ORIGINAL ARTICLE

# Accurate prediction of significant liver fibrosis using the Pentra score model in patients with chronic hepatitis C

Joanna Górka-Dynysiewicz[1], Monika Pazgan-Simon[2], Jolanta Zuwała-Jagiełło[1]

1 Department of Pharmaceutical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
2 Department of Infectious Diseases and Hepatology, Wroclaw Medical University, Wroclaw, Poland

**KEY WORDS**

chronic hepatitis C, liver fibrosis, Pentra score, predictive model, serum biomarker validation

**ABSTRACT**

**INTRODUCTION** Noninvasive methods are increasingly used in the clinical assessment of patients with chronic hepatitis C (CHC).

**OBJECTIVES** We aimed to develop a predictive model for the evaluation of significant fibrosis in patients with CHC, based on serum biomarkers. We compared the accuracy of our model in detecting significant fibrosis with currently known markers/models of fibrosis (such as the aspartate aminotransferase to platelet ratio index [APRI], the Fibrosis-4 [FIB-4] score, and the Forns index).

**PATIENTS AND METHODS** A total of 242 patients with CHC not receiving antiviral treatment were divided into 2 groups: training group (n = 150) and validation group (n = 92). Significant fibrosis was defined as F2 or higher on the Meta-analysis of Histological Data in Viral Hepatitis (METAVIR) scale.

**RESULTS** Multivariable analysis revealed that age ($P$ <0.001), pentraxin 3 (PTX3) levels ($P$ = 0.009), γ-glutamyl transpeptidase (GGT) to platelet count (PLT) ratio ($P$ = 0.08), and hyaluronic acid levels (HA) ($P$ = 0.07) were independent predictors of significant fibrosis. Based on that, we developed a model for predicting significant fibrosis: Pentra score = 0.176 × PTX3 (ng/ml) + 0.522 × HA (ng/ml) + 0.29 × GGT (IU/l) to PLT ($\times 10^9$/l) ratio + 0.14 × age (years) – 3.9346. Then, we compared our model with the biomarkers and models currently used to predict liver fibrosis. The Pentra score yielded the largest area under the receiver operating characteristic curve for predicting significant fibrosis in the training and validation groups (0.894 and 0.867, respectively). It also had the highest diagnostic accuracy in both groups (90.6% and 87.0%, respectively).

**CONCLUSIONS** Our model for detecting significant fibrosis in patients with CHC using pentraxin 3 and other serum biomarkers compares well with the existing and previously published indices. However, further validation in larger cohorts is needed.

Correspondence to:
Joanna Górka-Dynysiewicz, MSc, Department of Pharmaceutical Biochemistry, Wroclaw Medical University, ul. Borowska 211a, 50-556 Wroclaw, Poland, phone: +48 71 784 03 03, email: joanna.gorka-dynysiewicz@umed.wroc.pl
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**INTRODUCTION** Hepatitis C virus (HCV) affects approximately 200 million people worldwide. Hepatitis C virus infection is considered one of the major risk factors for liver disease. The World Health Organization reports that more than 71 million people are chronically infected with HCV globally, and approximately 0.4 million of those infected die due to HCV-related liver complications annually.[1-3] Chronic hepatitis C (CHC) is characterized by highly variable progression and—depending on the extent of liver fibrosis and inflammation—CHC can progress to cirrhosis and hepatocellular carcinoma.[4,5] Early treatment that prevents cirrhosis is the preferred strategy to avoid hepatocellular carcinoma. Proper management including monitoring of fibrosis progression and effective antiviral therapies (eg, direct-acting antivirals) has dramatically changed the outcome in patients with chronic HCV infection, and thereby improved liver histology, and prevented the progression to liver cirrhosis.[6]

Noninvasive approaches instead of liver biopsies are needed to determine the fibrosis stage and, therefore, improve prophylaxis and clinical management of patients with CHC. Until recently, liver biopsy was the "gold standard" for

## WHAT'S NEW?

Detecting significant fibrosis remains important in the treatment and follow-up of patients with chronic hepatitis C. Our study aimed to develop a noninvasive scoring system for the identification of significant fibrosis (ie, ≥F2 on the Meta-analysis of Histological Data in Viral Hepatitis [METAVIR] scale) in patients with chronic hepatitis C using serum-based biomarkers. Age, pentraxin 3 levels, γ-glutamyl transpeptidase/platelet count, and hyaluronic acid were found to predict significant fibrosis. Based on that, we developed a model for the evaluation of fibrosis, the Pentra score. We compared it with the currently used models (such as the aspartate aminotransferase to platelet ratio index [APRI], the Fibrosis-4 [FIB-4] score, and the Forns index): it had the largest area under the receiver operating characteristic curve and the highest diagnostic accuracy in the training and validation groups.

the evaluation of liver fibrosis. It has numerous limitations, such as invasive sampling error[7-9] and large variability among observers.[10] To overcome these limitations, noninvasive diagnostic methods are now increasingly used to assess liver tissue including those based on serum biomarkers[11,12] or on the measurement of liver stiffness by ultrasound and magnetic resonance elastography.[13,14] Functional imaging techniques, including magnetic resonance elastography and ultrasound elastography, are useful in assessing moderate to advanced liver fibrosis. Magnetic resonance elastography is considered the most accurate noninvasive imaging technique, and ultrasound elastography is currently the most widely used noninvasive means. However, these modalities are less accurate in early-stage liver fibrosis and some factors affect their accuracy.[15]

In addition, most of the available noninvasive models are less accurate in detecting intermediate fibrosis stages (ie, F1–F2 on the Meta-analysis of Histological Data in Viral Hepatitis [METAVIR] scale) compared with late-stage cirrhosis (F4).[16-18] Indeed, while various serum-based predictive models (such as the aspartate aminotransferase to platelet ratio index [APRI], the Fibrosis-4 [FIB-4] score, and the Forns index) for liver fibrosis have been proposed and validated,[10-14] their diagnostic accuracy remains hotly debated.[19,20] Therefore, we need more accurate noninvasive models to predict the evolution of liver fibrosis and precisely manage it in the light of personalized clinical medicine.

Pentraxin 3 (PTX3) is one of the serum biomarkers that has been investigated for its role in assessing liver fibrosis.[21] Physiologically, blood levels of PTX3 are quite low (<2 ng/ml), but its expression increases in response to inflammatory stimulation in many diseases, including hepatitis.[22] Specifically, in patients with nonalcoholic fatty liver disease and alcoholic hepatitis, PTX3 levels were shown to be associated with disease progression and a particular liver fibrosis stage.[23-25] Similarly, we demonstrated elsewhere[26] that PTX3 levels were related with the histologic stage of fibrosis, and that PTX3 serum concentrations showed high reliability for the diagnosis of significant fibrosis in patients with CHC before antiviral treatment.

The obtained results allowed us to hypothesize that incorporating PTX3 serum concentrations into a multidimensional model may be useful in predicting the fibrosis stage in patients with CHC. Here, we present (and validate) a novel predictive model (named the Pentra score) for the evaluation of significant fibrosis (ie, ≥F2 on the METAVIR scale) in patients with CHC using PTX3 levels and other serum biomarkers. We show that the Pentra score has diagnostic performance comparable with the existing indices and has additional advantages.

## PATIENTS AND METHODS

### Study population

We included a total of 242 patients with CHC who underwent liver biopsy and for whom stored sera were available admitted to the Department of Infectious Diseases and Hepatology of Wroclaw Medical University from October 2015 to April 2018. Patients were diagnosed with CHC infection based on the following signs: persistently elevated alanine aminotransferase (ALT) levels, anti-HCV and HCV RNA positivity for at least 6 months, and histopathologic features indicative of liver inflammation. Chronic hepatitis C was confirmed by measuring serum levels of HCV antibodies with an enzyme immunoassay and by the HCV RNA test using reverse transcriptase–polymerase chain reaction (Cobas Amplicor, Roche, San Francisco, California, United States). The patients in this study had not undergone antiviral therapy before.

### Patient characteristics

In total, 242 participants were included in the study: 135 men (55.79%) and 107 women (44.21%). The study group showed widespread intensification of liver fibrosis with all METAVIR stages. Overall, 21.07% of patients were classified as stage 0 (no fibrosis), and 22.31% as stage 1 (fibrosis restricted to the portal tract). The prevalence of significant fibrosis was 56.61%: 21.07% as stage F2 (a few septa extending beyond the portal tract but with intact architecture) and 35.54% as stages F3 or F4 (bridging fibrosis or cirrhosis, respectively).

Demographic data and full routine clinical assessment of chronic liver disease were obtained at the time of liver biopsy, including: sex, age, HCV genotype, HCV viral load, body mass index, levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyl transpeptidase (GGT), bilirubin, international normalized ratio, albumin, cholesterol, leukocytes, platelets (PLT), PTX3, hyaluronic acid (HA), and transforming growth factor $\beta_1$.

A training set (n = 150) was used to investigate the variables associated with significant fibrosis in patients with CHC based on univariate and multivariable analyses, and then to construct a predictive model. The reproducibility of the model was then tested in a validation set (n = 92).

We excluded patients dually infected with HCV and hepatitis B virus as well as patients with fatty liver disease. Patients with known substance (alcohol and / or intravenous drug) abuse and those with HIV, autoimmune or congenital metabolic liver conditions, malignancies, or treated with immunosuppressants were also excluded from the study. The purpose of each examination was fully explained and informed consent was obtained from all participants. The study protocol was approved by the bioethical committee of Wroclaw Medical University (no. KB-477/2017) and carried out in accordance with the 1975 Declaration of Helsinki (6th revision, 2008).

**Liver histology and quantification of liver fibrosis** Liver biopsies were performed under ultrasound guidance. Specimens were fixed with formalin, embedded in paraffin, and stained with hematoxylin and eosin. An expert pathologist blinded to patients' clinical characteristics evaluated the specimens according to the METAVIR scoring system, including the fibrosis score (F0, no fibrosis; F1, portal fibrosis alone; F2, portal fibrosis with rare septa; F3, portal fibrosis with numerous septa; and F4, cirrhosis) and the necroinflammatory activity score (A1, mild activity; A2, moderate activity; and A3, marked activity). In this study, we defined significant fibrosis as a score of F2 or higher. All patients with a score of F4 had compensated disease. There were no deaths associated with liver biopsies.

**Other staging models of liver fibrosis** Other prediction models used to assess liver fibrosis in this study were APRI,[27] the FIB-4 score,[28] and the Forns index,[16] calculated as follows: APRI = (AST [IU/l] / upper limit of normal) × 100/PLT ($10^9$/l); FIB-4 = age (years) × AST (IU/l) / PLT (×$10^9$/l) × (ALT [IU/l]$^{1/2}$); Forns index = 7.811 – 3.131 × ln(PLT) [×$10^9$/l] + 0.781 × ln(GGT) [IU/l] + 3.467 × ln(age) [years] – 0.014 × cholesterol [mg/dl].

**Statistical analysis** Data analysis was carried out using the Statistica 13.3 software (StatSoft, Kraków, Poland). Continuous variables were expressed as median (Q1–Q3) and compared using the Mann–Whitney test; categorical data were reported as percentages. Risk factors for liver fibrosis in patients with CHC were analyzed using binary logistic regression. Only existing data were used for the analysis (and numbers were given where appropriate). There was no imputation procedure concerning missing data.

A predictive model for identifying significant fibrosis was developed using a training set, and then validated with a separate, independent validation set. Patients were randomized into a training set and a validation set (62% to 38% ratio).

The goodness-of-fit tests (Akaike information criterion and Bayesian information criterion) were used to select the best model. In addition, to assess how well the data fits the model, the Hosmer–Lemeshow test was applied. Variables with $P$ value less than 0.01 in the univariable analysis were then included in a multivariable stepwise logistic regression analysis. Coefficients with $P$ value less than 0.1 in the multivariable analysis were then selected as components of a new equation for predicting significant fibrosis. First, univariate analysis was performed to detect candidate variables from different clinical factors that could be incorporated into a new predictive model.

Next, we tested the diagnostic accuracy of our new model derived from the training set using a validation set and determining the receiver operating characteristic curve (ROC). The area under the ROC (AUROC) and its 95% CI were calculated and the cutoff value determined using the Youden index. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of our model. All $P$ values were bilateral, and $P$ value of less than 0.05 was considered significant. Finally, we compared the diagnostic performance of our predictive model (Pentra score) with the following markers or models of liver fibrosis: PTX3, GGT, GGT to PLT ratio, HA, APRI index, FIB-4 score, Forns index.[16,27,28]

## RESULTS

**Patient characteristics** The model was constructed using data obtained from 150 patients (the training group) and validated using data of the remaining 92 patients (the validation group). No difference was found in baseline characteristics between both groups neither with respect to the assessed variables nor liver biopsy. Patients' characteristics at the time of liver biopsy, including the detailed demographic data and laboratory parameters, are shown in TABLE 1.

**Factors associated with significant liver fibrosis** In the univariate analysis, the following parameters were identified as positively related to significant fibrosis: age ($P$ <0.001), ALT ($P$ <0.01), AST ($P$ <0.01), GGT ($P$ <0.01), alkaline phosphatase ($P$ <0.01), PLT ($P$ <0.001), bilirubin ($P$ <0.001), GGT to PLT ratio ($P$ <0.001), PTX3 ($P$ <0.001), HA ($P$ <0.001), and transforming growth factor $\beta_1$ ($P$ <0.001) (TABLE 2).

When these 11 parameters were subsequently included in the multivariable analysis using forward stepwise procedures, age and PTX3 were found to be independent predictors of significant fibrosis. Additionally, the other 2 variables (GGT to PLT ratio and HA) were included in the model to improve its fit (TABLE 3).

Viral load and genotype were available in 109 patients from the training group. These patients did not differ in any of the analyzed variables from those for whom such data were not available.

**A novel model for the assessment of significant fibrosis** A predictive model was constructed by modeling the values of the independent variables and their regression coefficient. As age ($P$ <0.001)

**TABLE 1** Baseline characteristics of 242 patients with chronic hepatitis C at the time of liver biopsy: a comparison between the training and validation groups

| Variable | | Training group (n = 150) | Validation group (n = 92) | All patients (n = 242) |
|---|---|---|---|---|
| Sex, n (%) | Male | 87 (58) | 48 (52.17) | 135 (55.79) |
| | Female | 463 (42) | 44 (47.83) | 107 (44.21) |
| Age, y | | 55 (22–79) | 56 (22–76) | 55 (22–79) |
| HCV genotype 1b, n (%) | | 110 (73.33) | 68 (73.91) | 178 (73.55) |
| Viral load, mean $\times 10^5$ copies/ml | | 2.84 (0.019–7.13) | 3.06 (0.18–7.28) | 2.97 (0.019–7.28) |
| MELD score | | 7.6 (6.5–8.1) | 7.3 (6.6–7.9) | 7.5 (6.5–8.1) |
| BMI, kg/m$^2$, mean (SD) | | 22.2 (2.4) | 22.5 (2.62) | 22.3 (2.54) |
| ALT, IU/l | | 64 (13–278) | 63 (16–278) | 63 (13–278) |
| AST, IU/l | | 50 (17–242) | 50 (18–242) | 50 (17–242) |
| ALP, IU/l | | 82 (38–220) | 85.5 (38–201) | 83 (38–220) |
| GGT, IU/l | | 53 (12–352) | 58.0 (12–352) | 52.5 (12–352) |
| Bilirubin, mg/dl | | 0.83 (0.31–4) | 0.83 (0.35–4) | 0.83 (0.31–4) |
| INR | | 1.05 (0.92–1.38) | 1.04 (0.92–1.38) | 1.04 (0.92–1.38) |
| Albumin, g/dl | | 3.9 (2.41–4.72) | 3.8 (2.4–4.7) | 3.8 (2.4–4.72) |
| Cholesterol, mg/dl | | 154.2 (140.3–231) | 161.3 (151.3–228.7) | 154.2 (140.3–231) |
| Leukocytes, $\times 10^9$/l | | 6.24 (3.90–12.5) | 6.00 (1.87–12.5) | 6.08 (1.87–12.5) |
| PLT, $\times 10^9$/l | | 188.5 (121–360) | 189.0 (133–360) | 189.0 (121–360) |
| PTX3, ng/ml | | 4.80 (1.01–12.7) | 4.56 (1.29–12.69) | 4.7 (1.01–12.7) |
| HA, ng/ml | | 113.5 (7.9–826.9) | 114.4 (8.33–1036) | 114.36 (7.9–1036) |
| TGF-$\beta_1$, ng/ml | | 8.0 (2.12–31.5) | 7.56 (2.12–31.5) | 7.56 (2.12–31.5) |
| Fibrosis stage, n (%) | 0 | 32 (21.33) | 19 (20.65) | 51 (21.07) |
| | 1 | 34 (22.67) | 20 (21.74) | 54 (22.31) |
| | 2 | 30 (20) | 21 (22.83) | 51 (21.07) |
| | 3 | 25 (16.67) | 13 (14.13) | 38 (15.71) |
| | 4 | 29 (19.33) | 19 (20.65) | 48 (19.83) |

Data are presented as median (Q1–Q3) unless otherwise indicated.

SI conversion factors: to convert ALT, AST, ALP, and GGT to µkat/l, multiply by 0.0167; bilirubin to µmol/l, by 17.104; albumin to g/l, by 10; cholesterol to mmol/l, by 0.0259.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyl transpeptidase; HA, hyaluronic acid; HCV, hepatitis C virus; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; PLT, platelets; PTX3, pentraxin 3; Q1, lower quartile; Q3, upper quartile; TGF-$\beta_1$, transforming growth factor $\beta_1$

and PTX3 ($P$ = 0.009) were prognostic factors associated with significant fibrosis, and the GGT to PLT ratio ($P$ = 0.08) and HA ($P$ = 0.07) tended to be statistically significant, we constructed a model, named the Pentra score, expressed in the following formula: Pentra score = 0.176 × PTX3 (ng/ml) + 0.522 × HA (ng/ml) + 0.29 × GGT (IU/l) to PLT ($\times 10^9$/l) + 0.14 × age (years) – 3.9346.

**Predictive value and diagnostic accuracy of the Pentra score** We assessed the utility of our model for stratification of groups with mild (<F2) and significant (≥F2) liver fibrosis. Using the ROC analysis, an optimal cutoff value of 42.477 was determined for a diagnostic model based on the Pentra score, which means that patients with a Pentra score higher or equal to 42.477 had significant fibrosis, whereas those with a score lower than 42.477 had mild fibrosis. We then analyzed the predictive and diagnostic accuracy of the Pentra score and other 7 methods (PTX3, GGT, GGT to PLT ratio, HA, APRI index, FIB-4 score, Forns index) in the training group (**TABLE 4** and Supplementary material, *Figure S1*). Among them, the Pentra score could be highly predictive, with an AUROC of 0.894.

Considering the Pentra score, it was observed that the AUROC of the Pentra score (AUROC in the training group, 0.894; AUROC in the validation group, 0.867) differed from the results of the GGT to PLT ratio (AUROC, 0.648; $P$ <0.001 and AUROC, 0.618; $P$ = 0.005, respectively) and the FIB-4 score (AUROC, 0.77; $P$ = 0.005 and AUROC, 0.779; $P$ = 0.02, respectively). No difference was reported with regard to PTX3, HA, APRI index, and Forns index (Supplementary material, *Figure S1*).

The optimal cutoff value for each variable was adapted for the validation group. We then analyzed the predictive and diagnostic accuracy

**TABLE 2** Variables associated with significant fibrosis in the training group (150 patients) by univariate analysis

| Variable | | No significant fibrosis[a] (n = 66) | Significant fibrosis[b] (n = 84) | *P* value |
|---|---|---|---|---|
| Sex, n (%) | Male | 39 (59.09) | 52 (61.9) | 0.08 |
| | Female | 27 (40.91) | 32 (38.1) | |
| Age, y | | 44 (22–65) | 62 (32–79) | <0.001 |
| HCV genotype 1b, n (%) | | 48 (72.73) | 63 (75) | 0.09 |
| Viral load, mean $\times 10^5$ copies/ml | | 1.95 (0.019–6.44) | 2.98 (0.019–7.13) | 0.02 |
| BMI, kg/m$^2$, mean (SD) | | 21.9 (2) | 22.4 (1.9) | 0.14 |
| ALT, IU/l | | 40 (22–216) | 66 (13–278) | 0.008 |
| AST, IU/l | | 40 (17–190) | 67 (24–242) | 0.009 |
| ALP, IU/l | | 70 (43–134) | 87 (38–220) | 0.005 |
| GGT, IU/l | | 52 (12–175) | 77.5 (15–352) | 0.002 |
| Bilirubin, mg/dl | | 0.71 (0.31–4) | 1.03 (0.38–3.29) | 0.009 |
| INR | | 1.0 (0.8–1.15) | 1.06 (0.93–1.1) | 0.12 |
| Albumin, g/dl | | 4.0 (2.4–4.5) | 3.7 (2.6–4.7) | 0.16 |
| Cholesterol, mg/dl | | 175.4 (109.9–206.7) | 161.3 (140.3–231) | 0.27 |
| Leukocytes, $\times 10^9$/l | | 6.6 (4.87–12.5) | 5.99 (2.87–12.5) | 0.018 |
| PLT, $\times 10^9$/l | | 216 (183–360) | 178 (121–280) | <0.001 |
| PT, % | | 100 (76–119) | 92.0 (80–112) | 0.008 |
| GGT/PLT, IU/l / $\times 10^9$/l | | 0.25 (0.044–2.11) | 0.36 (0.01–6.62) | <0.001 |
| PTX3, ng/ml | | 3.26 (1.01–8.14) | 5.56 (1.91–12.69) | <0.001 |
| HA, ng/ml | | 46.88 (7.88–826.94) | 243.65 (69.37–1036) | <0.001 |
| TGF-$\beta_1$, ng/ml | | 3.05 (2.12–14.66) | 11.92 (2.75–31.5) | <0.001 |

Data are presented as median (Q1–Q3) unless otherwise indicated.

SI conversion factors: see **TABLE 1**

a Defined as a score of 0–F1 on the METAVIR scale

b Defined a score equal to or higher than F2 on the METAVIR scale

Abbreviations: METAVIR, Meta-analysis of Histological Data in Viral Hepatitis; PT, prothrombin time; others, see **TABLE 1**

**TABLE 3** Multivariable analysis of factors contributing to significant liver fibrosis in the training group

| Variable | Multivariable analysis OR (95% CI)[a] | *P* value |
|---|---|---|
| Age, y | 1.10 (1.04–1.16) | <0.001 |
| PTX3, ng/ml | 1.44 (1.09–1.92) | 0.009 |
| HA, ng/ml | 1.05 (0.99–1.1) | 0.07 |
| GGT/PLT, IU/l / $\times$ 10$^9$/l | 1.19 (1.08–1.3) | 0.08 |

a Logistic regression analysis

Abbreviations: OR, odds ratio; others, see **TABLE 1**

of the Pentra score and the other 7 methods in the validation group (**TABLE 4**, **FIGURE 1**).

In the validation group, all patients (53) with histopathologically confirmed significant fibrosis were identified using the Pentra score with a sensitivity of 100%. Moreover, among 53 patients with significant fibrosis (≥F2) identified according to the Pentra score, the disease was histopathologically confirmed in 45 cases, showing a PPV of 83.3%.

**DISCUSSION** Liver fibrosis is a serious life-threatening disease with high morbidity and mortality rates resulting from HCV infection. As mentioned above, liver biopsy—considered the "gold standard" for assessing liver fibrosis—has limitations in terms of invasiveness, costs, sampling and interobserver variability, and the dynamic process of fibrosis. The invasive nature of liver biopsy makes it unpractical, particularly in patients who require follow-up. Compelling evidence has demonstrated that all stages of fibrosis are reversible if the fibrotic factor is removed. Identifying HCV patients with fibrosis is of particular importance, as the choice of a treatment regimen, including the genotype-dependent one (direct-acting antivirals), depends on the severity of liver disease and/or prior therapy. According to the 2018 European Association for the Study of the Liver guidelines, there is a clear need for safe, effective, and reliable noninvasive assessment modalities to determine liver fibrosis and to manage it precisely in the light of personalized medicine. Nowadays, noninvasive methods excluding liver biopsy are considered as a reference standard in CHC.[3]

Some studies suggest that, compared with the use of single biomarkers or liver biopsy alone, combining multiple noninvasive methods using special algorithms could enable more

**TABLE 4** Area under the receiver operating characteristic curve analysis of 8 serum fibrosis markers and models in the training (n = 150) and validation (n = 92) groups

| Variable | AUROC (95% CI)[a] | Cutoff value[a] | Sensitivity, % | Specificity, % | PPV, % | NPV, % | ACC, % |
|---|---|---|---|---|---|---|---|
| Training group | | | | | | | |
| PTX3, ng/ml | 0.802 (0.727–0.877) | 4.48 | 73.0 | 75.5 | 84.4 | 60.7 | 73.9 |
| GGT, IU/l | 0.569 (0.469–0.668) | 101 | 22.7 | 93.9 | 80 | 39.3 | 48.2 |
| GGT/PLT, IU/l / × $10^9$/l | 0.648 (0.556–0.739) | 0.379 | 49.4 | 89.8 | 89.8 | 49.4 | 63.8 |
| HA, ng/ml | 0.891 (0.829–0.953) | 71.98 | 97.8 | 73.5 | 87 | 94.7 | 89.1 |
| APRI index | 0.831 (0.756–0.906) | 0.632 | 80.9 | 77.6 | 86.7 | 69.1 | 79.7 |
| FIB-4 score | 0.770 (0.69–0.851) | 1.862 | 77.5 | 65.3 | 80.2 | 61.5 | 73.2 |
| Forns index | 0.811 (0.739–0.883) | 5.67 | 84.1 | 65.3 | 81.3 | 69.6 | 77.4 |
| Pentra score | 0.894 (0.833–0.955) | 42.477 | 100 | 73.5 | 87.3 | 100 | 90.6 |
| Validation group | | | | | | | |
| PTX3, ng/ml | 0.753 (0.628–0.879) | 3.55 | 86.7 | 58.3 | 79.6 | 70 | 76.8 |
| GGT, IU/l | 0.525 (0.378–0.673) | 35 | 75.6 | 37.5 | 69.4 | 45 | 62.3 |
| GGT/PLT, IU/l / × $10^9$/l | 0.618 (0.482–0.753) | 0.5 | 44.4 | 83.3 | 83.3 | 44.4 | 58 |
| HA, ng/ml | 0.862 (0.772–0.952) | 69.37 | 97.8 | 62.5 | 83 | 93.8 | 85.5 |
| APRI index | 0.775 (0.649–0.901) | 0.632 | 73.3 | 75 | 84.6 | 60.7 | 73.9 |
| FIB-4 score | 0.779 (0.6–0.848) | 1.862 | 73.3 | 62.5 | 78.6 | 55.6 | 55.6 |
| Forns index | 0.779 (0.666–0.891) | 5.67 | 82.2 | 66.7 | 82.2 | 66.7 | 76.8 |
| Pentra score | 0.867 (0.778–0.956) | 42.477 | 100 | 62.5 | 83.3 | 100 | 87 |

a Calculated based on the ROC analysis for PTX3, GGT, the GGT/PLT ratio, HA, APRI index, FIB-4 score, Forns index, and Pentra score

Abbreviations: APRI, aspartate aminotransferase to platelet ratio index; AUROC, area under the receiver operating characteristic curve; FIB-4, Fibrosis-4; others, see TABLE 1

comprehensive first-line screening of liver fibrosis in patients with HCV.[29,30] Indeed, patients who are only assessed by aminotransferase levels could be misdiagnosed with severe fibrosis.[31,32] While assessing a combination of serum fibrosis biomarkers might be more accurate,[33,34] their routine use is somewhat limited as they are nonspecific to the liver and due to their high costs (ie, for patented tests). Despite this, serum biomarkers of fibrosis are well validated, have good reproducibility, and can be applied in outpatient clinics,[35-37] making them attractive for the noninvasive assessment of patients with CHC.

We developed a novel serum-based scoring system for the prediction of significant fibrosis in patients with CHC, the Pentra score, which includes age, PTX3, the GGT to PLT ratio, and HA. Pentraxin 3 is an established marker for detecting clinically significant and advanced fibrosis in patients with CHC. We demonstrated elsewhere[26] that PTX3 levels increased with the progression of liver fibrosis in patients with HCV. This is consistent with previous studies showing close associations among PTX3 levels, disease progression, stages of liver fibrosis in patients with nonalcoholic steatohepatitis and/or alcoholic hepatitis, and chronic HCV.[24,38,39] In the present study, we also found that PTX3 was useful as a single diagnostic marker. However, the combined Pentra score appeared to be superior to using PTX3 alone for predicting significant fibrosis in patients with CHC (ie, the AUROCs of 0.894 and 0.867 for the Pentra score in the training and validation groups, respectively, compared with 0.802 and 0.753 for PTX3). Moreover, the Pentra score had a negative predictive value of 100% and, therefore, could be used to identify patients without significant fibrosis in whom liver biopsy may be avoided.

Previous studies indicated that GGT levels are associated with the degree of liver fibrosis,[40-43] and our multivariable analysis confirmed that GGT levels were an independent predictor of significant fibrosis in patients with CHC. We also found out that platelet count was an independent predictor of significant fibrosis in patients with CHC. Similar to our findings in CHC, both GGT levels and platelet count were shown to be independent predictors of significant fibrosis (a score of F2 or higher) in patients with chronic hepatitis B virus infection.[40] Therefore, we incorporated both GGT and PLT into our final model.

Hyaluronic acid is a glycosaminoglycan mainly synthesized by hepatic stellate cells and degraded by the liver sinusoidal cells. The increased production of HA and its decreased degradation contributed to increased serum HA in patients with liver fibrosis. Several studies tested the predictive performance of HA and suggested that the HA level less than 60 ng/ml had good accuracy in ruling out patients with significant fibrosis or cirrhosis.[44,45] Other studies showed that a higher cutoff point values of HA level of 100 to 237 ng/ml could be used to identify cirrhosis.[46] Rosenberg et al[17] and Nishikawa et al[47] first showed in 2004 that applying a combination of biomarkers may improve the assessment of liver fibrosis compared with using a single factor alone. Their algorithm combining HA, N-terminal propeptide of type III

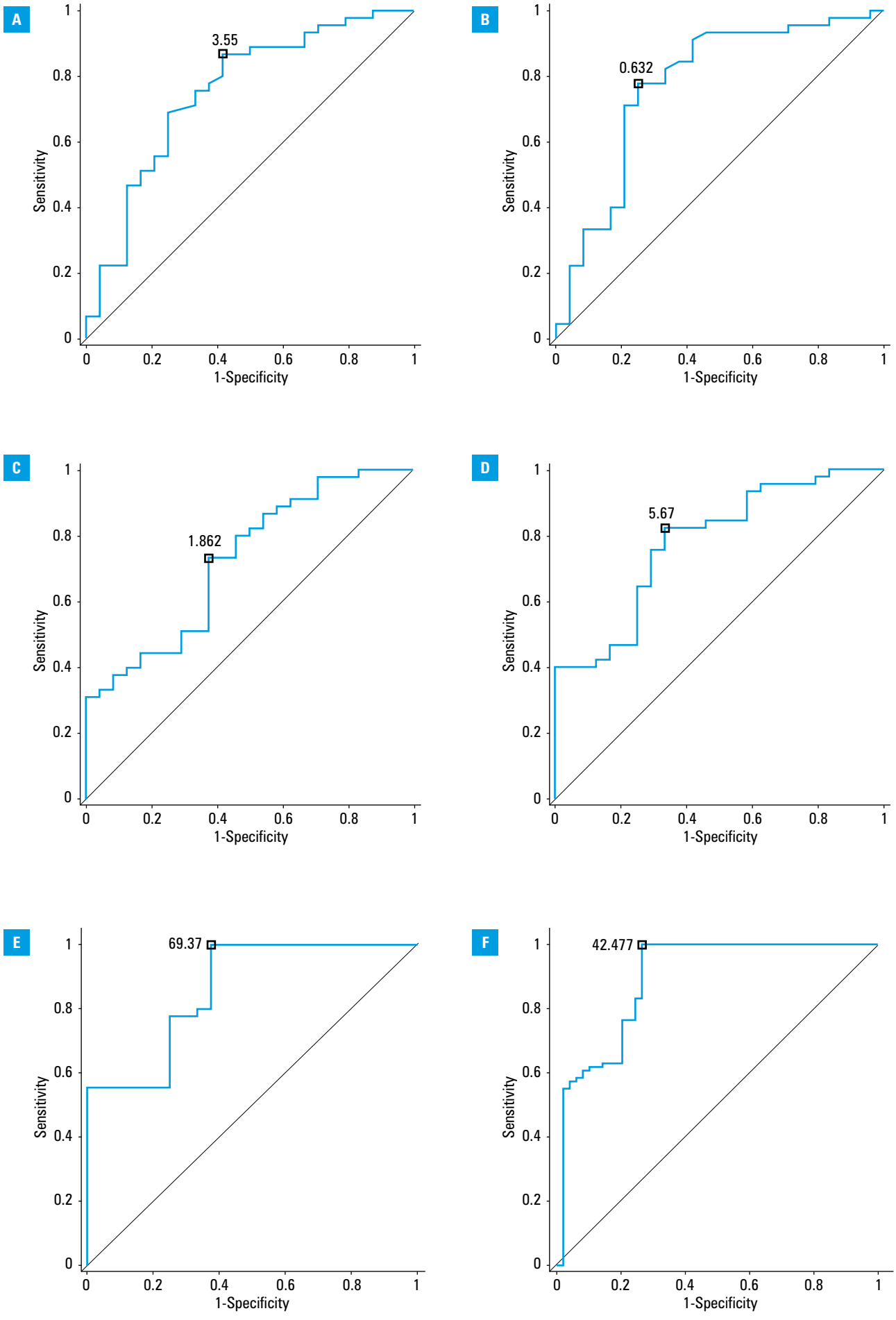


**FIGURE 1** Receiver operating characteristic curves of pentraxin 3 (**A**), the aspartate aminotransferase to platelet ratio index (APRI) (**B**), the Fibrosis-4 (FIB-4) score (**C**), the Forns index (**D**), hyaluronic acid (**E**), and the Pentra score (**F**) as markers for significant fibrosis in the validation group (n = 92)

collagen, and tissue inhibitor of matrix metalloproteinase 1 (as well as age) was predictive of significant fibrosis (a METAVIR stage F2 or higher) in patients with liver disease and showed a sensitivity of 90% and a NPV of 92%.[17] It was also revealed that the combination of serum levels of HA, N-terminal propeptide of type III collagen, and transforming growth factor β is more reliable to evaluate the degree of liver fibrosis in comparison with each marker alone.[48]

The Pentra score had slightly higher AUROCs for predicting significant fibrosis (0.87) compared with those obtained by Rosenberg et al[17] (0.804). However, as we did not test the levels of N-terminal propeptide of type III collagen or tissue inhibitor of matrix metalloproteinase 1 in this study, it is difficult to directly compare our results. In addition to serum biomarkers, previous studies have shown that age is an important risk factor in patients with CHC,[49,50] and thus both the FIB-4 score and the Forns index include age in their calculations. We also found age to be an important predictor ($P$ <0.001) associated with significant fibrosis and, therefore, included this factor in the Pentra score.

A major strength of our study is that we validated our model in an independent group. The coefficient of determination of the Pentra score was 85.4%, indicating that the formula explained over three-fourth of the variation in fibrosis in the analyzed sample. Moreover, the AUROC for the Pentra score was excellent (0.894). In addition, the Pentra score showed high sensitivity (100%) and specificity (73.5%), along with a high PPV (87.3%) and NPV (100%), which supports the efficacy of our model for identifying significant fibrosis in patients with CHC. Despite this, the score validation in an independent sample of HCV-positive patients demonstrated slightly less favourable results compared with the training group, particularly in terms of specificity (62.5%). The lower specificity of the score in the validation group is likely to be associated with a lower number of patients with mild fibrosis compared with the training group (39 vs 66). Nonetheless, our model showed good sensitivity (100%), PPV (83.3%), and NPV (100%) in the validation group. Furthermore, our model comprising all significant independent variables linked to liver fibrosis (ie, age, PTX3, the GGT/PLT ratio, and HA) was more accurate (in terms of AUROC analyses) than other noninvasive fibrosis indices we examined. Another advantage of the Pentra score over the currently available tests such as the APRI index, the FIB-4 score, or the Forns index is the absence of transaminases (ALT and AST) in its formula, which were supposed to possibly lead to false positive results in acute hepatitis.[31,32]

Our study has also some limitations. First, our sample size was relatively small (n = 242) and should be increased in the future to confirm our findings. Second, as we included only patients with CHC, the Pentra score requires further testing before it can be used for other types of chronic liver disease. Indeed, more validation studies including cohorts of patients with other liver diseases (such as chronic hepatitis B or autoimmune hepatitis) would be of interest. Third, our cohort of patients did not receive antiviral treatment and, therefore, whether the Pentra score will be valuable in monitoring regression after treatment requires further investigation.

In conclusion, the Pentra score is a new panel of biomarkers that can be used as a noninvasive method for the prediction of significant fibrosis in patients with CHC. It can be applied in clinics to assist physicians and patients in making the decision whether to embark on the treatment for HCV or wait for a more affordable therapy. This model can be applied in real-world clinical practice using existing medical records or as a broader web-based tool. This is of great clinical importance in the new era of antiviral therapy where fibrosis regression becomes one of the major treatment goals. The Pentra score could be used for the initial evaluation of the treatment priority in patients with newly diagnosed HCV infection.

## SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

## ARTICLE INFORMATION

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**CONFLICT OF INTEREST** None declared.



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