Universidade do Estado do Rio de Janeiro
Centro Biomédico
Faculdade de Ciências Médicas

Flavia Ferreira Fernandes

ELF (*Enhanced Liver Fibrosis*) como marcador não invasivo de fibrose hepática na hepatite C crônica

Rio de Janeiro
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Tese apresentada, como requisito parcial para obtenção do título de Doutor ao Programa de Pós-Graduação em Ciências Médicas, da Universidade do Estado do Rio de Janeiro.

Orientadora: Prof.ª Dra. Renata de Mello Perez

Coorientadora: Prof.ª Dra. Fátima Aparecida Ferreira Figueiredo

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Rio de Janeiro
2014

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RESUMO

FERNANDES, Flavia Ferreira. *ELF (Enhanced Liver Fibrosis) como marcador não invasivo de fibrose hepática na hepatite C crônica.* 2014. 91 f. Tese (Doutorado em Ciências Médicas) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2014.

A fibrose hepática é o aspecto mais relevante e o mais importante determinante de morbimortalidade na hepatite C crônica (HCC). Historicamente, a biópsia hepática é o método de referência para avaliação da fibrose causada pela HCC, apesar de apresentar limitações. O estudo de marcadores não invasivos, que possam obviar a necessidade da biópsia, é uma área de constante interesse na hepatologia. Idealmente, a avaliação da fibrose hepática deveria ser acurada, simples, prontamente disponível, de baixo custo e informar sobre o prognóstico da patologia. Os marcadores não invasivos mais estudados são a elastografia hepática transitória (EHT) e os laboratoriais. A EHT já foi extensamente validada na HCC e está inserida na rotina de avaliação destes pacientes. Dentre os laboratoriais, existem diversos testes em continua experimentação e, até o momento, nenhum foi integrado à prática clínica no Brasil, embora já aplicados rotineiramente em outros países. O *Enhanced Liver Fibrosis* (ELF), um teste que dosa no soro ácido hialurônico, pró-peptídeo amino-terminal do colágeno tipo III e inibidor tissular da metaloproteinase 1, tem se mostrado bastante eficaz na detecção de fibrose hepática significativa e de cirrose na HCC. Neste estudo o ELF teve o seu desempenho avaliado em relação a biópsia hepática e demonstrou apresentar boa acurácia na detecção tanto de fibrose significativa quanto de cirrose. Na comparação com a EHT apresentou acurácia semelhante para estes mesmos desfechos, com significância estatística. No entanto, foi observada uma superestimação da fibrose com a utilização dos pontos de corte propostos pelo fabricante. Este achado está em acordo com a literatura, onde não há consenso sobre o melhor ponto de corte a ser empregado na prática clínica. Com a ampliação da casuística foi possível propor novos pontos de corte, através da análise clássica, com a biópsia hepática como padrão ouro. O resultado obtido vai ao encontro do observado por outros autores. Em seguida, os novos pontos de corte do ELF foram reavaliados sem que a biópsia hepática fosse a referência, através da análise de classes latentes. Mais uma vez o ELF apresentou bom desempenho, inclusive com melhora de suas sensibilidade e especificidade em comparação com a análise clássica, onde a biópsia hepática é a referência. Assim sendo, é possível concluir que o ELF é um bom marcador não invasivo de fibrose hepática. No entanto, para detecção de fibrose significativa e cirrose, deve ser considerada a aplicação na prática clínica dos novos pontos de corte aqui propostos.

Palavras-chave: Hepatite C. ELF. Elastografia hepática transitória. Métodos não invasivos. Fibrose hepática. Pontos de corte. Análise de classes latentes.

ABSTRACT

FERNANDES, Flavia Ferreira. *ELF (Enhanced Liver Fibrosis) as a non invasive predictor of liver fibrosis in hepatitis C.* 2014. 91 f. Tese (Doutorado em Ciências Médicas) – Faculdade de Ciências Médicas, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2014.

Liver fibrosis is the most relevant issue concerning chronic hepatitis C (CHC) and determines its prognosis. Historically, liver biopsy has been the reference method for evaluating fibrosis related to CHC, though it presents many drawbacks. There is a continuing interest in the development of non invasive markers capable of replacing liver biopsy. The ideal surrogate for fibrosis evaluation should be accurate, simple, low cost and yield prognostic information. So far, the most well known non invasive methods are transient hepatic elastography (TE) and laboratory panels. TE has already been extensively validated and is integrated in patients routine. There is plenty of laboratory panels in continuing evaluation and some are already integrated in daily practice abroad. In Brasil, until the present moment, it is not a reality. Enhanced Liver Fibrosis (ELF) panel comprises the serum concentration of hyaluronic acid, tissue inhibitor of matrix metalloproteinases-1, and aminoterminal propeptide of type III procollagen and has demonstrated good performance in detecting significant fibrosis and cirrhosis in CHC patients. In the present study ELF had it's performance evaluated against liver biopsy and obtained satisfactory accuracy in detecting significant fibrosis and cirrhosis. In comparison to TE no statistically significant diference was observed, for the same endpoints mentioned before. However, the application of manufacturer's cutoff points produced overestimation of fibrosis stages. These findings are in accordance with other author's results, in that there is no consensus so far on the most adequate cutoff points for main clinical end points. Enlarging the data permited calculating new cutoff points, through the classical statistical approach, using liver biopsy as the gold standard. The results once more matched those published in literature. Following this, the ELF new cutoff points were evaluated in a statistical modeling where there are no gold standards, the latent classes analysis. Besides showing a satisfactory performance, in this new approach, ELF experimented an improvement in sensitivity and specificity, if compared with the classical analisys, with liver biopsy as reference. ELF panel has a good performance as a noninvasive fibrosis marker. However, new cutoff points need to be applied to improve its performance for the discrimination of different stages of fibrosis in CHC patients.

Keywords: Hepatitis C. ELF. Transient hepatic elastography. Non invasive methods. Kiver fibrosis. Cut-off points. Latent classes analysis.

LISTA DE ABREVIATURAS E SIGLAS

ACL	Análide de classes latentes
AH	Ácido hialurônico
ALT	Alanina aminotransferase
APRI	*Aspartate-to-Platelets Ratio Index*
AST	Aspartato aminotransferase
AUROC	Área sob a curva Roc
EHT	Elastografia hepática transitória
ELF	*Enhanced Liver Fibrosis*
GGT	Gamagutiltransferase
HCC	Hepatite C crónica
HCV	*Hepatitis C virus*
IC	Intervalo de confiança
IMC	Índice de massa corpórea
kPa	KiloPAscals
LN	Logaritmo Neperiano
MHz	MegaHertz
PIIINP	pró-peptídeo amino-terminal do colágeno tipo III
RVS	Resposta virológica sustentada
TIMP	inibidor tissular da metaloproteinase 1
USG	Ultrassonografia

LISTA DE SÍMBOLOS

cm	Centímetro
mm	Milímetro
%	Percentual
X	Multiplicação
=	Igual
-	Menos
≥	maior ou igual
α	Alpha
ɣ	Gama
Δ	Delta
<	Menor
≤	Menor ou igual

SUMÁRIO

INTRODUÇÃO

A hepatite C crônica (HCC) é uma doença prevalente no Brasil(1), com elevada morbimortalidade, manifesta como cirrose hepática, disfunção hepatocelular e hepatocarcinoma(2). A fibrose hepática é o aspecto mais relevante da HCC por ser o determinante desta morbimortalidade(3), além de ser o principal alvo de estudo de terapias que visam a controlar a progressão da doença(2). Até recentemente, no Brasil, a indicação de tratamento antiviral ainda era baseada no estágio de fibrose diagnosticado pela biópsia hepática(4). Somente em 2013 a elastografia hepática foi incorporada na avalição da fibrose hepática com vistas a indicação de tratamento da HCC(5).

A biópsia hepática

A biópsia hepática é o método de referência para avaliação da fibrose causada pela HCC, embora apresente limitações(6, 7). Trata-se de um método invasivo, que pode ser doloroso e apresentar morbidade e mortalidade associadas, apesar de raras(8, 9). Não é um bom método para ser utilizado de forma seriada e avaliar a progressão da fibrose(8). O principal fator relacionado à sua falha em classificar adequadamente a fibrose é o tamanho do fragmento. Fragmentos pequenos (menores de 2,0 cm) tendem a subestimar a fibrose(10, 11). Ainda que com amostras satisfatórias, representa apenas 1/50.000 do fígado e oferece representatividade limitada da doença hepática, principalmente devido ao caráter não homogêneo de distribuição da fibrose pelo parênquima hepático(12). Regev e cols(13). descreveram 33% de discordância entre biópsias realizadas em ambos os lobos hepáticos de pacientes com HCC. Bedossa e cols(10). demonstraram que um fragmento de biópsia hepática de 25mm de extensão pode apresentar avaliação incorreta da fibrose em até 25% dos casos, utilizando peças cirúrgicas como referência. Na sua leitura pode haver variabilidade tanto intra quanto interobservador (14-16), mesmo quando analisada por patologistas experientes. Isto se torna mais evidente na classificação da fibrose em estágios intermediários (F2 e F3 de Metavir)

que no diagnóstico de estágios extremos (F1 e F4 de Metavir) (17). Para normatização dos resultados são empregados escores semiquantitativos, baseados em alterações estruturais, que classificam de forma categórica a fibrose, o que não corresponde a real quantidade do tecido fibrótico hepático(18, 19).

O marcador não invasivo ideal

O método ideal para avaliar a fibrose hepática deveria ser simples, prontamente disponível, acurado e de baixo custo(20). Além disso, deveria contemplar tanto a descrição das alterações estruturais quanto a mensuração da quantidade de fibrose presente no parênquima, preferencialmente de forma não invasiva, e poder ser repetido durante a evolução da doença. Ainda idealmente, este método deveria ter também valor prognóstico(19, 21).

Diversos métodos alternativos à biópsia têm sido descritos nos últimos anos, utilizando-se a biópsia hepática com padrão–ouro, apesar das limitações já expostas(22). Dentre estes há os marcadores físicos (elastografia) e os laboratoriais.

Elastografia hepática transitória

Dentre os métodos físicos não-invasivos, a elastografia hepática transitória (EHT), obtida pelo Fibroscan (EchoSens, Paris, França), foi o mais estudado em grandes séries de pacientes. Trata-se de um método simples, de rápida aquisição, resultado imediato, curta curva de aprendizado, bem aceito pelos pacientes e que pode ser repetido inúmeras vezes, caso seja preciso. Baseia-se na medida de uma propriedade física intrínseca do parênquima hepático que é a sua elasticidade ou rigidez, de acordo com a quantidade de fibrose presente. Utiliza-se uma sonda que produz uma onda de baixa frequência (50 MHz) que é transmitida através do fígado. A esta sonda está acoplado um transdutor de ultrassonografia, que acompanha a onda vibratória, conforme ela se propaga pelo órgão. A velocidade dessa onda se

correlaciona diretamente com a rigidez hepática: quanto mais rápida sua propagação, mais rígido está o fígado. O resultado final representa o valor mediano das medidas válidas e é reportado em KiloPascals (kPa). A validade de cada medida da elasticidade é inteiramente definida pelo aparelho, sendo operador independente. A aplicabilidade ou interpretabilidade do método baseia-se no número total e proporção de medidas válidas e no grau de dispersão das medidas. A EHT consegue avaliar um volume de tecido hepático de, aproximadamente, 1 cm de diâmetro e 5 cm de comprimento, o que representa cerca de 100 vezes o volume de uma biópsia hepática percutânea (23).Tem sido empregada para obviar a indicação de biópsia hepática, mas em alguns casos, devido a características físicas dos próprios pacientes, não há êxito na obtenção das medidas. Fatores relacionados à redução do sucesso do exame são: obesidade, Diabetes tipo 2, sexo feminino, síndrome metabólica e idade(24). Apresenta excelente desempenho na detecção de cirrose, evitando a realização de biópsia em até 90% dos casos. Entretanto, não é tão satisfatória para discernir entres os estágios intermediários de fibrose (25). Fatores associados a resultados não fidedignos são: elevação de transaminases(26), colestase(27) e congestão hepática(28).

Na HCC, a AUROC para o diagnóstico de fibrose significativa (F≥2 Metavir) e cirrose hepática variou entre 0,79-0,83 e 0,95-0,97, respectivamente(8, 29, 30). Os estudos de Casterà et al(30) e Ziol et al(29) definiram 2 pontos de corte distintos para o diagnóstico da cirrose. O valor de 12,5 kPa (30) apresentou sensibilidade, especificidade e AUROC de 87%, 91% e 0.95, respectivamente. Enquanto 14,6 kPa(29) apresentou sensibilidade, especificidade e AUROC de 86%, 96% e 0,97, respectivamente.

Em 2013 a EHT passou a fazer parte da avaliação da fibrose como indicação de tratamento de pacientes com HCC conforme o Suplemento 2 ao Protocolo Clínico e Diretrizes Terapêuticas para Hepatite Viral e Coinfecções (Genótipo 1 do HCV e Fibrose Avançada)(5).

Marcadores laboratoriais

Apesar de não haver consenso quanto a uma classificação dos marcadores laboratoriais, estes exames realizados no soro podem ser divididos, didaticamente, em dois grupos: marcadores diretos ou indiretos. Os marcadores diretos visam a refletir a síntese e degradação da fibrose hepática. Já os marcadores indiretos são frequentemente compostos por exames de bioquímica, utilizados na rotina de acompanhamento dos pacientes com HCC, organizados na forma de índices como o *Aspartate-to-Platelets Ratio Index* (APRI)(31) e o Fibrotest (32).

Marcadores laboratoriais indiretos

O APRI (AST/limite superior da normalidade x 100/ plaquetas) é um método indireto, simples, que consegue predizer fibrose significativa (Ishak ≥ 3) em 51% dos casos e cirrose (Ishak ≥ 5) em 81% dos casos(31). Para populações com prevalência de fibrose significativa de 40% (como as dos centros de referência em hepatologia) apresenta um valor preditivo negativo de 80%, o que poderia reduzir o número de biópsias hepáticas em 35%. Para cirrose, com uma prevalência de 15%, o valor preditivo negativo seria de 91%, o que tornaria 75% das biópsias desnecessárias. Seu valor preditivo positivo, tanto para fibrose significativa quanto para cirrose, é baixo e sua principal aplicação é excluir estas condições(33). Na avaliação de valor prognóstico de complicações relacionadas à hepatopatia crônica se mostrou inferior a outros marcadores laboratoriais(21, 34). Além disso, parece sofrer ser influenciado por fatores metabólicos que levam a variações no valor de normalidade da AST, interferindo no seu desempenho(35). No entanto, é amplamente acessível, e se adequadamente empregado, principalmente se associado a outros métodos, pode somar na avaliação não invasiva da fibrose(36).

O Fibrotest inclui α2 macroglobulina, haptoglobina, apolipoproteina-A1, GGT e bilirrubina. No estudo original, demonstrou valor preditivo negativo para fibrose significativa (Metavir ≥ F2) de 100% e um valor preditivo positivo de 90%, para o mesmo grupo(32). Amplamente validado na literatura(21, 37) apresenta o

incoveniente de ser baseado em uma fórmula patenteada e comercializada, o que dificulta o acesso ao método.

Marcadores laboratoriais diretos

Os marcadores diretos mensuram componentes da matriz extracelular no soro, como glicoproteínas (laminina, ácido hialurônico), colágeno IV, pro-colágeno III, metaloproteinases, assim como citocinas envolvidas na fibrogênese. Nenhum dos marcadores diretos conhecidos satisfaz, até o momento, os requisitos para o marcador ideal, pois nenhum é específico do tecido hepático e todos sofrem interferências no seu metabolismo, *clearence* e excreção(18).

Enhanced Liver Fibrosis (ELF)

Em 2004, Rosenberg et al (38) avaliaram 1021 pacientes com hepatopatias crônicas de diversas etiologias, em um estudo multicêntrico que testou o desempenho de marcadores séricos diretos de fibrose, usando como padrão-ouro a biópsia hepática. Destes, 921 tiveram biópsias adequadas (mais de cinco espaços–porta). Nove mediadores - dentre eles constituintes da matriz extracelular e mediadores do remodelamento da matriz - foram testados em uma coorte randômica de 400 pacientes, dando origem a um algoritmo que foi, então, validado nos 521 pacientes subsequentes. Como resultado foi encontrado um algoritmo que inclui idade, ácido hialurônico (AH), o pró-peptídeo amino-terminal do colágeno tipo III (PIIINP) e o inibidor tissular da metaloproteinase 1 (TIMP-1), a saber: Scheuer = -.014*LN(idade) +.616 * LN(HA) +.586 * LN(PIIINP) + .472 _ LN(TIMP-1) - 6.38. A sensibilidade para detecção de fibrose significativa (F≥2 de Scheuer) foi de 90,3% (AUROC 0.78), com valor preditivo negativo de 92%. Para diagnóstico de cirrose (F≥4 de Scheuer) a sensibilidade foi de 90,7% com AUROC de 0,88 e valor preditivo negativo de 94,5%. Este escore foi denominado ELF (*European Liver Fibrosis*) e tem como principal crítica o fato de ter sido desenvolvido em uma coorte mista, com

hepatopatias crônicas de etiologias diversas, que apresentam processos fibróticos distintos. Mais tarde sua denominação foi mudada para *Enhanced Liver Fibrosis.*

Em pacientes com hepatite C, o ELF foi avaliado por Parkes et al(39) em 3 coortes distintas (2 prospectivas e uma retrospectiva), que juntas somavam 347 pacientes. Eles concluíram que é possível simplificar o escore retirando-se a idade (METAVIR = 7.412 + [ln(HA)*0.681] + [ln(PIIINP)*0.775] +[ln(TIMP1)*0.494] + 10), sem prejuízo ao seu desempenho. O escore simplificado consegue predizer fibrose significativa com AUROC de 0,85, o que evitaria 81% das biópsias. Este achado foi validado mais tarde por Catanzaro et al (40) que encontrou áreas sob a curva ROC (AUROC) para fibrose significativa e cirrose de 0,94, elevada sensibilidade para fibrose significativa (93%) e valor preditivo negativo de 92% para presença de cirrose.

Em comparação com outros métodos não invasivos Cobbold et al (41) avaliaram em uma coorte de 67 pacientes com HCC o desempenho do APRI, ELF, elastografia hepática transitória (EHT) e ultrassonografia (USG) convencional na determinação da gravidade da HCC, além das possíveis associações entre eles. Encontraram que o ELF e a EHT são os com melhor correlação entre si (coeficiente de correlação de 0,94). A associação tanto do ELF quanto da EHT com o APRI aumenta o desempenho deste último. No entanto, a associação do ELF com a EHT não melhora a predição de cada um deles isoladamente. A associação de três testes não melhora a detecção de fibrose, e portanto, não é custo-efetiva. A USG obteve o pior resultado, tanto isolado quanto em associação.

Outros autores estudaram a utilidade do ELF em cenários distintos. Na hepatite B crônica, Trembling et al (42) encontraram um bom resultado do ELF na detecção de fibrose avançada (AUROC 0,80) e cirrose (AUROC 0,83), porém aquém do observado para a EHT no mesmo grupo (0,90 para fibrose avançada e 0,95 para cirrose). Wong et al (43), também estudando pacientes com hepatite B, propuseram que sua associação com a EHT poderia melhorar o desempenho de cada um dos métodos em separado.Guha et al (44) validaram o ELF modificado (sem idade) em uma coorte de 196 pacientes portadores de esteato- hepatite não alcóolica. Eles observaram que, apesar de apresentar um bom desempenho na predição de ausência de fibrose e fibrose significativa (AUROC de 0,76 e 0,90, respectivamente), o ELF tem sua eficácia aumentada quando associado a outros fatores, como idade, índice de massa corporal (IMC), Diabetes, resistência insulínica, AST/ALT, plaquetas

e albumina. A adição destes marcadores ao ELF aumentou as AUROCs para ausência de fibrose e fibrose significativa para 0,84 e 0,98, respectivamente.

Em HCC, Martinez et al(45) demonstraram que pacientes que conseguem erradicar o vírus com o tratamento com Interferon Peguilado e ribavirina apresentam queda significativa no valor do ELF, o que representa o impacto do tratamento na matriz extracelular e, provavelmente, a melhora na fibrose hepática. Neste mesmo estudo o ELF foi comparado com outros maracdores não invasivos como o Forns escore, o APRI e o FIB-4. Para detecção de fibrose significativa (F≥2 de Metavir) os índices apresentaram desempenhos semelhantes. Apenas para detecção de cirrose o FIB-4 se mostrou superior aos demais.

Apesar de haver consenso sobre a boa performance do ELF como marcador não invasivo de fibrose e cirrose na HCC (40, 41, 46, 47), não há concordância sobre qual seria o melhor ponto de corte a ser aplicado na prática clínica. Xie(48) et al, em metanálise recente que incluiu nove estudos com o ELF, encontrou AUROC de 0.88, 0.86 e 0.87 para fibrose significativa, fibrose avançada e cirrose, respectivamente. No entanto, por serem os estudos muito heterogêneos quanto a etiologia da hepatopatia crônica, metodologia laboratorial, qualidade dos fragmentos de biópsia analisados e escores de classificação de fibrose empregados, concluiu não ser possível determinar um ponto de corte confiável.

Marcadores diretos e indiretos combinados

Escores que utilizam marcadores indiretos e diretos combinados também foram amplamente validados, dentre eles estão o Fibrometer (49) e o Hepascore (50). O Fibrometer é um escore que combina plaquetas, tempo de protrombina, AST, α2-macroglobulina, uréia e idade. Para detecção de fibrose significativa (≥ F2 de Metavir) a AUROC foi de 0,88, comparada com 0,80 para o Fibrotest, 0,82 para o Forns e 0,79 para o APRI(49).

O Hepascore utiliza bilirrubina, GGT, ácido hialurônico, α2-macroglobulina, idade e sexo, com AUROCs de 0,85 e 0,94 para fibrose significativa e cirrose, respectivamente(50).

A comparação desses métodos demonstrou que não há diferença significativa entre eles(51), com algumas poucas exceções: Fibrotest superior que o Hepascore para fibrose significativa e Fib-4 e Hepascore superiores ao APRI para cirrose(52).

A investigação de marcadores não invasivos de fibrose

A elastografia como opção não-invasiva de estadiamento da HCC já é uma realidade em nosso meio. No entanto, até o momento, marcadores não-invasivos laboratoriais não fazem parte da rotina de avaliação da HCC no Brasil(5), apesar de já amplamente utilizados em outros países(53).

Nos últimos anos, diversos autores têm questionado o papel da biópsia hepática como referência para o desenvolvimento dos marcadores não-invasivos de fibrose hepática(22, 54). O fato da biópsia hepática não ser um padrão ouro perfeito impede o progresso no estudo destes marcadores(6, 55). Para lidar com este obstáculo, tem surgido na literatura duas abordagens distintas no estudo dos marcadores não invasivos.

A primeira delas é a utilização de desfechos clínicos para avaliar a acurácia dos métodos não-invasivos. Boursier et al (56) ao compararem marcadores laboratoriais (APRI, FIB4, Fibrotest, Hepascore, FibroMeter, CirrhoMeter) com a biópsia hepática em pacientes com HCC puderam observar que, em um seguimento médio de 9,5 anos, os marcadores não invasivos se correlacionaram melhor com desfechos clínicos que o estagiamento da hepatopatia pela biópsia hepática. A estratégia que associou um marcador de fibrose com um de cirrose melhorou ainda mais este desempenho. Por sua vez Vergniol et al (34) observaram que a aplicação seriada da EHT e de marcadores laboratoriais como o Fib4, bem como suas variações (ΔFib4, por exemplo) através dos anos, foram capazes de, em um segmento de três anos, predizer sobrevida e complicações realacionadas a hepatite C crônica, principalmente naqueles com fibrose avançada e cirrose. Na cirrose biliar primária, Mayo et al (57) demonstraram que o ELF é um bom preditor de progressão da doença quando comparado com outros marcadores de prognóstico como a histologia, a bilirrubina, o MELD escore e o Mayo escore. Seu desempenho é tanto melhor quanto mais precocemente for aplicado. Já em portadores de HCC tratados

com Interferon Peguilado e ribavirina e que não obtiveram a resposta virológica sustentada (RVS), o ELF se correlacionou significativamente com a progressão para desfechos clínicos desfavoráveis como ascite, encefalopatia, hemorragia digestiva varicosa e carcinoma hepatocelular (58).

Outra forma de abordar o desempenho dos marcadores não invasivos é a aplicação de modelagem estatística que não dependa da biópsia hepática como padrão ouro. Um destes modelos é a análise de classes latentes (ACL). Trata-se de um método bastante utilizado em áreas do conhecimento onde não há um padrão ouro bem estabelecido, como as ciências sociais e a psiquiatria. Apenas recentemente vem sendo utilizado em hepatologia, ainda com poucos resultados publicados(59, 60). Este tipo de análise se baseia no paradigma de “classes latentes” no qual todas as variáveis incluídas estão sob risco de falso positivo ou falso negativo, inclusive a biópsia hepática (60). A adequação deste modelo exige que as variáveis analisadas sejam independentes entre si (suposição de independência condicional)(61). Para cada desfecho estudado o indivíduo pode ter dois possíveis resultados: “presença” ou “ausência” de doença. Um padrão de referência é contruído, baseado na combinação dos resultados estimados e observados para cada paciente. A partir daí, através do método matemático chamado *Standard Maximun Likelihood,* é possível se estimar a sensibilidade e especificidade de cada teste. Ainda pouco conhecido no nosso meio, é um método promissor de avaliação de métodos não invasivos.

1 OBJETIVOS

a) Avaliar o desempenho do ELF na detecção de fibrose significativa e cirrose em uma coorte de pacientes com hepatite C crônica, e comparar seus resultados com os da EHT, utilizando a biópsia hepática como referência.

b) Propor novos pontos de corte para o ELF na detecção de fibrose significativa e cirrose em pacientes com hepatite C crônica, utilizando a biópsia hepática como pacrão ouro.

c) Avaliar o desempenho diagnóstico do ELF, APRI, EHT e da biópsia hepática, aplicando os novos pontos de corte propostos para o ELF, utilizando um modelo matemático sem padrão ouro, como a análise de classe latentes.

2 ARTIGO 1- ENHANCED LIVER FIBROSIS PANEL AS A PREDICTOR OF LIVER FIBROSIS IN CHRONIC HEPATITIS C PATIENTS

Artigo aceito para publicação na revista

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ORIGINAL ARTICLE

Enhanced Liver Fibrosis Panel as a Predictor of Liver Fibrosis in Chronic Hepatitis C Patients

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Background: Evaluation of fibrosis is crucial in the assessment of chronic hepatitis C (CHC). The enhanced liver fibrosis (ELF) is a serological panel including hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and amino-terminal propeptide of type III procollagen (PIIINP) that has shown good results in predicting liver fibrosis in distinct scenarios of chronic liver diseases.

Aims: We aimed to assess the performance of ELF on the detection of fibrosis and cirrhosis in a CHC patient cohort and to compare the results of ELF and transient elastography (TE—Fibroscan) using liver biopsy as reference.

Patients and Methods: One hundred twenty patients were prospectively evaluated by TE and ELF using an ADVIA Centaur automated system. The ELF score was calculated using the manufacturer's algorithm. Biopsies were classified according to the METAVIR score. Receiver operator characteristic curve analyses were performed to evaluate the accuracy of ELF and TE.

Results: The area under the receiver operator characteristic curve (AUROC) of ELF for the diagnosis of significant fibrosis was 0.81 [95% confidence interval (CI), 0.73-0.87], for advanced fibrosis was 0.82 (95% CI, 0.74-0.88), and for cirrhosis was 0.78 (95% CI, 0.70-0.85). Using the proposed cutoffs, ELF overestimated fibrosis in 66% (81/120) of cases and underestimated in 3% (3/120). We found no statistically significant difference when comparing the AUROC of ELF and TE for diagnosing fibrosis or cirrhosis.

Conclusions: ELF panel is a good noninvasive fibrosis marker and showed similar results to TE in CHC patients. However, new cutoff points need to be established to improve its performance on patients with CHC.

Key Words: hepatitis C, liver fibrosis, liver biopsy, transient elastography, ELF score

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The authors declare that they have nothing to disclose.
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Evaluation of fibrosis is crucial in the assessment of chronic hepatitis C (CHC), not only for indicating antiviral therapy but also for prognostic information[1] and the development of new anti-fibrotic drugs.[2] Liver biopsy remains the reference method for staging fibrosis, despite presenting many drawbacks.[3,4] It is invasive, may be painful, and, in rare cases, this procedure may lead to serious complications.[5,6] Furthermore, even with adequate fragments, the accuracy of liver biopsy may be hampered by sampling error[7,8] and intraobserver and interobserver variability in histologic staging.[9,10]

In recent years much has been researched in the field of noninvasive markers for liver fibrosis.[11,12] These markers are based on either a biochemical or a physical approach.[12] Among the biochemical markers there are plenty of serological markers, either direct or indirect, isolated or organized in scores, such as APRI,[13,14] Forn's,[15] FIB-4,[16] Lok Index,[17] Fibrotest,[18] Fibrometer,[19] and Hepascore.[20] The most prominent representative of the physical approach to assess liver fibrosis is the transient elastography (TE; Fibroscan; EchoSens, Paris, France), which is based on the estimation of liver stiffness.[21] TE has been extensively validated as a noninvasive liver fibrosis marker in large cohorts of CHC patients.[22,23] It is a simple noninvasive examination of rapid acquisition, well accepted by the patients and that may be repeated as frequently as needed.[21] Although extremely accurate in detecting cirrhosis, it presents some limitations in differentiating intermediate stages of fibrosis.[22]

In 2004, a new serological panel including markers directly related to the synthesis and degradation of the extracellular matrix (ECM) was proposed.[24] The Original European Liver Fibrosis comprised age, hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and aminoterminal propeptide of type III procollagen (PIIINP).[24] These 3 components were derived from a panel of 9 molecules involved in ECM synthesis or degradation.[24] The elevation of serum HA and the other ECM components is due to an imbalance between synthesis and degradation of fibrotic tissue, mainly by hepatic stellate cells.[25] Some years later the Original European Liver Fibrosis was simplified by removal of the age, leading to the enhanced liver fibrosis panel (ELF; Siemens Diagnostics, NY).[26] So far, the ELF panel has been studied in distinct scenarios.[27–32] Besides its accuracy for the detection of moderate and advanced fibrosis in patients with varied etiologies,[27–32] ELF also performed well in predicting disease progression in a cohort of patients with primary biliary cirrhosis.[33]

When evaluated in a cohort of 512 CHC patients, using commercial reagents different from those developed by the manufacturer of the Siemens ELF test, ELF score performed

well to predict liver fibrosis or cirrhosis.[34] In other cohort of 162 CHC patients the ELF score was measured using the Siemens ELF test and presented good results in detecting both fibrosis and cirrhosis. The clearance of the virus by treatment with pegylated interferon and ribavirin in CHC patients was associated with a statistically significant change in the ELF score, reflecting the treatment impact on the ECM composition.[35] Its use replacing TE in combined strategies with other blood tests such as Fibrotest, Fibrometer, or Hepascore has proved to be as effective as those including TE in detecting fibrosis, besides being cost-effective.[36]

Recently 2 distinct studies worked upon the normal range of ELF values in healthy subjects and found divergent results[37,38] with respect to normal ranges (range, 5.95 to 8.73[37] vs. 6.7 to 9.8[38]) and influence factors. The association between ELF values, sex, and age was controversial. Lichtinghagen et al[38] found that sex and age need to be taken into account and that afternoon values did not differ significantly from morning values. Yoo et al[37] did not found such independent association.

ELF panel performance on diagnosing fibrosis and cirrhosis has already been compared with TE, mainly in mixed cohorts of patients with promising results,[29] as well as in Asiatic patients with chronic hepatitis B.[32]

Our main aims were to assess the performance of ELF on the detection of fibrosis and cirrhosis in a cohort of patients with CHC and to compare the results of ELF and TE in the same cohort, using liver biopsy as gold standard.

PATIENTS AND METHODS

Patients with CHC who were consecutively submitted to liver biopsy, in the period of January 2011 to July 2012, in 2 Liver Units in Rio de Janeiro, were evaluated. Those patients were referenced to assess the indication for treatment of CHC, and the liver biopsy was performed as part of this routine evaluation. Exclusion criteria were: human immunodeficiency virus and hepatitis B coinfection, alcohol daily intake >20 g for women and 40 g for men, cholestasis, chronic kidney failure, right-sided heart failure, fibrogenic drugs use, biopsies with <6 portal tracts, and refusal to sign the informed consent.

Liver biopsies were performed in both centers. Included patients were submitted to the TE measurement and a nonfasting blood sample was collected at the same day. The procedures were performed in the morning to avoid HA variability. TE and blood sample were performed in the same center so that the procedures were identical for all of them. The maximal interval between the biopsy and the noninvasive tests was 3 months.

The study protocol was conducted in agreement with ethical principles, under the guidance of Declaration of Helsinki and Good Clinical Practice Guidelines. The study was approved by the local Ethics Committee. All patients signed an informed consent.

Liver Histology

Ultrasound-guided percutaneous liver biopsies were performed using a 16-G disposable Menghini needle, under local anesthesia. The specimens were fixed in formalin and embedded in paraffin. Thereafter, 5 μm thick sections were stained with hematoxylin and eosin, reticulin, and Masson's trichrome. Biopsies were classified using the METAVIR[39] scoring system by the same experienced pathologist, who was blinded to patient data. Mild or absent fibrosis, significant fibrosis, advanced fibrosis, and cirrhosis were defined as METAVIR 0 to 1, ≥F2, ≥F3, and F = 4, respectively.

ELF

A 15 mL blood sample was collected and the serum was frozen at −70°C in an interval no longer than 3 hours. PIIINP, HA, and TIMP-1 were measured in all patients in a random-access automated clinical immunochemistry analyzer that performs magnetic separation enzyme immunoassay tests (ADVIA Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY). The ELF score was calculated using the algorithm recommended in the assay [ELF = 2.278 + 0.851 ln(HA) + 0.751 ln(PIIINP) + 0.394 ln(TIMP-1)]. The cutoff points suggested by the manufacturer were: <7.7, none to mild fibrosis; ≥7.7 to <9.8, moderate fibrosis; and ≥9.8, severe fibrosis. Intra-assay and interassay reproducibility was assessed in samples with low, intermediate, and high levels of HA, PIIINP, and TIMP by testing the same sample 5 times in the same day (intra-assay variability) and 5 times in consecutive days (interassay variability). The coefficient of variation ranged from 3.23 to 5.16, 2.86 to 5.35, and from 1.53 to 3.25 for the assays of HA, PIIINP, and TIMP, respectively.

TE

TE was performed using Fibroscan (EchoSens), using the M probe, by an experienced operator blinded to the biopsy and to the ELF panel results. Details of the technique and examination procedure have been published elsewhere.[21] The results were expressed in kPa and the median value of 10 acquisitions was considered for analysis. Only examinations with a success rate of at least 60% and an IQR/M ratio of 30% were considered. If the criteria described above were not fulfilled, the test was considered as invalid. If no valid measurements were achieved the examination was considered as failure. The cutoff values applied were: 7.1 kPa for F≥2, 9.5 kPa for F≥3, and 12.5 kPa for F≥4.[40]

Statistical Analysis

Statistical analyses were performed using the SPSS 17.0 (SPSS Inc., Chicago, IL) and MedCalc 12.2.1.0 (MedCalc Software). Continuous variables were reported as mean ± SD if normally distributed. Discrete variables were reported as absolute and relative frequency.

The Mann-Whitney test was applied for the comparative analyses of ELF score between stages of fibrosis. The Spearman coefficient was used to evaluate any correlations between

TABLE 1. Baseline Characteristics of Patients

Characteristics	Patients (n = 120)
Age (y)	53 ± 11.3
Sex (male) [n (%)]	41 (34)
BMI	27 ± 4.7
ELF	9.62 ± 1.20
TE	11.39 ± 9.04
Platelet count (×109/L)	217.19 ± 68.39
Albumin (g/L)	40.9 ± 7.8
AST	72.7 ± 77.0
ALT	84.0 ± 75.4

Continuous variables reported as mean ± SD.
Discrete variables reported as absolute and relative frequency.
ALT indicate alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; ELF, enhanced liver fibrosis; TE, transient elastography.

TABLE 2. Distribution of Liver Fibrosis According to ELF Manufacturer's Cutoff Points

METAVIR fibrosis	Absent to Mild Fibrosis F0-1	Moderate Fibrosis F2	Advanced Fibrosis F ≥ 3	Total
0	0	2	0	2
1	1	53	9	63
2	0	17	19	36
3	0	2	9	11
4	1	0	7	8
Total	2	74	44	120

ELF score was calculated from patients with chronic hepatitis C, which were histologically staged according to METAVIR[37] (F0, n = 2; F1, n = 63; F2, n = 36; F3, n = 11; F4, n = 4).
Manufacturer's cutoff points were applied: < 7.7, absent to mild fibrosis; ≥ 7.7 to < 9.8 moderate fibrosis, and ≥ 9.8, severe fibrosis.
ELF indicates enhanced liver fibrosis.

ELF, Fibroscan, and fibrosis according to METAVIR.[39] The performance of ELF and TE in predicting significant fibrosis, advanced fibrosis, and cirrhosis were estimated by the areas under the receiver operator characteristic curve (AUROC). Comparisons of AUROCs were performed according the DeLong method. To deal with a possible spectrum effect, Obuchowski's measure[41,42] was implemented to compare the accuracy of ELF and TE using statistical software R 2.12. Significance level was determined when *P*-value was ≤0.05, assuming 2-tailed tests.

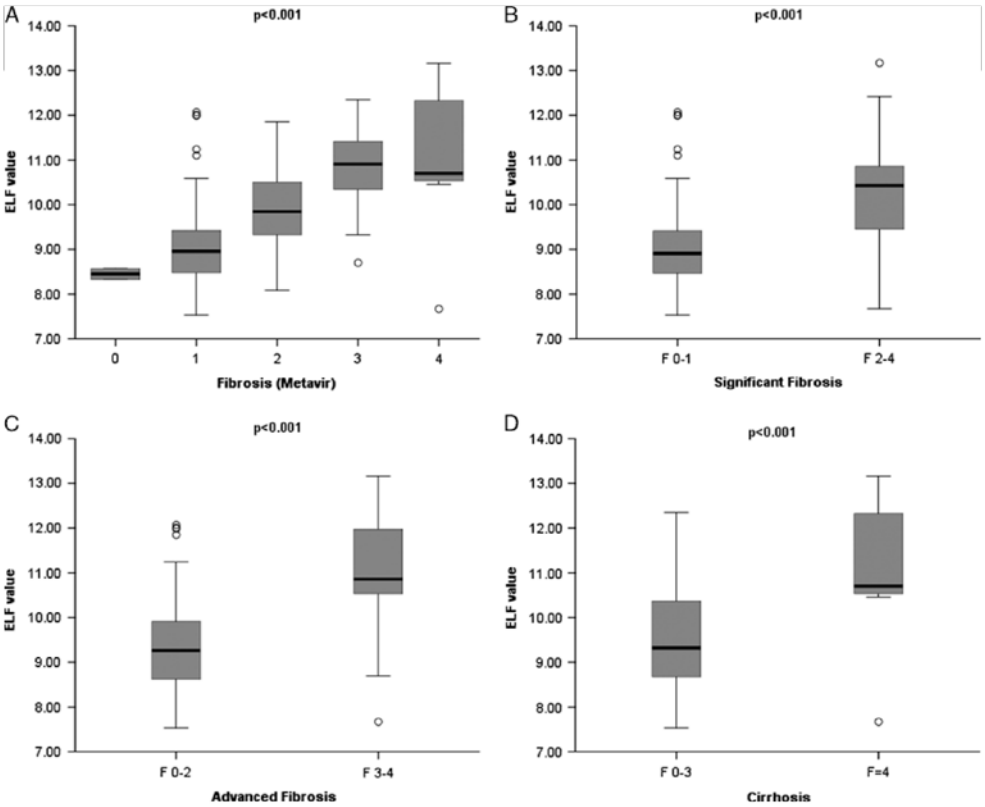

FIGURE 1. ELF according to liver biopsy stages of METAVIR. Box plot of ELF values according to the METAVIR stages of fibrosis (A) and to fibrosis categorized in significant (≥ F2) (B), advanced (≥ F3) (C), and cirrhosis (F = 4) (D). Boxes and horizontal lines within boxes represent interquartile range (IQR) and median values, respectively. The upper and lower whiskers indicate the 75th percentile plus 1.5 IQR and the 25th percentile minus 1.5 IQR respectively. ELF indicates enhanced liver fibrosis.

RESULTS

One hundred forty patients were evaluated and 20 were excluded. Among the latter, 9 patients refused to participate in the study and 11 had biopsies with < 6 portal tracts. Although it was possible to calculate the ELF in all 120 enrolled patients, in 2 of them the TE failed. The clinical and laboratorial characteristics of the 120 patients at the time of liver biopsy are shown in Table 1. The mean length of the included liver biopsies was 22.0 mm (SD ± 1.02) and the mean number of portal tracts was 11 (SD ± 4.0). Two patients presented moderate siderosis. The distribution of the whole cohort among METAVIR fibrosis stages was as follows: stage 0: 2%; stage 1: 52%; stage 2: 30%; stage 3: 9%; and stage 4: 7%.

Observing the results of ELF in our cohort, according to the manufacturer's cutoffs, we found: 2 (1%) patients with absent or mild fibrosis, 74 (62%) with moderate fibrosis, and 44 (37%) with advanced fibrosis. Using these proposed cutoffs ELF, the fibrosis status was overestimated in 66% (81/120) of cases and underestimated in 3% (3/120) with regard to the METAVIR score (Table 2). The Spearman correlation coefficient of ELF with the histologic staging was 0.57 ($P < 0.001$). There was significant difference between the median values of ELF observed for the 3 fibrosis categories studied (Fig. 1).

The diagnostic accuracy (AUROC) of ELF was 0.81 [95% confidence interval (CI), 0.73-0.87] for the diagnosis of significant fibrosis (F ≥ 2), 0.82 (95% CI, 0.74-0.88) for advanced fibrosis (F ≥ 3), and 0.78 (95% CI, 0.70-0.85) for cirrhosis. On the basis of the AUROC we calculated cutoff points for maximized sensibility and specificity, as well as the respective predictive negative and positive values, for each category of fibrosis (≥ F2, ≥ F3, and F4) (Table 3). We found no statistically significant difference when comparing the AUROC derived from ELF and TE for diagnosing significant fibrosis ($P = 0.13$), advanced fibrosis ($P = 0.08$), and cirrhosis ($P = 0.11$) (Fig. 2). We then reanalyzed the diagnostic accuracy of these 2 noninvasive markers with the Obuchowski measure, designed for nonbinary gold standards. Once again we found no statistically significant difference between ELF and TE. The overall estimate for ELF was 0.85 (95% CI, 0.81-0.90) and for TE 0.89 (CI, 0.86-0.93), $P = 0.12$.

DISCUSSION

In the present study, the ELF panel has proven to be an appropriate noninvasive marker for fibrosis in patients with CHC. Besides prospectively evaluating the Siemens ELF test in a cohort of 120 patients, we also compared it head-to-head to TE, using liver biopsy as a reference.

The ability of ELF panel to detect significant and advanced fibrosis was comparable with other studies, with AUROCs of 0.81 (95% CI, 0.73-0.87) and 0.82 (95% CI, 0.74-0.88), respectively.[26,34,28,35] However, for the detection of cirrhosis the ELF panel had an apparently inferior performance (AUROC of 0.78; 95% CI, 0.70-0.85) than those observed in other series, where AUROCs varied from 0.82 to 0.94.[24,28,34,35,43] This difference might be due to the low prevalence (6.7%) of cirrhotic patients in our series. Such lower prevalence may also explain the finding of a lower cutoff point for cirrhosis than for advanced fibrosis. Indeed, the low prevalence of cirrhotic patients is in accordance to our daily practice once cirrhotic patients are frequently diagnosed based on clinical or sonographic findings, obviating the need of biopsy.

TABLE 3. ELF Performance for Prediction of Significant Fibrosis, Advanced Fibrosis, and Cirrhosis

	AUC	95% CI	Cutoff	Se (%)	95% CI	Sp (%)	95% CI	PPV (%)	95% CI	NPV (%)	95% CI
Significant Fibrosis (F ≥ 2)	0.81	0.73-0.87	9.55	72.2	57.1-82.4	83.0	71.7-91.2	78.0	64.0-88.5	77.0	65.6-86.3
Advanced Fibrosis (F ≥ 3)	0.82	0.74-0.88	10.59	73.7	48.8-90.0	90.1	82.5-95.1	58.3	36.2-78.3	94.8	88.3-98.3
Cirrhosis (F4)	0.78	0.70-0.85	10.44	87.5	47.3-99.7	77.6	68.8-85.0	21.9	9.1-40.3	98.9	93.8-100

Area under the curves (AUC), diagnostic sensitivities, and specificities, positive and negative prospective values were calculated for ELF score. Cutoff values were calculated using receiver operating characteristics (ROC) analyses for the discrimination of significant fibrosis, advanced fibrosis, and cirrhosis, according to METAVIR.[37]

AUC indicates area under the curve; CI, confidence interval; ELF, enhanced liver fibrosis; NPV, negative prospective value; PPV, positive prospective value; Se, sensitivity; Sp, specificity.

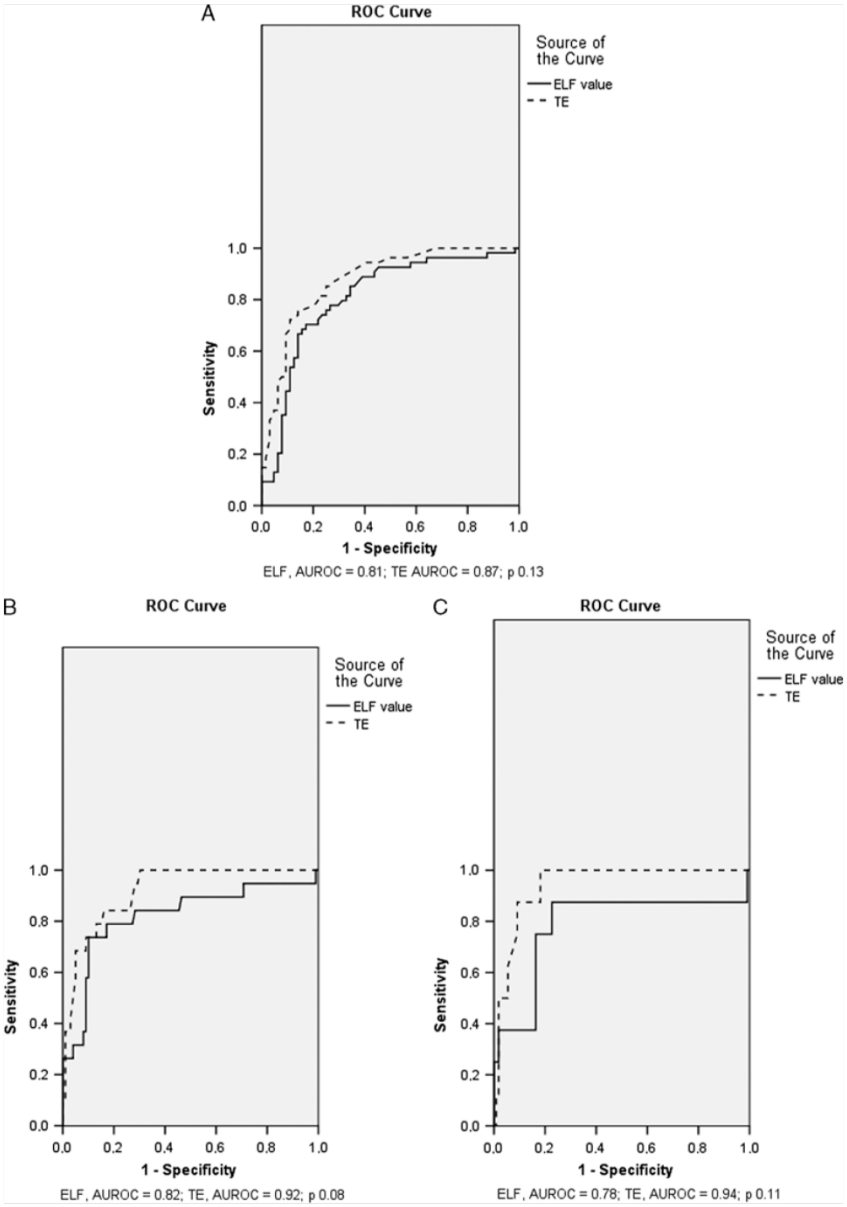

FIGURE 2. ROC analysis: ELF score versus TE. Receiver-operating characteristic (ROC) curves for ELF and TE diagnosis of significant fibrosis ($F \geq 2$) (A), advanced fibrosis ($F \geq 3$) (B), and cirrhosis (F4) (C). Areas under the curve (AUC) are indicated for both parameters.

Patients submitted to liver biopsy for evaluating treatment indication, as those of our cohort, usually present lower stages of fibrosis. Our study population is representative of the average CHC population in liver units where noninvasive markers are expected to be applied.

By using the Obuchowski measure,[41] a weighting scheme based on a reference distribution, we intended to eliminate the bias related to the distribution of fibrosis stages. The use of a weighting scheme and a penalty function prevents misclassifications with medical consequences.

With the analysis with the Obuchowski measure, ELF continued to show a good overall performance.

ELF panel had a similar performance to TE in the evaluation of liver fibrosis, without statistically significant difference. In contrast to TE, and as other serological tests, ELF offers the practical advantages of being readily available in the general laboratory, requirement of a simple blood sample, and no need for expensive investments in exclusive equipment.

To the best of our knowledge, the only study so far that compared head-to-head TE and ELF in a cohort composed exclusively of CHC patients was performed by Cobbold et al.[28] In that study 67 patients who had been submitted to liver biopsy in a maximum interval of 1 year were enrolled. Liver biopsies were considered representative based only on their length, without considering the number of portal tracts. The present study included a larger number of consecutively biopsied patients, in an interval no longer than 3 months between biopsy and blood sampling, which makes the ELF analysis more representative of the current histopathologic status.

Using the ELF panel cutoff points suggested by the manufacturer (insert package) we found overestimated results for fibrosis stage in comparison with the liver biopsy findings. We suppose this might have happened because of the mixed etiologies of liver disease included in the studies for establishment of the cutoff points. Recently Catanzaro et al[43] demonstrated a good performance of ELF panel for diagnosing fibrosis and cirrhosis in CHC patients, but the cutoff point found for cirrhosis was different from that proposed by the manufacturer. As emphasized by Sebastiani et al[44] the performance of noninvasive fibrosis biomarkers may be influenced not only by the prevalence of stages of hepatic fibrosis in the validating population sample but also by the etiology of chronic liver disease. Lichtinghagen et al,[38] after evaluating a cohort of 79 CHC patients, proposed cutoff points to significant fibrosis and advanced fibrosis identical to those indicated by the manufacturer. It is worth noticing that in their study the biopsies were classified according to Ishak and colleagues and that they correlated F0 with none to mild fibrosis, F1-4 with moderate fibrosis, and F5-6 with cirrhosis. Furthermore, 22 patients with decompensated end-stage cirrhosis were included.

Our results add extra complexity to this issue. The fact that the manufacturer's cutoff points were not adequate to our patients indicates the need of further studies dedicated to establish appropriate cutoff points for diverse clinical scenarios.

In conclusion, ELF panel had a good performance as a noninvasive fibrosis marker. The results obtained were comparable with those obtained by TE. However, there was an overestimation of fibrosis in comparison with the histologic evaluation, suggesting that new cutoff points need to be established to improve the performance of ELF for the discrimination of different stages of fibrosis in patients with CHC.

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2.1 Carta ao editor referente ao artigo 1- Important Issues in Determining The Cut-offs For Liver Fibrosis Index

Revista *Journal of Clinical Gastroenterology*

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Important Issues in Determining The Cut-offs For Liver Fibrosis Index

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Important Issues in Determining The Cut-offs For Liver Fibrosis Index

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Dear Editor,

We read with great interest the recently published article by Fernandes et al.[1] in which the authors assessed the performance of enhanced liver fibrosis (ELF) on the detection of fibrosis and cirrhosis in a chronic hepatitis C (CHC) patient cohort and to compare the results of ELF and transient elastography (TE—Fibroscan) using liver biopsy as reference. They found ELF panel as a good non-invasive fibrosis marker and showed similar results to TE in CHC patients. However, we think that there are some points that should be discussed about this study.

First, alcohol intake >20 g/day for women and >40 g/day for men was regarded as one of the exclusion criteria in the section of patients and methods. However, according to World Gastroenterology Organization Global Guidelines this cut-off should be considered as 20 g/day in women and 30 g/day in men.[2] This is critical because use of these improper alcohol consumption cut-offs may led to a bias in patient selection.

Second, in liver histology section, it is mentioned that biopsies were classified using the METAVIR scoring system by the same experienced pathologist, who was blinded to patient data. However, it is well known that the reproducibility of liver biopsy is poor owing to the heterogeneity of liver fibrosis and sample size as well as inter- and intrapathologist variability.[3,4] Therefore, it is generally recommended that at least two pathologists with appropriate experience should be involved in such studies involving histopathological scoring of chronic liver disease stage.[5]

Third, it is noted that the maximal interval between the biopsy and the noninvasive tests was 3 months. The results obtained from these non-invasiv tests can not specify the status of liver fibrosis in real-time when considering this long time interval

and short half-life of hyaluronic acid[6], tissue inhibitor of matrix metalloproteinases-1 (TIMP-1)[7] and amino-terminal propeptide of type III procollagen (PIIINP)[8] in the blood.

Fourth, authors stated that they found overestimated results [fibrosis status was overestimated in 66% (81/120) of cases] for fibrosis stage in comparison with the liver biopsy findings by using the ELF panel cut-off points suggested by the manufacturer, as shown in Table 2 in the original study. However, it would be more appropriate to determine optimum cut-offs with the highest sensitivity and specificity for significant fibrosis, advanced fibrosis and cirrhosis. If <7.7 is taken as basis, as suggested by the manufacturer for none to mild fibrosis classification, there were only two patients with F0-1, while the actual number of patients was 65 according to METAVIR classification. These findings reveal a requirement for a novel cut-off value (higher than 7.7) for determining none to mild fibrosis. A similar situation is true for another cut-off point which is suggested for severe fibrosis (≥9.8) by the manufacturer. Based on this cut-off, there were 44 patients with severe fibrosis (F3-4) while the actual number of patients was 19 according to METAVIR classification. These findings also reveal a requirement for a novel cut-off value (higher than 9.8) for determining patients with severe fibrosis.

In conclusion, determining more appropriate cut-off points specific to the pathologies involved in the etiology of liver fibrosis (like CHB) would be more convenient rather than using cut-off points suggested by the manufacturer(s).

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2.2 **Carta - Determining Cut-off points for Enhanced Liver Fibrosis Panel**

Carta submetida à revista *Journal of Clinical Gastroenterology*

Email de submissão da carta

07/01/2014

RE: JCG14335, entitled "Determining Cut-off points for Enhanced Liver Fibrosis Panel"

Dear Dr. Fernandes,

Thank you for your response. I am pleased to inform you that the Editorial Board has approved your manuscript for publication in The Journal of Clinical Gastroenterology. You will receive page proofs before the actual publication of your manuscript. PLEASE NOTE, HOWEVER, THAT NO DEFINITIVE DATE HAS BEEN SET FOR PUBLICATION OF YOUR MANUSCRIPT AT THIS TIME.

Thank you for submitting your work to our Journal, and we hope you will consider us again in the future.

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Sincerely,

Dr. Amir A. Qamar

Senior Associate Editor

Journal of Clinical Gastroenterology

Journal of Clinical Gastroenterology

Determining Cut-off points for Enhanced Liver Fibrosis Panel

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"Determining Cut-off points for Enhanced Liver Fibrosis Panel"

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Financial support

The study was supported by Siemens Healthcare by providing ELF test kits for the determinations of ELF score.

Conflict of Interest Disclosure

The authors disclosure no conflict of interest**.**

We acknowledge Dr. Sertoglu et al[1]. for their interest on our recent study of Enhanced Liver Fibrosis (ELF) Panel performance in Chronic Hepatitis C (CHC). We would like to add some comments to this valuable discussion. We agree that improper alcohol consumption cut-offs may lead to a bias in patient selection. The World Gastroenterology Organization Global Guidelines[2] has recently proposed an alcohol intake cut-off of 20 g/day in women and 30 g/day in men in order to distinguish non alcoholic fat liver disease from alcoholic liver disease. This is not the case in CHC population, where our aim was mainly to exclude alcoholic disease as the predominant etiology of chronic liver disease.

Much has been debated over the role of liver biopsy as a gold standard for liver fibrosis [(3,4)] and so far there is no better surrogate to replace it [5]. However, it has already been demonstrated by Bedossa et al[6] that METAVIR scoring provides a high degree of interobserver concordance for evaluation of fibrosis in hepatitis C patients. Furthermore, many other noninvasive tests have been validated in cohorts with only one experienced pathologist[7-10]. What outstands as a consensus is that the length of the liver fragment must be optimized in order to avoid misclassification [11-13]. It has been recommended that fragments should have at least 6 portal tracts and 25mm length[13]. In our study we excluded biopsies with less than 6 portal tracts and obtained a mean length of the included liver biopsies of 22.0mm (SD±1.02) and a mean number of portal tracts of 11(SD±4.0).

Regarding interval between the biopsy and the noninvasive tests, it is well known that liver fibrogenesis is a slow process and it's usually necessary at least 3 years for a progression of 1 point in liver stage in around 30% of patients [14,15]. Furthermore, the

study of Parkes et al[9] included patients whose biopsy had been performed within 6 months from the blood sample. Based in these concepts, we consider that an interval of up to three months may not hamper our results.

We strongly agree that it is necessary to establish new cut-off points for ELF as a predictor of liver fibrosis in CHC patients. That was the conclusion of our article. We did not proposed such cut-offs because cirrhosis was weakly represented in our population. We are already working in a large population to propose such cut-offs.

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3 ARTIGO 2 - ENHANCED LIVER FIBROSIS PANEL (ELF): NEW CUT-OFF POINTS FOR CHRONIC HEPATITIS C PATIENTS

Artigo submetido à revista *Journal of Clinical Gastroenterology*

Email de submissão do artigo 2

07/28/2014

Dear Dr. Fernandes,

Your submission entitled "Enhanced Liver Fibrosis Panel (ELF): new cut-off points for chronic hepatitis C patients" has been assigned the following manuscript number: JCG14438.

Please use the above manuscript number for all correspondence relating to your article.

Thank you for your interest in our Journal.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author.

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Sincerely,

Candace Peabody
Editorial Assistant
Journal of Clinical Gastroenterology

Enhanced Liver Fibrosis Panel (ELF): new cut-off points for chronic hepatitis C patients

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Conflict of Interest Disclosure

The authors disclosure no conflict of interest**.**

List of abbreviations

CHC	chronic hepatitis C
ELF	enhanced liver fibrosis
HA	hyaluronic acid
TIMP-1	tissue inhibitor of metalloproteinases 1
PIIINP	amino-terminal propeptide of type III procollagen
ECM	extra-celular matrix
TE	transient elastography
ROC	receiver operating characteristics
AUROC	area under the receiver operator characteristic curve
SD	standard deviation

Abstract

Determining liver fibrosis stage in chronic hepatitis C patients (CHC) is very important both for management as for prognosis of the disease. The enhanced liver fibrosis (ELF) is a serological panel including hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and amino-terminal propeptide of type III procollagen (PIIINP) that has shown good results in predicting liver fibrosis. However, new cutoff points need to be established to improve its performance on patients with CHC.

Aim: to propose new cut-off points of ELF panel for significant fibrosis and cirrhosis in CHC patients.

Patients and Methods: Four hundred and two patients were prospectively evaluated by ELF panel. Biopsies were classified according to the METAVIR score. Receiver operator characteristic curve analyses were performed to propose the best cut-off points for significant fibrosis and cirrhosis.

Results: For the diagnosis of significant fibrosis (Metavir F≥2) a cut-off value >9.37 provides a sensitivity 76% of and a specificity of 79%. The areas under the receiver operator characteristic curve, positive and negative predictive values were 0.81, 78% and 76%, respectively ($p<0.001$). For the diagnosis of cirrhosis (Metavir F=4) a cut-off value >10.31 provides a sensitivity of 81% and a specificity of 79%. The areas under the receiver operator characteristic curve, positive and negative predictive values were 0.79, 23% and 98%, respectively ($p<0.001$).

Conclusion: The new cut-off points proposed in this study shall improve the clinical applicability of this biomarker in the most clinically relevant situations, such as in the diagnosis of significant fibrosis and cirrhosis.

Key Words: Hepatitis C, Liver fibrosis, Liver Biopsy, cutoff points, ELF panel.

Introduction

For almost two decades non invasive fibrosis markers have been used to assess fibrosis stage in chronic hepatitis C (CHC) patients. Determining liver fibrosis stage is decisive for the appropriate management of the disease[1] and is a strong indicator of prognosis[2]. Ideally, the true gold standard for liver fibrosis stage would be the evaluation of the whole liver[3,4]. Since this is not feasible, liver biopsy has been adopted as the best possible reference[5]. However, several drawbacks of this approach have been pointed by many investigators[6-11]. Diverse surrogate serum biochemical biomarkers have been proposed as an alternative to liver biopsy. Most of them represent mathematical scores built on “indirect” serum markers of fibrosis, such as APRI[12], Fibrotest®[13], Fibrometer®[14], FIB-4[15] and Hepascore®[16].

Enhanced Liver Fibrosis (ELF) panel comprises the serum concentration of hyaluronic acid (HA), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), and aminoterminal propeptide of type III procollagen (PIIINP). When compared to scores based on “indirect” markers, ELF has the advantage of measuring only components directly involved in the fibrogenesis process and extra-cellular matrix (ECM) remodeling[17,18]. ELF has been validated for liver fibrosis staging in many clinical scenarios[19-2122-25].

In CHC patients ELF panel presented a good performance in detecting both fibrosis and cirrhosis[18,26-29]. A statistically significant reduction in ELF score was observed after the clearance of the virus by treatment with pegylated interferon and ribavirin, reflecting the treatment impact on the ECM composition[30]. ELF was also capable of stratifying the risk of subsequent progression to clinical outcomes in CHC patients[31]. Furthermore, ELF performed as well as transient elastography (TE) in CHC patients[26,32]. It was demonstrated that it is effective in replacing TE in combined strategies with other blood tests, such as Fibrotest®, Fibrometer®, or Hepascore® with the advantage of being cost-effective[33].

Recently, a meta-analysis has demonstrated that ELF has good diagnostic performance for assessing liver fibrosis, with receiver operator characteristic curve (AUROC) of 0.88, 0.86, and 0.87 for significant and severe liver fibrosis and cirrhosis, respectively. The study postulates that at least 74% of patients could have the liver biopsy avoided. One point of criticism was the lack of a consistent cut-off value

throughout the various studies[34]. In accordance to other groups, we recently showed that ELF performs well [32, 28, 27,29], but needs better cutoff points to improve its performance in clinical practice [28,29].

The aim of this study was to propose new cut-off points of ELF panel for the detection of significant fibrosis and cirrhosis in CHC patients.

Material and Methods

Patients with CHC submitted to liver biopsy were prospectively included in the study from January 2011 to July 2013 in three Liver Units in Rio de Janeiro, Brazil. Patients had been referred to the Liver Units to assess the indication for treatment and liver biopsy was performed as part of routine evaluation. Exclusion criteria were: human immunodeficiency virus and hepatitis B co-infection, alcohol daily intake >20g for women and 40g for men, cholestasis, chronic kidney failure, right-sided heart failure, use of fibrogenic drugs, biopsy specimens with less than six portal tracts, and refusal to sign the informed consent. Included patients had a non-fasting blood sample collected in a maximal interval of 3 months of liver biopsy. The study protocol was conducted in agreement with ethical principles, under the guidance of Declaration of Helsinki and Good Clinical Practice Guidelines. The study was approved by the local Ethics Committees. All patients signed the informed consent.

Liver Histology

Ultrasound-guided percutaneous liver biopsy was performed using a 16-G disposable Menghini needle, under local anesthesia. The specimens were fixed in formalin and embedded in paraffin. Thereafter, 5mm thick sections were stained with hematoxylin and eosin, reticulin, and Masson's trichrome. Histological specimens were classified using the METAVIR[35] scoring system by one experienced pathologist, who was blinded to patient data. Mild or absent fibrosis, significant fibrosis, advanced fibrosis, and cirrhosis were defined as METAVIR 0 to 1, F≥2, F≥3, and F4, respectively.

Determination of the Enhanced Liver Fibrosis (ELF) panel

A 15mL blood sample was collected and the serum was frozen at -70ºC in an interval no longer than 3 hours. PIIINP, HA, and TIMP-1 were measured in all samples in a random access automated clinical immunochemistry analyzer that performs magnetic separation enzyme immunoassay tests (ADVIA Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY). The ELF score was calculated using the algorithm recommended in the assay [ELF=2.278+0.851 ln(HA)+ 0.751 ln(PIIINP)+0.394 ln(TIMP-1)]. Intra-assay and inter-assay reproducibility was assessed in samples with low, intermediate, and high levels of HA, PIIINP, and TIMP by testing the same sample five times in the same day (intra-assay variability) and five times in consecutive days (inter-assay variability). The coefficient of variation ranged from 3.23 to 5.16 (for HA), 2.86 to 5.35 (for PIIINP), and from 1.53 to 3.25 (for TIMP-1).

Statistical Analysis

Statistical analyses were performed using the SPSS 17.0 (SPSS Inc., Chicago, IL) and MedCalc 12.2.1.0 (MedCalc Software). Continuous variables were reported as mean± standard deviation (SD) if normally distributed. Discrete variables were reported as absolute and relative frequency. The Mann-Whitney test was applied for the comparative analyses of ELF score between stages of fibrosis. The performance of ELF in predicting significant fibrosis, advanced fibrosis, and cirrhosis was estimated by the AUROC.

Results

The clinical and laboratorial characteristics at the time of liver biopsy of the 402 included patients are shown in Table I. The distribution of the whole cohort among METAVIR fibrosis stages was as follows: stage 0 - 3%; stage 1 - 47%; stage 2 - 27%; stage 3 - 16%; and stage 4 - 7%. The Spearman correlation coefficient of ELF with the histological staging was 0.57 ($p<0.001$). The median value of ELF in the fibrosis stages were: 8.63±0.60 in stage 0; 8.84±1.20 in stage 1; 9.63±1.28 in stage 2; 10.34±0.97 in stage 3 and 10.88±1.50 in stage 4 (Fig1). In the comparative

analysis, there was significant difference between the median values of ELF observed for the three fibrosis categories studied (Fig2). The diagnostic accuracy (AUROC) of ELF was 0.81 [95% confidence interval (CI), 0.77-0.85] for the diagnosis of significant fibrosis (≥F2), 0.81 (95% CI, 0.77-0.85) for advanced fibrosis (≥F3), and 0.79 (95% CI, 0.75-0.83) for cirrhosis (Fig3). On the basis of the AUROC we calculated cutoff points for maximized sensitivity and specificity, as well as the respective predictive negative and positive values, for each category of fibrosis (≥F2, ≥F3, and F4) (Table II).

Discussion

This study aimed to evaluate the best cut-off points of ELF score in a large cohort of patients with chronic hepatitis C submitted to liver biopsy. In our previous article we demonstrated a good performance of ELF panel in detecting both significant fibrosis and cirrhosis, but alerted to the fact that the manufacturer cut-off points overestimated the stages of fibrosis in a large proportion of patients[32]. In the present study, we propose adjusted ELF panel cut-off points that add value to the accuracy in determining the degree of fibrosis in CHC patients.

Some other groups have found results similar to ours[28,29]. In the Fibrostar Study Group with CHC patients, Guechot et al[28] found AUROC values of 0.78 and 0.85 for significant fibrosis and cirrhosis, respectively. In their cohort the best ELF panel cut-off points for significant fibrosis and cirrhosis would be 9.0 and 9.35, respectively, both of which different from the cut-off values originally proposed by the manufacturer. Lichtinghagen et al [29] observed that in CHC patients ELF panel values between 9.8 and 11.3 cannot be clearly attributed to a specific stage of fibrosis and proposed that an ELF score of 11.3 would be the appropriate threshold for cirrhosis.

The gray zone in the ELF panel may be explained by the dynamic nature of its components as well as by the relatively wide variation in the normal range for these parameters. In addition, HA, TIMP-1 and PIIINP are not only representative of ECM deposits. Conversely, they reflect a continuing process of deposition and degradation of ECM, which is also correlated to the histological inflammatory activity in the tissue[29]. This might indicate that ELF panel is not a good test for defining

intermediate stages of fibrosis, especially in conditions with a relatively high inflammatory component[36].

Based on our findings we recommend that new cut-off points should be adopted for the ELF panel specially when using the automated reagents for Siemens ELF™, which has the advantage be performed in a single analyzer with only one serum sample. We believe that the most important applicability of non-invasive tests is the detection of significant fibrosis and cirrhosis rather than staging fibrosis. As our results showed, the ELF panel can be used as a screening test to distinguish patients with significant liver fibrosis, usually indicated for treatment, from those with none or minimal fibrosis. With an AUROC of 0.81, which is very similar to other studies[18,20,28,36], the ELF panel cut-off of 9.37 could detect the presence of significant fibrosis with a sensitivity of 75%. For cirrhosis, although the AUROC was a little lower than other studies[18,20,28,29,36] the cut-off of 10.31 had a negative predictive value of 98%, which would be very useful in clinical practice.

A possible limitation of the present study is the low number of cirrhotic patients included. However, the low prevalence of cirrhotic patients is in accordance to the daily practice, since cirrhotic patients are frequently diagnosed based on clinical or sonographic findings, obviating the need of biopsy.

In conclusion, the ELF panel score has a good performance as a non-invasive marker of fibrosis in hepatitis C patients. The new cut-off points proposed in this study showed a better diagnostic performance and shall improve the clinical applicability of this biomarker in the most clinically relevant situations, such as in the diagnosis of significant fibrosis and cirrhosis.

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Table I: Baseline characteristics of patients

Characteristics	**Patients (n=402)**
Age (years)	51 ± 11.3
Gender (male/total,n,%)	164/405 (40)
ELF panel	9.43 ± 1.35
AST (U/L)	68.0± 59.90
ALT (U/L)	93.40 ± 68.80
Biopsy fragment length (cm)	2.32 ± 0.38
Fibrosis, METAVIR	
F0-F1	201 (50)
F2	109 (27)
F3	64 (16)
F4	28 (7)

Continuous variables reported as mean ± Standard Deviation. Discrete variables reported as absolute and relative frequency. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF, enhanced liver fibrosis panel.

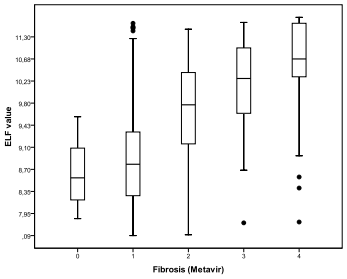
Table II

	AUC	95% CI	Cut-off	Se (%)	Sp (%)	PPV (%)	NPV(%)
Significant Fibrosis (F≥2)	0.81	0.77-0.85	9.37	76%	79%	78%	76%
Advanced Fibrosis (F≥3)	0.81	0.77-0.85	9.47	84%	67%	43%	93%
Cirrhosis (F4)	0.79	0.75-0.83	10.31	81%	78%	23%	98%

ELF, Enhanced Liver Fibrosis; AUC, area under the curve; CI, confidence interval; Se, sensitivity; Sp, specificity; PPV, positive prospective value; NPV, negative prospective value.

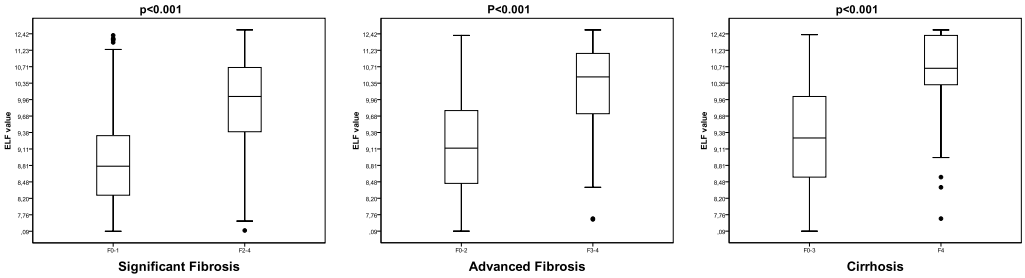
Area under the curves (AUC), diagnostic sensitivities and specificities, positive and negative prospective values were calculated for ELF score. Cut-off values were calculated using receiver operating characteristics (ROC) analyses for the discrimination of significant fibrosis, advanced fibrosis and cirrhosis, according to Metavir et al.

Figure 1: Median value distribution of ELF panel according to Metavir fibrosis stages



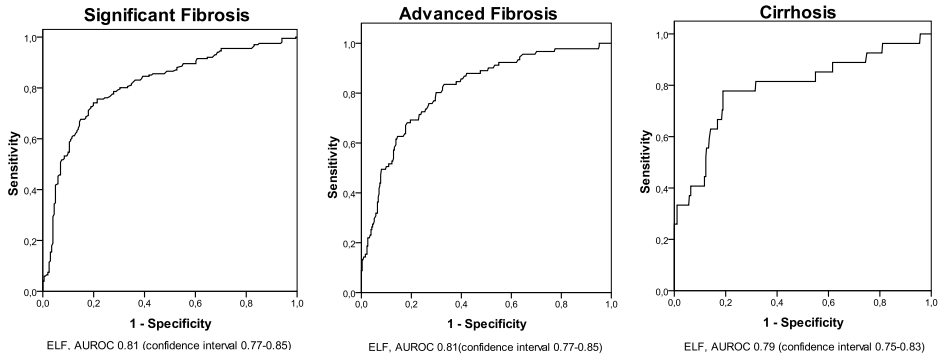
Box plot of ELF panel values according to the METAVIR stages of fibrosis. Boxes and horizontal lines within boxes represent interquartile range (IQR) and median values, respectively. The upper and lower whiskers indicate the 75th percentile plus 1.5 IQR and the 25th percentile minus 1.5 IQR respectively. ELF indicates enhanced liver fibrosis.

Figure 2: Median value of ELF according to the fibrosis categories



Box plot of ELF values according to the METAVIR stages of fibrosis. Boxes and horizontal lines within boxes represent interquartile range (IQR) and median values, respectively. The upper and lower whiskers indicate the 75th percentile plus 1.5 IQR and the 25th percentile minus 1.5 IQR respectively. ELF indicates enhanced liver fibrosis

Figure 3: Diagnostic accuracy of ELF according to the fibrosis categories



ROC analysis:.area under receiver-operating characteristic (AUROC) curves for ELF for significant fibrosis (F≥2), advanced fibrosis (F≥3) and cirrhosis (F4).

4 ARTIGO 3 - DIAGNOSTIC ACCURACY OF NON-INVASIVE METHODS AND LIVER BIOPSY BY LATENT CLASS ANALYSIS IN CHRONIC HEPATITIS C: AN APPROACH WITHOUT A GOLD STANDARD

Title page

Diagnostic accuracy of non-invasive methods and liver biopsy by Latent Class Analysis in chronic hepatitis C: an approach without a gold standard

Short title: A Latent Class Analysis for assessing liver fibrosis in CHC patients

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Abstract

Background and Aims: Non-invasive methods to assess liver fibrosis, such as enhanced liver fibrosis (ELF), aspartate-to-platelets ratio (APRI) and transient elastography (TE) have been validated in chronic hepatitis C (CHC) using liver biopsy as reference. The aim was to assess the diagnostic accuracy of TE, APRI, ELF and liver biopsy using a mathematical modelling without gold standard, such as Latent Class Analysis (LCA)

Methods: Consecutive CHC naive-patients submitted to ELF, APRI, TE and liver biopsy in a maximal delay of 3 months were eligible. TE was performed by an experimented operator blinded to tests results and biopsies were analyzed by a single experienced pathologist blinded to patient data. Significant fibrosis was defined as ELF≥9.37, APRI≥1.5, TE≥7.1 kPa or liver biopsy as METAVIR F≥2, respectively. Cirrhosis was defined as ELF≥10.31, APRI≥2.0, TE≥12.5 kPa or liver biopsy as METAVIR F=4, respectively.

Results: 117 patients [34% male, 55 years, BMI 26 Kg/m²; ALT 57U/L] were included. In the LCA model, sensitivity and specificity (95%CI) were 0.92(0.86-0.98) and 0.79(0.72-0.86); 0.47(0.40-0.54) and 0.99(0.95-1.00); 0.81(0.74-0.88) and 0.78(0.71-0.85); 0.86(0.68-1.00) and 0.91(0.79-1.00) for diagnosis of significant fibrosis and 0.92(0.76-1.00) and 0.94(0.91-0.94); 0.57(0.37-0.77) and 0.97(0.93-1.00); 0.94(0.84-1.00) and 0.88(0.82-0.94); 0.30(0.12-0.48) and 1.00 for cirrhosis by TE, APRI, ELF and liver biopsy, respectively. Using liver biopsy as a gold standard, the area under the receiver operator characteristic curve [AUROC (95%CI)] were 0.874 (0.811-0.937), 0.810 (0.732-0.887) and 0.807 (0.725-0.889) for diagnosis of significant fibrosis and 0.942 (0.890-0.993), 0.767 (0.585-0.948) and 0.783 (0.555-1.00) for cirrhosis diagnosis by TE, APRI, ELF, respectively.

Conclusion: In a model without a gold standard, non-invasive methods were accurate to stage fibrosis and liver biopsy should not be considered as a perfect gold standard.

Key words: accuracy; non-invasive methods; fibrosis; latent classes

Background

Chronic hepatitis C (CHC) remains a major public health issue representing one of the leading causes of cirrhosis worldwide [1]. Liver fibrosis assessment in CHC patients has implications for therapeutic and prognostic purposes [2]. Historically, liver biopsy has been used to assess hepatic fibrosis. However, it is an invasive method and might be associated with potential complications [3]. There is a continuous effort to develop non-invasive methods in order to avoid liver biopsy and its drawbacks. These methods might be classified as biological markers, such as FibroTest, FibroMeter, aspartate-to-platelet ratio index (APRI) and enhanced liver fibrosis (ELF) or physical methods, such as transient elastography (TE) [4]. Diagnostic accuracy of these non-invasive markers has been validated using liver biopsy as a reference. However diagnostic performance of this gold-standard has been challenged by the quality of histological specimen [5], sampling error [6] and intraobserver variability [7]. Therefore, diagnostic accuracy of non-invasive methods might be hampered because liver biopsy is not a perfect gold standard [8].

Latent Class Analysis (LCA) is a mathematical modelling currently applied to evaluate accuracy of diagnostic tests when a gold standard is lacking, as for qualitative social research [10]. In this methodology a reference standard is constructed based on the combination of observed and estimated tests results from each patient [9]. So far, this methodology has been used in very few studies in Hepatology.

The aim of the study was to assess the diagnostic accuracy of TE, APRI, ELF and liver biopsy using Latent Class Analysis, a mathematical modelling without a gold standard.

Material and Methods

Study design

This cross-sectional study was conducted in two centers (University of the State of Rio de Janeiro and Bonsucesso Federal Hospital) in Rio de Janeiro, Brazil. The inclusion criteria were age older than 18 years and presence of HCV-RNA in blood serum. The exclusion criteria were co-infection by chronic hepatitis B or human deficiency virus, self-reported excessive alcohol intake (>40g/day in men and >20g/day in women), chronic kidney disease, biopsy specimens with less than six portal tracts and non-applicability of tests.

Liver fibrosis staging was classified according to the METAVIR scoring [11]. Significant fibrosis and cirrhosis were defined as fibrosis stage F≥2 and F=4, respectively. Non-invasive tests and liver biopsy were performed in a maximum delay of 3 months. The study protocol was conducted in accordance with the Helsinki Declaration, and was approved by the local Ethics Committee. All patients signed an informed consent upon enrollment in the study.

Transient Elastography (TE)

TE was performed with a M probe of FibroScan® (EchoSens, Paris, France) by an experimented operator (>500 exams) blinded to others tests results, following a validated procedure [12]. TE was considered unreliable in presence of any of the following criteria: (i) < 10 successful measurements; (ii) an interquartile range higher than 30% of the median value; and (iii) a success rate, considered as the ratio between the number of valid and total measures, lower than 60% [13, 14]. Liver stiffness was considered as the median of all valid measurements.

Liver fibrosis staging, estimated by TE, was converted to the METAVIR scoring system [11] as proposed by Castera et al. [15]: <7.1 as F0F1; 7.1–9.4 as F2; 9.5–12.4 as F3 and >12.4 kPa as F4.

Aspartate-to-Platelets Ratio Index (APRI)

APRI was calculated according to the following formula: AST level (/ULN) / Platelets count (10^9/L) * 100. Liver fibrosis, estimated by APRI, was converted to the METAVIR scoring system [11] as proposed by Wai et al. [16]: ≥1.5 as F≥2 and ≥ 2 as F=4.

Enhanced Liver Fibrosis (ELF)

Calculation of ELF was performed in a frozen serum (-70°C) that was collected in the same day of TE. Serum amino-terminal propeptide of type III procollagen (PIIINP), hyaluronic acid (HA) and tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) were measured in a random access automated clinical immunochemistry analyzer that performs magnetic separation enzyme immunoassay tests (ADVIA Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY). The ELF score was calculated using the algorithm recommended by the manufacturer (Siemens, NY, USA): ELF=2.278+0.851 ln(HA)+0.751 ln(PIIINP)+0.394 ln(TIMP-1). Liver fibrosis,

estimated by ELF, was converted to the METAVIR scoring system [11] as proposed by Fernandes et al (ref pending): >9.37 as F≥2 and > 10.31 as F=4.

Liver biopsy

Percutaneous liver biopsies were performed using a 16-G Menghini needle guided by ultrasound under local anesthesia. The specimens were fixed in formalin, embedded in paraffin and cut in 5mm thick sections that were stained with hematoxylin and eosin, reticulin, and Masson's trichrome. Biopsies were classified using the METAVIR [11] scoring system by the same experienced pathologist, who was blinded to patient data. Patients having a liver biopsy specimen with less than 6 portal tracts were excluded.

Latent class analysis (LCA)

LCA is a mathematical modelling that estimates diagnostic accuracy of tests in a scenario where there is not a gold standard. The true disease status of an individual can be considered as a categorical latent variable such as "disease" or "no disease", which are named "latent classes". Through a mathematical method named *standard maximum likelihood* the modelling aims to obtain a unique solution for constructing a reference standard. Therefore sensitivities and specificities for each test can be estimated [17]. The assumption of conditional independence among tests must be respected and data must fit into the model (likelihood ratio goodness-of-fit value [likelihood squared (L^2)] significance > 0.05) [17].

In the present study, the LCA models were constructed upon four conditionally independents tests: APRI, ELF, TE and liver biopsy. APRI and ELF are both biological markers but include different parameters. TE is a physical method and liver biopsy is based on histological analysis. Two Latent Class (2LC)-models were fitted, one for presence or absence of significant fibrosis and other for presence or absence of cirrhosis. In each one of the models the patients status could be classified in two mutually exclusive groups: presence or absence of "disease". Using four tests with a dichotomous result (i.e. positive or negative) in each patient, there were 16 possible combinations for each clinical endpoint. The likelihood of observing each pattern of test results was calculated according to the probability for a positive or negative test. The number of expected cases estimated by LCA models was compared to observed cases for each of the patterns of test results.

Statistical Analysis

Continuous variables were reported as median [Inter-Quartil Range] and discrete variables were reported as absolute and relative frequency. Non-parametric tests, Mann-Whitney test for quantitative and Fisher's exact test for qualitative comparisons, were applied. Significance level was determined when $p \leq 0.05$ assuming two-tailed tests.

In the classical 2 x 2 analysis, the performances of TE, APRI and ELF were assessed using the fibrosis stage obtained by liver biopsy, the classical gold standard. The standard area under the Receiver Operating Characteristics Curve (AUROC) for each method was estimated by the empirical (non-parametric) method.

In the LCA, the sensitivity and specificity of each test, including liver biopsy, was assessed without a gold standard. For estimation of tests accuracy for significant fibrosis and cirrhosis by LCA, the 2LC-model that assumed the conditional independence among tests was compared to models with direct effect between tests. The model that better fits for LCA was chosen based on the following criteria: the p-value of the likelihood squared (L^2) had to be greater than 0.05 and the Bayesian information criterion (BIC) had to be the smallest among all competing models. Statistical analyses were performed using STATA statistical package for Windows (2012; StataCorp LP, College Station, TX, USA) and LEM, version 1.0 (Vermunt, 1997, unpublished).

Results

Among 131 consecutive CHC patients submitted to liver biopsy 117 patients [34% male gender, median (IQR) age of 55 (48-62) years and BMI of 26 (24-30) Kg/m²] were included. Patients were excluded due to low quality (less than 6 portal tracts) of liver biopsy specimen (n=11) or unreliable TE (n=3). It was possible to calculate APRI and ELF in all patients. According to liver biopsy, the prevalence of significant fibrosis and cirrhosis were 46% and 7%, respectively. Table 1 summarizes included patients characteristics.

Classical validity analysis using liver biopsy as a gold standard

Using liver biopsy as reference, for diagnosis of significant fibrosis the performance [AUROC (95%CI)] of TE, APRI and ELF were 0.874 (0.811-0.937),

0.810 (0.732-0.887) and 0.807 (0.725-0.889), respectively. In addition, the performance [AUROC (95%CI)] of TE, APRI and ELF for cirrhosis were 0.942 (0.890-0.993), 0.767 (0.585-0.948) and 0.783 (0.555-1.000), respectively. Sensitivities, specificities and positive likelihood ratio of non-invasive methods are summarized in Table 3.

Latent Class Analysis without a gold standard

The 2LC-model that respected the conditional independence among 4 tests (i.e. without direct effect between tests) was the model that better fitted data for LCA. This model presented the lower BIC among the competitive models and a non-significant L^2 p-value for diagnosis of significant fibrosis and cirrhosis (Table 2). The observed and estimated patient's distribution according to the 4 tests results for diagnosis of significant fibrosis and cirrhosis are shown in Table 3. The tests were perfectly concordant in 54 (46%) patients (all positive in 20 and all negative in 34 patients) for diagnosis of significant fibrosis and in 77 (66%) patients (all positive in 4 and all negative in 73 patients) for cirrhosis diagnosis.

For diagnosis of significant fibrosis the sensitivities (95% CI) were 0.92 (0.86-0.98), 0.47 (0.40-0.54), 0.81 (0.74-0.88) and 0.86 (0.68-1.00); the specificities (95% CI) were 0.79 (0.72-0.86), 0.99 (0.95-1.00), 0.78 (0.71-0.85) and 0.91 (0.79-1.00) for TE, APRI, ELF and liver biopsy, respectively. For cirrhosis the sensitivities were 0.92 (0.76-1.00), 0.57 (0.37-0.77), 0.94 (0.84-1.00) and 0.30 (0.12-0.48); the specificities were 0.94 (0.91-0.97), 0.97 (0.93-1.00), 0.88 (0.82-0.94) and 1.00 for TE, APRI, ELF and liver biopsy, respectively (Table 4).

Comparing to the classical analysis the diagnostic accuracy was increased for the non-invasive methods when analyzed by LCA. For the cirrhosis diagnosis, sensitivity of liver biopsy was reduced and its specificity was similar in LCA compared to classical analysis when liver biopsy was the gold standard (i.e. sensitivity and specificity = 1.00) (Table 4).

Discussion

This study highlights that diagnostic accuracy of non-invasive methods to estimate liver fibrosis can be assessed by a model without a gold standard. Historically, sensitivities and specificities of these methods must have been biased by limitations of liver biopsy which is an imperfect gold standard.

Liver biopsy has been used as an unquestionable gold standard for liver fibrosis staging. However, this method has been challenged by its feasibility and several limitations. LCA has been described as an accurate methodology to evaluate performance of tests in the absence of gold standard [18] when some specific criteria are respected [17]. Although extensively used in medical fields such as psychiatry, few studies have evaluated liver fibrosis assessment latent classes models.

Having liver biopsy as the reference, we found accuracy of TE, APRI and ELF for significant fibrosis and cirrhosis similar to described in previous meta-analyses [19-21]. According to our results, TE was the most sensitive and APRI the most specific non-invasive method for diagnosing significant fibrosis and cirrhosis. TE showed a better performance [AUROC (95%CI)] for diagnosis of significant fibrosis [0.874(0.811-0.937)] and cirrhosis [0.942(0.725-0.889)].

In our study, latent class rules were strictly respected. Four conditionally independent tests were analyzed, such as a physical method, a histological analysis and two biological algorithms that consist of totally different parameters. In addition, the 2LC-model without direct effect between tests was the model that data better fitted (lower BIC among competitive models and a non-significant L^2 p-value). Considering LCA, the sensitivity of non-invasive methods for diagnosis of significant fibrosis was increased compared to classical analysis. Similar results for TE and FibroTest, a biomarker to assess liver fibrosis, were also previously reported by authors that used the same mathematical model [8]. In the present study, according to LCA, TE and ELF were the most sensitive tests for diagnosis of significant fibrosis and cirrhosis, respectively and APRI was the most specific test for both diagnoses.

Previous authors also reported a decrease in the performance of liver biopsy in LCA [8]. In our study, sensitivity and specificity of liver biopsy decreased in LCA for diagnosis of significant fibrosis yielding 14% of false-negative and 9% of false-positive patients. However, we had a more important decrease in the sensitive of liver biopsy for cirrhosis diagnosis [0.30 (95%CI 0.12-0.48)] than previously reported. Poynard et al showed a sensitivity decrease from 1.00 (as a gold standard) to 0.51 when LCA was applied [8]. This might be explained by the difference among the studied populations. The accuracy of a test often varies along with the distribution of fibrosis stages, the so-called disease spectrum, which might interfere with sensitivity/specificity [22, 23]. We mostly included female patients with mild fibrosis (54%) and low prevalence of cirrhosis (7%). In the other hand, Poynard et al had a

more homogenous population, with 10-fold more patients, most male gender with 15% of cirrhotic patients [8]. In both studies, specificity of liver biopsy did not change in LCA compared to classical analysis (i.e. almost perfect).

Limitations of liver biopsy as a gold standard could be explained by sampling error, quality of specimen and interobserver variability of histological analysis. Regev et al reported a considerable discrepancy between liver biopsies performed in both hepatic lobes of the same patient [24]. Colloredo et al demonstrated that liver specimen length should follow the statement that "bigger is better" [5]. Bedossa et al have described that even the "classical 20mm-length" sample could misclassify liver fibrosis in a quarter of patients [6]. Mehta et al estimated the magnitude of the bias of diagnostic performance of non-invasive methods due to liver biopsy limitations [25]. Using liver biopsy as the reference, a non-invasive marker diagnostic performance may never reach the maximal AUROC value due to the gold standard issues [26]. Poynard et al, analyzing digitalized images of 27,869 virtual biopsies from surgical samples collected in patients submitted to partial hepatectomy, reinforced that liver biopsy is not perfect [27]. These results demonstrated that we must move forward in the field of evaluation of performance of non-invasive methods. This can be achieved by replacing liver biopsy by an indisputable gold standard, such as mortality in prospective studies [28] or using novels techniques without gold standard, such as applied in this study.

Non-invasive methods have also important limitations. TE performed by the M probe may be unreliable in 20% of patients [29] and this method may have a non-negligible interobserver variability [30]. In addition, liver stiffness may be overestimated in presence of necro-inflammatory activity [31], extra-hepatic cholestasis [32], hepatic congestion [33] and non-fasting status [34]. ELF includes serum markers involved in the synthesis and breakdown of the extra-cellular matrix that may be elevated in other systemic diseases not related to liver fibrosis [35]. Finally, APRI has a considerable variability due to laboratory upper limit for normal AST and this marker can be overestimated in presence of necro-inflammatory activity due to utilization of transaminases in its formula [36].

We acknowledged that our limited sample (n=117) with a low prevalence of cirrhosis (7%) is the main limitation of our study. The present study included consecutive naive CHC patients with indication of assessment of liver fibrosis for evaluation of therapeutic propose. The low prevalence of cirrhotic patients might be

due to selection of CHC naive-patients submitted to fibrosis staging for evaluation of therapeutic management. Other important limitation could be the absence of double blind histological analysis. However, Rousselet et al have demonstrated that a single pathologist specialized in liver histology with a long term practice in academic centers can accurately stage fibrosis in a liver specimen with a good quality [37]. In the present study, the histological analysis was performed by an experimented liver specialized pathologist (FC) in a 20 mm median specimen length with a median of 10 portal tracts.

The major strength of this study relies in the fact that we strictly respect the criteria for LCA and that data fitted very well in the model without co-linearity between non-invasive tests (2LC-model). In addition, the 2LC-model showed similar observed and estimated tests results, non-significant p value of L^2 and the lower BIC compared to others models. In a sensitive analysis, similar results for accuracy of the four tests were observed in models with direct effect between non-invasive methods (Supplementary Table 1).

In a model without a gold standard, TE and ELF were accurate to stage liver fibrosis and APRI to rule-out cirrhosis. To move forward the discussion of the performance of non-invasive methods mathematical models, such as latent class analysis, should be more studied in Hepatology research. The reference of liver biopsy as a gold standard must be reconsidered.

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Table 1. Baseline characteristics of the patients

	Patients (n=117)
Male gender	40 (34)
Age, years	55 [48-62]
BMI, kg/m²	26 [24-30]
ALT, U/L	57 [38-110]
AST, U/L	49 [34-81]
Alkaline Phosphatases, U/L	76 [62-99]
GGT, U/L	67 [37-129]
Platelets, $x10^9$/L	212 [174-260]
Non-invasive methods	
Transient elastography, kPa	8.3 [6.4-13.6]
F ≥ 2, TE ≥ 7.1 kPa	65 (56)
F = 4, TE ≥ 12.5 kPa	30 (26)
APRI	0.68 [0.43-1.37]
F ≥ 2, APRI ≥ 1.5	27 (23)
F = 4, APRI ≥ 2.0	18 (15)
ELF	9.39 [8.70-10.49]
F ≥ 2, ELF ≥ 9.37	59 (50)
F = 4, ELF ≥ 10.31	36 (31)
Liver biopsy	
Specimen length, mm	20 [10-30]
Portal tracts, n	10 [8-12]
Fibrosis, METAVIR	
F0F1	63 (54)
F2	35 (30)
F3	11 (9)
F4	8 (7)

Data expressed as median [Inter-Quartil Range] or absolute (%). ALT, alanine transaminase; APRI, aspartate-to-platelets ratio; AST, aspartate transaminase; BMI, body mass index; ELF, enhanced liver fibrosis; GGT, gamma- glutamyltransferase; TE, transient elastography. Castera et al [xx], Wai et al [xx] and Fernandes et al [xx] cut-offs were used for fibrosis staging based on transient elastography, APRI and ELF, respectively.

Table 2. Competitive comparison of latent classes models for assessment of liver fibrosis.

		Significant fibrosis (F≥2)		Cirrhosis (F=4)	
Model	**Model specification**	**L^2 (p value)**	**BIC**	**L^2 (p value)**	**BIC**
2LC	{X, TE|X, APRI|X, ELF|X, LB|X}	9.9504 (0.1268)	-18.6226	5.6494 (0.4636)	-22.9237
2LC with direct effect between TE and APRI	{X, TE APRI|X, ELF|X, LB|X}	7.9601 (0.0931)	-11.0886	4.7289 (0.3163)	-14.3198
2LC with direct effect between TE and ELF	{X, TE ELF|X, APRI|X, LB|X}	7.9397 (0.0938)	-11.1090	5.4854 (0.2410)	-13.5633
2LC with direct effect between APRI and ELF	{X, TE|X, APRI ELF|X, LB|X}	6.2539 (0.1810)	-12.7948	0.1466 (0.9988)	-18.9021

LC, latent class; L^2, likelihood squared; BIC, Bayesian information criterion; TE, transient elastography; APRI, aspartate-to-platelet ratio; ELF, enhanced liver fibrosis. 2LC was the model that data better fits for estimation of significant fibrosis and cirrhosis.

Table 3. Observed and estimated frequencies and standardized residual for 16 combinations estimated by the Latent Class Analysis (LCA) model for diagnosis of significant fibrosis and cirrhosis.

				Significant fibrosis (F≥2)			Cirrhosis (F4)		
TE	**APRI**	**ELF**	**LB**	**Observed**	**Estimated**	**Standardized residual**	**Observed**	**Estimated**	**Standardized residual**
1	1	1	1	20	17.158	0.686	4	3.973	0.013
1	1	1	0	2	2.790	-0.473	10	9.516	0.157
1	1	0	1	1	3.969	-1.490	0	0.236	-0.486
1	1	0	0	2	0.706	1.540	0	0.688	-0.829
1	0	1	1	18	19.618	-0.365	3	2.956	0.026
1	0	1	0	4	5.626	-0.686	7	7.642	-0.232
1	0	0	1	8	5.347	1.147	1	0.176	1.966
1	0	0	0	10	9.787	0.068	5	4.813	0.085
0	1	1	1	1	1.429	-0.359	0	0.356	-0.597
0	1	1	0	1	0.297	1.288	2	1.119	0.833
0	1	0	0	0	0.352	-0.594	0	0.021	-0.146
0	1	0	1	0	0.299	-0.547	2	2.090	-0.062
0	0	1	1	3	2.503	0.314	0	0.265	-0.515
0	0	1	0	10	9.579	0.136	10	10.173	-0.054
0	0	0	1	3	3.625	-0.328	0	0.016	-0.126
0	0	0	0	34	33.915	0.015	73	72.960	0.005

TE, transient elastography; APRI, aspartate-to-platelet ratio; ELF, enhanced liver fibrosis; LB, liver biopsy. 0, negative; 1, positive. Latent Class Analysis models that data better fitted for diagnosis of significant fibrosis [L^2 of 9.9504 (p value = 0.1268) / Bayesian information criteria = -18.6226] and cirrhosis [L^2 of 5.6494 (p value = 0.4636) / Bayesian information criteria = -22.9237].

Table 4. Performance of tests for diagnosis of significant fibrosis (F≥2) and cirrhosis (F=4) as estimated by classical 2 x 2 analysis (liver biopsy as gold standard) and Latent Class Analysis (without gold standard).

	Sensitivity (95%CI)		Specificity (95% CI)		Positive LR	AUROC (95% CI)
	Classical 2 x 2	LCA	Classical 2 x 2	LCA		
Significant fibrosis (F≥2)						
TE	0.87 (0.78-0.96)	0.92 (0.86-0.98)	0.71 (0.60-0.82)	0.79 (0.72-0.86)	2.8	0.874 (0.811-0.937)
APRI	0.41 (0.27-0.55)	0.47 (0.40-0.54)	0.92 (0.89-0.95)	0.99 (0.95-1.00)	5.1	0.810 (0.732-0.887)
ELF	0.78 (0.67-0.89)	0.81 (0.74-0.88)	0.73 (0.62-0.84)	0.78 (0.71-0.85)	2.9	0.807 (0.725-0.889)
Liver biopsy	1.00*	0.86 (0.68-1.00)	1.00*	0.91 (0.79-1.00)		
Cirrhosis (F4)						
TE	1.00	0.92 (0.76-1.00)	0.80 (0.71-0.89)	0.94 (0.91-0.97)	4.5	0.942 (0.890-0.993)
APRI	0.50 (0.16-0.84)	0.57 (0.37-0.77)	0.87 (0.81-0.93)	0.97 (0.93-1.00)	3.9	0.767 (0.585-0.948)
ELF	0.88 (0.68-1.00)	0.94 (0.84-1.00)	0.73 (0.64-0.82)	0.88 (0.82-0.94)	3.3	0.783 (0.555-1.000)
Liver biopsy	1.00*	0.30 (0.12-0.48)	1.00*	1.00		

* gold standard by definition. TE, transient elastography; APRI, aspartate-to-platelet ratio; ELF, enhanced liver fibrosis; CI, confidence interval; LCA, Latent Class Analysis; LR, likelihood ratio; AUROC, area under the receiver operator curve. Positive LR and AUROC were calculated by classical analysis using liver biopsy as gold standard.

Supplementary Table 1. Sensitivities and specificities of tests for diagnosis of significant fibrosis (F≥2) and cirrhosis (F=4) as estimated by Latent Class Analysis in models with co-linearity between non-invasive methods

	Sensitivity / Specificity		
Model	**2LC with direct effect between TE and APRI**	**2LC with direct effect between TE and ELF**	**2LC with direct effect between APRI and ELF**
	Significant fibrosis (F≥2)		
TE	0.92 / 0.75	0.93 / 0.82	0.94 / 0.81
APRI	0.46 / 0.96	0.46 / 1.00	0.46 / 0.98
ELF	0.84 / 0.78	0.82 / 0.82	0.79 / 0.77
Liver biopsy	0.91 / 0.91	0.82 / 0.91	0.86 / 0.92
	Cirrhosis (F4)		
TE	0.94 / 0.94	0.92 / 0.95	1.00 / 0.97
APRI	0.59 / 0.97	0.56 / 0.97	0.51 / 0.96
ELF	0.94 / 0.88	0.95 / 0.89	0.87 / 0.86
Liver biopsy	0.30 / 1.00	0.29 / 1.00	0.29 / 1.00

2LC, two latent classes; TE, transient elastography; APRI, aspartate-to-platelet ratio; ELF, enhanced liver fibrosis

5 DISCUSSÃO

A fibrose hepática é o aspecto clínico mais importante na HCC. Até recentemente houve grande preocupação com a precisão do estadiamento da fibrose na HCC, pois disto dependia a indicação de tratamento antiviral(62). À primeira vista, com a iminência da utilização em larga escala de drogas antivirais potentes, com taxas de RVS em torno de 90% (63), pode parecer que não será mais fundamental conhecer o estágio de fibrose na HCC. Espera-se que a RVS, principal objetivo no cuidado destes pacientes até o momento, torne-se um evento bem mais frequente que o observado. Subsequentemente, a fibrose hepática remanescente, seu retrocesso ou progressão, determinarão o prognóstico, mesmo naqueles que houverem alcançado a RVS(64). Assim sendo, o estudo da fibrose hepática na HCC é assunto atual, em constante evolução e ainda longe de estar completamente esgotado.

O objetivo inicial deste estudo foi avaliar o ELF como marcador não-invasivo da fibrose na HCC, utilizando como padrão ouro a biópsia hepática. Para melhor contextualizar sua aplicabilidade, foi proposta a comparação com outro método não invasivo já bem estabelecido na prática clínica, a EHT. Com a observação de que os pontos de corte até então utilizados não apresentavam bom desempenho, se fez clara a necessidade de proposta de pontos mais úteis à sua aplicação prática. Para mais uma vez colocar em contexto os novos pontos de corte propostos, nova análise, comparando o ELF com a EHT, a biópsia hepática e o APRI, foi realizada, desta vez sem que a biópsia hepática fosse considerada o padrão ouro de fibrose hepática.

No primeiro artigo (*Enhanced Liver Fibrosis Panel as a Predictor of Liver Fibrosis in Chronic Hepatitis C Patients(65))*, o ELF mostrou ser um bom marcador não invasivo de fibrose na HCC. Além da avaliação da sua acurácia tomando-se como referência a biópsia hepática, seu desempenho foi comparado ao da EHT para detecção de fibrose significativa, fibrose avançada e cirrose, sem que houvesse diferença estatisticamente significativa. ELF e EHT já haviam sido avaliados conjuntamente em populações de etiologias mistas(66-68) ou apenas de hepatite B crônica(42, 43). Até então a comparação de desempenho do ELF com a EHT em população estritamente de pacientes com HCC havia sido realizada apenas por

Cobbold et al (41). Este autor o havia feito em uma casuística mais restrita: 67 pacientes com fragmentos de biópsia hepáticas aquém do desejado e com intervalo de tempo entre os testes mais longo que o aqui apresentado. Para fibrose avançada a AUROC de ambos foi 0,82, enquanto para cirrose a AUROC do ELF foi de 0,91 e a da EHT 0,90.

No presente estudo, para a detecção de fibrose significativa e fibrose avançada o ELF obteve resultados semelhantes aos de outros estudos(39, 47, 69-71), com AUROCs de 0,81 (95% IC, 0,73-0,87) e 0,82 (95% IC, 0,74-0,88), respectivamente. No entanto, para a detecção de cirrose observou-se um desempenho mais discreto do ELF (AUROC de 0,78; 95% IC, 0,70-0,85), que o encontrado por outros autores cujas AUROCs encontradas variaram de 0,82 a 0,94 (39, 47, 66, 68, 71). Este pior desempenho no diagnóstico de cirrose pode ser explicado pela baixa prevalência de pacientes com cirrose histológica na coorte estudada (6,7%). Por tratar-se de uma população de pacientes submetidos à biópsia hepática, esta baixa prevalência de cirróticos está de acordo com a prática clínica. Mesmo antes da incorporação de métodos não invasivos como a EHT à rotina assistencial, pacientes com cirrose, muitas vezes, não eram submetidos à biópsia hepática por apresentarem alterações laboratoriais ou ultrassonográficas que firmavam o diagnóstico e permitiam a indicação de tratamento(4).

A distribuição desproporcional dos pacientes pelos cinco estágios de fibrose de METAVIR aqui observada está de acordo com a encontrada na prática clínica. A maioria dos pacientes em avaliação de fibrose apresenta-se nos estágios mais iniciais do espectro da doença (33). Com o intuito de evitar que este efeito de espectro pudesse gerar um viés na comparação entre o ELF e a EHT, aplicou-se o método estatístico de Obuchowski (71). A proposta deste tratamento estatístico é evitar que haja erros de classificação induzidos pela maior ou menor representação dos estratos de variáveis categóricas. As acurácias do ELF e da EHT, comparadas pelo método de Obuchowski, mantiveram-se significantemente semelhantes, reforçando o bom desempenho do ELF.

No entanto, foi possível observar que, ao empregar-se os pontos de corte propostos pelo fabricante do kit comercial (<7,7 = fibrose ausente a leve; de 7,7 a 9,8 = fibrose moderada; acima de 9,8 = fibrosis avançada), o ELF hiperestimou a presença da fibrose hepática (em 66% dos pacientes). Apesar de em pacientes sadios o valor do ELF ser significativamente maior para homens que para

mulheres(47), esta era uma população predominantemente feminina. Na casuística inicial de 120 pacientes o ponto de corte encontrado para fibrose avançada (≥F3) foi superior ao para cirrose (F=4). Infelizmente, devido à baixa prevalência de cirrose, não foi factível, neste primeiro momento, propor um ponto de corte mais adequado.

Existe na literatura uma lacuna no referente a um ponto de corte do ELF que possa ser aplicado na prática diária. Metanálise recente (48), incluindo nove estudos, demonstrou que o ELF apresenta acurácia diagnóstica para fibrose significativa, fibrose avançada e cirrose satisfatória suficiente (AUROCs de 0,88, 0,86, e 0,87, respectivamente) para que possa ser incorporado na rotina laboratorial de avaliação de pacientes com HCC. Este achado foi corroborado pelo de Zarski et al (46) que mostraram ser o ELF um método custo-efetivo ao ser associado a outros marcadores não-invasivos de fibrose. Em estratégias que utilizaram combinações de testes no intuito de reduzir a necessidade de biópsia hepática, o ELF pode substituir a EHT em associações com o Fibrotest, Fibrometer ou Hepascore, sem perda de acurácia (46). Segundo a metanálise já citada(48), a introdução isolada do ELF na avaliação destes pacientes reduziria em 74% a necessidade de biópsias hepáticas. No entanto, este estudo alertou para o fato de não haver um ponto de corte bem estabelecido.

O artigo original que propôs o ELF (38) foi baseado em uma população com múltiplas etiologias para a hepatopatia crônica. Já está bem demonstrado que a fibrose hepática não apresenta distribuição ou padrão semelhante em etiologias diferentes (72). Ao estabelecer-se pontos de corte para um método não-invasivo, visando à prática clínica, é fundamental que a população do estudo não seja mista, ou seja, que apenas uma etiologia para a fibrose esteja presente.

Outros grupos que estudaram o ELF encontraram boas acurácias, porém com pontos de corte mais elevados que os inicialmente propostos. Petersen et al (74), em estudo que comparou o ELF com o APRI para discriminação entre fibrose leve e significativa, propôs dois pontos distintos: um para ausência (≤8,19) e outro para presença (≥9,88) de fibrose significativa. No entanto 50,6% dos pacientes não puderam ser classificados, no que eles denominaram de *zona intermediária*. Com associação do APRI o número de pacientes na *zona intermediária* foi reduzido para 29.5%.No estudo de Guechot et al (70) observa-se que os pontos que melhor discriminariam fibrose significativa e cirrose seriam, respectivamente, 9,0 e 9,35. Por sua vez, Lichtinghagen et al (47) sugeriram que valores entre 9,8 e 11,3 não

poderiam ser atribuídos a nenhum estágio específico de fibrose. No entanto, valores acima de 11,3 apresentaram boa especificidade para cirrose (96%).

Neste contexto foi proposto o segundo artigo (*Enhanced Liver Fibrosis Panel (ELF): new cut-off points for chronic hepatitis C patients).* Seus resultados estão mais de acordo com estes autores que com o proposto pelo fabricante. Para fibrose significativa, o ponto de corte de 9,37 apresentou valor preditivo positivo de 78%, enquanto para cirrose, o ponto de corte de 10,31 mostrou valor preditivo negativo de 98%. A aplicação destes novos pontos pode representar uma real contribuição para a aplicabilidade do ELF na rotina laboratorial.

Infelizmente, o ponto discriminante entre F2 e F3 de Metavir não foi encontrado. Esta observação também vai ao encontro do anteriormente publicado. A restrita capacidade de discriminação de estágios intermediários é uma limitação comum a todos os marcadores não invasivos de fibrose (17, 52, 73). No caso específico do ELF a característica dinâmica de seus componentes explica, em parte, este achado (68). As concentrções séricas de HA, TIMP-1 e PIIINP não são diretamente proporcionais a quantidade de matriz extracelular já depositada. Pelo contrário, são marcadores que refletem o constante processo de deposição e degradação desta matriz. Além disso, O ELF também é influenciado pela atividade da doença e se correlaciona com marcadores de inflamação. Portanto, sua variabilidade é significativa e a superposição de seus valores séricos em estágios intermediários, inevitável (47). Este aspecto fica bastante patente ao observarmos o *box-plot* de distribuição do ELF pelos estágios de fibrose de acordo com METAVIR (figura 2 do segundo artigo). Apesar de haver diferença estatisticamente significante da mediana para cada estágio de fibrose, há uma nítida superposição de valores ao longo do espectro da hepatopatia.

Outro aspecto importante, já bastante debatido na literatura, que interfere na discriminação de estágios pelos marcadores não-invasivos de fibrose, é o fato destes serem validados em relação à biópsia hepática, que é um padrão ouro imperfeito (6, 10, 11, 56, 74). Em 2009, Mehta et al (6) já alertava para o fato de marcadores não invasivos não conseguirem ultrapassar acurácias de cerca de 80% pois a própria biópsia hepática impunha este limite. Bedossa (22) acrescentou que a avaliação de desfechos binários como presença ou ausência de cirrose, através do emprego de curva ROC, quando de fato a fibrose é um fenômeno de natureza contínua mas não linear, era outro fator de viés para a avaliação de marcadores

não-invasivos. No intuito de avaliar se os novos pontos de corte propostos para o ELF mantinham sua acurácia, quando avaliados em um contexto em que a biópsia não fosse referência, foi proposto um terceiro artigo (*Diagnostic accuracy of non-invasive methods and liver biopsy by Latent Class Analysis in chronic hepatitis C: an approach without a gold standard)*, aplicando a análise de classes latentes. Neste tipo de análise é possível calcular a sensibilidade e especificidade de métodos sem que haja uma referência dentre eles (61). Apesar de uma casuística modesta (117 pacientes) foram observadas as premissas metodológicas necessárias. Os quatro métodos não foram selecionados ao acaso. O ELF é composto apenas por integrantes da matriz extracelular; o APRI por AST e plaquetas; a EHT avalia um aspecto físico relacionado a elasticidade do parênquima hepático; e, finalmente, a biópsia hepática avalia alterações estruturais através de um score semiquantitativo (METAVIR). Não há qualquer colinearidade entre eles. Na análise tradicional, com a biópsia hepática como referência, a acurácia encontrada para a EHT, APRI e ELF foi semelhante à previamente relatada (48, 75). A EHT foi o método mais sensível e o APRI o mais específico, tanto para fibrose significativa quanto para cirrose. Em termos de acurácia avaliada pela AUROC (95%CI), a EHT teve o melhor desempenho para fibrose significativa [0.87 (0.81-0.93)] e para cirrose [0.94(0.72-0.88)]. Dentre os possíveis modelos de classes latentes, o de total independência entre os quatro testes foi o que melhor se adaptou aos dados, com o menor critério Bayesiano (tabela 2 do artigo 3). Com a aplicação da ACL houve um incremento na sensibilidade dos métodos não invasivos para detecção de fibrose significativa, comparando-se com a análise clássica, o que está de acordo com o previamente publicado (59). De acordo com a ACL, EHT e ELF foram os mais sensíveis para o diagnóstico de fibrose significativa e cirrose, enquanto o APRI foi o mais específico para ambos desfechos. Ao mesmo tempo, houve um descrécimo no desempenho da biópsia hepática, principalmente na sua sensibilidade (30%) para cirrose. No entanto ela manteve sua especificidade de 100% para cirrose. Poynard et al (59) também encontraram esta redução de sensibilidade, porém menos pronunciada (51%). Novamente, a baixa prevalência de cirrose na população avaliada pode explicar (76), ao menos em parte, este resultado, através do efeito de espectro. Além disso, retrospectivamente, vamos encontrar estudos que utilizaram a laparoscopia como referência, onde a sensibilidade da biópsia hepática para cirrose variava de 38,1% (78) a 68% (79), bastante aquém de 100%, como esperaria-se de um padrão ouro.

CONCLUSÕES

a) O ELF é um bom marcador não-invasivo de fibrose significativa e cirrose em pacientes com hepatite C crônica, quando avaliado em relação à biópsia hepática. Seu desempenho é comparável ao da elastografia hepática transitória
b) É necessário que novos pontos de corte, mais adequados à utilização do ELF na prática clínica, sejam adotados.
c) Propõe-se que 9,37 seja adotado como ponto discriminante para fibrose significativa e que 10,31 seja adotado para cirrose em pacientes com hepatite C crônica.
d) Os novos pontos de corte propostos para o ELF para discriminar fibrose significativa e cirrose em pacientes com hepatite C crônica apresentam bom desempenho quando analisados em um contexto em que a biópsia hepática não é considerada o padrão ouro, como na análise de classe latentes.

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