

Circulating microRNA Signatures as Non-Invasive Prognostic Biomarkers in Patients with Hepatocellular Carcinoma: A Review

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Abstract

Circulating microRNAs have been proposed as prognostic markers in hepatocellular carcinoma for well over a decade, and the case for them is easy to state: they are stable in serum, cheap to measure, and require only a blood draw. The case against is that almost every patient who develops hepatocellular carcinoma does so on a background of cirrhosis, and it is against that background, not against healthy controls, that any marker has to perform.

Objectives: To review the published evidence on circulating microRNAs in hepatocellular carcinoma: how well single markers and multi-marker panels perform, how that performance changes with the comparator population, and how much of the prognostic literature rests on circulating rather than tissue measurement.

Material and Methods: Narrative review of diagnostic accuracy studies, meta-analyses and prognostic cohorts indexed in PubMed and the major hepatology and oncology journals. Studies were eligible if they reported diagnostic performance of circulating microRNAs in hepatocellular carcinoma against a defined comparator, or outcomes stratified by circulating microRNA level. No new patient data were generated. Every accuracy estimate and effect size quoted is one published by the original investigators.

Results: Pooled across 13 studies covering 920 patients and 1,217 controls, serum miR-122 discriminated hepatocellular carcinoma from healthy controls with an area under the curve of 0.91, from hepatitis B or C infection with 0.87, and from cirrhosis or dysplastic nodules with only 0.74, where pooled sensitivity fell to 0.65. Multi-marker panels perform substantially better against the same difficult comparator: an eight-microRNA serum panel developed in 345 patients with hepatocellular carcinoma and 139 at-risk controls achieved an area under the curve of 0.99 with sensitivity of 97.7 per cent and specificity of 94.7 per cent, detecting 98 per cent of stage I cases. Prognostic evidence is thinner and less consistent, with individual cohorts reporting opposing directions of effect for the same microRNA.

Conclusion: The circulating microRNA literature in hepatocellular carcinoma is considerably stronger on detection than on prognosis. Accuracy figures are only interpretable in relation to the comparator used, and the comparator that matters clinically is the cirrhotic surveillance population, against which single markers perform poorly and panels perform well. Prognostic claims rest largely on small single-centre cohorts with inconsistent findings, and should be treated as provisional.

Abbreviations: HCC – Hepatocellular Carcinoma, miRNA – MicroRNA, AFP – Alpha-Fetoprotein, DCP – Des-Gamma Carboxyprothrombin, AUC – Area Under the Receiver Operating Characteristic Curve, ROC – Receiver Operating Characteristic, qRT-PCR – Quantitative Reverse Transcription Polymerase Chain Reaction, TACE – Transarterial Chemoembolization, OS – Overall Survival, PFS – Progression-Free Survival.

Keywords: MicroRNA; Hepatocellular Carcinoma; Liquid Biopsy; Prognostic Biomarkers; Liver Cirrhosis

Introduction

MicroRNAs are short non-coding RNAs that regulate gene expression post-transcriptionally, and their expression patterns differ systematically between tumour and normal tissue across human cancers [10]. What made them attractive as clinical markers was not the biology but the chemistry: they survive in serum, they tolerate freezing and thawing, and they can be quantified by methods available in any molecular laboratory.

In hepatocellular carcinoma the appeal is sharper than in most tumours, because the existing markers are weak. Alpha-fetoprotein has been used for surveillance since the 1990s despite well-documented limitations, including elevation in cirrhosis without tumour and failure to rise in a large proportion of small tumours [11]. Des-gamma carboxyprothrombin adds something but not enough [12,13]. Ultrasound, the other pillar of surveillance, is

operator-dependent and insensitive for small lesions.

The difficulty is that the clinical question is narrower than it first appears. Almost every patient who develops hepatocellular carcinoma does so on a background of chronic hepatitis or cirrhosis, and those patients are already under surveillance. A marker that separates patients with hepatocellular carcinoma from healthy volunteers answers a question nobody is asking. The question that matters is whether it separates cancer from cirrhosis, and as this review sets out, that distinction accounts for most of the variation in reported performance.

A second issue concerns what is being claimed. Much of the literature described as prognostic is in fact diagnostic, and a considerable proportion of the frequently cited prognostic associations were measured in resected tissue rather than in blood. The aim of this review is to separate those strands and report what the circulating measurement has actually been shown to do.

Material and Methods

Search strategy: PubMed and the archives of the major hepatology and oncology journals were searched for studies of circulating microRNAs in hepatocellular carcinoma. Search terms combined microRNA, miRNA and circulating or serum or plasma with hepatocellular carcinoma, liquid biopsy, diagnostic accuracy, prognosis, overall survival and cirrhosis. Reference lists of retrieved meta-analyses were screened by hand for primary sources.

Eligibility: Studies were included if they reported the diagnostic performance of one or more circulating microRNAs in hepatocellular carcinoma against a defined comparator group, or reported clinical outcomes stratified by circulating microRNA level. Studies measuring microRNA expression only in tumour tissue were excluded from the primary synthesis but are referred to where they are commonly cited as evidence for circulating markers. Studies without extractable numerical outcomes were excluded.

Data extraction and handling: Design, sample size, specimen type, assay method, comparator group and reported accuracy or effect estimates were extracted as published. Accuracy figures are reported alongside the comparator group used, because the two are not separable. No pooling or re-analysis was performed and no patient-level data were obtained. Figures 1 to 3 and Tables 1 and 2 reproduce published values only. This is a narrative synthesis and was not registered as a systematic review.

Results

Performance depends on the comparator, not the marker: The clearest demonstration comes from a meta-analysis of serum miR-122, the most abundant liver-specific microRNA, pooling 13 studies covering 920 patients with hepatocellular carcinoma and 1,217 controls. Against healthy controls the area under the curve was 0.91. Against patients with hepatitis B or C infection it was 0.87. Against all controls combined it was 0.82. Against cirrhosis or dysplastic nodule formation it fell to 0.74 (Figure 1) [2].

The same gradient appears in the component measures. Pooled sensitivity was 0.85 against healthy controls and 0.65 against cirrhosis, while specificity held between 0.75 and 0.83 throughout (Figure 2) [2]. In other words, the loss of performance in the clinically relevant comparison is driven by missed cancers rather than by false alarms, which is the worse of the two failure modes for a surveillance marker.

SERUM miR-122: DIAGNOSTIC ACCURACY BY COMPARATOR GROUP

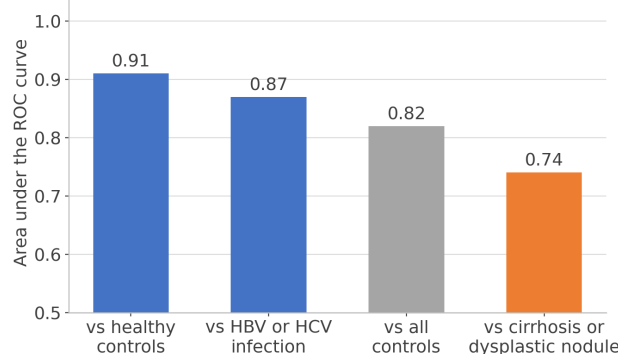


Figure 1: Pooled diagnostic accuracy of serum miR-122 for hepatocellular carcinoma against four comparator groups, from 13 studies [2]

SERUM miR-122: POOLED SENSITIVITY AND SPECIFICITY

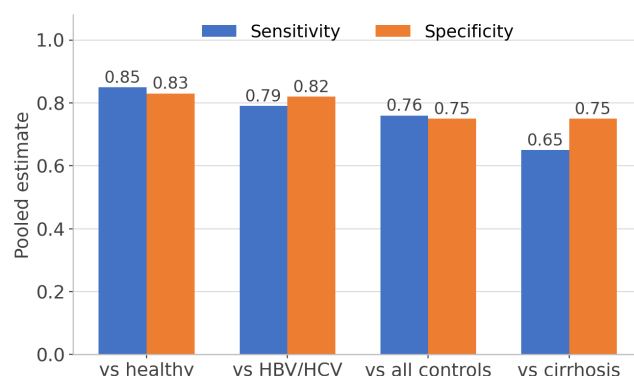


Figure 2: Pooled sensitivity and specificity of serum miR-122 by comparator group; sensitivity falls against cirrhosis while specificity holds [2]

Panels outperform single markers: The response to the single-marker problem has been to combine markers. In a study analysing serum from 345 patients with hepatocellular carcinoma, 46 with chronic hepatitis, 93 with cirrhosis and 1,033 healthy individuals by microarray, 52 candidate microRNAs were narrowed to an eight-marker panel comprising miR-320b, miR-663a, miR-4448, miR-4651, miR-4749-5p, miR-6724-5p, miR-6877-5p and miR-6885-5p. Against at-risk controls, meaning patients with chronic hepatitis or cirrhosis rather than healthy volunteers, the panel achieved an area under the curve of 0.99, with sensitivity of 97.7 per cent and specificity of 94.7 per cent, and detected 98 per cent of stage I cases (Figure 3) [1].

The contrast with the single-marker figures is the substantive finding of this literature. Performance against the difficult comparator moves from an area under the curve of 0.74 to 0.99 when eight markers are combined rather than one [1,2]. The same group has reported comparable panel performance in prostate, ovarian and bladder cancer using the same platform, which suggests the gain comes from the multi-marker approach rather than from anything specific to the liver [14,15,16].

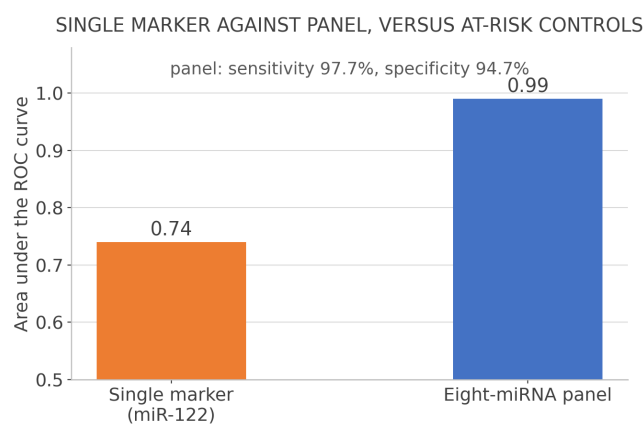


Figure 3: Diagnostic accuracy against at-risk controls for a single circulating marker and for an eight-microRNA panel [1,2]

Study	n	Specimen	Principal reported finding
Zhao et al. 2020 [2]	920 + 1,217	Serum	miR-122 AUC 0.91 vs healthy, 0.74 vs cirrhosis
Yamamoto et al. 2020 [1]	1,517	Serum	Eight-miRNA panel AUC 0.99 vs at-risk controls
Köberle et al. 2013 [3]	—	Serum	miR-1 and miR-122 prognostic for survival
Xu et al. 2015 [4]	—	Serum	High miR-122 associated with better survival
Pelizzaro et al. 2021 [7]	94	Whole blood	miR-21 ratio and miR-122 predict PFS after TACE
Zhang et al. 2015 [8]	327	Tissue	Seven-miRNA tissue signature predicts survival

Table 1: Principal studies reviewed, with specimen type and the finding as reported by the original investigators

The prognostic evidence is thinner than the diagnostic: Serum miR-1 and miR-122 were reported as prognostic markers in hepatocellular carcinoma a decade ago [3], and circulating microRNAs measured shortly after liver transplantation have been reported to predict recurrence and survival [6]. In patients treated with drug-eluting bead transarterial chemoembolization, the ratio of miR-21 before and after treatment and the absolute level of miR-122 both separated patients by progression-free survival ($p = 0.0002$ and $p = 0.02$), and the miR-21 ratio combined with alpha-fetoprotein identified early progressors among patients whose imaging showed a complete or partial response [7]. Plasma miR-122 has also been reported to predict treatment response after chemoembolization [5].

These are real findings, but they are small, single-centre and heterogeneous in design, and the direction of effect is not consistent between them. High serum miR-122 has been reported as independently associated with better overall survival in one cohort [4] and as a poor prognostic marker in hepatitis B-related disease in another. A marker whose reported prognostic direction depends on the cohort is not yet a prognostic marker.

It is also worth being precise about specimen type. Several of the most frequently cited prognostic signatures in this disease, including seven-microRNA models built from public sequencing data, were derived from resected tumour tissue rather than from blood [8]. Their performance says nothing directly about what a serum measurement would achieve, and conflating the two overstates how much prognostic evidence exists for the circulating compartment.

Comparator group	AUC	Sens.	Spec.
Healthy controls	0.91	0.85	0.83
HBV or HCV infection	0.87	0.79	0.82
All controls combined	0.82	0.76	0.75
Cirrhosis or dysplastic nodule	0.74	0.65	0.75

Table 2: Pooled performance of serum miR-122 by comparator group, from a meta-analysis of 13 studies [2]

Discussion

Two conclusions follow from the published data, and they pull in different directions. The first is that reported accuracy for a circulating microRNA is close to meaningless without the comparator attached. The same marker, measured in the same way and pooled across the same studies, moves from an area under the curve of 0.91 to 0.74 depending only on who the controls are [2]. Papers reporting performance against healthy volunteers are describing a test that would never be used that way.

The second is that the multi-marker approach appears to solve much of the problem. A panel of eight markers achieved near-perfect discrimination against exactly the at-risk population where single markers fail, and detected almost all stage I disease [1]. If that result replicates in independent prospective cohorts, it would represent a genuine advance over alpha-fetoprotein for surveillance.

Prognosis against detection

The prognostic case is weaker than the diagnostic one, and the gap is not always acknowledged. Detection studies in this field now run to hundreds or thousands of patients with separate discovery and validation sets [1]. Prognostic studies remain small, typically retrospective, frequently single-centre, and are often reported with p-values rather than effect sizes with confidence intervals, which makes them difficult to compare or to pool [5,7].

There is also a conceptual point. Circulating microRNA levels in this disease reflect hepatocyte injury as much as tumour burden, since miR-122 in particular is released from damaged liver whether or not a tumour is present. A marker whose level is driven jointly by cirrhosis severity and tumour biology will be difficult to interpret prognostically, because those two things move survival in the same direction for different reasons.

Study limitations

The limitations belong to the literature rather than to this synthesis. Pre-analytical handling is inconsistently reported across studies: haemolysis, choice of serum against plasma, extraction method and the reference gene used for normalisation all affect measured microRNA levels, and few reports document all four. Cut-off values are typically derived and tested in the same cohort, which inflates apparent accuracy. Comparator groups differ so widely that pooled figures require stratification to be interpretable, as the meta-analysis reviewed here demonstrates [2]. Publication bias toward positive findings is likely, although it was not detected by formal testing in that analysis [2].

Two gaps deserve naming. No study reviewed here compares a circulating microRNA panel against alpha-fetoprotein and ultrasound in a prospective surveillance cohort with clinical endpoints, which is the design that would establish clinical utility. And the prognostic literature contains no adequately powered study in which the microRNA measurement is tested against established prognostic staging rather than in isolation.

Conclusion

Circulating microRNAs discriminate hepatocellular carcinoma from healthy controls well, from viral hepatitis moderately, and from cirrhosis poorly when a single marker is used. Multi-marker panels close most of that gap and have reported near-complete detection of stage I disease against at-risk controls, which is the result worth pursuing. The prognostic literature has not kept pace: it rests on small cohorts, reports inconsistent directions of effect for the same marker, and is frequently conflated with tissue-based signatures that measure something else. Prospective validation against current surveillance practice, with pre-specified cut-offs and clinical endpoints, is the necessary next step before circulating microRNAs enter routine use.

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Conflict of Interest

No conflicts of interest are reported for any author.

Ethics Statement

Everything discussed here comes from studies already in print. No new patient information was collected and nobody took part as a research subject, so ethical clearance was unnecessary.

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