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Original article

Hepatocellular Carcinoma: Systematic Review of Relevant Biomarkers and the Impact on Liver Resections

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Abstract

Background: Hepatocellular carcinoma (HCC) is the primary cancer of the liver representing the 6th cause of cancer worldwide and the 3rd in terms of deaths per year. Recent advances have offered new treatment options for this disease. New links between biomarkers and patients' prognosis show a great potential for a more precise and targeted therapy. ***Method:*** A systematic review was performed following the guidelines outlined by The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Original articles published in the online database, Pubmed (Medline), Embase and Cochrane from January 2017 to February 2022 were screened after using specific keywords. ***Results:*** A total of 1300 studies was obtained by searching through online databases. Following the exclusion criteria, we included 18 studies in the systematic review. ***Conclusions:*** Biological markers are playing an important role for the prognosis of HCC. Alfa-fetoprotein (AFP) is still a valid biomarker, that can help stratify the risk for patients with HCC. Meanwhile, the current advancements in the field of miRNAs open new opportunities to analyse the role of miRNAs in HCC. Studies conducted on targeted therapies should also take into consideration the levels of these biomarkers, statistically linked with the prognosis of patients after liver resections.

Keywords: ***hepatocellular carcinoma, biomarkers, liver resections, surgical treatment, prognosis***

Introduction

Hepatocellular carcinoma (HCC) is the primary cancer of the liver, with an incidence of 4.7% of all cancers worldwide. The latest data from GLOBOCAN 2020 presents approximately 905 677 cases per year and 830 180 deaths caused by this pathology. Currently, it is the 6th cause of cancer worldwide and the 3rd cause in terms of deaths per year [1].

The highest incidence is observed in Asia (72.5%) with a mortality rate of 73.3%. Lower rates have been reported in Western Countries [1].

Advanced staged liver disease (ASLD) is considered to be the main risk for the development of hepatocellular carcinoma [2]. Even though the tumoral masses developed in a cirrhotic liver are most likely to become hepatocellular carcinomas, recent studies suggest that this type of cancer can develop on non-cirrhotic liver tissue or even in tissues without any objective signs of inflammation [3]. Therefore, in terms of social and economic impact, HCC is currently a burden for any medical system worldwide.

However, the main risk factors for developing ASLD remain chronic hepatitis C (HCV), hepatitis B (HBV) +/- delta, abusive alcoholic consumption, non-alcoholic steatohepatitis (NASH), autoimmune hepatitis, aflatoxins, diabetes, metabolic syndrome and genetic diseases [4,5].

In the past 5 years, there have been important advances both in hepatocellular cancer research and chronic viral hepatitis, with major improvements in terms of prevention and therapeutical approach [6].

Despite all this, for pathologists HCC is still a challenge and the main factors are represented by: tumour heterogeneity, hepatocellular differentiation, mixed hepato-cholangiocarcinoma, distinguishing HCC from its precursors or secondary malignancies [7]. Therefore, the treatment options are constantly changing based on the advancements in histopathological findings. Treatment armamentarium has gone through significant changes according to the latest updates of the BCLC guidelines from 2021, with further modifications proposed for 2022 [6].

Biomarkers such as α-fetoprotein (AFP) are associated with a more aggressive tumour phenotype and higher recurrence risk after

surgical resection, while a significant proportion of patients do not present elevation of AFP serum levels [8,9]. About 30-40% of the patients with HCC present low levels (<20 ng/ml) of this biomarker, however, even in these cases AFP levels are correlated with a poorer differentiation of the tumour, microvascular invasion, presence of satellite nodules and disseminated intrahepatic metastasis [9,10].

In order to increase the sensitivity and sensibility, several biomarkers have been proposed for the diagnosis and prognosis of HCC. Hence, the Japan Integrated System (JIS) has added the association between AFP and des-gamma-carboxyprothrombin (DCP) or Lens culinaris agglutinin (AFP-L3) to the latest recommendations [11].

Furthermore, glypican (GPC3), a heparan-sulphate proteoglycan is a promising biomarker due to the low expression in normal hepatocytes, focal nodular hyperplasia (FNH) and hepatocellular adenoma (HA) and relevant expression in HCC [8].

Osteopontin (OPN), a glycoprotein highly expressed in tumoral cells is associated with vascular invasion and undifferentiated HCC [12].

Golgi protein-73 and micro-RNAs are promising biomarkers in terms of prognosis, also conferring great potential for a targeted therapy [13].

The aim of the present systematic review is to offer an overview of the current, most relevant biomarkers in HCC in terms of their impact upon the prognosis of patients with HCC, undergoing liver resections.

Materials and Methods

Study design

The methodological algorithm for this systematic review consists of the definition of search strategies, selection criteria and data extraction. This study followed the guidelines outlined by The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (**see Figure 1**).

Search strategy

The original articles published on the online database, Pubmed (Medline), Embase and Cochrane since January 2017 until February 2022 were screened. Specific keywords were used to find eligible studies: (hepatocellular carcinoma) AND (biomarkers) AND (therapy) AND (surgery) AND (prognosis). The screening was performed by two independent reviewers, in order to further diminish bias.

Selection criteria

Eligible studies included data of current biomarkers in hepatocellular carcinoma and how they influence prognosis in patients with HCC, undergoing liver resections:

Exclusion criteria

1) Case series;
2) Letter to editors and brief reports;
3) Reviews;
4) Meta-analyses;
5) Non-English articles;
6) Non-surgical patients;
7) No liver resection, only liver transplant procedure;
8) No liver resection, only chemoembolization or ablation procedures.

Data extraction

Following a full review of the eligible studies, two independent reviewers performed data extraction and crosschecked all outcomes. During the process of selection and the extraction of data any disagreement between the two reviewers was discussed with a third and fourth reviewer. Reference lists from selected studies were searched for potential articles - "snowball" procedure.

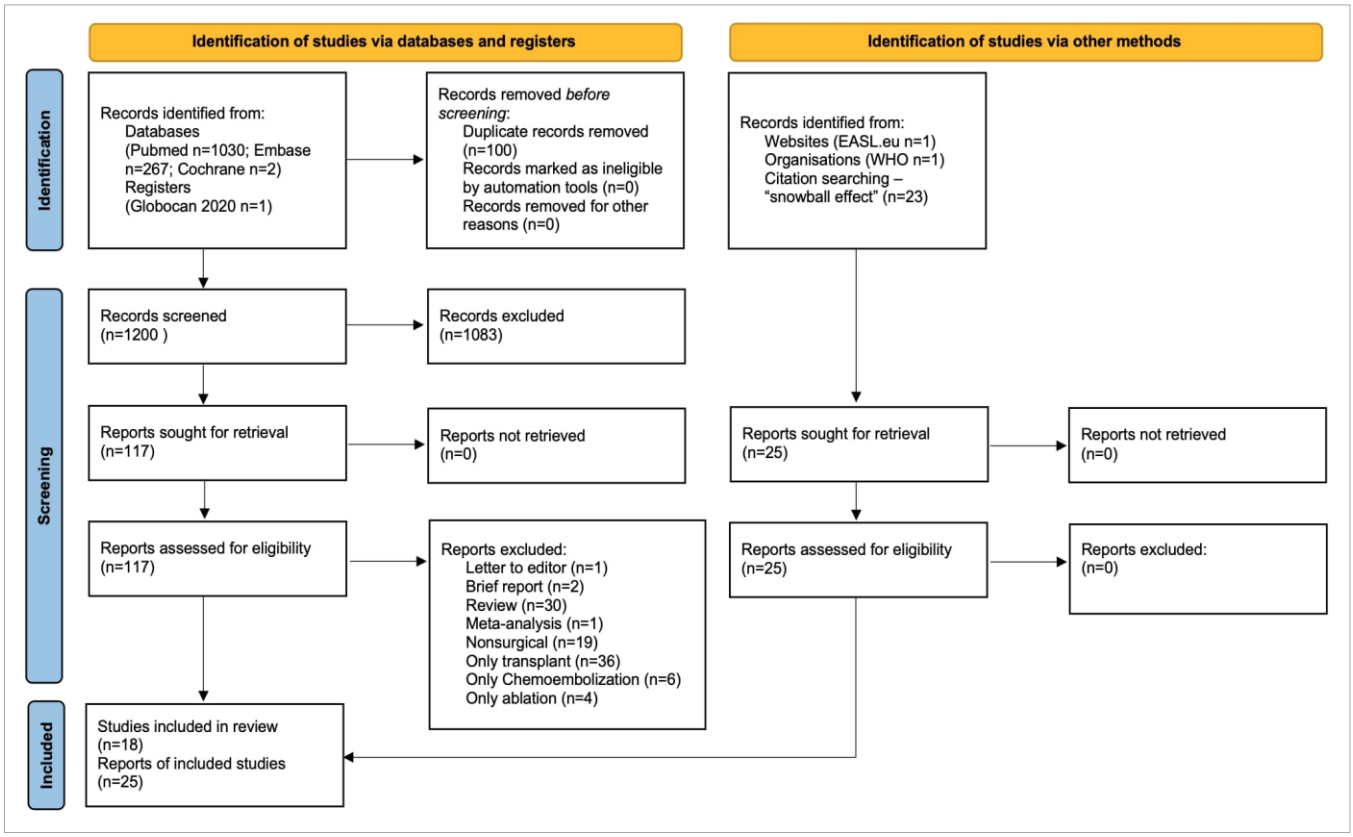

Figure 1: PRISMA flow chart for the selected studies included in the systematic review

Results and Discussion

Characteristics of the included studies

A total of 1300 studies was obtained by searching through Pubmed/Medline, Embase and Cochrane data bases. After exclusion of duplications, 1200 were screened for titles and abstracts. 1083 studies were excluded after screening, resulting in 117 articles, which were further fully reviewed. Following the exclusion criteria, we included 18 studies in the systematic review. 25 studies were identified via other methods (**see Figure 1**).

The included studies emphasize the role of several biomarkers in terms of prognosis, recurrence rate, tumour progression and overall survival. The characteristics of the included studies are depicted in table 1.

Five studies focused on the overall survival rate of the patients with HCC [14-18]. Tumour progression was evaluated in three studies [16,18,19]. Recurrence rate and prognosis were the main objectives in four studies [17,20-22], respectively eight studies [23-30]. Lee et al. studied the correlation between AFP values and the resection margins in patients with minor or major liver resections for HCC [31].

The included cohorts ranged from 40 patients up to 1572 subjects [20,23].

Table 1: General overview of the included studies

Author	Year	investigated variable	Number of patients	Objectives
Shen et al.	2017	AFP	280	Survival
Jung et al.	2017	Liver resections	1572	Prognosis
Von Felden et al.	2017	miRNA	40	Recurrence rate
Jones et al.	2018	mRNA	209	Recurrence rate
Fu et al.	2018	miRNA	318	Tumor progression and survival
Yoon et al.	2018	miRNA	93	Tumor progression
Ji et al.	2019	Glycoprotein 96	84	Prognosis
Zhou et al.	2019	AFP	710	Prognosis
Lee et al.	2019	AFP	534	Resection margins
Xiao-Long Li et al.	2019	AFP	841	Recurrence rate and survival
Manjiang Li et al.	2019	Risk factors	103	Prognosis
Ha et al.	2019	miRNA	289	Prognostic effect
McDonald et al.	2020	AFP	496	Survival
Cheng et al.	2020	Circular RNA 0016788	278	Tumor progression and survival
Huang et al.	2020	AFP	509	Prognosis
Tsilimigras et al.	2021	AFP	852	Recurrence rate
Gao et al.	2021	AFP	224	Prognosis
Hou et al.	2021	AFP, CA 19-9, CEA	128	Prognosis

RNA studies

The total number of studies conducted on RNAs was six (**see Table 2**). The RNAs were used as prognostic markers for recurrence rate and mortality, overall survival and tumor progression. Jones et al. and von Felden et al. investigated the role of miRNAs regarding the recurrence of hepatocellular carcinoma after curative liver resections [20,21]. Jones et al. included only a North American cohort with different underlying liver conditions demonstrating an association between lower tumoral miRNA-26a expression and the postoperative recurrence rate [21]. Von Felden et al. concluded that there is a significant association between high expression of miRNA-135 and early HCC recurrence [20]. The disease-free survival was 8.8 months in highly expressed subjects compared to 24.8 in low miRNA-135a expression. All subjects with early recurrence presented R1 margins of resection. In Yoon's et al. study, elevated miRNA-21 expression was linked to a significant poorer prognosis [19].

Fu et al. proved that a seven-miRNA signature was an independent prognostic factor after liver resections [16]. The applied model could predict the patients with stage I and II survival with statistically significant results.

In Ha's et al. study, the follow up median was 119 months and it highlighted that the low miR-122 expression group showed shorter recurrence-free survival (RFS) after curative surgery [27].

Cheng et al. investigated the role of circularRNA 0016788(circ_0016788) after liver resections, determining a statistically significant association between high expression of circ_0016788 and a shorter overall survival [18].

Different types of RNA extraction methods were used, including TaqMan MicroRNA Assays, Tect SYBR Green PCR Master Mix, High Capacity cDNA Reverse Transcription Kit in order to provide RNA samples from the liver tissue that was previously surgically resected.

The age of the subjects included in the studied cohorts ranged between 53 and 66.1 years [27,20]. The majority were stage I-II at the time of the diagnosis [16,20,21], while Yoon et al. reported more advanced disease [19]. The characteristics in regard of microinvasion are illustrated in table 3.

Table 2: Demographics, disease stage and the therapeutic strategy of the subjects included in the RNA studies

Author	Sex	mean Age (years)	BMI	Stage	Tumor-targeted therapy	Surgical intervention
Jones et al.	M – 72 F – 28	62.8	28.7	I/II – 53 III/IV – 20 UNK - 27	Yes – 28% No – 72%	Liver transplant – 49% Liver resections – 51%
von Felden et al.	M – 30 F – 10	66.1	Not Specified	I/II – 27 III/IV – 11	Yes – 22.5% No – 77.5%	Liver resections
Fu et al.	M – 213	65	Not Specified	I/II – 235	Yes – 16.9%	Liver resections

	F – 105			III/IV – 63 UNK – 20	No – 83.1%	
Yoon et al.	M – 81 F – 12	61.1	Not Specified	I/II – 34 III/IV – 59	Not Specified	Liver resections
Ha et al.	M – 238 F – 51	53	Not Specified	I/II – 238 III/IV – 51	Not Specified	Liver resections
Cheng, et al.	M – 223 F – 55	58.4	Not Specified	Not Specified	Not Specified	Liver resections

Pathological aspects of the surgical specimens and tumor characteristics (differentiation grade, size, number of tumors resected and existence of microinvasion) are depicted in table 3.

Table 3: Tumor analysis in RNA studies

Author	**Differentiation grade**	**tumor Size (cm)**	**Number of tumors**	**Microinvasion**
jones et al.	G1 – 8 G2 – 66 G3 – 6 Missing – 20	5.9 cm	2	Yes – 29 No – 54 UNK - 17
von Felden et al.	G1 – 10 G2/G3 – 29 Missing – 1	Not Specified	Not Specified	Yes – 37 No – 3
Fu et al.	Not Specified	Not Specified	Not Specified	Yes – 3 No – 229 UNK – 86
Yoon et al.	Not Specified	< 5 – 53.8% > 5 – 46.2%	Single – 50 Multiple - 43	Not Specified
Ha et al.	Not Specified	< 5 – 66.1% > 5 – 33.9%	Single – 270 Multiple – 19	Yes – 159 No – 130
Cheng et al.	Not Specified	< 5 – 55.4% > 5 – 44.6%	Single – 152 Multiple – 126	Not Specified

**Unknown-UNK*

All the included studies implied sample collection from surgically resected specimens. The percentage of patients undergoing tumor-targeted therapies are shown in table 2. Jones et al. reported that more patients with viral-related and alcohol-related HCC benefited from tumor-targeted therapies compared to other etiologies of the liver disease (40% and 36%, respectively; with significant P = 0.02). Fu et al. showed that only 6.3% of patients followed adjuvant chemotherapy alone, all the rest needed systemic treatment combined with minimally invasive ablation, embolization or radiation therapies.

AFP studies

There is a total number of nine studies on AFP as a biomarker for hepatocellular carcinoma (**see Table 1**). Shen el al. demonstrated AFP serum levels to be statistically significant in regard of prognosis, meaning AFP-positive HCC patients, who exceeded the Milan criteria, were independently associated with poorer both disease-free survival (DFS) and overall survival (OS) [14]. In Zhou's et al. study, an AFP serum level decrease of 9% significantly discriminated OS and DFS, with a potential role of improving the BCLC guidelines [25]. Huang et al. proved novel normograms using independent risk factors in AFP-negative HCC patients, which proved to be predictive in terms of survival and recurrence after radical resection [28].

Lee et al. reported a strong association between optimal resection margins and preoperative AFP levels: 0.5 cm margins being advised for AFP values of 15-200 ng/ml and 1 cm for values > 200ng/ml [31] (**see Table 4**).

Table 4: AFP studies design

Author	**tumor marker**	**Sex**	**mean age (years)**	**Preoperative serum level (ng/ml)**	**Objectives**	**Surgical treatment**
Shen et al.	AFP	Male – 86.8% Female – 13.2%	> 60 – 20.7% <60 – 79.3%	> 400	Survival	Yes
ZhoU et al.	AFP	Male – 565 Female - 145	51.69	Not Specified	Prognosis	Yes
Huang et al.	AFP	Male – 90.4% Female – 9.6%	55	Not Specified	Prognosis	Yes
Lee et al.	AFP	Male – 428 Female - 106	> 60 – 214 <60 – 320	>200	Resection margins	Yes
Xiao-Long Li et al.	AFP	Male – 366 Female - 86	52.8	230	Recurrence rate and survival	Yes
McDonald et al.	AFP	Male – 56% Female – 44%	32	>150	Prognosis	Yes

Tsilimigras et al.	AFP	Male – 76.1% Female – 23.9%	67	> 400	Recurrence rate and prognosis	Yes
Gao et al.	AFP	Male – 75% Female – 25%	> 60 – 43%% <60 –57%	Not Specified	Prognosis	Yes
Hou et al.	AFP	Male – 78.9% Female – 21.1%	51	Not Specified	Prognosis	Yes

Xiao-Long Li. et al proposed AR (AFP response) as a predictive tool in surgically treated hepatocellular carcinoma. The tumor marker was measured twice, before surgery (a-fetoprotein0) and 1 week after (a-fetoprotein7), in order to evaluate its predictive value for oncological effect of surgical resection for HCC with positive a-fetoprotein [17] (**see Table 5**).

McDonald's et. al study focused on fibrolamellar HCC, showing a potential association between AFP abnormal values and poor survival of these patients [15].

AFP values were higher when presented with tumor recurrence, following HCC resection. Moreover, these aspects were highlighted by Tsilimigras et al., showing a AFP's prediction role in overall survival [22]. On the other hand, the prediction role of AFP seems to have little to no value in small HCC or in non-cirrhotic patients, who undergo liver resection [29].

Hou et al. proposes a new tumor marker score (TMS) with optimal prognostic value for combined HCC and cholangiocarcinoma (CHCC). TMS includes AFP, CEA and CA19.9 with different cut-off values, that cand eventually predict prognosis after surgery [30].

Table 5: Tumor characteristics in AFP studies

Author	**MEAN Tumor diameter (cm)**	**number of tumors**	**Diferentiation grade**	**Satelite lymphnodes**
Shen et al.	8.4	Solitary – 67.5% Multiple – 32.5%	G1/G2 – 52.5% G3 – 47.5%	Yes – 16.8% No – 83.2%
Zhou et al.	4.89	Solitary – 554 Multiple – 156	G1/G2 – 697 G3 – 13	Not Specified
Huang et al.	Not Specified	Solitary – 91.9% Multiple – 9.1%	Not Specified	Yes – 18.3% No – 81.7%
Lee et al.	>3 cm – 223 < 3cm – 311	Not Specified	Not Specified	Yes – 139 No – 395
Xiao-Long Li et al.	>5 cm – 139 <5cm – 313	Solitary – 401 Multiple – 51	Not Specified	Yes – 188 No – 264
McDonald et al.	Not Specified	Solitary – 134 Multiple – 34	G1/G2 – 77 G3 – 65	Yes – 33 No – 97
Tsilimigras et al.	4.8	Not Specified	G1/G2 – 641 G3 – 168	Yes – 37% No – 63%
Gao et al.	Not Specified	Not Specified	G1/G2 – 137 G3 – 21	Not Specified
Hou et al.	>5 cm – 42.97 <5cm – 57.03	Solitary – 73.44 Multiple – 26.56	G1/G2 – 67.97% G3 – 32.03%	Yes – 26.56% No – 73.44%

Both HBs Ag (Hepatitis B Antigen) and HBV (Hepatitis B viremia) status were analysed in three studies [14,25,28]. HBV load was considered alone in other three studies [22,30,31] and Xiao-Long et al. reported only HbS Ag status. McDonald et al. and Gao et al. did not specify Hepatitis B status.

Other biological markers like albumin, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are described in table 6. The association between these markers and OS are analyzed by Shen et al., showing their lack of independent impact on the OS. On the contrary, AFP serum levels, tumor size, satellite lymphnodes and MVI (microvascular invasion) proved a statistically significant impact on the OS [14]. Huang et al. confirms the relationship between these factors and OS, adding ALP (alkaline phosphatase), liver cirrhosis and Edmonson-Steiner grade as further independent factors [28].

Table 6: Biochemical values in AFP studies

Author	**HBS Ag**	**HBV load**	**AFP (ng/ml)**	**ALB (g/dl)**	**NLR**	**PLR**	**ALT mean (IU)**	**AST Mean (IU)**
Shen et al.	Positive – 89.3% Negative – 10.7%	Positive – 43.6% Negative – 56.1%	>400 – 71.4% <400 – 28.6%	>3.5 – 90.7% <3.5 – 9.3%	>2 – 70% <2 – 30%	>150 – 22.5% <150 – 77.5%	59.8	53.2
Zhou et al.	Positive – 89.3% Negative – 10.7%	Positive – 609 Negative – 101	Not Specified	4.7	Not Specified	Not Specified	43.9	Not Specified

Huang et al.	Positive – 89.2% Negative – 10.8%	Positive – 41.7% Negative – 58.3%	Not Specified	4.22	Not Specified	Not Specified	36.3	30.3
Lee et al.	Not Specified	Positive – 280 Negative – 254	> 200 – 140 <200 - 354	>4 – 331 <4 – 166	Not Specified	Not Specified	Not Specified	Not Specified
Xiao-Long Li et al.	Positive – 88% Negative – 12%	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified
McDonald et al.	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified
Tsilimigras et al.	Not Specified	Positive – 25.6% Negative – 74.4%	>400 – 18.6% <400 – 81.4%	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified
Gao et al.	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified
Hou et al.	Not Specified	Positive – 78.13% Negative – 21.87%	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified	Not Specified

HBsAg=hepatitis B surface antigen; HBV=hepatitis B viremia; ALB=albumin; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; ALT=alanine aminotransferase; AST=aspartate aminotransferase

Other studies

Jung et al. assessed the advantages of anatomical resections (AR) over non-anatomical resections (NAR) in patients with HCC according to the ADV score [23]. ADV score measures AFP, des-gamma-carboxyprothrombin (DCP) and tumor volume (TV), multiplying the values and expressing the result in a logarithmic scale (log 10). ADV was related to a prognostic value for AR when the score was < 4 log or in absence of MVI.

Manjiang et al. studied the prognosis and risk factors in patients with re-resection in patients with recurrent HCC. AFP values > 20ng/mL, portal hypertension (PH) and gross vascular invasion (GVI) were independent risk factors for poorer survival [26].

Glycoprotein 96(GP96), a novel biomarker starting to prove utility in the prognosis of HCC, was studied by Ji et al. showing an association with positive outcomes in patients with normal AFP values and early HCC stages after curative liver resections [24].

Discussions

Hepatocellular carcinoma is still facing challenges in terms of diagnosis, treatment options and prognosis. Different biochemical markers have been studied during the past two decades and their association with prognosis and overall survival have been analysed aiming to offer targeted therapies for liver cancer.

We reviewed the biomarkers, which currently impact the prognosis of HCC patients, proposed for liver resections. Two main biomarkers are related to OS and prognosis: AFP and RNA. Alongside these two, GP96 and DCP are novel molecules that may prove utility in the clinical practice.

From eighteen studies, six focused on the role of RNAs, molecules that have shown to statistically impact prognosis of patients with HCC. Five studies were conducted on miRNA and one study on circularRNA. This group of non-coding RNAs with typically 18-22 nucleotides in length, regulate gene expression by binding to the messenger RNA [32]. Several studies have evaluated their prognostic role in different gastrointestinal malignancies [33,34]. Five studies focused on the prognostic role and the impact on OS of miRNAs.

MiRNA-26a was studied by Jones et al., showing that a prevalence of lower mi-RNA-26a was present in 68% of the subjects included in the cohort. The patients with lower biomarker levels had significantly higher tumor recurrence (hazard ratio [HR], 2.45; 95% confidence interval [CI], 1.18 to 5.1; P = 0.016) and higher mortality of borderline significance (HR, 1.51; 95% CI, 0.94 to 2.41; P = 0.086). An important aspect of the included cohort is that few subjects presented chronic viral hepatitis B as their underlying risk factor for HCC development, comparing to previous studies on Asian patients. Meanwhile, most of the patients were overweight or obese.

Downregulation of miRNA-122 was also studied by the working group of Ha et al. with a shorter recurrence-free survival (RFS) and disease-free survival (DFS) in a cohort of 289 patients. Both studies, showed a poorer prognosis when downregulation of these miRNAs is present in tissue samples of HCC tumors after liver resections.

Two other studies, highlighted that the upregulation of miRNA-21 and miRNA-135a is associated with poorer prognosis of the affected patients after liver resection. Tumor targeted therapies after HCC may influence tumor progression, high levels of biomarkers as mi-RNA135a being linked to early recurrence and need for urgent adjuvant therapy after surgical resection. High tumoral expression of miR-135a was associated with high risk of recurrence (HR = 4.2, p = 0.024, time to recurrence 8.8 ± 2.0 vs. 24.8 ± 4.4 months in patients with low miR-135a expression) in Ha et al. study. The study also highlights that tumoral biomarkers should be considered in trials that are conducted adjuvant therapy in patients with resectable HCC [35]. MiRNA-21upregulation was also associated with poor prognosis in a prospective study with 93 HCC patients, showing high expression values comparing to adjacent nontumor tissues. The statistically significant impact of these

biomarkers on prognosis also suggests a potential role for targeted therapies in HCC patients.

As novel biomarkers miRNAs have also been studied as combinations of molecules, a seven mi-RNA signature (miR-187, miR-9-3, miR-490, miR-1258, miR-3144, miR-551a and miR-665) being identified as potential independent prognostic factor for OS in early-stages of HCC(P=0.007) [16]. The role of these miRNAs in different malignancies has already been highlighted by several studies [36,37,38].

The current review also included one study focusing on circular RNA 0016788 which was analyzed on a 278 cohort after liver resection in patients with HCC. The results showed significant correlation between high expression on tumor tissue comparing to adjacent tissue but also an association with larger tumor size and more advanced BCLC stages [18].

An important characteristic of the included cohorts was that most cases were stage I-II [16,20,21] which suggest the role of these biomarkers after surgical resection in early HCC.

These studies show a great potential of RNA molecules and that further studies could confirm the role of a new prognosis tool for HCC patients that could be implemented in clinical practice.

Alfa-fetoprotein is a 70kD glycoprotein, similar to the molecular structure of albumin [12]. It is the the most widely accepted serum biomarker for HCC diagnosis and follow-up, although due to the lack of high sensitivity and specificity, its value is still challenged [39]. The controversaries regarding its role are also linked to the significant proportion of HCC patients, that do not present elevated AFP levels [8]. Another pitfall of this biomarker is represented by its elevated levels in cirrhotic patients with flares of HCV and HBV infections [40]. AFP was also proposed for a new criterion, aiming to include patients at high risk of early recurrence of HCC. On a cohort of 166 AFP-UTC (alpha-fetoprotein-adjusted-to-HCC-size) was correlated to a 5-year overall survival above 70% which highlight a potential adjustment for the Milan criteria [41]. These data suggest AFP still plays an important role for HCC, but there is still place for improvement in terms of survival and recurrence after liver transplantation.

The nine studies focused on the role of AFP in correlation with surgical resection in HCC patients. In most cases AFP values were measured preoperatively, but changes in its values also influenced the prognosis of the included cohorts. A daily postoperative decrease of AFP with a cut-off percentage of 9% compared to preoperative levels was shown to be an independent prognostic factor for patients undergoing hepatectomies in the study published by Zhou et al [25]. Despite promising results, the limitations of the study were represented by including only Chinese patients and that the results could not apply for HCC patients with normal AFP levels. AFP dynamic changes were also assessed by Shen et al. showing that normalization AFP levels were significantly influencing OS. Factors that independently and negatively influenced AFP normalization were preoperative AFP level (>400 ng/mL), tumor size, differentiation grade and satellite nodules.

Recurrent disease is common in patients with HCC [42]. Due to the poor OS of these patients, a prognosis tool would be needed. AFP levels were analyzed at the time of recurrence in patients with HCC in a study published by Tsilimigras et al. [22] showing that AFP>10 ng/mL was linked to a worse 3-year post-recurrence survival compared to individuals with AFP<10 ng/mL (28.7% vs. 65.5%, p=0.001). These results could help could help by stratifying risk and OS in patients with recurrent HCC. In cases of repeated hepatectomy for recurrent disease AFP values > 20ng/mL at first resection, portal hypertension and gross vascular invasion were shown to be independent factors for worse overall survival [26].

Another challenge in terms of prognosis is represented by HCC in patients without cirrhosis, undergoing curative resection. The association between biomarkers and prognosis is still unclear. In Gao et al. study, in patients with single HCC nodules <3cm treated with curative resections, AFP levels were shown to have no predictive value [29]. Further studies should be conducted on the role of biomarkers in non-cirrhotic HCC patients.

Due to the significant proportion of patients with normal AFP levels, other biomarkers should be considered in order to predict OS after liver resections in patients with HCC. Huang et al. proposed normograms comprising liver function, tumor status and clinicopathological characteristics for OS and RFS in AFP-negative HCC. Comparing to BCLC staging system, consisting of tumor stage, liver function, performance status and cancer-related symptoms, the proposed normograms in the abovementioned study showed a had a higher prediction accuracy for OS and RFS. The variables of the normograms significantly linked to OS and RFS prediction were alkaline phosphatase, liver cirrhosis, tumor size, satellite lesions, microvascular invasion and Edmondson-Steiner grade.

Another aspect that was highlighted in our review was the resection margin for HCC patients with different AFP levels. Lee et al. showed that AFP could be used in guiding resection margins: a resection margin ≥0.5cm was advised for the patients with AFP between 15 and 200ng/ml and ≥1cm for the patients with AFP over 200ng/ml. In patients with normal AFP levels, tumor free margins were shown to be enough [28].

The negative impact of elevated AFP levels on OS was also shown in fibrolamellar HCC [15] and the combination with carcinoembryonic antigen (CEA) and human carbohydrate antigen 19.9 (CA 19.9) levels could be used to provide optimal prognostic values in combined HCC-cholangiocarcinoma patients [30]. Combinations with des-gamma-carboxy prothrombin (DCP) and AFP could also be used to calculate scores that can predict survival in small HCC with low tumor marker expression [23].

All the included studies suggest that AFP still plays an important role in the prognosis of HCC patients that undergo liver resections.

Of the included studies, one study focused on GPC3, a heparan sulphate proteoglycan, that is promising biomarker by showing an association with positive outcomes in patients with normal AFP values and early HCC stages after curative resection. Encouraging data also suggest a potential role in diagnosing early HCC, as GPC3 is not correlated to the tumor size [43].

Conclusions

In conclusion, AFP and miRNAs are related as prognostic biomarkers of hepatocellular carcinoma. They play important roles in tumor recurrence and overall survival, alfa-fetoprotein being the most feasible one in patients undergoing curative liver resections.

Although, despite the plentitude of studies and the promise of many classes of biomarkers, it appears that, presently there is need for a combination of serum markers in order to detect early HCC or recurrent tumors after receiving curative resection or chemo/immunotherapy.

To evaluate the various types of medicines addressed, future prospective multicenter trials in identified at-risk groups are needed.

It's difficult to identify HCC using just one biomarker, because of the multidimensional pattern of the disease. Biomarker combinations under investigation could provide more accurate and relevant information for future personal HCC diagnosis and prognosis. Despite the fact that new biomarkers are being developed at an increasing rate, additional studies for their clinical utility are in

high demand worldwide. We believe that discovering novel cost-effective biomarkers or biomarker combinations for HCC early detection would be fruitful.

Ethics approval and consent to participate

Not applicable.

List of abbreviations

HCC: Hepatocellular carcinoma
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
AFP: Alfa: Fetoprotein
CA 19.9: Carbohydrate antigen 19.9
ASLD: Advanced staged liver disease
HCV: Chronic hepatitis C
NASH: Non-alcoholic steatohepatitis
JIS: Japan Integrated System
DCP: Des-gamma-carboxyprothrombin
AFP-L3: Lens culinaris agglutinin
FNH: Focal nodular hyperplasia
HA: Hepatocellular adenoma
OPN: Osteopontin
RFS: Recurrence-free survival
DFS: Disease-free survival
BCLC: Barcelona Clinic Liver Cancer
TMS: Tumor marker score
CHCC: Cholangiocarcinoma

Data Availability

Not applicable.

Conflicts of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

HB contributed with the conceptualization, methodology and validation; AC contributed with the data curation, the original draft preparation, review and editing; FVZ contributed with the original draft preparation and editing; RAC contributed with the formal analysis and investigation; NAH contributed with the conceptualization, reviewing and supervision. The individual contributions of authors to the manuscript should be specified in this section. All authors read and approved the final manuscript.

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Supplementary Materials

Not applicable.

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