Significant reduction of heart rate variability is a feature of cirrhosis acute decompensation most notable in acute-on-chronic liver failure and predicts 90-day mortality

Running title: Loss of HRV in cirrhosis decompensation

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Acknowledgements: We thank Isansys Limited for their technical support in delivering this study.

Financial support: This study was supported by grants from the Deutsche Forschungsgemeinschaft (SFB TRR57 to P18), Cellex-Foundation and UCL Business award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest: UCL Business has filed a patent application on the use of Heart Rate Variability in cirrhosis decompensation.

Author contributions:

Guarantor of article: RPM
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Analysis and interpretation of data: DC, RPM, KLT, RS, CJ, HJ, JT
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Statistical analysis: KLT, RS, DC, RPM, CJ, JT, HJ
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Abbreviations:

ACLF	Acute-on-chronic liver failure
AD	Acute decompensation
AUROC	Area under Receiver Operating Characteristic
CI	Confidence interval
CLIF-C AD	CLIF Consortium Acute Decompensation Score
CP Score	Child Pugh Score
CRP	C-reactive protein
ECG	Electrocardiography
HE	Hepatic encephalopathy

HRV	Heart rate variability
IQR	Inter-quartile range
INR	International Normalized Ratio
MELD	Model for End-stage Liver Disease
NAFLD	Non-alcoholic fatty liver disease
NN	Normal beat-to-beat variation of R-R intervals
SD	Standard deviation
SDNN	Standard deviation of all NN intervals
WBC	Total white blood cell count

Summary

Background & Aims: Heart rate variability (HRV) is reduced in cirrhosis and in conditions of systemic inflammation but its association with cirrhosis decompensation and development of acute-on-chronic liver failure (ACLF) is unknown. This study aimed to validate wireless remote monitoring of HRV in cirrhosis decompensation, and whether HRV loss is a surrogate for progression and inflammation and if its measurement can determine prognosis in acute decompensation of cirrhosis.

Methods: 111 patients at risk of cirrhosis decompensation at 2 clinical sites were monitored for HRV. Standard deviation of all normal beat-beat intervals (SDNN) reflecting HRV was assessed using remote monitoring (Isansys Lifetouch®) and/or Holter ECG recording. Clinical outcomes and major prognostic scores were recorded during follow up in all patients.

Results: Reduction of HRV, denoted by lower baseline SDNN, correlated with severity of decompensation (14 vs. 33ms; $p<0.001$, acute decompensated (AD) vs. stable out-patient cirrhosis). Furthermore, SDNN was significantly lower in patients developing ACLF compared to those with only AD (10 vs. 16 ms, $p=0.02$), and correlated inversely with MELD, and Child-Pugh scores and CRP (all $p<0.0001$) and white cell count ($p<0.001$). SDNN predicted disease progression on repeat measures and was an independent predictor of 90-day mortality, where a cut-off of 13.25 ms had a 98% negative predictive value.

Conclusions: This study clearly demonstrates that HRV wireless remote monitoring identifies cirrhosis patients at high risk of developing ACLF and death, and suggests that HRV monitoring might help guide need for early intervention in such patients.

Word count: 244

Clinical Trial number: NIHR clinical research network CPMS ID 4949

Key words: Heart rate variability; inflammation; cirrhosis acute decompensation

Introduction:

Under normal physiological conditions, cardiac responses to physical activity and stress (increased cardiac output) are regulated largely by the autonomic nervous system. The interplay between sympathetic and parasympathetic autonomic nervous system activity is reflected in heart rate variability (HRV), the variation in normal heart beat-beat intervals, with greater parasympathetic modulation promoting increased HRV in a linear manner.[1] Loss of normal HRV is a feature of systemic inflammation and is associated with increased morbidity and mortality, particularly in conditions such as after cardiac ischaemia and in diabetes mellitus.[2, 3]

Patients with cirrhosis are known to have numerous cardiac abnormalities including prolongation of the QTc interval [4, 5] and altered baroreceptor responses.[6] Moreover, a reduction in HRV has been described in patients with cirrhosis and it has been suggested that a further loss occurs with increasing severity of cirrhosis, and in the presence of hepatic encephalopathy.[7, 8] However, such observations have been largely based in stable cirrhosis outpatients with limited 5-minute electrocardiography (ECG) assessments. The change in HRV with acute decompensation of cirrhosis and development of acute-on-chronic liver failure (ACLF) and the prognostic significance of any such changes are unclear. Assessment of HRV in dynamic situations like cirrhosis, in which acute alterations in physiology and inflammation modify day-to-day changes in HRV, ideally requires continuous monitoring. The Isansys Lifetouch® system enables continuous, wireless collection of heart rate and cardiac performance data, sampling at a rate of 1000Hz, using a simple non-invasive skin surface sensor, which attaches to the precordium and communicates with an accompanying wireless gateway. The heart beat-beat intervals recorded by the device can then be calculated through automatic QRS identification and extracted from the gateway following the recording period.

We hypothesized that in patients with cirrhosis hospitalized for acute decompensation (AD), the development of ACLF will be associated with a further reduction in HRV, reflecting the degree of hepatic decompensation and level of inflammatory response. This study aimed to validate wireless remote monitoring of HRV in AD patients and establish whether HRV

measurement is a surrogate for progression and inflammation, and if its measurement can determine prognosis in AD.

Methods

Study design and patient selection

Patients with an established diagnosis of cirrhosis based on clinical, ultrasound and/or laboratory/histological criteria of cirrhosis were included at 2 sites; the Liver and Transplantation Unit at the Royal Free hospital, London, UK and the Department of Internal Medicine I, University of Bonn, Germany.

London Patients

The study was approved by the local governing Research Ethics Committee (London – Harrow; REC Ref: 08/H0714/8) and all patients (or a family member if without capacity) provided informed consent in accordance with the 1975 Helsinki Declaration. Patients were recruited prospectively between March 2013 and July 2015 at the Royal Free Hospital, London, as part of an on-going biomarker study of cirrhosis decompensation. This study screened patients presenting with acute decompensation of cirrhosis including those presenting with hepatic encephalopathy, infection (SBP and other), new onset jaundice, variceal bleeding, new renal impairment and increasing ascites. After a period of 48 hours during which the patient would receive specific treatment for their precipitating event, if there were no signs of clinical or biochemical improvement, and the patient had no study exclusion criteria and was deemed a candidate for intensive care support for organ failure should they require it, the patient was included into the parent biomarker study. Key exclusion criteria included: Admission for reasons other than acute decompensation of cirrhosis (other co-morbid diseases, especially established cardiovascular or intrinsic renal disease); Malignancy (extra-hepatic or a hepatocellular carcinoma); Patients who had undergone major surgery or had unresolved surgical problems; Pregnancy.

Only patients full filling the above criteria and willing to also undergo monitoring, were approached for the HRV sub-study protocol. In addition, patients were excluded from the HRV sub-study study if they had significant cardiac rhythm abnormalities including atrial fibrillation or/and cardiac pacemakers, or were taking negative chronotropic medications such as beta-blockers, adrenergic receptor blocking agents or anti-arrhythmics. Patients were recruited sequentially based on availability of the Isansys Gateways for monitoring.

Bonn Patients

These comprised patients that were clinically stable, ambulant, outpatients, with either controlled ascites or previously treated decompensation events but who were now in a stable clinical state, to be maintained in the community. They were prospectively recruited as part of an observational study on cardiac function from February 2014 to January 2015, including Holter examination. The local ethics committee of the University of Bonn approved the study (No. 121/14). The main exclusion criteria were as per the patients from London. These patients provided a comparable stable out-patient cohort who were at risk of decompensation, to draw comparisons with those hospitalised for acute decompensation, and to determine if measurement of HRV (SDNN) could predict risk of poor outcome even in outpatients.

Follow up of the patients

All patients had clinical, demographic and laboratory parameters recorded at baseline with further serial measures during their period of follow up till 90 days. All patients underwent a baseline ECG before initiating continuous HRV assessment as described below.

The Chronic Liver Failure (CLiF) Consortium criteria were used to define the development of ACLF during admission in AD patients.[9] Previously validated scores including Child-Pugh (CP) classification, Model for End Stage Liver Disease (MELD) and the CLiF Consortium Acute Decompensation (CLIF-C AD) score [9, 10] were also applied to assess disease severity. During the follow-up, evolution of MELD score and development of organ failure, ACLF and death were recorded.

All patients underwent routine clinical care with management according to local hospital guidelines and directed by the treating clinicians. This included intensive care unit and specific organ support, such as renal filtration, when indicated in patients with acute decompensation. Such patients were observed until hospital discharge, with further follow-up data collected to 90 days.

Heart rate variability evaluation

An initial pilot study was performed on twenty healthy adults without liver disease, and on no concomitant medication, nor with a significant past medical history, to test the feasibility of wireless remote monitoring of HRV using the Isansys Lifetouch® system. Using systems integrated into the LifeTouch® gateway and Kubios HRV Version 2.0, three separate, 5 minute, artefact-free, continuous R-R interval sessions, during the waking hours of 8am and

7pm, were selected for analysis using Kubios HRV Version 2.0.[11] These sessions were used to establish standard deviation of all NN intervals (SDNN), for a given subject for that corresponding day. Artefacts were identified through assessment of RR interval data, graphically and computationally represented in Kubios, and denoted if recordings deviated greatly from the average baseline RR interval during the selected time period.

Having demonstrated feasibility of measurement and reproducibility of HRV data with the Lifetouch system in controls, a further assessment was performed on the first 13 cirrhosis in-patients studied at the Royal Free site, to establish if there was good concordance between remote device measurement and standard ECG /Holter R-R interval recording. Once proven, all subsequent HRV data upon hospitalization was evaluated using the Isansys Lifetouch® system through generation of SDNN values, as described above. In some patients, repeat measurements of HRV were availed during decompensation or development of ACLF, by leaving a device on the patient for up to 5 days. For stable out-patients, SDNN was derived from Holter devices at the time of review.

One individual (DAC) performed all the SDNN analysis and was blind to individual patient data and outcomes, and adopted the same filtering techniques for artefact and time window analysis across patients from both sites, for consistency. As we wished to assess the impact of ambulant patients and 'real-life' activities that would reflect utility of monitoring in daily practice, we elected to study waking-hour time windows to assess differences in HRV that were then standardized across all patients.

Plasma IL-6 and IL-8 measurement

IL-6 and IL-8 were measured using standard ELISA kits as per manufacturer's instruction (BD Biosciences).

Statistical analysis

Given the concordance in SDNN availed from ECG and Lifetouch system data outputs and that we were seeking to explore the practical utility of HRV denoted by SDNN as a 'real life' measure of risk of cirrhosis decompensation and mortality, we amalgamated the data sets from London and Bonn and considered the application of baseline SDNN across all included patients.

Regarding data analysis, continuous variables were expressed as median and interquartile range (IQR) and categorical variables as numbers and percentages. Uni-variate analysis was carried out to identify the baseline factors associated with occurrence of death. Differences in continuous variables between survivors and non-survivors were accessed using the Mann-Whitney test, whereas categorical variables were tested using Pearson χ^2 test.

As this was an aetiological research study design, we used logistic regression to examine the impact of SDNN levels in predicting 90-day mortality, whilst adjusting for confounding variables by including all the relevant potential confounders of an association between SDNN and 90-day mortality, as elaborated in the results section. Correlations were analysed with the Spearman's rank correlation. P-values <0.05 were considered statistically significant. Using ROC curve analysis to predict mortality, a cut-off for SDNN was found and the cohort was divided in two groups. Differences between mortality in each group were displayed by Kaplan-Meier curves and tested using the log-rank test. All statistical analysis was performed by means of Stata® 14.

The study is reported in line with STROBE guidance for Observational Cohorts

Results

Baseline characteristics of patients

Of the 145 patients assessed for eligibility for inclusion in the study, 111 patients (74 male) were included in London and Bonn and analysed for HRV as described in Figure 1. Amongst these, 49 had acute decompensation of cirrhosis based on development of hepatic encephalopathy, and/or renal dysfunction or increasing ascites and were admitted to the hospital. By comparison, the 62 cirrhosis out-patients without AD were clinically stable, under regular follow-up with no new changes in clinical condition or medication. There were no significant differences in gender between stable out-patients and AD patients with predominantly male subjects in both groups but AD patients were significantly younger (53 vs. 59 years of age, $p<0.02$). About 70% of patients in both groups were of alcoholic aetiology (actively consuming alcohol), the remainder split between viral, NAFLD and other causes, with no differences between the groups. Alcoholic hepatitis (AH) [as defined by a bilirubin > *5mg/dl* in an active drinker (>40g alcohol/day), with AST>50, AST>ALT ratio of 1.5, and/or histological criteria when present] was the precipitant for AD in 41% and infection in 27%. In 12% of AD patients both infection and AH were present, whilst in 20% of cases, no precipitant cause was noted. As anticipated, AD patients had significantly greater derangement in INR, were more hypoalbuminaemic, and had significantly higher plasma bilirubin compared to stable out-patients, along with higher aminotransferases (see Table 1). As a consequence, AD patients had significantly greater MELD scores (20 vs. 9, $p<0.001$). Moreover, AD patients had a median CLIF-C AD score of 55, suggesting an appreciable risk of mortality based on findings of the CANONIC study.[10] The principle organ dysfunction in AD patients was hepatic encephalopathy, in 41% of cases. As expected, amongst the 62 stable out-patients, most (84%) were Child A (5 [5-6]- median and IQR), whilst AD patients had significantly higher Child scores (9 [9-10]) split equally between Child B and C disease. AD patients also had significantly higher white blood cell counts (7.1 vs. 5.7 $x10^9$/L; $p=0.01$) and CRP levels (20 vs. 5 mg/L; $p<0.001$) compared to out-patients.

Nine patients who were admitted with AD met criteria for acute-on-chronic liver failure (ACLF) at the time of monitoring: 2 with ACLF1, 5 with ACLF2 and 2 with ACLF3. [9] Two patients required terlipressin whilst undergoing monitoring, 1 noradrenaline, and 2 who had been on beta-blockers, had these stopped upon admission.

Validation of remote monitoring for HRV measurement in healthy volunteers and cirrhosis patients

Having established pilot HRV data collection in 20 healthy controls (40% male, mean age 33.5 ± 9.23 years), 13 consecutive enrolled cirrhosis patients had R-R intervals derived from ECG traces compared with the Isansys Lifetouch® system to derive SDNN data. This showed good concordance with the SDNN values obtained through remote, continuous monitoring, as demonstrated by the Bland-Altman Plot in Supplementary Figure 1. Subsequent data collection to assess HRV in all cirrhosis patients was evaluated through measuring SDNN.

Using the remote monitoring system, it was possible to analyse HRV in all monitored decompensated in-patients. Monitoring was performed continuously and there were no statistically significant differences between day and night measures of SDNN (10.3 (6.7-15.0) vs. 13.9 (5.0-16.7), p=0.63).

Amongst the out-patients with Holter ECG monitoring, HRV was interpreted in 89% of patients. Due to the difficulty in obtaining an artefact-free, 5-minute period at the predetermined analysis time set out in the blinded analysis protocol, and the resulting computational errors in calculating HRV parameters, 8 out-patients were excluded at the time of HRV analysis. However, importantly, there were no statistically significant differences in SDNN evaluated between the sites as exemplified by patients with MELD >16: Bonn SDNN 14 (10-16) vs. RFH 12 (10-15); p=0.60, and similarly, Child C patients: Bonn SDNN 11 (10-16) vs. RFH 11 (9-13); p=0.48. Also worthy of note there was no significant correlation noted between QT intervals and SDNN: Spearman's rho=0.02; p=0.85.

Association between HRV and acute decompensation and progression to ACLF

All cirrhosis patients had considerably lower HRV as assessed by SDNN, compared to healthy subjects in whom median SDNN was 55ms. Baseline SDNN was noted to be significantly lower in AD patients compared to cirrhosis out-patients (14 vs. 33ms; p<0.001). By contrast the baseline heart rate was significantly elevated in AD patients (82 vs. 64 bpm; p=0.03). There were no differences in mean arterial pressure between cirrhosis out-patients and AD patients.

As can be seen in Figure 2A, there is a clear relationship between reduction in HRV as denoted by lower SDNN, and progression of liver disease from stable out-patients to AD

patients in hospital. Moreover, as patients progress to ACLF, there is a further significant reduction in median SDNN [IQR] (AD vs. ACLF: 16 [11-24] vs. 10 [9-12] ms; p= 0.02), as shown in Figure 2B. This marked reduction in SDNN with AD progression and ACLF, reflecting further impairment of HRV, is demonstrated by the examples of Poincaré plots shown in Supplementary Figure 2 A-D. This representative data shows there is significantly reduced HRV compared to healthy subjects when liver disease progresses, most evident, from stable out-patients to AD patients. The magnitude of reduction in HRV is best exemplified in Supplementary Figure 2D, reflecting a case of a patient who developed ACLF, with multi-organ failure and succumbed in the intensive care unit, with almost negligible HRV, despite the high physiological stress and use of inotropes in this setting.

Across all the studied patients (Figure 3A-C), SDNN correlated strongly and inversely with CP score (Rho = -0.67; $p < 0.0001$), MELD score (Rho = -0.66; $p < 0.0001$), and in AD patients, CLIF-C AD score (Rho = -0.63; $p<0.0001$). SDNN also correlated inversely and significantly with heart rate (Supplementary Figure 3), suggesting a potential relationship between the hyperdynamic circulation and reduced HRV.

Evaluation of factors that predicted mortality on uni- and multi-variate analysis

All patients had data collected on 90-day mortality, status of liver disease severity and development of organ dysfunction. There were 12 deaths (7 male) that occurred within 90 days. No differences in age, gender distribution nor aetiology were noted between survivors and non-survivors. Non-survivors had higher bilirubin (6.6 vs. 1.2 mg/dl; p=0.06), significantly higher INR (1.6 vs. 1.1; $p<0.01$) and lower albumin levels (31 vs. 35 g/L; p=0.02) compared with those alive at 90 days (Table 2). Similarly, WBC (9.8 vs. 5.8; $P<0.02$) and CRP (40 vs. 8 mg/L; $p<0.001$) were significantly elevated in non-survivors. As anticipated, non-survivors had higher MELD scores and were predominantly Child C disease. In relation to hepatic encephalopathy, there was a greater number of non-surviving patients presenting with hepatic encephalopathy compared to survivors. In patients that died, infection was the main injurious trigger in at least 50% of cases, often associated with subsequent multi-organ failure. However, there were no statistically significant differences in renal dysfunction (defined as increase in serum creatinine by more than 0.3mg/dl within 48 hours or to more than 1.5 fold at the time of monitoring, compared to baseline levels at admission) between the groups.

SDNN was significantly lower in non-survivors than survivors (11 vs. 26 ms; p<0.0001). The repeat measurement of SDNN over a mean period of 5 days demonstrated a 55% decrease in SDNN from baseline in AD patients that died within 90 days, whilst there was no significant change from baseline in survivors. However, in the sub-group who developed ACLF, a 25% decrease from baseline SDNN was observed in those patients that died, as opposed to a 30% increase in SDNN in patients that survived their ACLF, despite all patients being managed according to the same standard protocols.

By using logistic regression analysis, the crude odds ratio (OR) for SDNN in predicting 90-day mortality was 0.83 (95% confidence interval (CI): 0.74 - 0.93; P=0.002). We then performed multivariate analyses and introduced potential confounders for analysis in a logistic regression model. We adjusted for confounding by patients gender, age, presence of HE and bilirubin, INR, albumin, sodium, creatinine, CRP and WBC levels and chose to introduce the actual variables, instead of the composite scores. The impression that SDNN was associated with 90-day mortality persisted after confounder adjustment; the adjusted OR for SDNN was 0.79 (95% CI: 0.65 - 0.97; P=0.02) and the analyses disclosed baseline SDNN as the only independent predictor of 90-day mortality (Supplementary Table 1).

Utility of SDNN in predicting 90-day mortality

The predictive utility of baseline SDNN in determining 90-day mortality was evaluated using Area under Receiver Operating Characteristic (AUROC) curve analysis. Given the clinical use of other scoring systems and their strengths on univariate analysis, we also compared performance of SDNN to CP score (AUC: 0.85; 95%-CI: 0.76-0.93), MELD score (AUC: 0.77; 95%-CI:0.64-0.90), MELD-Na score (AUC: 0.76; 95%-CI:0.61-0.90), CRP-values (AUC: 0.85; 95%-CI:0.76-0.93) and WBC (AUC:.0.70; 95%-CI:0.53-0.88). Interestingly, baseline SDNN had the highest AUROC for 90-day mortality (AUC: 0.87; 95%-CI:0.79-0.96), as shown in Figure 4A.

Using an optimal sensitivity and specificity from the ROC curve, a baseline SDNN cut-off value of 13.25 ms had a 98% negative predictive value (sensitivity 83%, specificity 86%) for predicting 90-day mortality. This cut-off could distinguish between survivors and non-survivors at 90 days, as shown in the Kaplan Meier analysis (Figure 4B).

When patients were compared applying an SDNN cut-off above and below 13.25 ms, the principle variables that differed between the groups were that patients with an SDNN <13.25

ms had significantly elevated bilirubin (11.1 vs. 1 mg/dl, p<0.0001); INR (2.1 vs. 1.1, p<0.0001); MELD score (25 vs. 11, p<0.0001) and CLIF-C AD score (59 vs. 46, p<0.0001), as shown in Table 3. Moreover, those with an SDNN <13.25ms had significantly higher heart rates compared to those with SDNN >13.25ms: [87 (80-100) vs. 72 (64-85), p=0.02]. Of note, there were no differences in renal function or encephalopathy, nor aetiological factors for liver disease, nor age in patients above and below the SDNN cut-off of 13.25ms. However, infection was more commonly noted in 67% of cases as a factor during decompensation, in those with SDNN <13.25 ms.

Systemic inflammation and its association with HRV

As might be expected, stable out-patients had considerably lower CRP values compared to patients with AD (10 vs. 5 mg/L, p<0.001), as was their WBC. Of interest, SDNN was found to strongly and inversely correlate with CRP (Spearman Rho = -0.56; p < 0.0001). SDNN also correlated inversely with WBC (Rho = -0.34; p < 0.001), Supplementary Figures 4A and 4B. Moreover, in patients with SDNN <13.25 ms, WBC (10.5 vs. 5.8, p<0.001) and CRP (40 vs. 7, p<0.0001) were markedly elevated, compared to patients with SDNN values above this threshold (Table 3). Assessment of the pro-inflammatory cytokines IL-6 and IL-8, also showed these to be markedly increased in patients with an SDNN cut-off <13.25 ms compared to those ≥13.25 ms (Table 3; Supplementary Figures 4C and 4D).

Discussion

Reduction in HRV, as a manifestation of autonomic system dysfunction, has been described in many conditions including cardiac ischaemia, diabetes mellitus, sepsis [2, 3, 13] and chronic liver disease.[7]

The key findings reported in this study are:

(1) Continuous, wireless remote monitoring of HRV is feasible in patients with cirrhosis, and measuring SDNN reflects changes in HRV in this population. (2) SDNN is reduced significantly with progression of cirrhosis to decompensation and is further reduced in patients with ACLF. (3) Reduction in SDNN, reflecting loss of HRV, negatively correlates with disease severity scores such as MELD and CP score and importantly, also with indicators of inflammation (CRP and WBC). (4) Measurement of SDNN shows utility in predicting 90-day mortality and a SDNN cut-off of <13.25 ms we show has a negative predictive value of 98% in determining outcome.

Numerous factors affect HRV measurement including respiration, intrinsic cardiac abnormalities, circadian rhythm, age and genetic factors. Moreover, given significant inter- and intra-individual differences in HRV, reliable measurement of HRV has historically been deemed to be complex and requisite of specific conditions and necessitating special equipment. In this study, we describe a continuous, ambulatory, remote measurement of HRV in patients with established cirrhosis, in whom autonomic dysfunction is common (up to 80%).[14, 15] Traditional ECG measurement of HRV encompasses short-term 5 minutes ECG segments being interpreted separately as reflecting HRV in that specific time window, under stable physiological conditions. The Lifetouch system by contrast, not only facilitates continuous monitoring irrespective of the individuals' daily activity or physical ill-health but also helps negate the short coming of limited ECG time capture, where artefact and premature beats caused by these factors over the 5 minutes of analysed R-R interval, make further interpretation difficult. This is particularly the case in patients with cirrhosis, in whom high respiratory rates, inflammation and impaired baroreceptor responses interfere considerably with standard ECG R-R interpretation.[4, 16-18] Using the remote monitoring device, we were able to interpret HRV in all monitored in-patients with acute decompensation.

HRV data can be described in both time domain and frequency domain variables. SDNN is the most commonly described HRV time domain measurement in part, through its simple evaluation without the need for complex analytical systems, unlike frequency domain variables.[19] In addition, many frequency domain measures are influenced by high respiratory rate and gender, and necessitate significant short-term data filtering.[20, 21] As a consequence, we elected to focus on SDNN in this study given the increased respiratory rate and systemic inflammation in our patient cohort, and in order to reduce the filtering requirements on each data set.

A key finding in this study was the demonstration that HRV as assessed by SDNN was significantly lower in patients hospitalized with acute decompensation as compared to stable out-patients. Moreover, HRV was further reduced in patients with ACLF. SDNN levels in decompensated cirrhosis patients fell to approximately 25% of the median level in controls, and to half the value seen in compensated patients. There was also a strong inverse correlation between SDNN and prognostic scores such as MELD and CP and also importantly, the CLIF-C AD score. The latter has been shown to most accurately prognosticate in those patients with cirrhosis decompensation at risk of progression to ACLF and mortality.[10] It is therefore noteworthy that in this study, SDNN was the only independent predictor of 3-month mortality. Moreover, repeat measurement of SDNN during a patient's journey through hospital admission, demonstrated that *in those who manifest a significant increase in SDNN, there was a potential for recovery, whilst a further decrease in SDNN was more commonly associated with mortality.* This suggests a future potential for continuous monitoring whereby an increase in SDNN following intervention, might inform of favourable response to therapy, whilst conversely, failure to recover SDNN might indicate patients with a higher risk of death.

A further interesting observation was that HRV as assessed by *SDNN demonstrated a good inverse correlation with CRP values and also with WBC.* Moreover, there was also a weak but statistically significant association between low SDNN and increased concentrations of the pro-inflammatory cytokine IL-6 but only a trend of an association with IL-8. This is notable given that 50% of our studied patients that died did so from infection, and also the relationship between low SDNN and the development of the inflammatory syndrome of ACLF. The loss of HRV as indicated by reduction in SDNN may, therefore, reflect a response

to inflammation, whether driven by infection or a sterile inflammatory response, as often seen in ACLF.[22, 23] This is supported by the wider literature on HRV, where for example after unstable angina pectoris, high levels of CRP correlate inversely with loss of HRV.[24] Similarly, following traumatic brain injury, there is a reduction in HRV and this is associated with a change in immune responses,[25, 26] whilst in patients treated for sepsis, reduced SDNN has been linked to poor outcome.[13]

The recent literature in decompensated cirrhosis patients has highlighted the pathophysiologic significance of systemic inflammatory response, as demonstrated by raised leucocyte counts and more recently elevated CRP, as being associated with poor outcome.[10, 27] Of interest, in this study, only SDNN was an independent predictor of mortality. However, the measurement of SDNN may potentially serve as a surrogate of inflammatory activity that can be availed remotely in patients at risk of acute decompensation. If SDNN decreases further, this may indicate a need for closer clinical review of the patient to look for development of ACLF or new infection, before organ failure ensues, and when early intervention with antibiotics or organ support may have most optimal effect. Clearly, this assertion requires further evaluation in a controlled study and also further mechanistic evaluation, to determine whether reduction in HRV reflecting impaired autonomic function, is causally related to poor outcome.

ROC curve analysis demonstrated SDNN has good predictive utility for 90-day mortality (AUC of 0.87) and the SDNN cut-off of >13.25 ms had a negative predictive value of 98% in determining outcome. Patients with an SDNN <13.25 ms invariably had worse liver function (MELD), state of liver decompensation (CLIF-C AD score) and inflammatory indices (CRP and WBC) than patients with SDNN >13.25 ms. This is aligned with a study demonstrating that septic patients on the intensive care unit had an increased risk of death in lower SDNN ranges, even after adjusting for other organ failure severity scores.[13] The need for a larger validation cohort for the SDNN cut-off of 13.25 ms for 90-day mortality is one limitation of our study.

There are few other limitations to consider when interpreting the data presented in this study: Firstly, the patients were recruited at two different sites with a mix of compensated and decompensated patients. Whilst there was heterogeneity in the population, we feel this

reflects the ‘real-life’ situation of patients presenting to the hospital who are at risk of decompensation or/and have had prior decompensation. Furthermore, there were no clear differences noted in liver disease aetiology, renal function or blood pressure between compensated and decompensated patients at baseline. The only clear differences observed between these groups were in liver prognostic scores and SDNN, which was the focus of the study.

A second point of note is the constraint placed by limited availability of the Lifetouch monitors such that it was not possible to accrue repeat measures over long periods, during every patient’s treatment journey. As such, only a limited number of patients had repeated measurements beyond baseline SDNN, and from this we were able to learn that changes in SDNN may indicate a trajectory of improvement or deterioration. However, given the limited sample size and thereby statistical under-powering for this aspect of the data analysis, clearly a dedicated study of long-term SDNN monitoring is required, to determine its value in predicting recovery or progression from a decompensation episode.

In conclusion, we describe the wireless, remote monitoring of HRV and demonstrate that a significant reduction in SDNN (reflecting changes in HRV), in patients with cirrhosis, correlates with severity of liver decompensation and inflammation, deteriorates further in those with ACLF, and acts as an independent prognostic factor for mortality. Our data suggests that using such tools to measure HRV remotely may facilitate a means of monitoring cirrhosis patients at risk of decompensation and high mortality. This will help guide appropriate early intervention if SDNN drops below a determined threshold, and to assess response to therapy, with potential for health economic benefit. The data presented supports the need for further controlled studies of remote HRV monitoring in cirrhosis patients at risk of decompensation.

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Table 1. Baseline patient characteristics

	All patients (n=111)	Stable out-patient cirrhosis (n=62)	Acute decompen-sation (n=49)	P value
Predisposition				
Male gender, n (%)	74 (67%)	40 (65%)	34 (69%)	0.59
Age (years) (range)	56 (19-89)	59 (19-89)	53 (28-77)	0.02
Aetiology: Alc/viral/NASH/other (n)	78/19/7/7 (71/17/6/6%)	43/12/4/3 (69/19/7/5%)	35/7/3/4 (72/14/6/8%)	0.82
Precipitant factor: AH/inf/both/unknown (n)	–	N/A	20/13/6/10 (41/27/12/20%)	–
Laboratory Parameters				
ALT (U/L)	33 (23-59)	35 (24-59)	32 (20-56)	0.34
AST (U/L)	43 (32-74)	36 (23-45)	59 (43-108)	<0.001
Bilirubin (mg/dL)	1.3 (0.7-4.2)	0.8 (0.6-1.3)	5.9 (1.5-16.1)	<0.001
Albumin (g/dL)	35 (29-40)	36 (33-43)	32 (25-35)	<0.001
INR	1.2 (1.0-1.6)	1.1 (1.0-1.1)	1.6 (1.3-2.2)	<0.001
Creatinine (mg/dL)	1.0 (0.8-1.4)	1.0 (0.8-1.4)	1.0 (0.7-1.4)	0.33
Inflammatory response				
CRP (mg/L)	10 (4-32)	5 (3-10)	20 (11-52)	<0.001
WBC ($x10^9$/L)	6.0 (4.4-9.2)	5.7 (4.3-7.3)	7.1 (5.1-12.4)	0.01
Severity and organ injury				
Child-Pugh A/B/C (n)	54/34/23 (49/30/21%)	52/10/0 (84/16/0%)	2/24/23 (4/49/47%)	<0.001
MELD	12 (8-19)	9 (7-12)	20 (14-26)	<0.001
CLIF-C AD score (n=49)	–	N/A	55 (46-60)	–
HE (+/-)	22/89 (20/80%)	2/60 (3/97%)	20/29 (41/59%)	<0.001
Renal dysfunction (+/-)	22/89 (20/80%)	13/49 (21/79%)	9/40 (18/82%)	0.73
Heart rate (bpm)	80 (68-98)	64 (52-88)	82 (72-100)	0.03
MAP (mmHg)	85 (78-92)	87 (79-97)	85 (77-92)	0.087
SDNN (ms)	25 (14-38)	33 (25-42)	14 (11-23)	<0.001

Alc: alcohol, AH: alcoholic hepatitis, ALT: alanine aminotransferase, AST: aspartate aminotransferase, both: refers to AH and infection, CLIF-C AD: CLIF Consortium Acute Decompensation, CRP: C-reactive protein, HE: hepatic encephalopathy, inf: infection, INR: international normalized ratio, MAP: mean arterial pressure, MELD: Model for End-Stage Liver Disease, NASH: non-alcoholic steatohepatitis, SDNN: standard deviation of all NN intervals, WBC: white blood cell count.

Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.

Table 2. Patient characteristics in accordance with 90-day survival

	90-day survival		
	Yes (n=99)	**No (n=12)**	**P value**
Predisposition			
Male gender, n (%)	67 (68%)	7 (58%)	0.52
Age (years)	58 (47-65)	58 (49-68)	0.91
Aetiology:			
alcohol/viral/NASH/other (n)	70/17/6/6 (71/17/6/6%)	8/2/1/1 (67/17/8/8%)	0.98
Laboratory Parameters			
ALT (U/L)	32 (23-53)	50 (22-76)	0.33
AST (U/L)	40 (32-68)	61 (35-114)	0.18
Bilirubin (mg/dL)	1.2 (0.7-2.7)	6.6 (1.2-10.9)	0.06
Albumin (g/dL)	35 (30-41)	31 (25-35)	0.02
INR	1.1 (1.0-1.5)	1.6 (1.3-2.2)	<0.01
Creatinine (mg/dL)	1.0 (0.8-1.4)	1.0 (0.7-1.4)	0.99
Inflammatory response			
CRP (mg/L)	8 (4-20)	40 (26-65)	<0.001
WBC ($x10^9$/L)	5.8 (4.3-8.5)	9.8 (5.7-16.5)	0.02
Severity and organ injury			
Child-Pugh A/B/C (n)	54/29/16 (55/29/16%)	0/5/7 (0/42/58%)	<0.001
MELD	12 (8-16)	21 (16-24)	<0.01
CLIF-C AD score (n=49)	49 (45-58)	57 (55-60)	0.11
HE (+/-)	17/82 (17/83%)	5/7 (42/58%)	0.04
Renal dysfunction (+/-)	20/79 (20/80%)	2/10 (17/83%)	0.77
Heart rate (bpm)	79 (68-98)	87 (68-110)	0.28
MAP (mmHg)	85 (77-92)	90 (82-93)	0.32
SDNN (ms)	26 (17-38)	11 (10-12)	<0.0001

AH: alcoholic hepatitis, ALT: alanine aminotransferase, AST: aspartate aminotransferase, both: refers to AH and infection, CLIF-C AD: CLIF Consortium Acute Decompensation, CRP: C-reactive protein, HE: hepatic encephalopathy, INR: international normalized ratio, MAP: mean arterial pressure, MELD: Model for End-Stage Liver Disease, NASH: non-alcoholic steatohepatitis, SDNN: standard deviation of all NN intervals, WBC: white blood cell count.

Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.

Table 3. Patient characteristics in accordance with baseline SDNN above or below the threshold of 13.25 ms

	SDNN <13.25 ms (n=24)	**SDNN ≥13.25 ms (n=87)**	**P value**
Predisposition			
Male gender, n (%)	15 (63%)	59 (68%)	0.63
Age (years)	53 (43-62)	59 (49-67)	0.07
Aetiology:			
alcohol/viral/NASH/other (n)	15/5/2/2 (63/21/8/8%)	63/14/5/5 (72/16/6/6%)	0.82
Laboratory Parameters			
ALT (U/L)	34 (24-66)	32 (23-53)	0.50
AST (U/L)	76 (44-125)	38 (28-56)	<0.001
Bilirubin (mg/dL)	11.1 (4.3-26.3)	1.0 (0.7-1.8)	<0.0001
Albumin (g/dL)	33 (27-35)	35 (30-41)	0.03
INR	2.1 (1.4-2.4)	1.1 (1.0-1.3)	<0.0001
Creatinine (mg/dL)	1.1 (0.7-1.8)	1.0 (0.8-1.4)	0.86
Inflammatory response			
CRP (mg/L)	40 (19-59)	7 (3-16)	<0.0001
WBC ($x10^9$/L)	10.5 (5.7-16.3)	5.8 (4.4-7.8)	<0.001
IL-6 (pg/ml)	21 (14-58)	11 (7-24)	0.06
IL-8 (pg/ml)	65 (34-96)	20 (12-55)	0.02
Severity and organ injury			
Child-Pugh A/B/C (n)	1/7/16 (4/29/67%)	53/27/7 (61/31/8%)	<0.0001
MELD	25 (19-29)	11 (7-13)	<0.0001
CLIF-C AD score (n=49)	59 (55-64)	46 (42-53)	<0.0001
HE (+/-)	6/18 (25/75%)	16/71 (18/82%)	0.47
Renal dysfunction (+/-)	7/17 (29/71%)	15/72 (17/83%)	0.19
Heart rate (bpm)	87 (80-100)	72 (64-85)	0.02
MAP (mmHg)	88 (78-93)	85 (78-90)	0.37

AH: alcoholic hepatitis, ALT: alanine aminotransferase, AST: aspartate aminotransferase, both: refers to AH and infection, CLIF-C AD: CLIF Consortium Acute Decompensation, CRP: C-reactive protein, HE: hepatic encephalopathy, IL: interleukin, INR: international normalized ratio, MAP: mean arterial pressure, MELD: Model for End-Stage Liver Disease, NASH: non-alcoholic steatohepatitis, WBC: white blood cell count.

Continuous variables are presented as median (IQR) and categorical variables as numbers and percentages.

Figure legends

Fig. 1. Consort diagram.

Consort diagram showing study design, numbers of patients recruited and analysed.

Fig. 2A. SDNN in healthy subjects, stable out-patient cirrhosis and acute decompensated cirrhosis patients in hospital.

SDNN in healthy subjects (n=20), stable out-patient cirrhosis (n=62) and acute decompensated cirrhosis patients (n=49). The solid horizontal lines indicate the median values, the boxes the IQR and the error bars min-max.

As cirrhosis progresses to acute decompensation, there is a clear reduction in HRV as noted by the statistically significant reduction in SDNN.

Fig. 2B. SDNN in acute decompensated cirrhotic patients with and without ACLF.

SDNN in acute decompensated cirrhotic patients with no ACLF (n=40) and with ACLF (n=9). The solid horizontal lines indicate the median values, the boxes the IQR and the error bars min-max.

Fig. 3. Correlation between SDNN and CP score (A), MELD score (B) and CLIF-C AD score (C).

Correlation between SDNN and Child-Pugh (CP) score (rho=-0.67; P<0.0001) (A), Model for End-stage Liver Disease (MELD) score (rho=-0.66; P<0.0001) (B) and CLIF-C AD score (rho=-0.63; P<0.0001) (C). The linear regression line shows the correlation.

Fig. 4A. ROC curve analyses for predicting 3-months mortality.

Receiver operating characteristic (ROC) curve analyses of SDNN, C-reactive protein (CRP), Child-Pugh (CP) score, Model for End-stage Liver Disease (MELD) score, MELD-sodium (Na) score and white blood cell count (WBC) as predictors for 3 months mortality.

Fig. 4B. Survival curves for patients according to SDNN.

Three months survival curves for patients with SDNN ≥13.25 ms (n=87, solid line) and <13.25 ms (n=24, dashed line) (P<0.0001).