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COMBINED GENETIC RISK SCORE FOR LIVER CIRRHOSIS PREDICTION IN CHRONIC HEPATITIS C: COMPARATIVE EVALUATION OF FIVE POLYMORPHISMS AND THEIR CUMULATIVE CONTRIBUTION

Qaxarov Abduqaxar Nabijanovich
Andijan State Medical Institute, Uzbekistan
abdukahar721@gmail.com

Aleynik Vladimir Alekseyevich
Andijan State Medical Institute, Uzbekistan
Aleynik.vladimir@mail.ru

Vasilevskiy Eduard Aleksandrovich
Andijan State Medical Institute, Uzbekistan
ed1970doctor@mail.ru

Dadabayev Omonjon Tolibjonovich
Andijan State Medical Institute, Uzbekistan
omonjondadabayev76@gmail.com

ABSTRACT

Background and Aim. Individual genetic polymorphisms demonstrate only moderate predictive accuracy for liver cirrhosis (LC) in chronic hepatitis C (CHC). Polygenic approaches that combine multiple loci covering distinct pathogenetic pathways may substantially improve risk prediction. This study aimed to develop a combined genetic risk score based on five polymorphisms (TNF-α G308A, IL28B C/T, VEGFA C936T, MMP9 Gln279Arg, SOD2 Ala16Val) and to evaluate its diagnostic performance in comparison with individual loci and standard clinical indices.

Materials and Methods. 93 CHC patients were enrolled: Group I comprised 48 patients without cirrhosis (F0–F3 by METAVIR) and Group II comprised 45 patients with verified cirrhosis (F4). All five loci were genotyped by real-time PCR with TaqMan probes. A cumulative score (0–10) was calculated by assigning 0–2 points per locus (0 = protective homozygote, 1 = heterozygote, 2 = risk homozygote). ROC analysis with AUC calculation was performed. AUC comparisons were made using the DeLong method.

Results. A score ≥6 was found in 64.4% of cirrhosis patients vs. 16.7% of non-cirrhotic patients (χ^2=22.87; p<0.001; OR=9.29; 95% CI 3.37–25.6; RR=3.86; 95% CI 1.89–7.90). The combined score AUC was 0.79 (95% CI 0.70–0.87), significantly exceeding each individual polymorphism (p=0.003–0.04). Integration with APRI >1.0 yielded AUC=0.86 (95% CI 0.78–0.93), specificity 91.7%, and PPV 87.5%. The genetic score correlated with MELD (r=0.47; p<0.001), APRI (r=0.52; p<0.001), and liver stiffness by elastography (r=0.56; p<0.001).

Conclusion. The combined genetic risk score substantially outperforms individual polymorphism analysis and provides clinically meaningful prediction for LC formation.

Integration with APRI achieves AUC=0.86. A three-tier risk stratification algorithm is proposed for practical clinical application.

Keywords: Genetic risk score, polygenic prediction, liver cirrhosis, chronic hepatitis C, ROC analysis, APRI, MELD, risk stratification, TNF-α, IL28B, VEGFA, MMP9, SOD2.

Introduction

Progression of chronic hepatitis C to liver cirrhosis is determined by a complex interplay of viral, environmental, and host genetic factors that collectively shape each patient's individual risk profile [1, 2]. In our previous studies, we comprehensively analyzed the associations of five single nucleotide polymorphisms (SNPs) with LC formation in CHC: TNF-α G308A (rs1800629), IL28B C/T (rs12979860), VEGFA C936T (rs3025039), MMP9 Gln279Arg (rs17576), and SOD2 Ala16Val (rs4880). Each polymorphism demonstrated a statistically significant association with cirrhosis; however, the diagnostic accuracy of individual loci remained moderate, with AUC values ranging from 0.60 to 0.67 [3, 4].

The limited predictive value of single genetic markers in polygenic diseases is a well-established phenomenon in genetic epidemiology. Each SNP explains only a small fraction of heritable variability, and only the cumulative contribution of multiple loci can approach a clinically meaningful level of prediction [5]. Huang et al. (2007) proposed a 7-gene signature for LC prediction in CHC, demonstrating the substantial superiority of a combined approach over individual gene analysis [6]. However, accessible and validated risk scores adapted to specific populations and not requiring expensive sequencing panels remain virtually unavailable.

A fundamental advantage of our five-polymorphism panel is its coverage of distinct yet complementary pathogenetic pathways of fibrogenesis: chronic inflammation (TNF-α), innate antiviral immune response (IL28B), pathological angiogenesis (VEGFA), extracellular matrix remodeling (MMP9), and oxidative stress (SOD2). This complementarity ensures an additive, and potentially synergistic, effect when combined into a unified prognostic score. Importantly, these five genes were selected not arbitrarily but based on their individual statistical significance in our population-specific analysis, ensuring that every component of the score contributes independently to risk prediction.

An additional area of interest is the integration of genetic data with routine clinical indices, primarily APRI (AST-to-Platelet Ratio Index) and MELD (Model for End-Stage Liver Disease). The genetic score is a stable parameter determined once and not subject to dynamic fluctuations, whereas APRI and MELD reflect the current functional state of the liver. Their combination potentially merges information about genetic susceptibility with data on the current stage of hepatic damage, providing a more comprehensive risk assessment than either approach alone.

The aim of this study was to develop a cumulative genetic risk score based on five polymorphisms, to evaluate its diagnostic performance in comparison with individual loci and

standard clinical indices, and to determine the optimal combination for practical LC prediction in CHC.

Materials and Methods

This study was conducted at the clinic of Andijan State Medical Institute from 2019 to 2024. The protocol was approved by the local ethics committee; all patients provided written informed consent. A total of 93 CHC patients were enrolled: 51 males (54.8%) and 42 females (45.2%), mean age 48.6±11.2 years. The diagnosis was confirmed by detection of anti-HCV antibodies by ELISA and HCV RNA by real-time PCR. Fibrosis stage was verified by transient elastography (FibroScan, Echosens, France).

Group I included 48 (51.6%) patients without cirrhosis (F0–F3 by METAVIR). Group II comprised 45 (48.4%) patients with verified cirrhosis (F4). Child-Pugh classification in Group II: class A in 21 (46.7%), class B in 17 (37.8%), and class C in 7 (15.5%). Exclusion criteria: HBV or HIV co-infection, alcohol abuse, autoimmune liver diseases, and hepatocellular carcinoma.

Genotyping of all five loci was performed by real-time PCR with allele-specific TaqMan probes on a CFX96 thermal cycler (Bio-Rad). Genomic DNA was extracted from venous blood using commercial kits (GeneJET, Thermo Fisher Scientific). Each sample was analyzed in duplicate for each of the five loci.

Genetic risk score calculation. For each polymorphism, a score was assigned based on genotype: 0 points for the protective homozygote, 1 point for the heterozygote, and 2 points for the risk allele homozygote. Protective and risk alleles were defined based on the results of our association analysis [3, 4]. The maximum possible score was 10. The scoring scheme is presented in Table 1.

Table 1. Genetic risk score calculation scheme

Polymorphism	0 points (protective homozygote)	1 point (heterozygote)	2 points (risk homozygote)	Pathogenetic pathway
TNF-α (G308A)	GG	GA	AA	Inflammation
IL28B (C/T)	CC	CT	TT	Immune response
VEGFA (C936T)	CC	CT	TT	Angiogenesis
MMP9 (Gln279Arg)	AA (Gln/Gln)	AG	GG (Arg/Arg)	Matrix remodeling
SOD2 (Ala16Val)	TT (Ala/Ala)	TC (Ala/Val)	CC (Val/Val)	Oxidative stress

Clinical indices were calculated as follows: APRI = (AST/ULN) / Platelets ($\times 10^9$/L) × 100; MELD = 3.78 × ln(bilirubin mg/dL) + 11.2 × ln(INR) + 9.57 × ln(creatinine mg/dL) + 6.43.

Statistical analysis included Pearson's χ^2 test, Fisher's exact test, OR, RR, 95% CI, PPV, and NPV. ROC analysis with AUC, sensitivity, and specificity was performed for each

polymorphism individually, for the combined score, for APRI, MELD, and for the combination of score plus APRI. AUC comparisons were made using the DeLong method. Correlation analysis used Spearman's coefficient. Significance was set at $p<0.05$. Software: SPSS 26.0, MedCalc 20.0.

Results

Genetic risk score distribution. The distribution of patients by genetic score categories is presented in Table 2 and Figure 1.

Table 2. Distribution of the combined genetic risk score by groups

Score	Group I (n=48)	Group II (n=45)	χ^2	p	OR (95% CI)
0–2	18 (37.5%)	3 (6.7%)	12.67	<0.001	0.12 (0.03–0.43)
3–5	22 (45.8%)	13 (28.9%)	2.88	0.09	0.48 (0.21–1.12)
6–8	7 (14.6%)	20 (44.4%)	10.16	0.002	4.67 (1.73–12.6)
9–10	1 (2.1%)	9 (20.0%)	7.87	0.005	11.8 (1.43–97.1)
≥6 total	8 (16.7%)	29 (64.4%)	22.87	<0.001	9.29 (3.37–25.6)

Note: OR is calculated for Group II compared to Group I.

At a threshold of ≥6 points: sensitivity 64.4%, specificity 83.3%, PPV 78.4%, NPV 71.4%. OR=9.29 (95% CI 3.37–25.6; p<0.001); RR=3.86 (95% CI 1.89–7.90). Patients with a score ≥6 had nearly tenfold increased odds of cirrhosis compared to those scoring below 6. The most pronounced differences were observed at the extremes: at 0–2 points, cirrhosis was present in only 6.7%, whereas at 9–10 points, cirrhosis was identified in 90.0% (9 out of 10 patients).

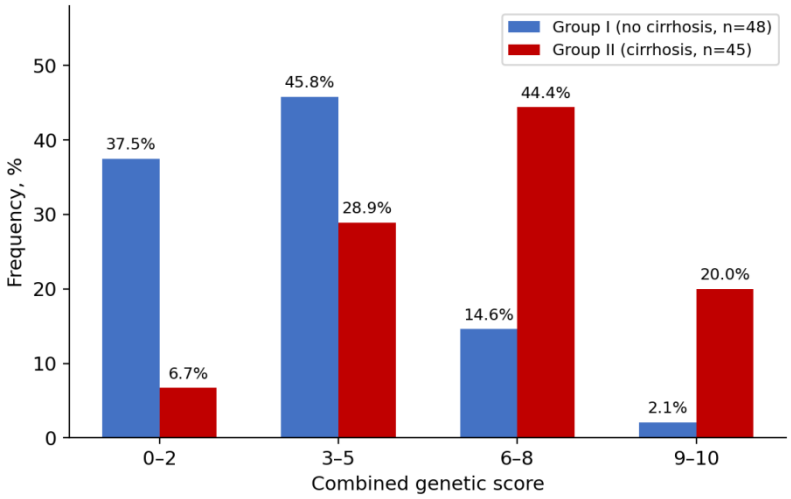

Figure 1. Distribution of the combined genetic risk score in Groups I and II

Comparative evaluation of diagnostic performance. ROC analysis was performed for each predictor individually and in combinations. The results are presented in Table 3 and Figures 2–4.

Table 3. Comparative diagnostic performance of cirrhosis predictors

Parameter	Score ≥6	Score ≥6 + APRI >1.0	APRI >1.0	MELD	IL28B	SOD2	TNF-α	MMP9	VEGFA
AUC	0.79	0.86	0.82	0.78	0.67	0.64	0.62	0.61	0.60
95% CI	0.70-0.87	0.78-0.93	0.73-0.90	0.69-0.87	0.57-0.77	0.54-0.74	0.52–0.72	0.50-0.71	0.49-0.70
Sensitivity	64.4%	62.2%	66.7%	60.0%	53.3%	42.2%	55.6%	48.9%	51.1%
Specificity	83.3%	91.7%	85.4%	83.3%	79.2%	72.9%	62.5%	68.7%	66.7%
PPV	78.4%	87.5%	81.1%	77.1%	70.6%	59.4%	58.1%	59.5%	58.9%
NPV	71.4%	72.1%	73.2%	69.0%	64.4%	57.1%	62.5%	58.9%	59.3%

The combined genetic score (AUC=0.79) significantly outperformed each individual polymorphism. Pairwise AUC comparisons using the DeLong method yielded the following results: difference with IL28B Δ12=0.12 (z=2.05; p=0.04); with SOD2 Δ15=0.15 (z=2.58; p=0.01); with TNF-α Δ17=0.17 (z=2.74; p=0.006); with MMP9 Δ18=0.18 (z=2.89; p=0.004); with VEGFA Δ19=0.19 (z=2.97; p=0.003). The genetic score was comparable in diagnostic performance to APRI (AUC=0.82; Δ03=0.03; z=0.66; p=0.51) and MELD (AUC=0.78; Δ01=0.01; z=0.16; p=0.87). A comparative visualization of AUC values is presented in Figure 2.

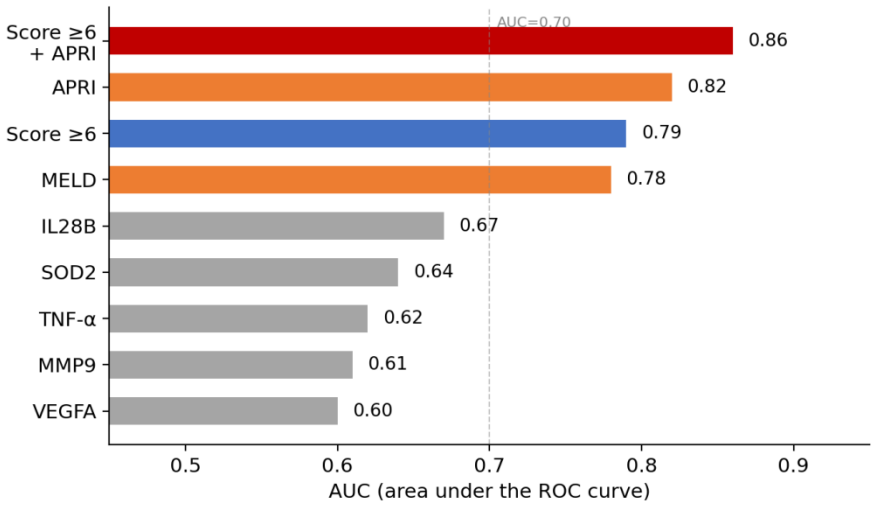

Figure 2. Comparative AUC values of cirrhosis predictors

Diagnostic performance of individual polymorphisms with 95% confidence intervals is shown in Figure 3. All five polymorphisms had AUC values significantly above 0.50 ($p<0.05$), confirming their individual prognostic significance, although none exceeded the 0.70 threshold commonly considered the minimum for clinical utility.

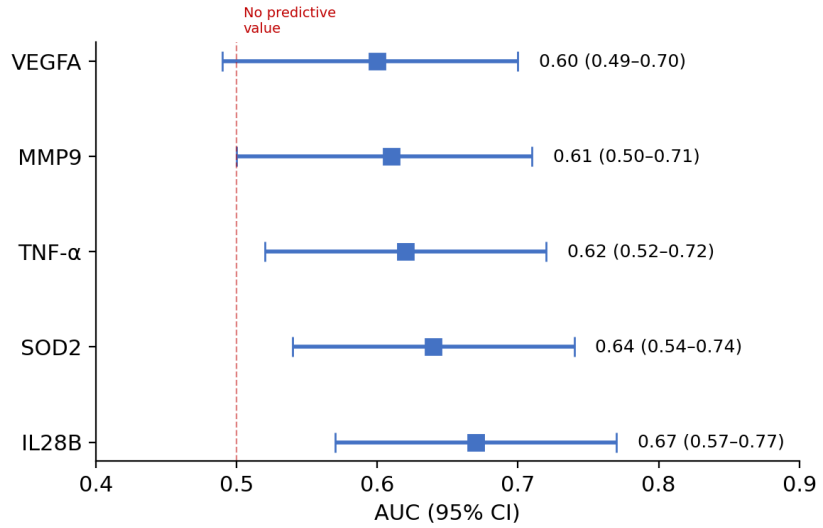

Figure 3. AUC of individual polymorphisms with 95% CI (dashed line = AUC 0.50)

Combination of genetic score with APRI

The highest diagnostic performance was achieved by combining the genetic score ≥6 with APRI >1.0: AUC=0.86 (95% CI 0.78–0.93), sensitivity 62.2%, specificity 91.7%, PPV 87.5%, NPV 72.1%. The AUC difference between the combination and APRI alone was Δ0.04 ($z=1.01$; $p=0.32$); between the combination and the genetic score alone was Δ0.07 ($z=1.56$; $p=0.12$). Although the AUC increment did not reach statistical significance, the clinically meaningful improvement was the increase in specificity from 83.3% to 91.7% and PPV from 78.4% to 87.5%. A comparison of diagnostic metrics is shown in Figure 4.

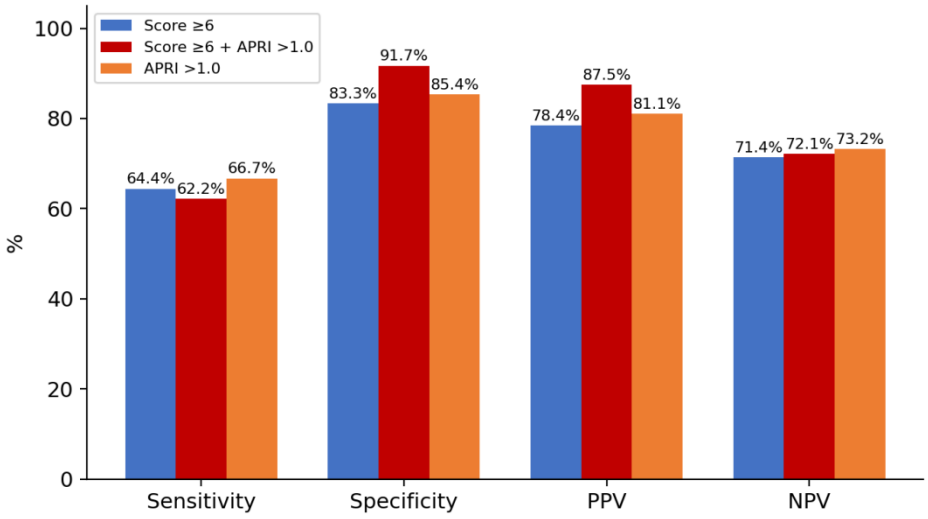

Figure 4. Comparison of sensitivity, specificity, PPV, and NPV across predictive approaches

Risk stratification. Based on the obtained data, a three-tier risk stratification system is proposed (Figure 5):

- **Low risk (0–2 points):** probability of cirrhosis 6.7%. Standard surveillance at 12-month intervals. This category included 37.5% of Group I patients.
- **Intermediate risk (3–5 points):** probability of cirrhosis 37.1%. Shortened monitoring intervals to 9 months with mandatory elastography. This category included 45.8% of Group I patients.
- **High risk (≥6 points):** probability of cirrhosis 78.4%. Monitoring every 6 months, immediate initiation of antiviral therapy, and upper endoscopy for esophageal varices assessment. This category included 64.4% of Group II patients.

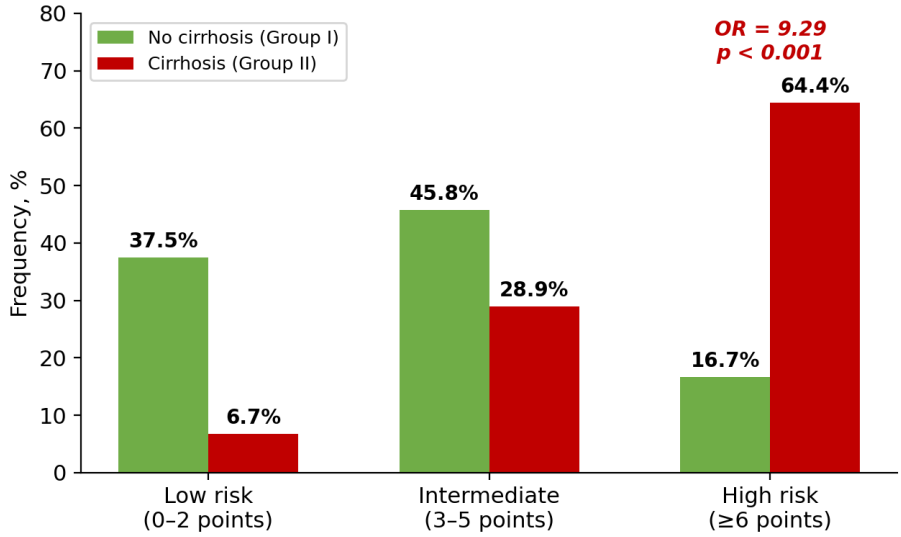

Figure 5. Risk stratification by combined genetic score (OR=9.29 for score ≥6)

Correlation analysis. The combined genetic score correlated significantly with clinical indices: Spearman's coefficient with MELD r=0.47 ($p<0.001$), with APRI r=0.52 ($p<0.001$), and with Child-Pugh score r=0.44 ($p<0.001$). Correlation with elastography data (liver stiffness, kPa): r=0.56 ($p<0.001$). The moderate correlation strength confirms that the genetic score and clinical indices reflect different but complementary aspects of the disease: genetic susceptibility and current functional status, respectively.

Discussion

The present results demonstrate the fundamental advantage of a polygenic approach to LC prediction in CHC. The combined genetic score AUC (0.79) significantly exceeds each of the five individual polymorphisms (0.60-0.67) and approaches the performance of routinely used clinical indices such as APRI (0.82) and MELD (0.78). The AUC increment of 0.12 to 0.19 when transitioning from monogenic to polygenic analysis confirms the additive contribution of genetic variants affecting distinct pathogenetic pathways.

Each of the five polymorphisms modulates a separate but pathogenetically interconnected mechanism of fibrogenesis. TNF-α determines the intensity of the inflammatory cascade and

hepatocyte apoptosis [7]. IL28B modulates the efficiency of the innate antiviral immune response through the interferon-lambda-3 system [8]. VEGFA controls pathological angiogenesis in fibrous septa, which is directly linked to portal hypertension progression [9]. MMP9 determines the intensity of extracellular matrix remodeling and the balance between collagen degradation and synthesis [10]. SOD2 regulates mitochondrial antioxidant defense and hepatocyte resistance to oxidative damage [11]. The accumulation of unfavorable variants across multiple mechanisms creates a synergistic effect, multiplying the risk of disease progression.

The high OR (9.29) for the ≥6-point threshold indicates a strong association and confirms the clinical significance of the score. For comparison, the OR for the strongest individual marker (IL28B TT) was 2.89, and for SOD2 Val/Val it was 2.33 [3, 4]. Thus, the combined analysis increases the strength of association more than threefold compared with the best individual marker, illustrating the power of integrating information across multiple biological pathways.

The combination of the genetic score with APRI deserves particular attention. Although the AUC increment (Δ0.07) did not reach statistical significance, the clinically critical improvement was the increase in specificity to 91.7% and PPV to 87.5%. High specificity is essential for a screening test as it minimizes false-positive results and, consequently, unnecessary invasive procedures. The principal advantage of this combination is the merging of a stable genetic profile, determined once and independent of the patient's current condition, with a dynamic clinical index reflecting the actual severity of hepatic damage.

The proposed three-tier risk stratification has direct practical implications. Low-risk patients (0–2 points, cirrhosis probability 6.7%) can be followed with standard surveillance intervals, reducing the burden on healthcare systems. High-risk patients (≥6 points, cirrhosis probability 78.4%) require intensive monitoring and urgent initiation of antiviral therapy. This differentiation enables rational allocation of limited resources, concentrating attention on patients with the highest progression risk.

The economic accessibility of the panel is an important aspect of its practical applicability. The cost of genotyping five loci by real-time PCR is approximately 450,000 UZS (approximately $35 USD), which does not exceed the cost of a single MRI examination and is substantially lower than the cost of liver biopsy. Crucially, the obtained genetic information retains its value for life and does not require repeat testing, unlike laboratory and instrumental parameters that require regular reassessment.

Our results are consistent with the polygenic prediction concept proposed by Huang et al. (2007), who developed a 7-gene signature using a similar approach [6]. Our score differs in using fewer genes (5 vs. 7), which simplifies and reduces the cost of analysis while maintaining clinically meaningful accuracy (AUC=0.79). Furthermore, our panel has been specifically adapted to the Central Asian population, accounting for local allele frequencies that may differ substantially from European or East Asian reference populations.

Study limitations include the single-center design, relatively small sample size (n=93), lack of prospective validation, and absence of an external validation cohort. The sample size precluded a comprehensive multivariate analysis adjusting for all covariates (age, sex, HCV genotype, duration of infection). Promising future directions include: (1) multicenter prospective

validation across different Central Asian regions; (2) panel expansion to 7–10 genes, incorporating PNPLA3 (rs738409), TM6SF2 (rs58542926), and HFE (rs1800562) to improve AUC; (3) development of a mobile calculator application for automated score computation and risk stratification; (4) evaluation of the score in subgroups of patients receiving and not receiving antiviral therapy.

Conclusions

1. The combined genetic risk score (0–10 points) based on five polymorphisms (TNF-α, IL28B, VEGFA, MMP9, SOD2) achieves AUC=0.79 (95% CI 0.70–0.87), significantly outperforming each individual polymorphism (p=0.003–0.04).
2. At a threshold of ≥6 points: OR=9.29 (95% CI 3.37–25.6; p<0.001), sensitivity 64.4%, specificity 83.3%, PPV 78.4%, NPV 71.4%.
3. The combination of the genetic score ≥6 with APRI >1.0 provides maximum diagnostic performance: AUC=0.86 (95% CI 0.78–0.93), specificity 91.7%, PPV 87.5%.
4. The genetic score is comparable in performance to APRI and MELD but represents a stable, once-determined parameter that retains its predictive value for life.
5. The three-tier risk stratification (low 0–2, intermediate 3–5, high ≥6 points) enables differentiated patient management and rational resource allocation.
6. The score is recommended for practical application in gastroenterology and infectious disease practice for risk stratification of CHC progression to liver cirrhosis.

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