Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Pre-procedural Heart Rate Variability Predicts Recurrence-Free Survival in Hepatocellular Carcinoma after Microwave Ablation: A Retrospective Cohort Study with Proteomic Exploration

Qiqi Liu
Eighth Affiliated Hospital of Sun Yat-sen University
Yannan Luo
Eighth Affiliated Hospital of Sun Yat-sen University
Zehao Chen
Shenzhen Institutes of Advanced Technology
Jiahui Dai
Eighth Affiliated Hospital of Sun Yat-sen University
Haiyu Kang
Eighth Affiliated Hospital of Sun Yat-sen University
Huahui Liu
Eighth Affiliated Hospital of Sun Yat-sen University
Shuang Liang
Eighth Affiliated Hospital of Sun Yat-sen University
Zhiming Zhang
Eighth Affiliated Hospital of Sun Yat-sen University
Ke Liu
Eighth Affiliated Hospital of Sun Yat-sen University
Erjiao Xu
xuerjiao@mail.sysu.edu.cn

Eighth Affiliated Hospital of Sun Yat-sen University

Research Article

Keywords: Hepatocellular carcinoma, Heart rate variability, Microwave ablation, Prognosis, Recurrence-free survival, Proteomics

Posted Date: September 27th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10795006/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background & Aims: Heart rate variability (HRV) reflects autonomic function and has prognostic value in several cancers. We evaluated whether pre-procedural HRV predicts recurrence-free survival (RFS) after microwave ablation (MWA) for hepatocellular carcinoma (HCC).

Methods: This retrospective cohort included 101 HCC patients who underwent MWA (2021-2024). Time-domain HRV parameters (SDNN, RMSSD) were derived from pre-procedural 10-second ECGs. RFS was assessed by Kaplan-Meier and multivariable Cox regression. Exploratory serum proteomics was performed in a matched subset (n=25) as a hypothesis-generating analysis.

Results: Over a median follow-up of 12.0 months, 44 patients (43.6%) relapsed. Multivariable Cox regression confirmed SDNN ≥18.59 ms as an independent predictor of prolonged RFS (HR=0.384, 95%CI: 0.197-0.752, P= 0.005), along with CRP (HR=1.058, 95%CI: 1.025-1.092, P= 0.001) and recurrent tumor status (HR=2.407, 95%CI: 1.176-4.926, P= 0.016). In exploratory proteomics (n=25), the high-HRV group showed enrichment of proteins annotated to extracellular matrix organization and coagulation pathways, and relative downregulation of glycolysis/hypoxia pathway proteins; these associations require validation in independent cohorts.

Conclusions: Higher pre-procedural SDNN is independently associated with prolonged RFS after MWA for HCC. This simple, non-invasive metric from routine ECGs may serve as a complementary prognostic biomarker to identify high-risk patients.

1. Introduction

For patients with early-stage hepatocellular carcinoma (HCC) who are ineligible for surgical resection, microwave ablation (MWA) is a first-line curative treatment [1]. However, post-ablation recurrence remains frequent, highlighting the need for additional prognostic tools [2, 3].

Accumulating evidence indicates that autonomic nervous system (ANS) activity influences tumor biology through neuro-immune interactions [4]. Excessive sympathetic activity can promote tumor growth and metastasis [5], whereas vagal activation exerts anti-inflammatory effects via the cholinergic anti-inflammatory pathway, and may enhance anti-tumor immunity [6]. These findings suggest that preserved vagal tone could confer a systemic environment less permissive to tumor recurrence.

Heart rate variability (HRV), the beat-to-beat variation in cardiac intervals, provides a non-invasive window into ANS function [7, 8]. Higher HRV reflects greater vagal tone and autonomic flexibility and has been proposed as a systemic biomarker of neuro-immune regulation. In oncology, lower HRV has been associated with adverse outcomes across several malignancies [9]. In HCC specifically, prior studies have linked reduced HRV to poorer overall survival (OS) [10]; however, its prognostic value for recurrence-free survival (RFS) after curative-intent thermal ablation has not been investigated.

Notably, the validity of HRV parameters derived from ultra-short (e.g., 10-second) ECGs remains debated. While Munoz et al. reported substantial agreement for both SDNN and RMSSD between 10-second and 5-minute recordings in a large cohort (N = 3,387) [11], Nussinovitch et al. found that RMSSD, but not SDNN, is reliable from ultra-short recordings [12]. Given this unresolved methodological controversy, we prespecified SDNN and RMSSD as co-primary HRV indices and interpreted findings with caution.

We hypothesized that higher pre-procedural HRV would be independently associated with prolonged RFS in HCC patients undergoing MWA. To explore potential biological mechanisms linking autonomic function to recurrence, we performed exploratory, hypothesis-generating serum proteomic profiling and gene set enrichment analysis (GSEA) in a subset of patients with high versus low HRV.

2. Methods

2.1 Study Design and Patient Selection

This retrospective cohort study included patients diagnosed with HCC who underwent curative-intent MWA in our hospital between January 1, 2021 and December 31, 2024. Diagnosis was confirmed by histopathology or non-invasive imaging criteria according to current guidelines. MWA procedures aimed to achieve a 5-mm safety margin whenever technically feasible. Data were extracted from electronic medical records.

Inclusion criteria: (1) availability of a standard resting 10-second ECG recorded within one week before MWA and suitable for HRV analysis; (2) complete preoperative clinical and laboratory data; (3) regular postoperative imaging follow-up.

Exclusion criteria: (1) active secondary malignancy; (2) history of severe arrhythmia, heart failure, myocardial infarction, or implanted cardiac pacemaker; (3) long-term use of medications that significantly affect ANS function (e.g., beta-blockers).

The study was approved by the Ethics Committee of the Eighth Affiliated Hospital of Sun Yat-sen University (approval number: 2023-068-01), and informed consent was waived due to the retrospective design.

2.2 Data Collection and Follow-up

Demographic, clinical, and preoperative serum biochemical parameters were collected, including complete blood count, liver function tests, α-fetoprotein (AFP), C-reactive protein (CRP), and Child-Pugh score. Follow-up lasted from the date of MWA until radiologically confirmed tumor recurrence, death, or study end (December 31, 2025). RFS was defined as the time from MWA to first detection of recurrence, assessed every 3 months by contrast-enhanced imaging, including contrast-enhanced computed tomography (CECT), contrast-enhanced magnetic resonance (CE-MR), or contrast-enhanced ultrasound (CEUS). OS was defined as the time from MWA to last follow-up or death.

2.3 Heart Rate Variability Analysis

HRV was derived from 10-second resting ECGs using time-domain methods. Primary parameters were: SDNN, standard deviation of all normal-to-normal intervals; RMSSD, root mean square of successive differences between adjacent NN intervals. ECG recordings were obtained after at least 5 minutes of rest in the supine position.

2.4 Proteomic Profiling and Gene Set Enrichment Analysis

Exploratory serum proteomic profiling was performed in a subset of patients selected from the top and bottom tertiles of SDNN. Patients were matched by age (± 5 years), sex, and Child-Pugh class (A or B), yielding a high-HRV group (n = 12) and a low-HRV group (n = 13). Baseline characteristics including tumor size, tumor number, and AFP did not differ between groups (all $P > 0.05$).

Serum samples collected within one week before MWA were processed using a high-throughput platform with protein enrichment magnetic beads, followed by trypsin digestion. Peptides were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an EvosepOne system coupled to an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific) in data-independent acquisition (DIA) mode. Raw data were processed with Spectronaut (v19.6) against the human UniProtKB proteome. Label-free quantification (LFQ) intensities were $\log_2$-transformed and median-normalized.

Gene set enrichment analysis (GSEA) was performed by ranking all quantified proteins by $\log_2$ fold change (high-HRV vs. low-HRV) and testing against the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database using the clusterProfiler R package (4.10.1) with 1,000 gene set permutations. For each pathway, we obtained the normalized enrichment score (NES), nominal P value, and two complementary false discovery rate (FDR) estimates: p.adjust (Benjamini-Hochberg method) and q values (Storey's method, which models the null distribution of P values for refined FDR control). Pathways with $|NES| > 1.5$ and q value < 0.25 were considered significantly enriched. Sensitivity analysis using p.adjust < 0.25 yielded consistent results. Quality control was ensured by interspersed pooled reference samples, with a median coefficient of variation (CV) < 15% across runs.

Detailed procedures for sample processing, LC-MS/MS acquisition, data processing, and GSEA are provided in Additional file 1: Supplementary Methods S1.

2.5 Statistical Analysis

Categorical variables are presented as numbers (percentages); continuous variables as mean ± SD or median (IQR). Group comparisons used Student's t-test, Mann-Whitney U test, chi-square test, or Fisher's exact test as appropriate. RFS differences across HRV subgroups were visualized by Kaplan-Meier curves and compared by log-rank test. Univariate Cox regression identified predictors of RFS; variables with $P < 0.05$ were entered into a multivariable Cox model using forward stepwise selection (entry $P < 0.05$). Results are reported as hazard ratios (HR) with 95% confidence intervals (CI). Two-sided $P < 0.05$ was considered significant. No a priori sample size calculation was performed because all eligible

patients during the study period were included; the available sample (n = 101, 44 events) was sufficient for the primary analysis of SDNN with adjustment for three covariates. All analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY) and R version 4.2 (for GSEA analyses).

3. Results

3.1 Patient Characteristics

Of 145 initially screened HCC patients, 101 met inclusion criteria (Fig. 1). Baseline characteristics are shown in Table 1. The median age was 60 years (range 31–78), and 84 patients (83.2%) were male. Median tumor diameter was 19.0 mm (IQR 13.5–28.5). Median SDNN was 18.59 ms (IQR 14.23–25.30), and median RMSSD was 22.71 ms (IQR 17.03–33.20).

Table 1
Baseline characteristics of the HCC cohort (N = 101).

Variables	Value (N = 101)
Demographics	
Male gender, n (%)	84 (83.17)
Age ≥ 65 (years), n (%)	42 (41.58)
Clinical characteristics	
Heart rate (bpm), median (IQR)	74.18 (67.33, 81.93)
Systolic blood pressure (mmHg), median (IQR)	130.00 (117.00, 142.00)
Diastolic blood pressure (mmHg), median (IQR)	80.00 (75.00, 89.00)
Aetiology (Normal/Alcohol/Viral), n	3/1/97
Child-Pugh score (A/B/C), n	85/15/1
Liver cirrhosis (yes/no), n	77/24
Heart rate variability	
SDNN (ms), median (IQR)	18.59 (14.23, 25.30)
RMSSD (ms), median (IQR)	22.71 (17.03, 33.20)
Tumor characteristics	
Tumor number, median (IQR)	1 (1, 2)
Tumor maximum diameter (mm), median (IQR)	19.0 (13.5, 28.5)
History of prior HCC treatment, n (%)	59 (58.42)
History of open surgery, n (%)	34 (33.66)
Prior chemotherapy, n (%)	25 (24.75)
Laboratory parameters	
α-fetoprotein (ng/mL), median (IQR)	8.41 (3.21, 55.4)
C-reactive protein (mg/L), median (IQR)	2.56 (1.49, 6.05)
Albumin (g/dL), median (IQR)	39.88 (36.56, 42.69)

Values are median (IQR) or n (%). IQR = interquartile range; SDNN = standard deviation of normal-to-normal intervals; RMSSD = square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals.

Stratified analysis (Table 2) showed that patients ≥ 65 years had significantly lower SDNN and RMSSD than those < 65 years (P = 0.025). Patients with recurrent disease (history of prior curative therapy) also had lower HRV than treatment-naïve patients (P < 0.05). No significant differences were observed by sex or prior systemic therapy (P > 0.05).

Table 2
Stratified analysis of HRV parameters.

Variables	SDNN, ms	RMSSD, ms
Sex	P = 0.86	P = 0.81
Male	18.71 (14.1, 24.8)	22.86 (17.1, 33.7)
Female	18.54 (15.3, 31.1)	22.83 (15.9, 33.4)
Age	P = 0.025	P = 0.025
< 65 years	19.98 (15.2, 30.9)	25.43 (18.6, 36.9)
≥ 65 years	16.41 (12.6, 20.5)	20.72 (14.9, 28.5)
Treatment history	P = 0.049	P = 0.004
Naïve	21.436 (14.1, 41.1)	28.47 (19.0, 63.5)
Recurrent	17.919 (14.2, 21.9)	21.82 (14.9, 28.0)
Systemic therapy	P = 0.23	P = 0.20
yes	16.814 (14.3, 21.7)	21.820 (16.6, 26.2)
no	19.068 (14.2, 31.2)	23.006 (17.1, 36.9)

Values are median (IQR). P values were calculated using the Mann-Whitney U test. SDNN = standard deviation of normal-to-normal intervals; RMSSD =square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals.

3.2 Risk Factors for Recurrence-Free Survival

During a median follow-up of 12.0 months (IQR 5.98–17.00), recurrence occurred in 44 patients (43.6%). Univariate analysis identified SDNN ≥ 18.59 ms, RMSSD ≥ 22.71 ms, CRP, recurrent tumor status, and tumor number as significant predictors of RFS (Table 3). In multivariable Cox regression, SDNN ≥ 18.59 ms (HR = 0.384, 95% CI: 0.197–0.752, P = 0.005), CRP (HR = 1.058, 95% CI: 1.025–1.092, P = 0.001), and recurrent tumor status (HR = 2.407, 95% CI: 1.176–4.926, P = 0.016) remained independent predictors.

Table 3
Univariable and multivariable Cox assessment to identify predictors of RFS in hepatocellular carcinoma patients.

Variables	Univariable		Multivariable	
	HR (95%CI)	P	HR (95%CI)	P
SDNN ≥ 18.59 ms	0.389 (0.200–0.759)	0.006	0.384 (0.197–0.752)	0.005
RMSSD ≥ 22.71 ms	0.430 (0.227–0.815)	0.010	-	-
C-reactive protein	1.059 (1.025–1.095)	0.001	1.058 (1.025–1.092)	0.001
Recurrent tumor status	2.260 (1.113–4.591)	0.024	2.407 (1.176–4.926)	0.016
Number of tumors	1.362 (1.044–1.778)	0.023	-	-

HR = hazard ratio; CI = confidence interval; SDNN standard deviation of normal-to-normal intervals; RMSSD = square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals.

3.3 Association between HRV and Survival

Kaplan-Meier analysis showed that patients with low SDNN (< 18.59 ms) had significantly worse RFS than those with high SDNN (log-rank P = 0.0039). A similar pattern was observed for RMSSD (log-rank P = 0.0083) (Fig. 2A, B). Neither SDNN nor RMSSD was significantly associated with OS (log-rank P > 0.05 for both) (Fig. 2C, D).

3.4 Proteomic Signatures Associated with HRV Status

In this exploratory analysis (n = 25), GSEA identified 33 pathways with |NES| > 1.5 and q value < 0.25 enriched in the high-HRV group (SDNN ≥ 18.59 ms, n = 12), and 9 pathways with |NES| > 1.5 and q value < 0.25 enriched in the low-HRV group (SDNN < 18.59 ms, n = 13) (Additional file 4: Table S1). Pathways enriched in the high-HRV group were predominantly related to coagulation and fibrinolysis, extracellular matrix (ECM) organization, integrin signaling, and platelet activation (Fig. 3). Representative pathways included REACTOME_EXTRACELLULAR_MATRIX_ORGANIZATION (NES = 2.06, q value = 8.32×10^{-3}), PID_INTEGRIN3_PATHWAY (NES = 2.02, q value = 1.15×10^{-2}), WP_ BLOOD_CLOTTING_CASCADE (NES = 1.99, q value = 2.35×10^{-2}), and BIOCARTA_ FIBRINOLYSIS_PATHWAY (NES = 1.93, q value = 2.35×10^{-2}). Core enriched proteins consistently included fibrinogen chains (FGB, FGG, FGA), thrombospondin 1 (THBS1), and several collagens (COL6A2, COL6A3).

Pathways enriched in the low-HRV group were dominated by glycolysis, hypoxia, and keratinization (Fig. 3). WP_AEROBIC_GLYCOLYSIS (NES = − 1.83, q value = 3.98×10^{-2}),

WP_HIF1A_AND_PPARG_REGULATION_OF_GLYCOLYSIS (NES = − 1.83, q value = 3.98×10^{-2}), and BUFFA_HYPOXIA_METAGENE (NES = − 2.05, q value = 4.78×10^{-2}) were significantly downregulated in the high-HRV group. Core proteins included glycolytic enzymes (GAPDH, LDHA, LDHB) and the glucose transporter SLC2A1.

The observed association of high HRV with coagulation/ECM pathways and low HRV with glycolysis/hypoxia does not imply causation; these patterns suggest systemic metabolic and matrix-related differences that merit further investigation.

4. Discussion

4.1 Principal Clinical Findings

This study demonstrates that higher pre-procedural SDNN is independently associated with prolonged RFS in patients with HCC undergoing curative-intent MWA. Autonomic function measured by a routine ECG provides prognostic information beyond conventional clinical parameters.

4.2 Comparison with Prior Literature

Our results align with evidence linking autonomic dysfunction to adverse cancer outcomes. Sympathetic overdrive promotes a pro-tumorigenic microenvironment, whereas vagal activation exerts anti-inflammatory effects via the cholinergic pathway [13]. Higher HRV, reflecting greater autonomic flexibility, may indicate a systemic state less permissive to tumor recurrence. A systematic review of 19 studies concluded that higher HRV is associated with better cancer prognosis [14].

In patients with cirrhosis, reduced HRV correlates with elevated inflammatory markers and independently predicts mortality [7]. Although cirrhosis is not equivalent to HCC, the association between HRV and inflammation in the cirrhotic setting suggests that autonomic dysregulation might influence HCC prognosis through chronic inflammatory pathways. In advanced cancer, low HRV independently predicts shorter survival [14]. Previous HCC studies have linked low HRV to poorer overall survival at diagnosis [10]; our data extend this finding to RFS after curative-intent local therapy.

Only SDNN—not RMSSD—retained independent significance in the multivariable analysis. While RMSSD is considered a more specific index of vagal tone, SDNN from short recordings captures both sympathetic and parasympathetic influences. The lack of independent significance for RMSSD tempers a purely vagal interpretation. Potential explanations include: (1) limited statistical power (post-hoc power for RMSSD at HR = 0.70 was 0.54); (2) lower reliability of 10-second RMSSD compared with SDNN in our population [12]; (3) the protective effect captured by SDNN may reflect a composite of sympathetic and parasympathetic influences rather than vagal tone alone. Given the well-documented methodological concerns regarding ultra-short RMSSD, we lean toward the interpretation that this negative finding is at least partially attributable to measurement instability rather than a true lack of vagal protection.

4.3 Integrative Physiology: Proteomic Insights

A key contribution of this study is the integration of HRV with circulating proteomics, although the following observations are exploratory and require replication in independent cohorts. Higher HRV was associated with enrichment of pathways involved in extracellular matrix (ECM) organization, integrin signaling, coagulation, and platelet activation. These processes support vascular integrity and immune cell trafficking. Platelets contribute to immune modulation within the tumor microenvironment, and their activation state can influence cancer progression and immune surveillance [15].

Of note, coagulation and platelet activation are generally associated with tumor metastasis, yet their enrichment in the high-HRV group was accompanied by longer RFS. Potential explanations for this apparent paradox include: (i) the dual role of platelets in tumor immunity—moderate platelet activation can recruit natural killer (NK) cells and enhance anti-tumor immunity; (ii) the enriched pathways predominantly involve fibrinogen and early components of the coagulation cascade, which may reflect maintenance of vascular integrity rather than pathological thrombosis. Concurrent enrichment of ECM and integrin pathways suggests that preserved vagal tone might modulate ECM composition and organization, creating a microenvironment relatively unfavorable to tumor cell migration and colonization. These interpretations await functional validation.

This interpretation aligns with the observed downregulation of glycolysis and hypoxia pathways in the high-HRV group. Hypoxia-inducible factor 1α (HIF-1α) drives metabolic reprogramming (the Warburg effect) to facilitate tumor growth[16]. Nevertheless, the cross-sectional proteomic design precludes causal inference: low HRV and glycolytic signatures may be parallel consequences of the same underlying disease state (e.g., advanced tumor burden). For example, progressive tumors can release inflammatory cytokines that act on central autonomic nuclei to reduce HRV, while simultaneously exhibiting a Warburg effect that elevates serum glycolytic enzymes. Thus, low HRV and glycolysis signatures may both be non-causal markers of tumor burden.

4.4 Psychological and Behavioral Confounders

In patients undergoing liver resection, preoperative depressed mood has been associated with reduced HRV [17]. Psychological distress (depression, anxiety) is linked to both lower HRV and worse oncologic outcomes, suggesting a potential confounding or mediating pathway. Future HCC studies should incorporate validated psychological measures to assess whether psychological factors influence the prognostic value of HRV.

4.5 Clinical Utility and Future Directions

Our findings have potential implications for post-MWA management, but these remain hypothetical pending prospective validation. First, SDNN derived from a routine 10-second ECG—a test already performed in most preoperative evaluations—could identify patients at elevated recurrence risk without additional cost or time, provided that future prospective studies address the methodological concerns regarding ultra-short SDNN. Second, for patients with low SDNN (< 18.59 ms), closer imaging surveillance (e.g., shortening the interval from 3 months to 2–3 months) or consideration of adjuvant therapies (e.g., immunotherapy) may be warranted, but this requires prospective validation. Third, the

proteomic signature of low HRV—enrichment of glycolysis and hypoxia pathways—suggests that interventions targeting metabolic reprogramming or hypoxia signaling pathways (to be validated in preclinical models) might have particular value in this subgroup. However, no clinical recommendation can be drawn from this hypothesis- generating finding.

4.6 Strengths and Limitations

Strengths include a well-defined clinical scenario, a simple non-invasive biomarker derived from routine ECGs, rigorous adjustment for confounders, and exploratory integration of proteomic data.

Several limitations should be acknowledged. (1) The single-center retrospective design risks selection bias and unmeasured confounders (e.g., psychological stress, physical activity, respiratory rate during ECG recording). (2) The modest sample size (n = 101, 44 events) limits subgroup analyses. (3) Ten-second ECGs cannot capture full autonomic circadian profiles and are sensitive to transient respiratory influence. Nevertheless, previous studies have shown moderate population-level correlation between single resting HRV and 24-hour HRV, and our results are directionally consistent with those expected from prospective studies using standard recordings, partially mitigating this concern [18, 19]. (4) Exploratory proteomics in a subset (n = 25) without an independent validation cohort precludes causal inference; GSEA results were controlled using q value < 0.25 to manage the false discovery rate, but remain hypothesis-generating.

5. Conclusions

Pre-procedural autonomic function, quantified by SDNN from a routine ECG, is an independent predictor of tumor recurrence in HCC patients undergoing MWA. Reduced SDNN identifies a subgroup at elevated risk. These promising findings require validation in prospective cohorts using standard HRV recordings before clinical translation.

Abbreviations

AFP = Alpha-fetoprotein; ANS = Autonomic nervous system; CECT = Contrast-enhanced computed tomography; CE-MR = Contrast-enhanced magnetic resonance imaging; CEUS = Contrast-enhanced ultrasound; CRP = C-reactive protein; ECG = Electrocardiogram; FDR = False discovery rate; GSEA = Gene set enrichment analysis; HCC = Hepatocellular carcinoma; HR = Hazard ratio; HRV = Heart rate variability; IQR = Interquartile range; MWA = Microwave ablation; NES = Normalized enrichment score; RFS = Recurrence-free survival; RMSSD = Root mean square of successive differences between adjacent NN intervals; SD = Standard deviation; SDNN = Standard deviation of all normal-to-normal intervals.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the Eighth Affiliated Hospital of Sun Yat-sen University (approval number: 2023-068-01). Informed consent was waived due to the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The proteomics raw data are not publicly available due to institutional data protection policies but can be requested from the corresponding author with appropriate ethics approval.

Competing interests

The authors declare no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82272011), General Program of the Provincial-Enterprise Joint Fund (No. J2025A1515220141), the Shenzhen Science and Technology Program (No. JCYJ20220530144210022), the Guangdong Basic and Applied Basic Research Foundation (No. 2023A1515011409), and the Futian Health care Research Project (FTWS2026029, FTWS2026032).

Authors' contributions

QL and YL contributed equally to this work. QL: conceptualization, data curation, formal analysis, methodology, writing – original draft. YL: data curation, investigation, visualization, writing – original draft. ZC: proteomics data analysis, bioinformatics, writing – review & editing. JD: investigation, resources. HK: data curation, validation. HL: formal analysis, software. SL: resources, supervision. ZZ: investigation. KL: conceptualization, funding acquisition, supervision, writing – review & editing. EX: conceptualization, project administration, funding acquisition, supervision, writing – review & editing. All authors read and approved the final manuscript.

Acknowledgements

None.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63. 10.3322/caac.21834.
2. Gu H, Seo Y, Chung DJ, et al. Microwave ablation vs. liver resection for patients with hepatocellular carcinomas. J Liver Cancer. 2025;25(1):99–108.
3. Giuffrè M, Zuliani E, Visintin A, et al. Predictors of Hepatocellular Carcinoma Early Recurrence in Patients Treated with Surgical Resection or Ablation Treatment: A Single-Center Experience. Diagnostics (Basel). 2022;12(10):2517. 10.3390/diagnostics 12102517.
4. Dattaro L. Pancreatic cancer is evasive. Is the nervous system the reason why? Nature. 2025;648(8093):S49–50.
5. Zhi X, Li B, Li Z, et al. Adrenergic modulation of AMPK-dependent autophagy by chronic stress enhances cell proliferation and survival in gastric cancer. Int J Oncol. 2019;54(5):1625–38.
6. Taha AM, Elrosasy A, Mohamed AS, et al. Effects of Non-invasive Vagus Nerve Stimulation on Inflammatory Markers in COVID-19 Patients: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Cureus. 2024;16(10):e70613. 10.7759/cureus.70613.
7. Abid N, Mani AR. The mechanistic and prognostic implications of heart rate variability analysis in patients with cirrhosis. Physiol Rep. 2022;10(8):e15261. 10.14814/phy2.15261.
8. Kemp AH, Quintana DS, Gray MA et al. Impact of depression and antidepressant treatment on heart rate variability: a review and meta-analysis. Biol Psychiatry. 2010;67(11):1067–1074. 10.1016/j.biopsych. 2009.12.012.
9. Guan W, Wang Y, Zhao H, et al. Prediction models for lymph node metastasis in cervical cancer based on preoperative heart rate variability. Front Neurosci. 2024;18:1275487.
10. Ciurea A, Gheonea DI, Schenker M, et al. The Prognostic Correlation of Heart Rate Variability at Diagnosis with Survival of Patients with Hepatocellular Carcinoma. Diagnostics (Basel). 2021;11(5):890. 10.3390/diagnostics11050890.
11. Munoz ML, van Roon A, Riese H, et al. Validity of (Ultra-)Short Recordings for Heart Rate Variability Measurements. PLoS ONE. 2015;10(9):e0138921. 10.1371/journal. pone. 0138921.
12. Nussinovitch U, Elishkevitz KP, Katz K, et al. Reliability of Ultra-Short ECG Indices for Heart Rate Variability. Ann Noninvasive Electrocardiol. 2011;16(2):117–22. 10.1111/j.1542-474X.2011. 00421. x.
13. De Couck M, Caers R, Spiegel D et al. The Role of the Vagus Nerve in Cancer Prognosis: A Systematic and a Comprehensive Review. J Oncol. 2018; 2018:1236787. 10.1155/2018/1236787
14. Kloter E, Barrueto K, Klein SD, et al. Heart Rate Variability as a Prognostic Factor for Cancer Survival - A Systematic Review. Front Physiol. 2018;9:623. 10.3389/fphys.2018.00623.
15. Rab SO, Altalbawy FMA, Bishoyi AK, et al. Targeting platelet-tumor cell interactions: a novel approach to cancer therapy. Med Oncol. 2025;42(7):232.

16. Zeng Y, Tao Y, Du G, et al. Advances in the mechanisms of HIF-1α-enhanced tumor glycolysis and its relation to dedifferentiation. Prog Biophys Mol Biol. 2025;197:1–10.
17. Kim E, Park J, Lee SM, et al. Preoperative depressed mood and perioperative heart rate variability in patients with hepatic cancer. J Clin Anesth. 2016;35:332–8.
18. Krause E, Vollmer M, Wittfeld K, et al. Evaluating heart rate variability with 10-second multichannel electrocardiograms in a large population-based sample. Front Cardiovasc Med. 2023;10:1144191. 10.3389/fcvm.2023.1144191.
19. Orini M, van Duijvenboden S, Young WJ, et al. Long-term association of ultra-short heart rate variability with cardiovascular events. Sci Rep. 2023;13(1):18966. 10.1038/s41598-023-42208-9.

Figures

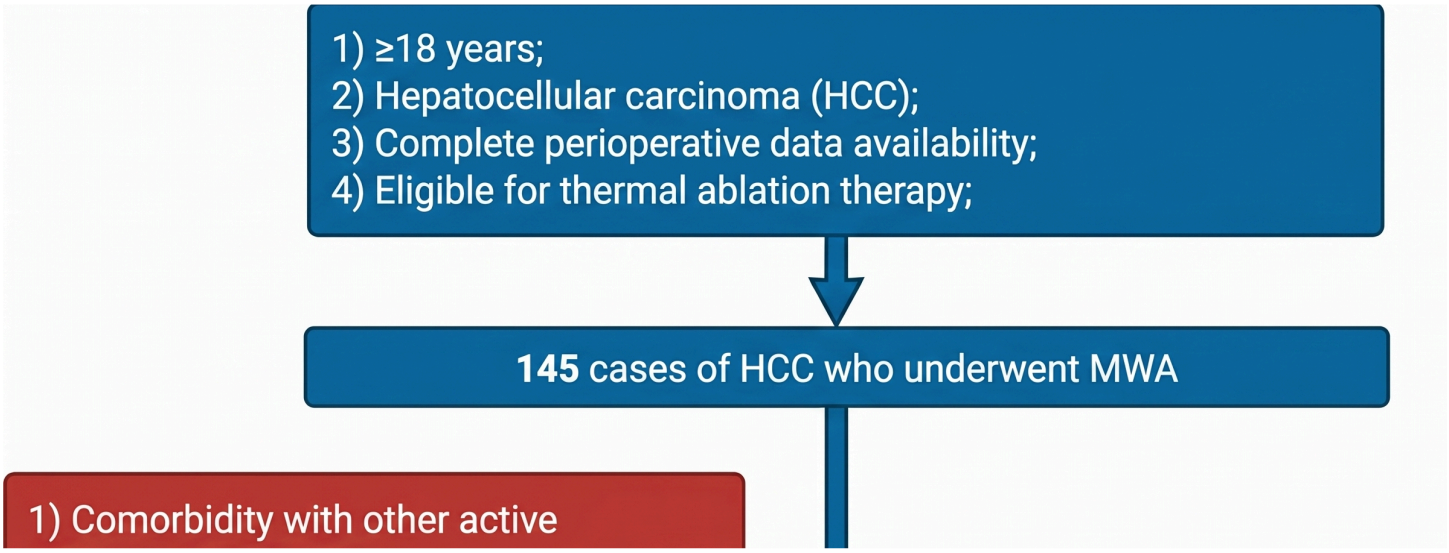


Figure 1

Study Flowchart. Selection process for the final study cohort of patients with hepatocellular carcinoma (HCC) undergoing microwave ablation (MWA). HRV, heart rate variability.

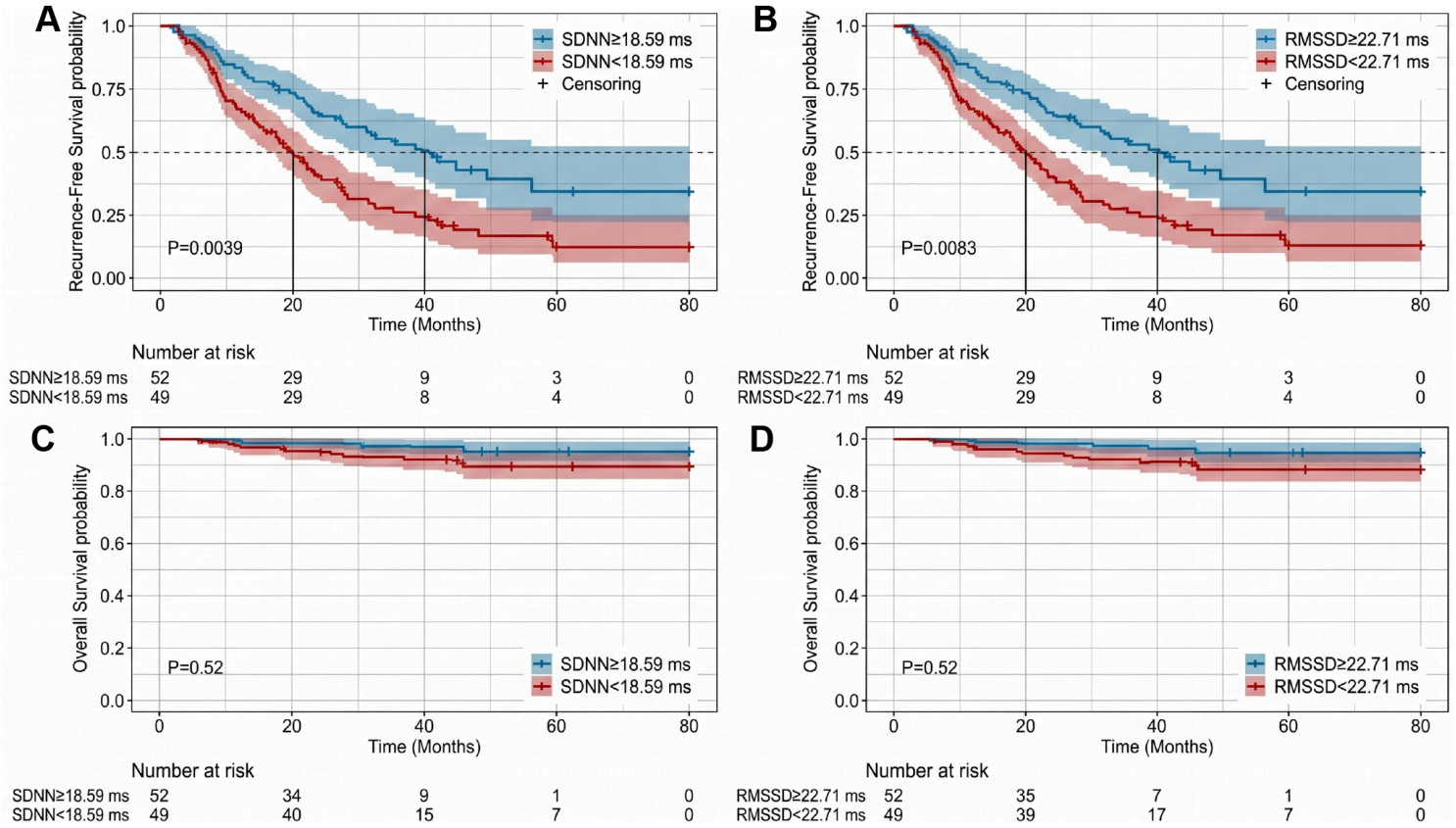


Figure 2

Recurrence-free survival (RFS) and overall survival (OS) stratified by heart rate variability (HRV) indices. Kaplan-Meier curves for (A) RFS by standard deviation of normal-to-normal intervals (SDNN), (B) RFS by root mean square of successive differences between adjacent normal-to-normal intervals (RMSSD), (C) OS by SDNN, and (D) OS by RMSSD. Patients were dichotomized by median HRV values (SDNN ≥18.59 ms, RMSSD ≥22.71 ms). RFS was better in high-HRV groups (log-rank P = 0.0039 for SDNN; P = 0.0083 for RMSSD). OS did not differ significantly (log-rank P > 0.05 for both). SDNN, standard deviation of normal-to-normal intervals; RMSSD, root mean square of successive differences between adjacent normal-to-normal intervals.

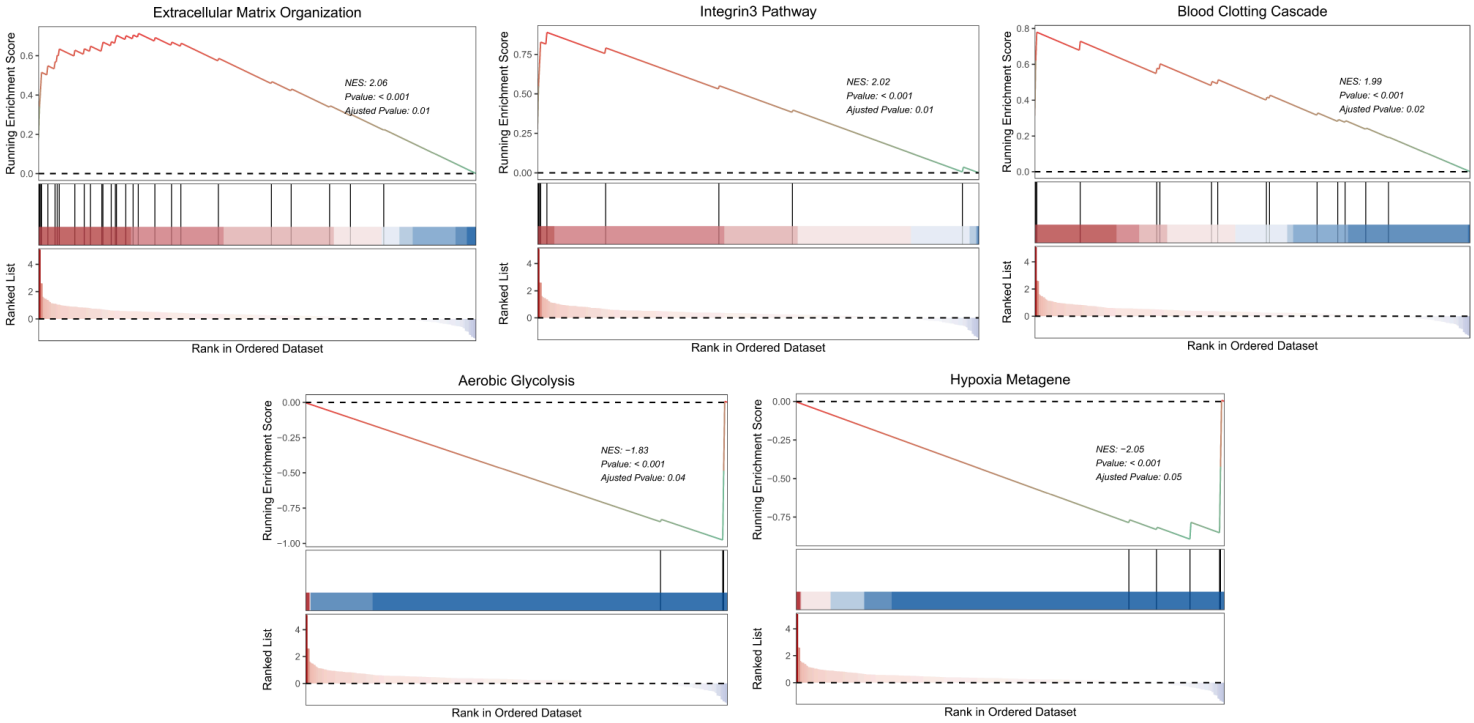


Figure 3

Gene set enrichment analysis (GSEA) reveals distinct pathway signatures associated with high and low heart rate variability (HRV) in patients with hepatocellular carcinoma (HCC) after microwave ablation (MWA). The high-HRV group was enriched in pathways related to coagulation, fibrinolysis, extracellular matrix organization, integrin signaling, and platelet activation. The low-HRV group showed enrichment of glycolysis, hypoxia, and keratinization pathways (downregulated in the high-HRV group). Pathways with |normalized enrichment score (NES)| > 1.5 and q value < 0.25 were considered significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1SupplementaryMethodsS1.docx
- Additionalfile2Fig.S1.jpg
- Additionalfile3Fig.S2.jpg
- Additionalfile4TableS1.docx