

Combined serum IFN- γ and IL-22 levels as predictive biomarkers for hepatocellular carcinoma risk: A clinical investigation

HUI HUANG¹, XUEYING SUN¹, QIQI ZHANG¹, CHUNYAN LIU¹, XU CAO^{1,2},
DUO ZHANG¹, GONGCHEN WANG¹ and CHUNWEN PU¹⁻³

¹Department of Biobank, Dalian Public Health Clinical Center, Dalian, Liaoning 116001, P.R. China;

²Dalian Clinical Medical Research Center for Viral Hepatitis, Dalian, Liaoning 116001, P.R. China;

³Dalian Public Health Research Institute, Dalian, Liaoning 116001, P.R. China

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Abstract. Hepatocellular carcinoma (HCC) is predominantly diagnosed at advanced stages due to a lack of reliable biomarkers for early detection. Consequently, identifying predictive biomarkers for HCC development in patients with chronic liver diseases holds significant potential for improving early diagnosis and clinical outcomes of this aggressive malignancy. Serum levels of immune checkpoint proteins and cytokines were quantified by ELISA in patients with chronic hepatitis B, liver cirrhosis, and HCC, with healthy subjects serving as controls. Cytokine profiles were compared between patients with HCC and without HCC using one-way ANOVA and multivariate logistic regression. The diagnostic performance of interferon (IFN)- γ , interleukin (IL)-22, and α -fetoprotein (AFP) was evaluated by receiver operating characteristic (ROC) curve analysis. The serum levels of programmed cell death protein 1 and programmed death-ligand 1 progressively increased with advancing stages of liver disease. IFN- γ emerged as a protective factor against HCC [odds ratio (OR)=0.991; 95% CI, 0.984-0.997], while IL-22 was identified as a risk factor (OR=1.020, 95% CI, 1.004-1.036). The combined evaluation of IFN- γ and IL-22 demonstrated superior diagnostic accuracy [area under the curve (AUC)=0.863; 95% CI, 0.755-0.971] compared with AFP alone (AUC=0.644; 95% CI, 0.483-0.804). The combination of IFN- γ and IL-22 exhibited enhanced diagnostic performance compared with AFP alone in predicting and detecting early-stage HCC, suggesting its potential utility as a novel biomarker panel for HCC screening.

Introduction

Hepatocellular carcinoma (HCC) is a classic example of inflammation-associated malignancy, predominantly arising in patients with pre-existing chronic liver disease (1). The pathogenesis of HCC involves a complex, multistep process influenced by multiple etiological factors. Early-stage diagnosis remains particularly challenging because initial disease progression is asymptomatic. Consequently, there is a pressing clinical need for reliable diagnostic approaches capable of differentiating among distinct stages of HCC progression, which could significantly improve patient outcomes and survival rates.

Chronic hepatitis B virus (HBV) infection is a well-established risk factor for HCC development. As a non-cellular pathogenic agent, HBV induces chronic liver injury through aberrant immune-mediated mechanisms. Growing evidence supports the pivotal role of immune pathogenesis in HBV-related HCC progression. Recent studies indicate that immune escape mechanisms, mediated by the suppression of various immune cell subpopulations, significantly contribute to HCC initiation and progression (2). A comprehensive understanding of immune landscape alterations during HCC progression may provide valuable insights into developing early diagnostic strategies.

Immune checkpoints represent critical regulatory mechanisms that maintain immune homeostasis through both positive and negative regulation of immune cell activity. During tumorigenesis and progression, dysregulation or functional impairment of immune checkpoint proteins may facilitate tumor immune evasion and promote malignant progression (3). Programmed cell death protein 1 (PD-1), predominantly expressed on activated T cells, B cells, and myeloid cells, exerts immunosuppressive effects by binding to its ligands programmed death-ligand 1 (PD-L1) and PD-L2, thereby inhibiting T-cell activation and cytokine production. In HCC, tumor cells frequently exhibit upregulated PD-L1 expression, which engages PD-1 expressed on tumor-infiltrating T cells and suppresses their anti-tumor activity. Activation of the PD-1/PD-L1 axis significantly impairs T cell-mediated immune responses by inhibiting the secretion of key cytokines, including IFN- γ and TNF- α (3).

Correspondence to: Dr Chunwen Pu, Department of Biobank, Dalian Public Health Clinical Center, 269 Guibai Road, Ganjingzi, Dalian, Liaoning 116001, P.R. China
E-mail: 2295050337@qq.com

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Furthermore, cytokines such as IL-6 and TGF- β can modulate tumor immune evasion through regulation of immune checkpoint expression and function. Nevertheless, the precise interplay between PD-1 signaling and cytokine networks during HCC progression remains poorly understood. Recent research has expanded beyond PD-1 to investigate the role of T-cell immunoglobulin and mucin domain-containing protein 3 (Tim-3) as a novel immune checkpoint molecule in various pathological conditions (4). However, the expression dynamics and functional significance of Tim-3 in HCC pathogenesis remain elusive. Given these knowledge gaps, systematic analyses of immune checkpoint proteins and cytokine expression profiles are necessary to identify reliable biomarkers for early HCC detection.

To identify potential diagnostic biomarkers for different stages of HCC progression, the expression profiles of immune checkpoint proteins and cytokines were systematically analyzed in serum samples from healthy donors and patients with chronic hepatitis B (CHB), liver cirrhosis (LC), and HCC. Through statistical analysis using one-way analysis of variance (ANOVA) and multivariate logistic regression, interferon (IFN)- γ and interleukin (IL)-22 emerged as significantly differentially expressed cytokines. Receiver operating characteristic (ROC) curve analysis demonstrated that the combination of IFN- γ and IL-22 exhibited superior diagnostic performance compared with conventional α -fetoprotein (AFP) in predicting HCC. These findings provide a robust theoretical foundation for developing early clinical diagnostic strategies for HCC.

Patients and methods

Patient characteristics. The present study received approval [approval ID: 2023-029 (KY)-001] from the Ethics Committee of Dalian Public Health Clinical Center (Dalian, China). A total of 80 participants from Dalian Public Health Clinical Center were enrolled between January 2016 and December 2019. Written informed consent was obtained from all participants prior to inclusion. Demographic and clinical characteristics of the study population are presented in Table I.

Participants were stratified into four distinct groups: Healthy donors (n=17), patients with CHB (n=23), patients with LC (n=20), and patients with HCC (n=20). The diagnosis of CHB, LC, and HCC was established according to the standardized diagnostic criteria of the Asian Pacific Association for the Study of the Liver (5,6). Patients were excluded if they had coexisting viral hepatitis (such as HCV/HDV), HIV, non-viral liver disease, severe extrahepatic comorbidities, or were pregnant/lactating. Healthy donors were defined as individuals without evidence of hepatic disease or malignancy, as confirmed by comprehensive medical evaluation.

Quantification of cytokine and immune checkpoint protein expression. Cytokine and immune checkpoint protein levels were quantified using commercially available ELISA kits (Jiangsu Meibiao Biotechnology Co., Ltd.), according to the manufacturer's instructions. Specifically, IL-1 β was measured using the human IL-1 β ELISA kit (product no. MB-0181A), IFN- γ with the human IFN- γ ELISA kit (product no. MB-0033A), and PD-L1 with the human PD-L1 ELISA

kit (product no. MB-3913A). PD-1 levels were determined using the human PD-1 ELISA kit (product no. MB-3912A), while IL-22 was assessed with the human IL-22 ELISA kit (product no. MB-3810A). Additional cytokine measurements included IL-13 (product no. MB-0062A), IL-10 (product no. MB-3705A), IL-9 (product no. MB-0087A), IL-4 (product no. MB-0051A), and IL-6 (product no. MB-0049A). Furthermore, phosphatidylinositol 3-kinase (PI3K) levels were analyzed using the human PI3K ELISA kit (product no. MB-15917A), and Tim-3 was quantified with the human Tim-3 ELISA kit (product no. MB-15801A).

Statistical analysis. Statistical analyses were performed using SPSS version 22.0 (IBM Corp.) and graphs were generated using GraphPad Prism version 7 (Dotmatics). Continuous variables with normal distribution were analyzed by one-way ANOVA or unpaired Student's t-test, as appropriate. Scheffe and Games Howell were chosen to test the homogeneity of variance in the data for the post hoc comparison. Following the initial screening of variables based on the results obtained from one-way ANOVA, the selected variables were subsequently incorporated into a multivariate logistic regression analysis. A threshold of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Serum expression profiles of immune checkpoint proteins in healthy donors and patients with CHB, LC, and HCC. The expression levels of PD-1 and PD-L1 progressively increased with advancing liver disease, being lowest in healthy donors and highest in patients with HCC patients (Fig. 1). Comparative analysis revealed significant upregulation of both PD-L1 and PD-1 expression in patients with HCC relative to healthy controls ($P < 0.05$). By contrast, Tim-3 expression remained relatively stable across all groups, with no statistically significant differences (Fig. 1).

Serum cytokine expression profiles in healthy donors and patients with CHB, LC, and HCC. Comparative analysis of cytokine expression revealed distinct patterns across study groups. Serum levels of IL-1 β and IL-22 were significantly elevated in patients with HCC compared with the other groups (Fig. 2A and C). Conversely, IFN- γ and IL-10 concentrations were significantly decreased in patients with HCC than in healthy controls, CHB, and LC groups (Fig. 2B and H). Furthermore, IL-4 expression was significantly elevated in patients with HCC compared with healthy and LC groups (Fig. 2D), while IL-9 levels were significantly increased compared with healthy controls and patients with CHB (Fig. 2E). Notably, IL-13 expression was significantly higher in patients with HCC compared with the LC group (Fig. 2F). Among analyzed cytokines, only IL-6 exhibited no statistically significant differences between groups (Fig. 2G).

The PI3K signaling pathway plays a crucial regulatory role in fundamental biological processes, including cell growth, proliferation, and differentiation (7). Dysregulation and excessive activation of this pathway have been strongly implicated in tumorigenesis and cancer progression (7). To investigate its potential involvement in liver disease progression, PI3K

Table I. Patients clinical characteristics.

Characteristics	Healthy donors (N=17)	CHB (N=23)	LC (N=20)	HCC (N=20)
Age (years)	43.35±7.42	48.48±11.15	54.85±10.69	57.8±7.82
Sex (M/F)	5/12	20/3	14/6	12/8
WBC (10 ⁹)	5.8 (4.8-7.15)	4.96 (3.87-6.17)	4.27 (3.50-5.25)	4.69 (3.39-6.05)
ALT (IU/l)	14.2 (10.6-22.3)	54.3 (28-143.7)	34 (20.73-53.8)	25.5 (16-36.1)
AST (IU/l)	16.5 (15.25-20.55)	30.6 (17-79.4)	34.7 (26.38-46.28)	27.9 (18.38-37.15)
Total bilirubin	9.9 (6.9-13)	14.8 (10.8-18.1)	21.2 (12.9-30)	16.4 (10.28-21.7)
AFP >100 ng/ml	0	0	0	4

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; M, male; F, female; WBC, white blood cell; ALT, alanine aminotransferase; AST, aspartate aminotranferase; AFP, α -fetoprotein.

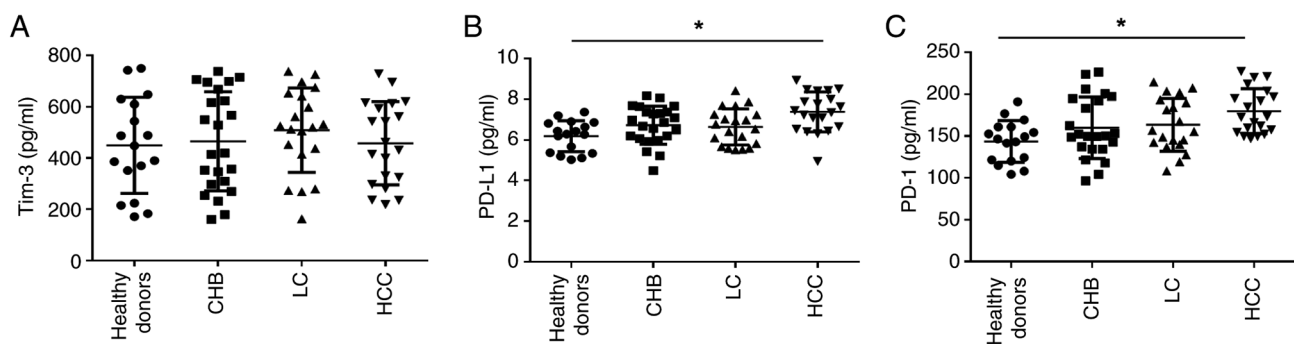


Figure 1. Serum expression profiles of immune checkpoint proteins in healthy donors and patients with CHB, LC, and HCC. (A) Tim-3 expression levels across study groups. (B) PD-L1 expression profiles among different cohorts. (C) PD-1 concentration variations in the study populations. * $P<0.05$. CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; Tim-3, T-cell immunoglobulin and mucin domain-containing protein 3; PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein 1.

expression levels were quantitatively analyzed in serum samples from healthy volunteers and patients with CHB, LC, and HCC. The results revealed progressive elevation of PI3K levels with advancing disease stages, with significantly higher expression in patients with HCC compared with the other groups (Fig. 2I).

Dual roles of cytokines in HCC pathogenesis: IFN- γ as a protective factor and IL-22 as a risk factor. In the multivariate logistic regression analysis, cytokine expression patterns were systematically evaluated using CHB, LC, and HCC as respective reference groups, incorporating all measured cytokines into the model. With HCC as the reference group, significant differential expression was observed for all proteins except IL-6 and Tim-3 ($P<0.05$; Table II). Notably, no statistically significant differences in cytokine profiles were detected among healthy donors, CHB patients, and LC patients.

Given that the majority of HCC cases develop from CHB infection, the subsequent analysis focused on hepatitis B-related disease progression by excluding the healthy control group. The remaining cohorts were stratified into HCC and non-HCC groups for comparative analysis. Through multivariate logistic regression analysis of differentially expressed proteins between these groups, IFN- γ was identified as a significant protective factor against HCC development [odds ratio (OR), 0.991; 95% CI, 0.984-0.997], while IL-22 emerged

as a distinct risk factor for HCC progression (OR, 1.020; 95% CI, 1.004-1.036) (Table III).

Diagnostic performance evaluation: ROC curve analysis of IFN- γ , IL-22, and AFP in HCC detection. To evaluate the diagnostic potential of IFN- γ and IL-22 in HCC, a comparative analysis of these biomarkers alongside AFP in patients with HCC, was performed. ROC curve analysis revealed that both IFN- γ (AUC, 0.810; 95% CI, 0.696-0.924) and IL-22 (AUC, 0.784; 95% CI, 0.661-0.907) demonstrated superior diagnostic performance compared with AFP (AUC, 0.644; 95% CI, 0.483-0.804) (Fig. 3 and Table IV). Furthermore, the IL-22/IFN- γ ratio exhibited enhanced diagnostic capability (AUC, 0.857; 95% CI, 0.747-0.967) relative to AFP. Notably, the combination of IFN- γ and IL-22 showed the highest diagnostic accuracy (AUC, 0.863; 95% CI, 0.755-0.971) among all tested parameters. The hierarchical order of diagnostic efficacy based on AUC values was as follows: IFN- γ combined with IL-22 > IL-22/IFN- γ ratio > IFN- γ > IL-22 > AFP.

Discussion

Current clinical detection of HCC primarily relies on imaging modalities, including ultrasound, MRI, CT, and AFP measurement (8). Although AFP is widely regarded as the most reliable serum biomarker for HCC diagnosis (9), its diagnostic accuracy

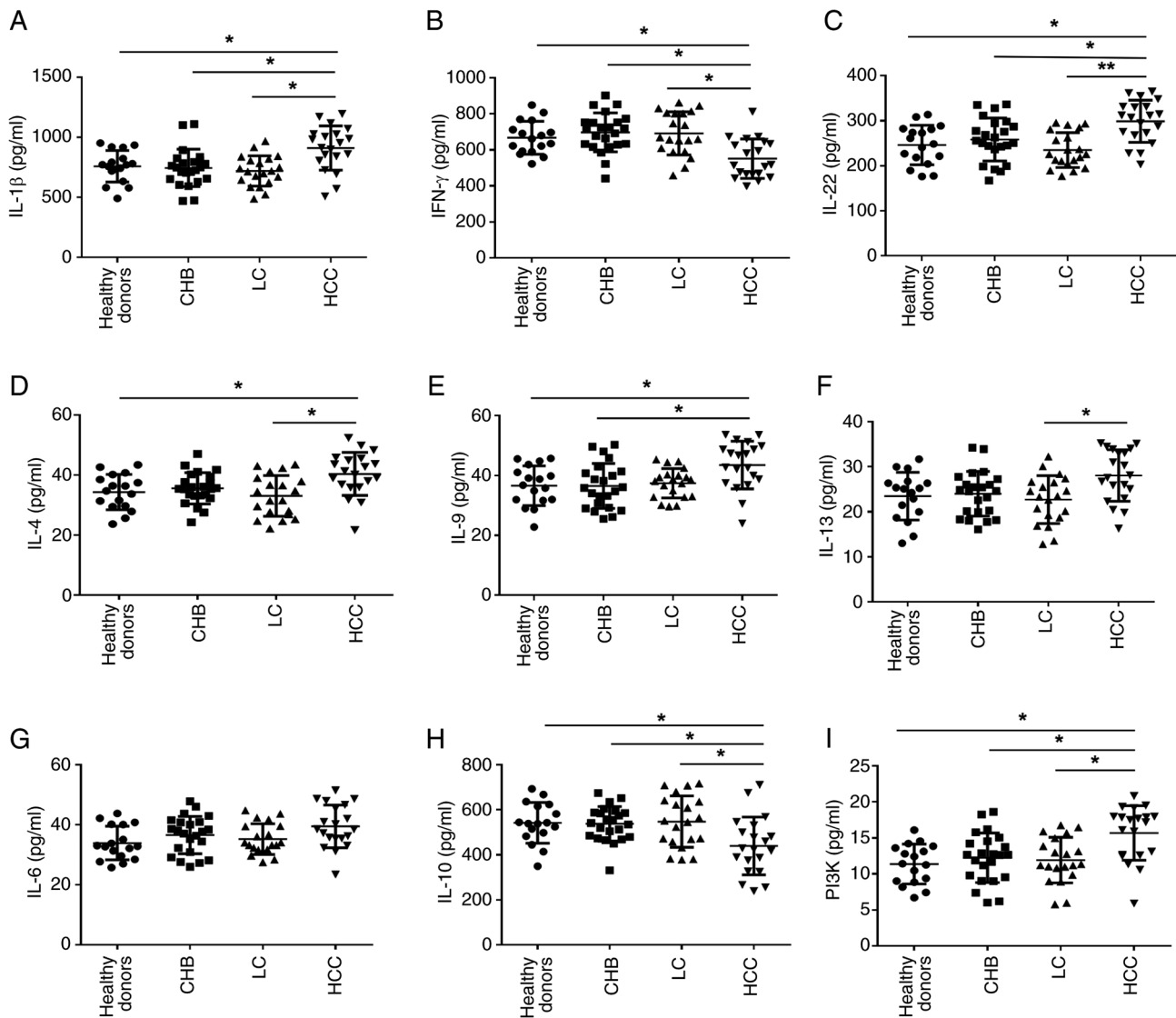


Figure 2. Serum cytokine expression profiles in healthy donors and patients with CHB, LC, and HCC. (A-H) Quantitative analysis of eight distinct cytokine expression profiles: (A) IL-1 β , (B) IFN- γ , (C) IL-22, (D) IL-4, (E) IL-9, (F) IL-13, (G) IL-6 and (H) IL-10. (I) PI3K expression levels across study groups. * $P < 0.05$ and ** $P < 0.01$. CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; IL-, interleukin; IFN- γ , interferon- γ ; PI3K, phosphatidylinositol 3-kinase.

remains limited, especially in early-stage HCC with tumors smaller than 2 cm, where AFP levels often remain below clinical detection thresholds (9). This limitation was apparent in the cohort of the present study, where only 4 out of 20 patients with HCC exhibited AFP levels exceeding 100 ng/ml. These findings underscore the critical need to develop more sensitive, non-invasive serological diagnostic approaches for early HCC detection.

HCC typically develops in the context of chronic liver disease, contributing to an immunosuppressive hepatic microenvironment and T-cell dysfunction (10,11). During hepatocarcinogenesis, the anti-tumor immune surveillance mechanisms of the liver become impaired, with immune checkpoint pathways, particularly the PD-1/PD-L1 axis, playing a pivotal role in this process (12). In the present study, serum PD-L1 and PD-1 levels were quantified using ELISA in samples from healthy donors and patients with CHB, LC, and HCC. Results revealed a progressive increase in PD-1 and

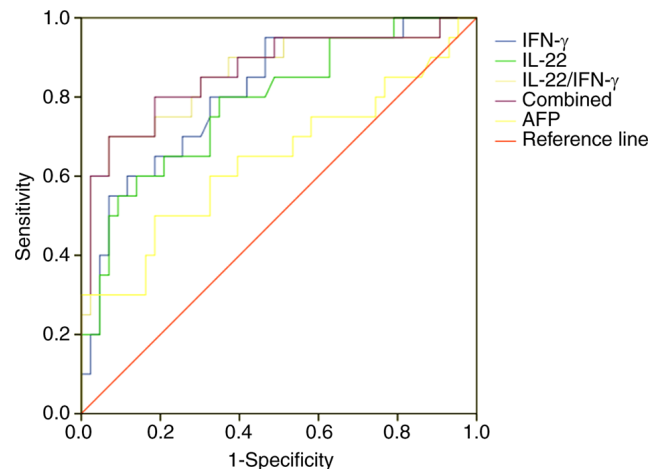


Figure 3. Comparative diagnostic performance of IFN- γ , IL-22, and AFP in HCC detection. ROC curve analysis comparing the diagnostic efficacy of IFN- γ , IL-22, and AFP in patients with HCC. IFN- γ , interferon- γ ; IL-22, interleukin-22; AFP, α -fetoprotein.

Table II. Statistical comparison of cytokines and immune checkpoint proteins using one-way ANOVA.

Characteristics	Control (N=17)	CHB (N=23)	LC (N=20)	HCC (N=20)	F	P-value
IL-1 β	759.71 $\pm$ 132.11 ^a	745.88 $\pm$ 155.74 ^a	720.63 $\pm$ 126.14 ^a	912.09 $\pm$ 184.46	6.499	0.001
IFN- γ	668.23 $\pm$ 91.62 ^a	697.89 $\pm$ 108.05 ^a	691.96 $\pm$ 119.93 ^a	551.93 $\pm$ 109.55	8.088	<0.001
PD-L1	6.18 $\pm$ 0.76 ^a	6.73 $\pm$ 0.94	6.64 $\pm$ 0.89	7.38 $\pm$ 0.98	5.564	0.002
PD-1	143.48 $\pm$ 24.86 ^a	159.92 $\pm$ 36.6	163.41 $\pm$ 31.55	179.78 $\pm$ 26.85	4.326	0.007
IL-22	246.24 $\pm$ 44.02 ^a	258.43 $\pm$ 47.83 ^a	234.75 $\pm$ 38.7 ^b	298.86 $\pm$ 46.85	7.73	<0.001
IL-13	23.49 $\pm$ 5.3	24.01 $\pm$ 4.96	22.72 $\pm$ 5.33 ^a	28.08 $\pm$ 5.72	4.031	0.01
IL-10	542.23 $\pm$ 90.53	538.21 $\pm$ 77.11 ^a	547.87 $\pm$ 114.29 ^a	439.82 $\pm$ 128.58	4.898	0.004
IL-9	36.61 $\pm$ 6.64 ^a	36.53 $\pm$ 7.49 ^a	37.44 $\pm$ 4.9	43.53 $\pm$ 7.93	4.783	0.004
PI3K	11.38 $\pm$ 2.77 ^a	12.25 $\pm$ 3.44 ^a	11.93 $\pm$ 3.16 ^a	15.69 $\pm$ 3.79	6.679	<0.001
IL-6	33.95 $\pm$ 5.63	36.61 $\pm$ 6.24	35.25 $\pm$ 5.12	39.51 $\pm$ 7.12	2.89	0.041
IL-4	34.33 $\pm$ 5.9 ^a	35.59 $\pm$ 5.21	33.06 $\pm$ 6.8 ^a	40.39 $\pm$ 7.19	5.11	0.003
Tim-3	449.18 $\pm$ 187.64	464.81 $\pm$ 193.19	508.38 $\pm$ 164.7	457.07 $\pm$ 162.4	0.43	0.732

^aP<0.05 and ^bP<0.01. CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; IL-, interleukin; IFN- γ , interferon- γ ; PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein 1; PI3K, phosphatidylinositol 3-kinase; Tim-3, T-cell immunoglobulin and mucin domain-containing protein 3.

Table III. Multivariate logistic regression analysis of HCC and non-HCC groups.

Characteristics	Multivariate analysis		
	OR	95% CI	P-value
IFN- γ	0.991	0.984-0.997	0.004
IL-22	1.020	1.004-1.036	0.014

HCC, hepatocellular carcinoma; IFN- γ , interferon- γ ; IL-, interleukin; OR, odds ratio; CI, confidence interval.

PD-L1 expression, with the lowest levels observed in healthy donors and the highest expression in patients with HCC, reflecting disease progression. PD-L1 and PD-1 expression were significantly elevated in patients with HCC compared with healthy controls (P<0.05). These results align with previous research demonstrating that HCC is characterized by high PD-L1 expression (13). Furthermore, observations of PD-1 upregulation in patients with HCC are consistent with reports of PD-1 induction in circulating monocytes from patients with HBV-associated HCC (13).

Although Tim-3 expression was assessed across the four study groups, no significant differences were observed among the cohorts. This suggests Tim-3 may have less influence on liver disease progression compared with the PD-1/PD-L1 pathway. However, the precise role of Tim-3 in liver disease pathogenesis requires further investigation. Unlike previous research utilizing cell lines (14), the present research used patient serum samples, providing more clinically relevant data to support the development of non-invasive diagnostic strategies for HCC detection.

HBV infection represents a global health challenge, affecting over 2 billion individuals worldwide and is one of

the primary etiological factors for liver disease (15,16). A total of ~350 million infected individuals progress to CHB, with one-third developing LC, and >75% eventually progressing to HCC (17). This disease continuum of CHB, LC, and HCC is widely recognized as the natural progression of chronic HBV infection (18). While immune-mediated chronic inflammation is established as a critical driver of HCC pathogenesis, the precise molecular mechanisms underlying this progression remain poorly understood. In this investigation, expression profiles of eight cytokines across different stages of chronic HBV infection were systematically analyzed. Initial screening using one-way ANOVA identified statistically significant cytokines, which were subsequently incorporated into multivariate logistic regression analysis. Two particularly noteworthy cytokines emerged: IFN- γ and IL-22. One-way ANOVA demonstrated significant upregulation of IL-22 in patients with HCC compared with healthy donors, CHB, and LC groups, whereas IFN- γ exhibited significant downregulation in patients with HCC relative to these groups. Given that most HCC cases develop from HBV-related CHB, the analysis focused on disease progression by excluding healthy donors and stratifying the remaining cohorts into HCC and non-HCC groups. Multivariate logistic regression analysis of differentially expressed proteins between these groups identified IFN- γ as a protective factor against HCC development (OR=0.991; 95% CI, 0.984-0.997), while IL-22 emerged as a significant risk factor (OR=1.020; 95% CI, 1.004-1.036).

IFN- γ , a pleiotropic cytokine, plays crucial roles in immune regulation, antiviral defense, and anti-proliferative processes (19). Previous research demonstrated that sustained IFN- γ exposure could suppress hepatocarcinogenesis despite concurrent liver injury (20). The findings of the present study revealed a progressive decrease in IFN- γ expression associated with HCC development. This downregulation might be mediated by Tim-3, which has been shown to inhibit IFN- γ production in T cells (21). However, the precise molecular mechanisms require further investigation. Notably, Li *et al* (20)

Table IV. Diagnostic accuracy assessed by AUC of ROC curves.

Variable	AUC	Std. error	P-value	95% CI
Combined	0.863	0.055	<0.001	0.755-0.971
IL-22/IFN- γ	0.857	0.056	<0.001	0.747-0.967
IFN- γ	0.810	0.058	<0.001	0.696-0.924
IL-22	0.784	0.063	<0.001	0.661-0.907
AFP	0.644	0.082	0.068	0.483-0.804

AUC, area under the curve; ROC, receiver operating characteristic; Std., standard; CI, confidence interval; IL-, interleukin; IFN- γ , interferon- γ ; AFP, α -fetoprotein.

reported that patients with HCC with low serum IFN- γ levels or negative IFN- γ receptor expression in tumor tissue exhibited larger tumor volumes, increased metastatic potential, and higher recurrence rates following treatment.

IL-22, a member of the IL-10 cytokine family, plays a significant role in antimicrobial defense, tissue homeostasis, and repair mechanisms (22). As a hepatocyte survival factor, IL-22 has substantial effects in various liver pathologies, including hepatitis, cirrhosis, and HCC, through interactions with IL-22R1 and IL-10R2 receptors (22). Although IL-22 demonstrates hepatoprotective properties during hepatic inflammation (23), emerging evidence suggests its dual role in liver pathophysiology. Previous research established an association between elevated levels of IL-22 and alterations in the hepatic microenvironment, with progressive increases observed from normal tissue to cirrhotic liver and subsequently to tumor tissue (24), a trend consistent with the findings of the present study in the sera of patients with LC and HCC. Furthermore, excessive IL-22 levels may promote hepatocarcinogenesis in chronic hepatitis patients, potentially facilitating tumor development through regulatory mechanisms in viral hepatitis (25).

To evaluate the diagnostic potential of these cytokines, comparative analyses using ROC curves for IFN- γ , IL-22, and AFP were performed in patients with HCC. ROC curve analysis demonstrated superior diagnostic performance of both IFN- γ and IL-22 compared with conventional AFP testing. Notably, the combination of IFN- γ and IL-22 exhibited enhanced diagnostic accuracy compared with AFP alone. These findings suggest that the combination of IFN- γ and IL-22 holds promise for early HCC detection, providing a theoretical foundation for clinical application. However, further research is warranted to elucidate the underlying mechanisms and validate these findings in larger clinical cohorts.

Furthermore, additional cytokines, including IL-4 and IL-9, showed significant differential expression during the progression from CHB and LC to HCC. IL-4 is a pleiotropic cytokine predominantly secreted by activated T helper 2 cells. As a key immunomodulatory molecule, IL-4 serves crucial functions in both mediating and regulating immune and inflammatory responses. This cytokine not only orchestrates humoral and cellular immune responses but also acts as a critical regulator of B cell, T cell, and macrophage functions in combating infections and malignant cellular processes (26). Current research indicates that IL-4, along with IL-13, functions as

a prototype inducer of M2 macrophages, an alternatively activated macrophage phenotype that has been demonstrated to facilitate HCC progression (26). IL-9 is a pleiotropic cytokine capable of modulating diverse cellular functions. Emerging evidence highlights the dual role of IL-9 in tumorigenesis, establishing it as a molecular double-edged sword in cancer biology. This cytokine exhibits context-dependent immunomodulatory effects in tumor immunity: It functions as a lymphocyte growth factor that promotes progression in hematological malignancies, while in certain solid tumors, it exhibits tumor-suppressive activity by activating both innate and adaptive immune responses. Notably, these antitumor effects are not universal, as IL-9 has been shown to facilitate tumor progression in some solid malignancies (27). Despite these advances, the diagnostic potential of IL-9 in early-stage HCC remains largely unexplored. Consequently, further investigation is required to fully elucidate the mechanistic role of IL-9 in HCC pathogenesis and its broader implications in tumor immunology. The potential utility of these cytokines as predictive biomarkers for HCC development warrants further comprehensive investigation.

While the findings of the present study highlight the diagnostic potential of the IFN- γ /IL-22 cytokine panel, several limitations should be acknowledged. On the one hand, the study cohort was derived from a single center, potentially limiting the generalizability of the results across diverse ethnic populations and etiologies of chronic liver disease. On the other hand, the mechanistic insights into the protective role of IFN- γ and the tumor-promoting effects of IL-22 remain observational. Further *in vitro* and *in vivo* functional studies are needed to elucidate the underlying signaling pathways. To address these limitations, multi-center validation in prospective, longitudinal cohorts is needed to confirm the reproducibility of the panel across different clinical settings. Mechanistic investigations using knockout models or cytokine-neutralizing antibodies are required to establish causality between IFN- γ /IL-22 and HCC pathogenesis. Future studies should also explore dynamic changes in cytokine levels during therapeutic interventions (such as immunotherapy or resection), which may further refine their utility as predictive biomarkers. Furthermore, immune checkpoint inhibitors show stage-specific variations in therapeutic efficacy across different liver disease phases. Through systematic analysis of immune checkpoint protein and cytokine expression profiles at various stages of HCC development, a distinct predictive molecular signature could

be established. This approach holds significant promise for achieving early and accurate HCC diagnosis, potentially revolutionizing current diagnostic paradigms.

In conclusion, the findings of the present study demonstrated that IFN- γ acts as a protective factor against HCC progression, while IL-22 serves as a significant risk factor for HCC development. Notably, the combination of IFN- γ and IL-22 exhibited superior diagnostic accuracy compared with conventional AFP testing for detecting HCC. These results suggest that the combination of IFN- γ and IL-22 holds substantial promise as a novel diagnostic tool for HCC, potentially improving early detection and clinical outcomes.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

CP conceived and designed the study. CL and GW contributed to data acquisition. XC and DZ contributed to data analysis. QZ and XS performed the formal analysis and data interpretation. HH analyzed the data and drafted the manuscript. CP and HH confirm the authenticity of all the raw data. All authors contributed to the writing, review and editing of the manuscript, reviewed the results, as well as read and approved the final version of the manuscript.

Ethics approval and consent to participate

The samples in this study were obtained with informed consent. The study was approved [approval ID: 2023-029 (KY)-001] by Ethics Committee of the Dalian Public Health Clinical Center (Dalian, China). The study complies with the Declaration of Helsinki.

Patient consent for publication

All patients involved in this study signed informed consent forms and agreed to the publication of the article.

Competing interests

The authors declare that they have no competing interests.

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