Glycomics as prognostic biomarkers of hepatocellular carcinoma: A systematic review

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Abstract. Hepatocellular carcinoma (HCC) is one of the most lethal malignancies, which is associated with a low 5-year survival rate. The importance of effective disease monitoring and prognostic evaluation is undeniable. For the present study, a systematic review was performed using extensive searches in Medline, Embase, Web of Science and Scopus up to December 29, 2023. The aim of the present study was to examine whether N-glycomics could predict the risk of developing HCC in adults with chronic liver disease and, if HCC was present, predict overall survival. As a secondary outcome, the prediction capability of HCC recurrence was assessed. After deduplication, 3,904 studies were identified, of which 30 were included. Overall, the median size of the study cohort was 144 patients, with a median follow-up time of 63.6 months. Three studies explored N-glycomics in whole serum, whereas the rest focused on individual glycoproteins, with Mac-2 binding protein glycosylation isomer (M2BPGi) being the most commonly studied. Most articles investigated baseline M2BPGi values as predictors for the development of HCC and demonstrated a median area under the curve of 0.83 with a cut-off index value of 1.8. In conclusion, it was revaled that N-glycan changes exhibit added value in determining patient prognosis in terms of survival, monitoring HCC development and recurrence.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies endangering global health, while the diagnostic assessment and routine follow-up measures are far from satisfactory (1). In general, the foremost significant risk factor for developing HCC is underlying liver cirrhosis (LC). Hence, biannual HCC surveillance strategies in daily medical practice are broadly recommended, including imaging with abdominal ultrasonography (US) with or without monitoring serum alpha-fetoprotein (AFP) (2). This screening tool, nevertheless, has inadequate performance characteristics for early HCC detection and evaluation due to modest early-stage sensitivity, interobserver variation and limited patient adherence (3).

While most cancers have decreasing mortality, HCC continues to be one of the leading causes of cancer-related death, with an overall five-year survival of 15% (1). Inadequate early detection, the lack of curative options for individuals found at advanced stages and conflicting risks of death from concomitant LC all contribute to this high mortality rate (1,3,4). Curative treatment modalities, such as radiofrequency ablation (RFA), liver surgery and transplantation, are only available for early-stage disease (5), with five-year survival rates above 60%. Relapse within those five years is prevalent, even after receiving optimal treatment. Accordingly, vigilant monitoring with attention to HCC development and prediction of recurrence is required to decrease disease-related mortality.

Serum is a useful tool for detecting HCC because many serum proteins are produced and secreted by the liver, and aberrant serum proteins may act as molecular indicators of liver disease progression and carcinogenesis. In the last decades, several serum proteins have been described as possible biomarkers for HCC diagnosis and prognosis, but most require further validation before being applicable in routine clinical practice. Lens culinary agglutinin-reactive fraction of AFP (AFP-L3), des-gamma-carboxyprothrombin (DCP) and cell-free DNA, for example, have all been studied (3). In cancer,

protein glycosylation has emerged as a major field of interest since it plays various roles in cellular activities (6). Humans experience two main forms of glycosylation: N- and O-linked glycosylation. The most common N-linked type involves sugar molecules attached to a nitrogen atom in an asparagine residue as part of a specific protein sequon. Different monosaccharides can be consecutively attached to each other and processed by several glycan-modifying enzymes without the use of a template, resulting in the dynamic manufacture of substantial glycopeptide heterogeneity. When cancer cells begin to develop abnormally, cell-cell interactions change and altered N-glycan structures become apparent during cancer progression, such as core fucosylation, β1,6-GlcNAc branching, bisecting GlcNAc and sialylation (6-8).

Consequently, detecting and quantifying specific glycans associated with tumour progression in patients with liver disease provides insight into cancer growth and could be a promising approach for personalised HCC management. In this study, we performed a systematic review of the potential value of N-glycomics as prognostic biomarkers in HCC.

Materials and methods

Protocol. This systematic review was performed concordant with the PRISMA 2020 statement (https://www.prisma-statement.org/prisma-2020), and was registered on PROSPERO (https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=283324).

Literature search. A scoping review was performed to exclude existing systematic reviews on the same topic using the search terms 'glycosylation' and 'hepatocellular carcinoma'. A comprehensive literature search was conducted through the databases Medline (PubMed interface), EMBASE (embase.com interface), Web of Science Core Collection and Scopus through December 20, 2022, with no start date restriction. An update was executed through August 17 and December 29, 2023. With the assistance of an experienced librarian (N.P.), optimised search terms for the concepts 'glycosylation', 'hepatocellular carcinoma' and 'biomarker' were identified. Searches were limited to studies in English. Complementary, cited references (e.g., reference list of recent review articles) were explored, and grey literature (e.g., Google Scholar, Biblio UGent) was screened for eligibility. The search strategy for all databases is detailed in Table SI. The search databases used are available at the following URL*s*: https://pubmed.ncbi.nlm.nih.gov/; https://www.embase.com/#advancedSearch/; https://www.webofscience.com; https://www.scopus.com/search/form.uri?display=basic#basic.

Eligibility criteria and study selection. Published studies of any design, except review articles and guidelines, were included. Animal studies, studies on children (<18 years) and abstracts without the availability of a full-text paper were excluded. Eligible studies assessed the potential of serum N-glycomics as prognostic biomarkers for HCC regardless of aetiology, disease stage, comorbidities or treatment. After deduplication, using the Endnote software application, all records were screened independently by at least two authors (N.S., E.B. or X.V.) in Rayyan (doi: 10.1186/s13643-016-0384-4), with discrepancies resolved by consensus.

Outcome measures. The primary goal was to evaluate the capability of N-glycomics to predict the risk of developing HCC in adults with chronic liver disease and, if HCC was present, to predict the overall survival or survival rate. As a secondary goal, the potential to predict HCC recurrence was assessed. The predictability of the glycomics-based biomarker was expressed as the area under the receiver operating characteristic curve (AUC) with its optimal cut-off index (COI) value obtained by the maximised Youden index and corresponding sensitivity and specificity. P-values of the log-rank test, comparing high and low levels of the biomarker in the Kaplan-Meier analysis, were reported when available. Statistical significance was set at a two-tailed Pvalue of <0.05.

Data extraction. Relevant data were extracted from the full-text articles by two independent authors (N.S. and E.B.) using a standardised form designed a priori. The following information was retrieved from each study: author, year, study design, sample size of different study cohorts (training, control, validation), patient and disease characteristics, HCC aetiology, glycoprotein or N-glycan with its analytical technique and statistical methods.

Quality assessment. The QUALSYST quality assessment tool (doi: 10.7939/R37M04F16) was applied to assess the overall risk of bias. As such, the methodological quality of the included studies was determined based on 14 criteria. A score was given depending on the extent to which the specific criterion was disclosed (yes=2; partial=1; no=0; N/A=not applicable). Table SII outlines the summary score created for every article by adding the scores obtained across the rated criteria. The quality of the included articles was assessed by two independent reviewers (E.B. and N.S.), with discrepancies resolved by consensus.

Results

General results. A database search generated 8,386 studies, while 32 studies emerged through other methods. In total, 3,936 paper abstracts were reviewed, and 279 were considered eligible. Following a full-text evaluation, 249 publications were omitted, providing 30 studies examining serum N-glycomics as a predictive biomarker in HCC (Fig. 1). The features of the included reports are tabulated by the main examined outcome: prediction of HCC development in chronic liver disease (Table I), survival or mortality (Table SIII) and recurrence (Table SIV) in HCC. The most relevant descriptives are highlighted for all articles and the subset of articles on M2BPGi and for each outcome by itself (Table SV). Fig. 2 illustrates an overview of the investigated glycans and glycoproteins per outcome.

HCC prediction in chronic liver disease. In whole serum, a high baseline GlycoCirrhoTest, calculated as the logarithmic ratio of the bigalacto core-α-1,6-fucosylated bisecting biantennary glycan NA2FB to the triantennary glycan NA3 and GlycoHCCRiskScore (based upon six altered glycans) was investigated as a predictor for developing HCC in cirrhotic patients (9). Both glycotests outperformed AFP (AUC=0.56) at a COI value of 5.75 ng/ml, with no significant

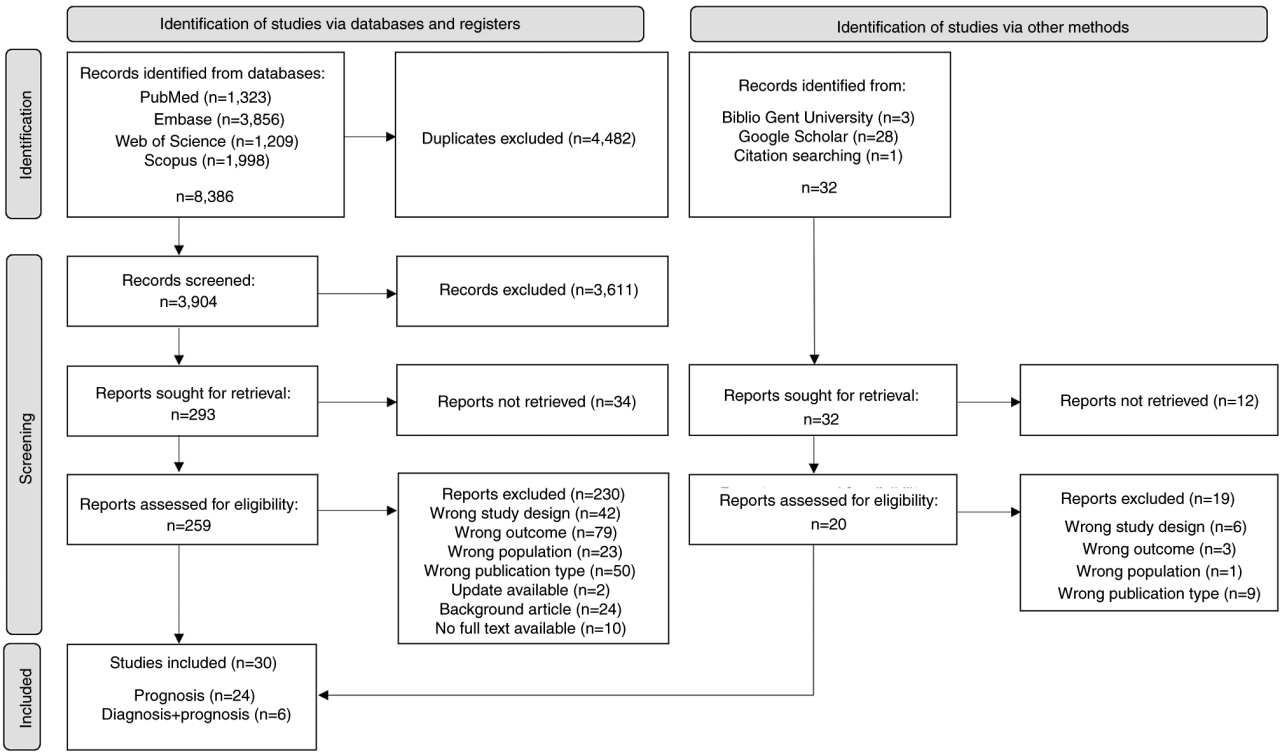

Figure 1. PRISMA flow diagram of the study selection process.

difference between the GlycoCirrhoTest (AUC=0.71) and GlycoHCCRiskScore (AUC=0.73) themselves.

Furthermore, particular glycoproteins associated with HCC development were investigated, such as Mac-2 binding protein, thrombospondin-2 (TSP-2) and fucosylated haptoglobin. The role of the serum Mac-2 binding protein glycosylation isomer or Wisteria floribunda agglutinin-positive Mac-2 binding protein (M2BPGi or WFA$^+$-M2BP), a well-known glycomarker of hepatic fibrosis, was studied in 19 articles. Eighteen of them investigated viral HCC aetiology, such as chronic hepatitis B (CHB) virus (10-20) and hepatitis C virus (HCV) infection (21-27), while only a single research group considered other underlying liver diseases (28). In terms of outcome measures, the majority addressed the impact of baseline M2BPGi/WFA$^+$-M2BP value in the development of HCC (10,12,13,15,16,21,22), and the remainder aligned pre- or post-treatment values with overall survival (23,24,27), recurrence (14,20,27,28) or death (24). Remarkably, all studies used the same chemiluminescent enzyme-linked immunoassay by a two-step sandwich method to analyse the M2BPGi/WFA$^+$-M2BP concentration in the serum (29).

Viral hepatitis remains a significant global health issue, exposing patients at increased risk of developing cirrhosis, hepatic decompensation and eventually HCC. Highly successful antiviral medications have reduced liver inflammation and suppressed virus replication; nevertheless, these agents can only minimise but not prevent the risk of developing HCC. Even after long-term viral suppression, this liver cancer type can occur (12,17,25). As such, HCC risk prediction has become imperative in the clinical monitoring and disease management of CHB and HCV patients. In the past few years, the significance of M2BPGi/WFA$^+$-M2BP as a feasible predictive marker for the development of HCC in viral hepatitis has been explored. Elevated baseline values of this glycoprotein were able to discriminate CHB individuals at high risk for HCC from those at low risk before treatment (10-13), during treatment with nucleoside analogues (NA) (14) such as Entecavir, and in treatment-naive patients (both cirrhotic and non-cirrhotic) (11,13,15,16). The same results were obtained when M2BPGi was assessed as an independent predictor during viral remission following NA treatment (17,18), even in those with low AFP (P<0.001) (19). This prognostic potential was also achieved in the hepatitis C population, in which both the baseline value (in mostly untreated patients) (21-23) and the post-therapeutic value after direct-acting antivirals (DAAs) or interferon (IFN) at the time of virological elimination (24-26), were considered to predict HCC development.

Subsequently, six studies compared M2BPGi/WFA$^+$-M2BP directly to AFP and their combination in predicting the development of HBV- and HCV-related HCC. They showed that baseline M2BPGi and the combination with AFP (model, AUC=0.81) was superior to AFP alone for long-term prediction until five years (M2BPGi, AUC=0.74) (15) and ten years (M2BPGi, AUC=0.84) (16) in treatment-naive CHB. Equivalently, baseline WFA-M2BP (AUC=0.87) and the combination with AFP (AUC=0.91) served as a better marker for short-term prediction within the first year in treatment-naive HCV (21). At time of sustained viral response (SVR) after antivirals in HCV, M2BPGi/WFA$^+$-M2BP with COI above 2 was able to predict the development of HCC, with an AUC of 0.97 [26], even up to ten years after treatment (AUC=0.71) (25). Similarly, elevated TSP-2 level - a glycoprotein produced by the fibrotic liver - in HCV patients who achieved SVR with DAAs was found to have a function as

Table I. Prediction of HCC development in chronic liver disease.

First author, year of publication	Patients with HCC	Controls	External validation	Analytical technique	Biomarker characteristics			(Refs.)
					Glycan	Prognostic value	AUC	
Verhelst *et al*, 2017	n=125 LC n=34 HCC Median FU time: 66.7 m	N/A	N/A	DSA-FACE	Whole serum GlycoCirrhoTest=Log [NA2FB]/[NA3] GlycoHCCRiskScore =[(NGA2FB x 0.137) + (NA2FB x -0.044) + (NA3 x -0.216) + NA3Fb x 0.158) + (NA3Fbc x 0.796) + (NA4 x -0.764)]	High baseline GlycoCirrhoTest (≥0.2) and GlycoHCCRiskScore are predictive for developing HCC in LC	GlycoCirrhoTest AUC=0.71 GlycoHCCRisk Score AUC=0.73 AFP AUC=0.56	(9)
Liu *et al*, 2017	n=357 HBV-related HCC FU-time: 7 y	n=713 CHB	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High baseline M2BPGi (≥2) and the prediction model (M2BPGi + AFP + HBsAg) are predictive for developing HCC in treatment-naive CHB	1-2 years model: AUC=0.84 M2BPGi: AUC=0.79 AFP: AUC=0.69 2-5 years model: AUC=0.81 M2BPGi: AUC=0.74 AFP: AUC=0.63	(15)
Jun *et al*, 2019	n=692 CHB n=47 HCC Median FU time: 6.8 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High baseline M2BPGi (>1.09) is predictive for developing HCC intreatment-naive CHB	10 years CHB M2BPGi: AUC=0.84 AFP: AUC=0.75	(16)
Mak *et al*, 2019	n=207 CHB n=14 HCC Median FU time: 13.1 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High M2BPGi (≥0.68) within 3 years after HBeAg seroconversion is predictive for developing HCC in treatment-naive CHB	AUC=0.88	(10)
Heo *et al*, 2016	n=95 CHB n=7 HCC Median FU-time: 45 m	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	WFA^+-M2BP	High baseline WFA^+-M2BP (≥1.8) is predictive for developing HCC in CHB (82% antiviral therapy)	P=0.016	(13)

Table I. Continued.

First author, year of publication	Patients with HCC	Controls	External validation	Analytical technique	Biomarker characteristics			(Refs.)
					Glycan	Prognostic value	AUC	
Murata *et al*, 2020	n=147 CHB n=14 HCC Median FU time: 6.6 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High M2BPGi (>1.5) at 48 weeks NA therapy is predictive for developing HCC in CHB	AUC=0.83 P<0.001	(18)
Su *et al*, 2020	n=126 LC n=20 HBV-related HCC Mean FU time: 50.3 m	N/A	n=145 LC n=30 HBV-related	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High M2BPGi (≥3) at antiviral therapy-induced VR is predictive for developing HCC in CHB	AUC=0.79 P<0.0001	(17)
Shinkai *et al*, 2018	n=234 CHB n=24 HCC Median FU time: 51 m	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High M2BPGi (≥1.21) at 48 weeks of NA therapy (VR) is predictive for developing HCC in CHB	P<0.001 Low AFP: P=0.011	(19)
Mak *et al*, 2019	n=100 HBV-related HCC Median FU time: 7.1 y	n=185 CHB	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High baseline M2BPGi (≥1.15) is predictive for developing HCC in CHB on entecavir therapy	AUC=0.64 P=0.0370	(11)
Tseng *et al*, 2020	n=899 CHB n=64 HCC Median FU time: 7 y	N/A	n=384 CHB n=36 HCC HBV-related	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High baseline M2BPGi (≥1.73) is predictive for developing HCC in CHB on entecavir therapy	P<0.001	(12)
Kawaguchi, 2018	n=141 CHB n=17 + 71 HCC FU-time: 10 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	WFA^+-M2BP	High WFA^+-M2BP (≥0.8) during NA treatment is predictive for HCC recurrence and combi (≥1.05) with HBcrAG (≥3) is predictive for HCC development in CHB	M2BPGi recurrence AUC=0.71 P=0.0110 M2BPGi + HBcrAg development P<0.0001	(14)

Table I. Continued.

First author, year of publication	Patients with HCC	Controls	External validation	Analytical technique	Biomarker characteristics			(Refs.)
					Glycan	Prognostic value	AUC	
Lin *et al*, 2018	n=921 HCV n=122 Median FU-time: 21.7 y	n=799 HCV	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	WFA^+-M2BP	High baseline WFA^+-M2BP (≥1.5) and in combination with + AFP + age + sex +ALT + AST/ALT ratio are predictive for developing HCC intreatment-naïve HCV	1 year model: AUC=0.91 M2BPGi: AUC=0.87 AFP: AUC=0.82 M2BPGi overall AUC=0.76	(21)
Yamasaki *et al*, 2014	n=707 HCV n =110 HCC Mean FU-time: 8.2 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	WFA^+-M2BP	High baseline WFA^+-M2BP (≥4.0) is predictive for developing HCC in HCV (53% antiviral therapy)	P<0.001 3 years M2BPGi: AUC=0.83 AFP: AUC=0.77 5 years M2BPGi: AUC=0.86 AFP: AUC=0.80 7 years M2BPGi: AUC=0.82 AFP: AUC=0.80	(22)
Sasaki *et al*, 2015	n=238 HCV n=16 HCC Median FU time: 9.1 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	WFA^+-M2BP	High WFA^+-M2BP (>2) at SVR is predictive for developing HCC in HCV after IFN therapy	P<0.0001 3 years M2BPGi: AUC=0.91 AFP: AUC=0.88 5 years M2BPGi: AUC=0.81 AFP: AUC=0.78 10 years M2BPGi: AUC=0.71 AFP: AUC=0.63	(25)
Nagata *et al*, 2016	n=119 HCV n=8 HCC Mean FU-time: 7.1 y	N/A	N/A	Chemiluminescent enzyme-linked immunoassay (HISCL-5000)	M2BPGi	High M2BPGi (≥2.2) at SVR is predictive for developing HCC in HCV after therapy	M2BPGi: AUC=0.97 AFP: AUC=0.75 P=0.0007	(26)
Matsumae *et al*, 2023	n=786 HCV n=24 HCC Median FU-time: 41.5 m	N/A	n=262 of 786 HCV	ELISA	TSP-2 AFT = TSP-2 + FIB-4 + AFP	High TSP-2 (>86.95 ng/μl) at SVR is predictive for developing HCC in HCV after DAAs therapy	TSP-2: AUC=0.70 AFT: AUC=0.83 AFP: AUC=0.72 P<0.0001	(30)

Table I. Continued.

First author, year of publication	Patients with HCC	Controls	External validation	Analytical technique	Biomarker characteristics			(Refs.)
					Glycan	Prognostic value	AUC	
Asazawa *et al*, 2015	n=19 TNM I (<2 cm) FU time: 10 y	N/A	N/A	Lectin ELISA	Fuc-Hpt elevation rate = change in Fuc-Hpt values at 2 time points during 10-year FU)	High Fuc-Hpt elevation rate (>498.2%) is predictive for developing HCC in LC	Fuc-Hpt elevation rate AUC=0.75	(31)

M, months; y, years; AFP, alpha-fetoprotein; LC, liver cirrhosis; HBV, hepatitis B virus; CHB, chronic hepatitis B; HCV, hepatitis C virus; FU, follow up; (S)VR, (sustained) virological remission; DAAs, direct-acting antiviral agents; IFN, interferon; M2BPGi, Mac-2 binding protein glycosylation isomer; WFA^+-M2BP, Wisteria floribunda agglutinin positive Mac-2 binding protein; TSP-2, Trombospondin-2; Fuc-Hpt, fucosylated haptoglobin.

a biomarker for the development of HCC (AUC=0.70), which further improved (AUC=0.83) when combined with AFP and the Fibrosis-4 (FIB-4) index (30).

Finally, Asazawa *et al* (31) determined the elevation rate of fucosylated haptoglobin (Fuc-Hpt) as the difference in Fuc-Hpt levels at two time points over a ten-year follow-up period to predict the development of HCC in cirrhotic patients (AUC=0.75) and found that an increase of more than 498.2% had 100% specificity for HCC occurrence.

Survival prediction in HCC. The Car-G risk score (32) was constructed to predict survival in AFP-negative HCC patients based on a whole serum biomarker panel of 13 N-glycan abundances using logistic regression. After HCC resection, 30% of high-risk patients with a baseline Car-G risk score of more than 1.80 had shorter overall survival (Log Rank, P=0.025), and 35% had lower recurrence-free survival (Log Rank, P=0.046) than low-risk patients.

The role of the glycosylated AFP specifically garnered attention in prognostic biomarker research, just as it is valued in diagnostic settings. In 1998, Aoyagi *et al* (33) defined the fucosylation index (FI) of AFP as the percentage of the lectin-reactive fraction of AFP (AFP-L3) to total AFP when assessing overall survival in HCC patients receiving transarterial locoregional treatment based on baseline FI, AFP and their combination. They elucidated that patients with FI scores >18% and AFP concentrations >200 ng/ml were at risk for low overall survival; in terms of separating high-risk and low-risk individuals, the combination of FI and AFP concentration excelled the individual indices (Log Rank, P=0.0003). Similarly, a more recent study (34) demonstrated that baseline AFP-L3 prognostic performance was successful, even in patients with low AFP serum concentrations (≤20 ng/ml). The COI of AFP-L3 was 10% for AFP above 20 ng/ml, and the five-year overall survival of HCC patients with an AFP-L3 level above this COI was 28.3% lower than those with an AFP-L3 level below 10% (Log Rank, P=0.001). Five-year recurrence-free survival was not substantially different between both groups in a subcohort of 129 HCC patients treated with curative RFA.

Toyoda *et al* (28) and Fujiyoshi *et al* (27) questioned whether WFA^+-M2BP could act as a potential marker for the assessment of survival after curative resection and displayed that high baseline levels were able to predict low overall survival (Log Rank, P=0.013, COI >4.615) (27) or survival rate (Log Rank, P=0.0187, COI >3) (28). Similar results were seen in treatment-naive HCV patients or patients who did not achieve SVR after IFN therapy (23). Subsequently, an association was found between higher M2BPGi levels (cut-off 1.8-2.2) after DAA-induced SVR and mortality in HCV (Log Rank, P=0.02) (24). In addition, low overall survival (Log Rank, P=0.010, AUC=0.69) and high carcinogenesis rate (Log Rank, P<0.006, AUC=0.76) in HCV-related cirrhosis have been linked to augmented serum levels of WFA^+-colony stimulating factor 1 receptor (WFA^+-CSF1R, COI ≥310 ng/ml) and WFA^+-CSF1R% (COI ≥35%), respectively (35). The comparison for both outcomes was made with AFP and AFP-L3, but no significant results could be retained for both markers. The Kaplan-Meier technique with the Log Rank test was used to conduct survival

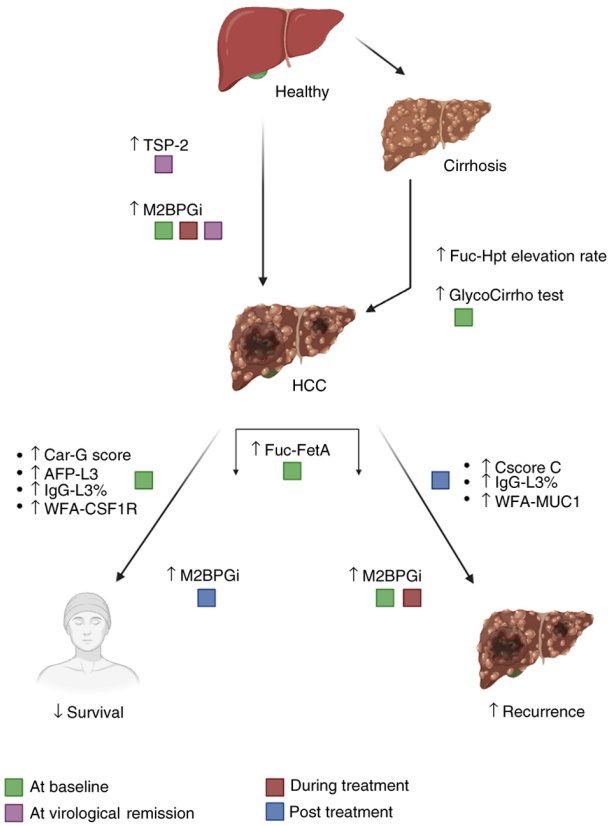

Figure 2. Overview of the most common investigated glycans and glycoproteins before, during or after treatment for HCC (created with BioRender.com). TSP-2, trombospondin-2; M2BPGi, Mac-2 binding protein glycosylation isomer; Fuc-Hpt, fucosylated haptoglobin; HCC, hepatocellular carcinoma; Fuc-FetA, fucosylated fetuin-A; AFP-L3, lens culinary agglutinin-reactive fraction of alpha-fetoprotein; WFA-CSF1R, Wisteria floribunda agglutinin-positive-colony stimulating factor 1 receptor; Cscore C=[(-0.3829 x NG1A2F) + (0.4214 x NA3Fb) + 0.9539]; WFA-MUC1, Wisteria floribunda agglutinin-positive-mucin 1.

studies. Corresponding P-values may also be found in Tables SIII and SIV.

Recurrence prediction in HCC. In whole serum, Fang *et al* (36) displayed that the logarithm of the ratio of the branching α-1,3-fucosylated triantennary glycan NA3Fb to the single monogalacto core-α-1,6-fucosylated biantennary glycan NG1A2F and the Cscore C performed better in monitoring HBV-related HCC recurrence after surgical resection, regarding the appearance of vascular invasion. As to AFP at COI of 200 ng/ml (AUC=0.61), the specificity of the log ratio improved by 16% compared to the same sensitivity level, with an AUC reaching 0.706 (Cscore C=[(–0.3829 x NG1A2F) + (0.4214 x NA3Fb) + 0.9539], AUC=0.703).

Towards research on predictive glycoproteins, three research teams revealed that an increased level of WFA$^+$-M2BP before curative resection (14,20,29) or RFA (combined with or without transarterial chemoembolisation [TACE]) (14) in viral hepatitis or other aetiologies (28) was associated with a high recurrence rate of HCC. Furthermore, three other glycoproteins are reported to play a potential role in predicting relapse after HCC treatment, namely fetuin-A (FetA) (37), immunoglobulin G (IgG) (38) and mucin 1 (MUC1) (39). Two studies questioned whether the risk for recurrence and low survival rates following curative liver resection in HBV-related HCC patients could be related to alterations in the glycomarker concentration. High preoperative fucosylated FetA (Fuc-FetA, COI >1.105) and IgG (IgG-L3%, COI >28%) levels were shown to be predictive of low recurrence-free survival (P=0.018) [37] and overall survival (P=0.023), whereas a postoperative increase in IgG-L3% predicted HCC recurrence (P=0.003) (38). Tamaki *et al* (39) disclosed that elevated serum Wisteria floribunda agglutinin-positive sialylated mucin 1 (WFA$^+$-sialylated MUC1, COI ≥900 μl/ml) levels could indicate a high recurrence rate (P=0.020) and less fortunate type of recurrence (P=0.020) in RFA-cured early-stage HCC patients.

Discussion

In this systematic review, we demonstrated that serum N-glycomics might be a valuable biomarker for predicting de novo HCC development in chronic liver disease or recurrence after treatment. Within the current shift towards personalised medicine, the prediction of disease progression and response to therapy is undeniable. To date, routinely performed liver biopsy has been discarded as the gold standard for diagnosis of HCC recurrence due to its invasiveness, sampling error and associated complication risks (13,40). Surveillance imaging at a well-defined interval, with or without serum AFP, is generally recommended (41). However, these methods do not always seem adequate for early HCC detection and disease monitoring. Although imaging can be sensitive for detecting a lesion, a certain tumour load is required before it becomes apparent, implying we are continuously falling behind. Similarly, the historically used marker AFP in diagnosing early HCC (with traditional COI of 20 ng/ml) is known for its poor sensitivity (ranging from 39 to 64%) with limited specificity (ranging from 76 to 97%), meaning that it can be a false positive in non-malignant conditions with active hepatocyte regeneration or false negative in the presence of HCC (4,42). In analogy with other malignant tumours, such as soft tissue sarcomas (43), recent reports have uncovered inflammatory parameters that may influence tumour aggressiveness and outcomes, such as neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein (CRP) and cytokines (44). However, the biomarkers often remain specific within a given setting (e.g., immunotherapy) and they have not been widely implemented clinically to date. The major unmet need in HCC management remains the lack of validated and clinically feasible noninvasive biomarkers with appropriate sensitivity and specificity, providing prompt information concerning HCC progression and prognosis compared to conventional biomarkers and allowing treatment decisions to be guided more effectively.

Serum N-glycomics might be able to meet this demand since it is known that alterations in the serum protein glycomic profile reflect a disbalance of the hepatocyte homeostasis, where the glycan product is mostly generated. Glycosylation, the most prominent posttranslational modification of proteins, depends on the expression of glycosyltransferases, which are dynamically regulated depending on the cell state. These glycome alterations disrupt the control of cell adhesion, migration and proliferation, resulting in pathological

processes that lead to cancer development (7). The metastatic potential of tumour cells, for example, has been linked to increased sialylation of cell surface glycoproteins, occurring as a result of the addition of terminal sialic acids to newly formed branches in the synthesis of multi-antennary glycans (↑ GlcNAc-transferase V). Other known glycomic changes linked to HCC include aberrant outer arm fucosylation of highly branched N-glycans (↑ α-1,3-fucosyl-transferase), synthesis of AFP-L3 (↑ α-1,6-fucosyl-transferase) and upregulated GlcNAc-transferase III, resulting in increased formation of bisecting GlcNAc (7,9,45). Fucose can be conjugated to N-glycans controlled by different fucosyltransferases (FUTs) in various ways. It has been displayed that α-1,6 core fucosylation of N-glycan GlcNAc residues, produced by FUT8, has a key function in modulating growth factor signalling pathways promoting tumourigenesis, notably those mediated by TGFβR, EGFR, VEGFR and c-Met1 (45-47). FUT3 to FUT7 and FUT9 to FUT11 facilitate α-1,3/4 branch fucosylation, acknowledged for generating a number of Lewis antigens and enhancing metastatic ability. In particular, E-selectin, expressed on endothelial cells, is bound by increased sialyl-Lewis X (sLe X) in cancer cells. Thus, the capacity of circulating tumour cells to extravasate from the vessels into neighbouring tissues may be improved (47,48).

We investigated 30 reports studying serum N-glycomics as omics-based prognostic biomarkers for the development and recurrence of, and overall survival in, HCC. Recent technology breakthroughs have resulted in multi-omics data, representing the biological heterogeneity of HCC and their potential as biomarkers. Proteomics research has focused, among many others, on the tumour markers AFP-L3, DCP and M2BPGi. An early stage of liver cancer is frequently diagnosed using the proportion of AFP-L3 to total AFP (AFP-L3%), which is believed to be an HCC-specific glycoprotein (32-34). Together with DCP, it is incorporated in the BALAD-2 prognostic model, which has been confirmed as an effective model for predicting survival in HCC patients by an international study (49). Both glycomarkers also seem to have prognostic value for survival after treatment (50) and waitlist dropout among HCC patients awaiting liver transplantation (51). Consequently, elevated levels of DCP, a non-functional prothrombin precursor linked to tumour angiogenesis and vascular proliferation, were considered effective for HCC prognosis in general (20,28,33,34). According to a recent review (52), DCP has a specificity of 81 to 98% and a sensitivity of 48 to 62% as a predictive marker in HCC, identifying advanced-stage individuals who may benefit from (first-line) sorafenib treatment and playing a prognostic role in the detection of portal vein invasion. Alternatively, the extensively glycosylated form of Mac-2 binding protein (M2BP), so-called M2BPGi or WFA+- M2BP, garnered a lot of attention as a novel biomarker of hepatic fibrosis progression, one of the strongest predictors of HCC development (28). Briefly, M2BPGi serves as a messenger for the activation of hepatic stellate cells throughout the advancement of fibrosis by triggering sinusoidal cell dysfunction. Since the sugar chain structure of M2BP would alter in response to the evolution of fibrosis, the potential of M2BPGi to predict HCC may be ascribed to its pertinent ability to distinguish different fibrotic stages. Moreover, the increased serum level of M2BPGi reflects underlying hepatocellular carcinogenesis, given its properties of activating the mammalian target of rapamycin (mTOR) signalling pathway by binding galectin-3. Besides being an indirect marker for hepatic fibrosis, serum M2BPGi could indicate early HCC detection during disease monitoring. Nevertheless, M2BPGi levels may not be used alone in HCC prediction, as elevated levels may also be seen in chronic cardio-pulmonary diseases (13,15,26,53).

Since hepatic fibrosis progression is considered to be one of the strongest predictors of HCC development, the investigated glycomics-based biomarkers were often compared to other indirect biochemical markers like the FIB-4 index and aspartate aminotransferase-to-platelet ratio index (APRI) (9,10,14,16,19,26,29,35). It is known that these evaluation methods are hampered by limited specificity in distinguishing fibrosis state, which was also confirmed in this systematic review. Individual laboratory values, whether or not linked to demographic characteristics like age and gender, are frequently used in prognostic HCC models to increase predictive accuracy. For example, we see that albumin and bilirubin are included in the BALAD-2 score (49), while platelet count is taken into account in the PAGE-B score (46). Not surprisingly, many glycomics studies in this systematic review also include (one of) these factors within their uni- or multivariate analysis.

Despite the potential use of serum N-glycomics as predictive biomarkers in HCC, various limitations must be acknowledged. First, specific study population groups were selected regarding aetiology in many of the included articles. Most studies considered only Asian patients with viral aetiology, making it difficult to extrapolate these results to the overall HCC population. Although viral hepatitis continues to be the leading cause of HCC globally, alcohol abuse and nonalcoholic steatohepatitis (NASH) are increasingly responsible for the rise in HCC incidence in Western countries. Second, most of the glycoproteins investigated are markers for fibrosis and, by extension, cirrhosis. It is unclear whether these indicators are equally effective in predicting the development of HCC in individuals who lack a cirrhotic background. Third, due to insufficient sample sizes and often retrospective study design, the test performances might be overestimated, and selection bias might be present. Further studies involving all aetiologies and expanding their sample sizes are required to resolve these issues. Fourth, not all research included statistical comparisons with 'a gold standard' like AFP, making it challenging to assess the benefit of the new biomarker across studies and obscuring its significance. More comprehensive studies are necessary to confirm the clinical application of N-glycan markers in this predictive setting. Finally, heterogeneity among the reports was too extended for a meta-analysis to be performed. Statistical analysis was considered on the subdomain related to the glycoprotein M2BPGi, since most articles investigated this glycoprotein. Of the eighteen M2BPGi-related publications there was access to hazard ratios (or the data from which the ratios could be calculated), seven articles focused on univariate analysis and eleven on multivariate analysis - with only seven articles correcting for the same variable in the latter. Consequently, the numbers and power were too small to conduct a proper meta-analysis. Moreover, a conscious decision was made not to concentrate

the research on specific glycoproteins, as the purpose of this systematic review is to provide all available data on predictive glycomics-based biomarkers in HCC.

To the best of our knowledge, no systematic review have been performed on this subject. In addition to the latest comprehensive analyses of non-invasive biomarkers for HCC screening (3) and immunotherapy (44), this review offers an outline of the updated knowledge regarding glycomics-based biomarkers in HCC, which could encourage current interest in the epigenetics of cancer and personalized medicine. Therefore, our findings may contribute to the research field as they provide a fresh perspective on the present state of expertise on glycomics in HCC.

In conclusion, it is generally accepted that early detection of HCC lesions can significantly improve long-term survival, and several HCC biomarkers have been studied for this purpose. However, none have obtained broad clinical usage, except for AFP, the current HCC biomarker that is approved on a global scale. The significant false positive rate of AFP in LC and the inadequate sensitivity in detecting early-stage HCC imply that new biomarkers are necessary. Aberrant N-glycosylation of serum proteins is known to contribute to HCC development. In particular, increased levels of M2BPGi may reflect underlying hepatocellular carcinogenesis. Aberrant N-glycosylation of serum proteins may ultimately have value as predictive biomarkers for the development, recurrence and survival of HCC. However, more research with a refined study design and patient selection is essential to validate this.

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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

NS, XV, HVV, LM and NC conceptualized the study and developed the theoretical framework. NS, EB and NP designed and constructed the methodology of the study, with extensive support from SL, LG, AG, SR and LD. NS, EB and XV curated data for the study (study selection was performed by NS and XV; full text selection by NS and EB; data extraction by NS and EB). NS wrote the original draft of the study. XV, LM, NC and HVV provided general supervision, project administration and funding acquisition. NS and XV confirm the authenticity of all the raw data. All authors discussed the results, and have read and approved the final version of the manuscript.

Ethics approval and consent for participation

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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