
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6.3 CARTA DE ANUÊNCIA DA INFECTOLOGIA

Termo de Anuência

Declaro estar ciente do projeto de pesquisa: “AVALIAÇÃO ULTRASSONOGRÁFICA DE PACIENTES COM FEBRE AMARELA AGUDA E NA CONVALESCENÇA”, sob a responsabilidade do Dr. Yuri Costa Sarno Neves (pesquisador executante) e do Dr. Fernando Linhares Pereira (pesquisador secundário) e sob a orientação da Dra. Maria Cristina Chammas.

Autorizo a execução do projeto na UTI-DMIP.

Declaro também apoiá-lo e dele participar, assim que o mesmo for aprovado pela CAPESQ.

Atenciosamente,

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7. REFERÊNCIAS BIBLIOGRÁFICAS

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