# Network approach to prognosis in cirrhosis

*by*

Tope Oyelade

[redacted]

A thesis submitted in the fulfilment of the requirements for the degree of Doctor of Philosophy (Ph.D.)

*From*

University College London (UCL)

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# Abstract

Definitive treatment following decompensation of cirrhosis is limited to liver transplantation based on prognosis. Although useful, current prognostic models do not consider the interaction between organ systems and treat them as isolated units. Approaches to improve these models through the addition of more biomarkers still does not resolve the limitations of the simplistic approach. This work tests the hypothesis that organ systems' functional connectivity is reduced in cirrhosis and may hold independent prognostic values.

Indeed, the prognostic values of physiologically integrative approaches such as heart rate variability measures are well established. However, *while integrative physiological indices such as HRV analysis have prognostic value in cirrhosis, they do not reveal details of organ systems network interactions potentially driving patient outcomes.* To assess the functional connectivity of organs, I used a network physiology approach and developed a novel method for physiological network mapping at the individual patient's level to assess the interaction between routine clinical/biochemical biomarkers. The method is known as Parenclitic network analysis and measures the deviation of a pair of patient variables from the expected relationship based on a model population (e.g., healthy, survivors, treatment responders, etc.). The results show that parenclitic network mapping can predict mortality independent of MELD (Model for End-stage Liver Disease) in two independent cohorts of patients with decompensated cirrhosis. Also, this novel method was found to predict response to targeted albumin therapy in a large, multicentre group of patients admitted to the hospital for decompensated cirrhosis. Finally, the Parenclitic network analysis predicted prognosis in paracetamol-induced acute liver failure patients independent of the sequential organ failure score (SOFA) as well as the King's College Criteria (KCC). Importantly, the parenclitic network analysis is based on routine data and significantly improved the prognostic models currently used in cirrhosis and acute liver failure.

In sum, this thesis shows that organ system decoupling is linked with survival and may predict response to therapy in patients with liver disease.

## Impact Statement

Current prognostic models in cirrhosis does not sufficiently address the multi-organ involvement of decompensation and improvement is urgently needed to capture subsets of patients incorrectly prognosticated. In this thesis, I assessed the predictive value of network physiologic measures in patients with cirrhosis.

My systematic review of Heart Rate Variability (HRV) in cirrhosis uncovered high interstudy variability which makes it hard to draw a generalizable conclusion from the myriads of studies published on the topic. Further, Heart Rate Turbulence indices, an index of autonomic nervous and baroreflex coupling with the cardiac cycle, were assessed for prognostic value, for the first time in patients with cirrhosis. Also, a novel method for physiological network mapping at individual patients' level, the parenclitic network analysis was developed and applied for the prediction of survival in cirrhosis patients in a first-of-its-kind approach showing significant prognostic values as well as the ability to predict patients likely to respond to targeted albumin therapy. Specifically, decoupling along the white cell counts and C-reactive protein axis predicted 6-month survival in patients admitted for decompensated cirrhosis independent of MELD, the current prognostic model.

Further, to assess whether network mapping can predict survival in patients with paracetamol-induced acute liver failure, I developed a code using structured query language (SQL) to extract clinical and laboratory data of this patient group from a large clinical database of intensive care unit (ICU) admissions (MIMIC-III). The parenclitic network analysis showed differences in network clusters between p-ALF patients who survived 28-day ICU stays compared with nonsurvivors linked with pH regulation. Also, the network indices predicted survival independent of the current prognostic models (i.e., SOFA and KCC scores).

Finally, I mapped the physiological coupling of pairs of parallel physiological signals by computing the transfer entropies between the variables. Specifically, transfer entropies between heart rate, respiratory rate, and oxygen saturation were computed for ICU patients with cirrhosis and analysed for prognostic value. The result showed that transfer entropy, which measures causal links between time series was not linked with 28-day ICU survival in cirrhosis.

Overall, network mapping of organ systems coupling was found in this thesis to predict survival independent of current prognostic indices. This novel method also predicted patients with decompensated cirrhosis who did not benefit from targeted albumin infusion. Importantly, the addition of the network mapping of routine clinical and laboratory variables significantly improved the performance of current prognostic model while providing pathophysiological insights into the factors that potentially drives patients' outcomes. Indeed, network analysis of patients' routine data provides a fertile platform for big data analysis and results may offer clinical insights likely to drive targeted, personalised treatment for complex disease in future. Also, this technique can be incorporated into mobile application for bedside use with the potential to guide clinical decision-making.

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## Acknowledgment

My gratitude goes to Kevin, my primary supervisor for giving me the opportunity to do this research and to Alireza who is first and foremost a friend, my secondary supervisor, and my manager. Thanks to both of you for your support, guidance, motivation, and direction and for making this work possible in the first place.

I would like also to extend my sincere appreciation to all the students and staff members of the UCL Institute for Liver and Digestive Health and UCL Division of Surgery and Interventional Science for their support, friendship, and insights and for providing an enabling environment for creativity, cutting edge research, and paving ways.

Special thanks to Steven Buckingham for introducing me to the team and the all-able Jill Norman, for your continued support, encouragement, and assistance.

I would like to also thank all the collaborators: the unseen hands that kept the wheel of this research spinning through their invaluable contribution and for providing a platform for the improvement of science and innovation.

Finally, I would like to thank every member of my family who have made priceless sacrifices, one way or the other, to support, love and tolerate me through this journey. And to my parents, you are a source of motivation.

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# Chapter 1

# Introduction

Physiological systems comprise multiple connected subsystems interacting to maintain homeostasis in an ever-changing environment. Thus, disruption in the complexity or connectivity changes the unique and collective inherent ability of the system to adapt due to the reduced complexity of various physiological variables [1]. This failure to adapt or respond appropriately can be transient and mild, or it can be devastating, for example with sepsis and multiple organ failure [2, 3]. Reduced complexity in physiological variables such as cardiac rhythm [4], blood oxygen saturation [5, 6], and skin or core body temperature [7, 8] are associated with increased mortality. Further, reduced functional connectivity between organ systems significantly and independently predicts survival in cirrhosis [9, 10] or sepsis [11, 12]. Understanding the interaction between these units (and/or subunits) may help us to understand the dynamics of complex diseases and direct early intervention to improve outcomes.

Simple models that assess organ systems in isolation remain the typical methods to estimate prognosis in many complex diseases such as decompensated cirrhosis (e.g., MELD, model for end-stage liver disease), sepsis (e.g., SOFA, sequential organ failure assessment score), and others. These models do not consider the complex interaction between the individual units. Thus, many prognostic models fail to optimize their value.

The network approach provides an alternative approach based on the complex interactions between individual organ systems within a physiological system. Network physiology identifies the dynamics of connections between individual organ systems and improves clinical evaluation and assessment of prognosis [13, 14]. Patients with decompensated cirrhosis or sepsis, are at high risk of developing multi-organ dysfunction, failure, and death. Recent advances in network physiology have paved the way for the application of network mapping to physiological data with the hope of early intervention and improved outcomes [9-12, 15].

Decompensated cirrhosis is a late stage of liver cirrhosis characterized by multiple organ dysfunction with patients developing ascites, hepatic encephalopathy, portal hypertension, kidney failure, cardiomyopathy, abnormal pulmonary function, immune dysfunction, or impairment of circadian rhythms [16-20]. The development of hepatic decompensation marks a pivotal stage in the clinical evolution of cirrhosis and is

associated with poor prognosis. The complex interaction between systems may generate unexpected outcomes with directed therapy [21-24]. For example, targeting nitric oxide to regulate the hyperdynamic circulation in patients with decompensated cirrhosis was expected to lead to clinical improvement, and yet was associated with mental status deterioration with restlessness, confusion, and disorientation [25, 26]. Likewise, the use of terlipressin, a synthetic vasopressin analogue, to improve kidney function in patients with the hepatorenal syndrome was unexpectedly observed to be associated with pulmonary oedema in some patients [27]. In a recent multinational study aimed at the targeted replacement of plasma albumin in patients hospitalized with decompensated cirrhosis and hypoalbuminemia, China et al found no significant benefit. However, a significant increase in pulmonary oedema was observed in the treatment arm [28]. This evidence, and others beyond the scope of this introduction, highlights the failure of isolated targeting of unique organ systems without much consideration for the system-wide physiological context within which such organ system operates.

In the clinical course of cirrhosis, the compensated stage may last many years as the gradual breakdown in the liver's ability to perform its pivotal roles in maintaining homeostasis is balanced by other organ systems. Untreated cirrhosis may then result in total breakdown in liver function following liver injury due to sepsis or other insults and being complicated by other secondary events such as variceal bleeding, ascites, or hepatic encephalopathy. In some, there may be a sufficient recovery of liver function following cessation of alcohol consumption in the alcoholic, treatment of hepatitis C in the patient with chronic viral hepatitis, or treatment of autoimmune liver disease by immunosuppression. However, once a patient passes the point of decompensation, the prognosis is often poor, and the only definitive treatment option is limited to liver transplantation [29, 30].

Regardless, it is important to have and develop accurate scoring systems that predict survival. This is for the prioritisation of liver donation and enhanced survival of the recipients. Recently, the MELD 3.0 was introduced as a replacement for the MELD-Sodium (MELD-Na) score which was the gold standard for short-term (90-day) prognosis in patients with decompensated cirrhosis. The MELD-Na score comprises a calculation based on serum sodium, bilirubin, and creatinine, together with the prothrombin time

(international normalized ratio, INR) [31]. While the MELD 3.0 adds serum albumin level as well as gender to the MELD-Na [32]. Advances in our understanding of decompensated liver disease implicate other factors such as age, cholesterol, hospital length of stay, white blood cell count, and albumin as determinants of patients' outcomes [33]. As research continues to unravel the physiological depth of cirrhotic decompensation, it is becoming clearer that simply considering a few surrogate biomarkers, especially as isolated independent variables may not be sufficient for accurate prediction of clinical outcomes in complex diseases including cirrhosis.

## The liver is highly central in the physiological network.

The liver has direct and indirect synthetic, metabolic, and immune functions. The synthetic function of the liver makes it an essential modulator of microcirculation (through the synthesis of albumin) and haemostasis (through the synthesis of coagulation factors). The liver plays a crucial role in glucose/energy metabolism and the hepatocytes' oscillatory clock gene expression modulates central circadian rhythms and behaviours [34]. The liver is an important systemic barrier and clears a variety of different endogenous (e.g., hormones) and exogenous compounds (e.g., xenobiotics, gut-derived bacterial lipopolysaccharide endotoxins) with implications in the pathophysiology of diseases. Aside, various recent works have established links between the liver and other organ systems, especially the enteric and nervous systems (the gut-brain axis). Indeed, the translocation of pathogen-associated molecular patterns (e.g. gut bacterial lipopolysaccharides) into the systemic circulation (due to increased gut permeability or reduced hepatic clearance) remains one of the key drivers of decompensatory events (e.g. encephalopathy) and mortality in patients with liver failure [35-37] [38, 39]. The crosstalk between the liver and the nervous system has been shown to regulate the hepatic metabolism of lipids and glucose as well as glycogen storage [40-42]. Furthermore, the contribution of gut microbiome dysbiosis to the development, prognosis, and treatment of liver disease has received significant attention in recent years. Indeed, altered intestinal microbiomes have been linked with the development of various aetiologies of liver disease [43, 44]. For instance, the development of non-alcoholic fatty

liver disease (NAFLD) and non-alcoholic steatohepatitis was linked with dysregulation in the gut microbiota [45, 46] as well as other components of the gut-brain axis [47].

While the communication between the gut and the liver is driven by anatomical connections (e.g., directly via the portal vein and the enterohepatic circulation) [48, 49], there are various complex communication pathways between the liver and brain including the neural pathways, humoral signalling, circulating cytokine and monocyte-to-brain signalling [50, 51]. For instance, the vagal neural pathways, including the afferent and efferent vagal arms can respectively communicate and trigger pathophysiological changes in the visceral organs to maintain homeostasis [52-54] or influence brain-derived motivational states and sickness behaviours [55]. Indeed, impaired metabolism and clearance of ammonium ions via disruption to the urea cycle [56] and reduced enzymatic activities of the glutamine synthase [57] due to reduced liver function or circulatory bypass via portosystemic shunting are hallmarks of decompensated cirrhosis [58]. The resulting hyperammonaemia combined with systemic accumulation of the gut-derived false neurotransmitters (e.g., octopamine) [59] and endotoxins [60] plays a role in impaired neural function observed in patients with cirrhosis.

Further, reduced clearance of gut-derived bacterial lipopolysaccharides seen in liver failure leads to a systemic increase in circulating cytokines (IL-1β, IL-6, and TNFα) and may induce peripheral as well as cerebral endothelial cells to produce nitric oxide and prostaglandins. The production of these mediators is linked with the activation of sickness behaviours as well as cognitive and behavioural changes [61, 62]. Circulating cytokines may penetrate via the circumventricular organs of the brain thereby influencing nervous activities during systemic inflammation linked with liver disease [63, 64]. For instance, in a model of liver disease, circulating monocytes were observed to alter the excitability of neurons and initiate sickness behaviours [65]. Evidence also shows that dysregulation in the gut-brain axis is significantly linked with increased alcohol cravings and decreased social cognition [66] (*Figure 1.1*).

This connected role of the liver makes is a good model for network physiology and continued research along this line is likely to open new frontiers of understanding regarding the critical communication axes or nodes that could be best pharmacologically

or non-pharmacologically (e.g., through nerve stimulation, fractal-like ventilation [67]) targeted to improve the prognosis of patients with decompensated cirrhosis.

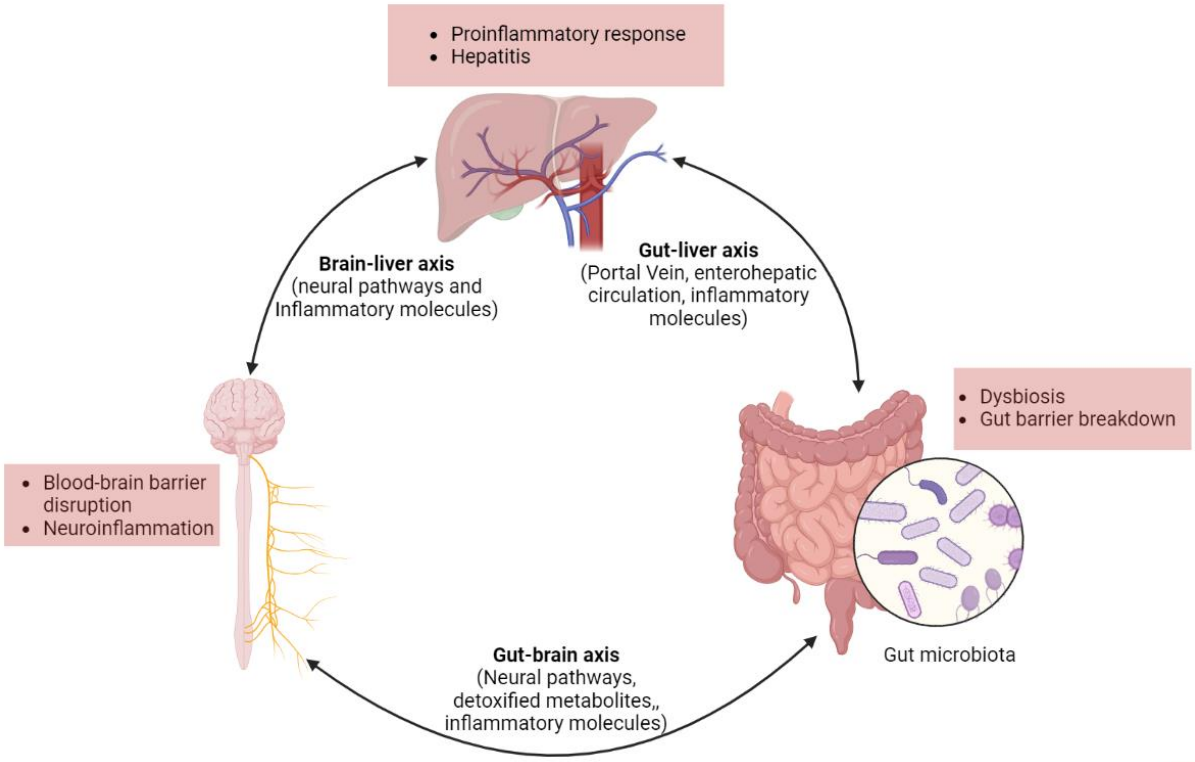


***Figure 1.1.*** *The gut-liver-brain axis is connected via diverse pathways and regulates the activities of each other. Image created using Biorender.*

## Cirrhotic decompensation – a network problem.

With approximately 2 million global annual deaths, liver disease remains one of the major diseases of high epidemiological significance [68]. This number highlights the complexity of decompensated cirrhosis and the implication of this on clinical management. Indeed, this complexity also makes decompensated cirrhosis a prime candidate for network analysis especially due to its extra-hepatic involvements.

The trigger for decompensation of cirrhosis has been explained by various classical hypotheses including the "vascular underfilling" and "overflow" theories [69], the "peripheral arterial vasodilation" hypothesis (PAVH) [70], and the "systemic inflammation" hypothesis (SIH) [71]. Irrespective of this, the decompensation event remains a pivotal stage in the clinical history of cirrhosis and is associated with high mortality. While patients with compensated cirrhosis may survive for over 12 years, decompensation results in a significant reduction in survival time to approximately 2 years [21]. Indeed, the risk of mortality is significantly linked with the number of organ-systems involved in cirrhotic decompensation [72].

Accordingly, decompensation is driven by a clinically significant increase in portal pressure and a decline in liver function [21, 73]. This combined breakdown often results in jaundice, gastrointestinal bleeding, encephalopathy, and ascites, which are clinical hallmarks of the decompensatory phase of cirrhosis. These events may also herald other extrahepatic complications such as autonomic dysfunction, hepatorenal syndrome, hepato-pulmonary syndrome, cirrhotic cardiomyopathy, and rebleeding resulting finally in multiorgan failure [74-78]. Curiously, most of the extrahepatic complications of decompensated cirrhosis are not associated with significant structural damage to the involved organ and have been reported to be reversible by liver transplantation [79-82]. These findings further support the need to focus more on organ systems interaction rather than the individual units.

Decompensation is a critical stage of cirrhosis characterized by a cascading breakdown of multiple extra-hepatic organ systems [83] (*Figure 1.2*). This often culminates in multiple organ failure and a significant increase in mortality risk [83-85]. Indeed, recent studies have shown that decompensated cirrhosis is associated with network disruption.

Specifically, loss of coordination between organ systems was found by Tan et al to predict the survival of patients with decompensated cirrhosis independent of MELD [10, 15]. The exact mechanism for physiological network disruption in decompensated cirrhosis is not well understood. However, evidence from experimental (*in vitro, ex vivo,* and *in vivo*) studies indicate impaired end-organ responsiveness to physiologic signals in animal models of decompensated cirrhosis [17, 86-88]. For example, cardiomyocytes, cardiac pacemaker cells, and vascular smooth muscle cells exhibit a blunted response to adrenergic and cholinergic stimuli in experimental decompensated cirrhosis [88-91]. Other reports support disruption of the physiological control in decompensated cirrhosis by demonstrating reduced controllability of the physiological sub-systems in decompensated cirrhosis using advanced statistical techniques used in analysis of complex systems [92, 93]. For example, Shirazi et al., used a computational approach and showed that cardiac rhythm in patients with decompensated cirrhosis keeps a significantly longer memory about its past decelerating events compared to those with compensated cirrhosis and healthy volunteers [92]. Intuitively, it is more difficult to control a system that holds a long memory of its past and thus increased memory can make the physiological network less adaptable to both environmental and intrinsic changes [94]. To confirm this finding, Satti et al., used an alternative method (the extended Poincare plot analysis of physiological signals) and showed a significantly longer auto-correlation and memory in patients with decompensated cirrhosis in comparison with compensated cirrhosis and healthy individuals [4]. These findings are in line with impaired physiological control and network disruption in decompensated cirrhosis. However further investigations are required to determine the mechanism behind the transition from compensated to decompensated cirrhosis in terms of organ systems disconnection.

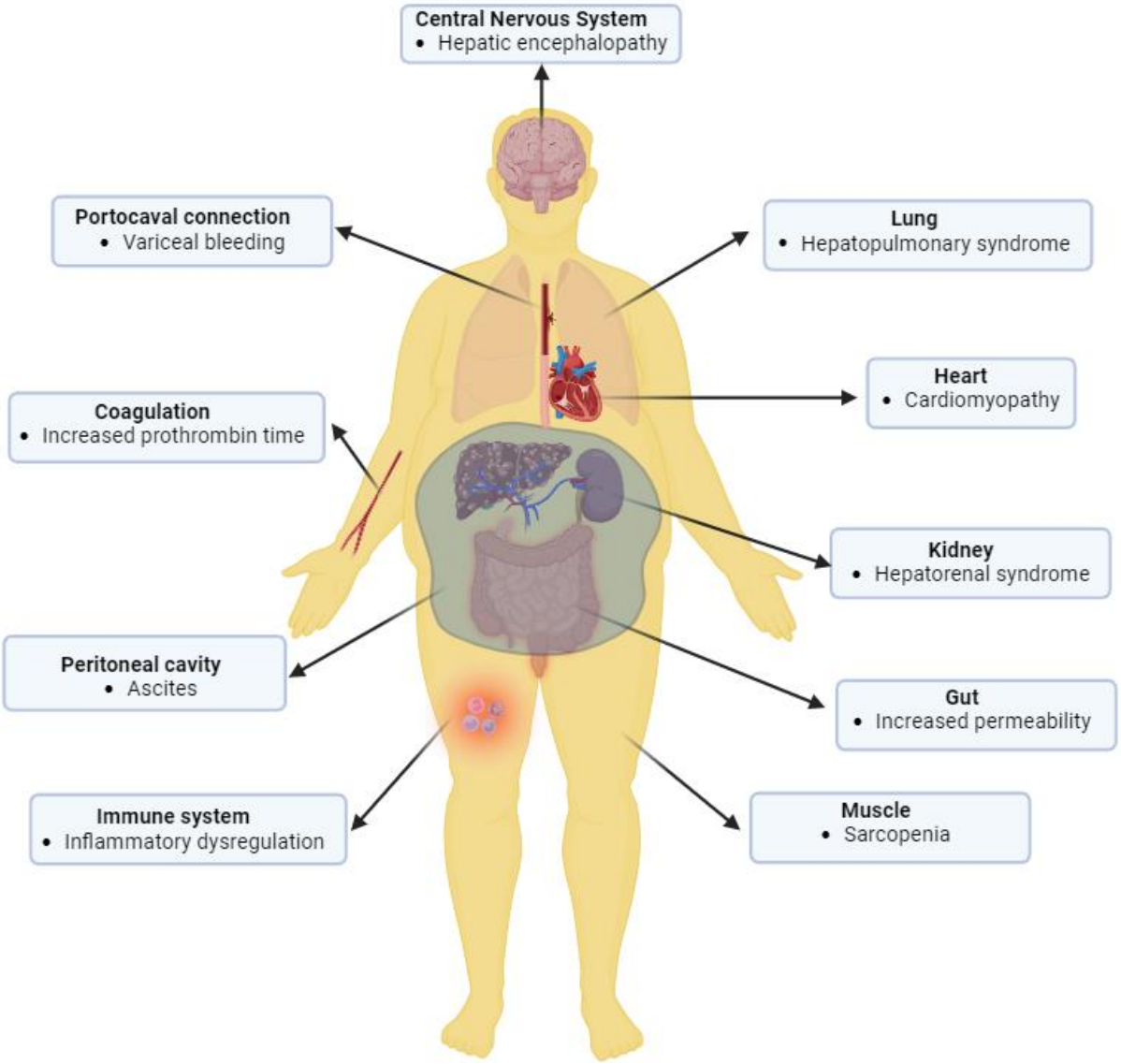


***Figure 1.2.*** *The decompensation stage of cirrhosis is heralded by extrahepatic organ involvement affecting various organ systems including the circulatory system (cirrhotic cardiomyopathy) the nervous system (hepatic encephalopathy and dysregulated autonomic cardiac regulation), the kidney (hepatorenal syndrome), the respiratory system (hepatopulmonary syndrome), digestive system (intestinal injury, and increased permeability of the intestinal wall), blood coagulation and immune system. Also, fluid build-up in the peritoneal cavity (ascites) may result from increased portal tension. Image created using Biorender.*

## Trends in prognostic model in cirrhosis

The trends in prognostic models for liver disease in the past decades provide a basis for a shift in paradigm towards network physiologic and holistic approaches. Once decompensation occurs, liver transplantation remains the definitive treatment option. However, due to scarcity, patients are prioritized based on the severity of liver disease and prediction of survival using prognostic models. The very first model used was the Child- Turcotte-Pugh (CTP) score, developed in 1964 primarily to predict survival in patients undergoing TIPS (Trans-jugular Intrahepatic Portosystemic Shunt) for variceal haemorrhage [95, 96]. Initially, CTP is based on five physiological variables i.e., ascites, hepatic encephalopathy, bilirubin, serum albumin, and nutritional status. A modified version was proposed in 1973 for risk stratification in patients undergoing surgical transection for variceal bleeding, whereby the included parameter "nutritional status" was replaced by "prothrombin time" or "international normalized ratio" [97]. Over the years and until recently, the CTP has been used for the prioritization of patients with decompensated cirrhosis for liver transplantation as well as for the prediction of surgery outcomes [98]. However, both the CTP and its modified form were never statistically validated clinically until its later replacement.

The CTP score was dropped because of the subjectivity in the classification of some of the included markers such as ascites and hepatic encephalopathy as well as a limitation regarding the "ceiling effect" since patients could not be classed above Child C even if they have worse severity and prognosis [99]. Also, the CTP score does not include renal function, a crucial aspect of cirrhotic decompensation [100]. To overcome these limitations, the MELD (Model for End-stage Liver Disease) score was proposed as the reference tool for prognostication in patients with liver disease and includes patients' INR (international normalized ratio), total bilirubin and serum creatinine levels [101]. The first version of the MELD was proposed in the year 2000 as a tool for predicting 3-month survival in 231 patients following TIPS placement [102]. Originally, this model included the serum level of bilirubin, creatinine, international normalised ratio (INR), and the etiology of cirrhosis (e.g., viral/others = 1; alcohol/cholestatic = 0) combined using a Cox proportional hazard regression analysis. The MELD was compared with the CTP and found to be superior in the classification of patients with cirrhosis as well as predicting survival in patients with renal dysfunction who had been classified as CTP class B [102, 103].

The MELD was later updated for use in the severity scoring of liver disease and to estimate the risk of mortality in patients on the liver transplantation waiting list [104]. Compared with the CTP score, the MELD was validated in various cirrhosis populations with different aetiology using the c-statistic, a statistical prognostic tool based on the area under the Receiver Operator Characteristic (ROC) curve and established to be superior to the CTP for organ allocation [105, 106]. The superiority of the MELD to the CTP was later confirmed by other authors and was described as a movement toward evidence-based medicine from a highly subjective, sometimes exaggerated scoring system (i.e., the CTP) [107-111].

However, limitations regarding inter-laboratory variability in the measurement of creatinine level and INR as well as gender bias resulted in the proposal of modified MELD scores [112]. Also, the MELD score was later reported to be limited in predictive value, especially in patients with relatively low scores. For instance, compared with other decompensatory events such as hepatic encephalopathy, hyponatraemia (low serum sodium), glomerular filtration rate, as well as the CTP score, the MELD score was reported to perform statistically less in terms of predicting mortality in patients with end-stage liver disease on the transplant waiting list [113], where hyponatraemia and presence of ascites [114-116] were reported to be more effective. Further, when compared with MELD alone, the addition of serum sodium level was associated with a 7% [115] reduction in waiting list mortality in patients with end-stage liver disease. Thus, in 2006 Biggins et al proposed a replacement of the MELD with the MELD-Na score after reporting an improved prognostic accuracy in the latter [117]. Recently, Kim et al introduced the MELD 3.0 in 2021 as the gold standard for short term prognosis in decompensated cirrhosis. Specifically, the MELD 3.0 adds gender, serum albumin level to the MELD-Na as well as statistical interactions between albumin-creatinine and bilirubin-sodium with a reported increase (~9%) in net reclassification and better discrimination especially reducing the gender disparity associated with MELD-Na [32].

Importantly, modifications of the MELD score have been in the inclusion of more biomarkers shown to have independent prognostic values in the patient population and as shown in MELD 3.0, the realization that biomarkers do interact to drive disease outcomes. For instance, the United Kingdom Model for End-Stage Liver Disease (UKELD)

score incorporates serum sodium into the MELD score with improved predictive accuracy [118]. Further, Montagnese et al showed a significant improvement in the prognosis value of MELD following the addition of the mean dominant frequency of the patient's electroencephalogram (EEG) and proposed the MELD-EEG model as a better alternative to MELD alone for predicting survival in patients with cirrhosis [119]. Recently, the MELD-Plus score was proposed as a better prognostic model and adds albumin, sodium, white cell count (WCC), total cholesterol, age, and length of hospital stay to the traditional MELD variables [33]. However, even the MELD-plus does not incorporate all possible extra-hepatic decompensation events observed in critically ill patients with cirrhosis who develop acute-on-chronic liver failure (ACLF). ACLF is a syndrome associated with a significantly poor short-term prognosis and is clinically characterised by multiple extra-hepatic organ failures in patients with acute decompensation of cirrhosis [38]. Consequently, the European Foundation for the study of chronic liver failure (CLIF) developed the CLIF organ failure (CLIF-OF) score [120], a derivative of the sequential organ failure assessment (SOFA) score [121] to capture the poorer prognosis due to the sequential breakdown in organ systems function characteristic of late stage decompensated cirrhosis [122]. Recently, the CLIF-C (Chronic Liver Failure Consortium) MET prognostic model was also developed and positively validated by the CLIF group incorporating biomarkers from metabolomics analysis associated with systemic inflammation, mitochondrial dysfunction, and sympathetic nervous activation [123].

The trends in prognostic models for patients with decompensated cirrhosis have followed the continued inclusion of more organ systems (through representative markers) and proportionately reflect the increasing acceptance of the multi-organ, extra-hepatic implications of decompensated cirrhosis. However, current prognostic scores/models still consider the organ systems as separate, independent units instead of coordinated functioning parts constantly communicating as a network to maintain homeostasis.

## Omics, and system biology in liver cirrhosis

As more research continues to clarify the complex pathophysiology of decompensated cirrhosis, attention is being gradually diverted toward a holistic view of prognosis. In recent years, scientists have successfully employed machine learning and artificial intelligence approaches for prognosis in liver disease [124-127]. Various omics analyses have been performed in cirrhosis resulting in new insights into the pathophysiology of cirrhosis as well as biomarkers of significant prognostic values. For instance, blood metabolomics of patients with decompensated and compensated cirrhosis revealed that mitochondrial dysfunction via systemic inflammation may drive organ failure in chronic liver disease [128]. Also, Claria et al performed an untargeted lipidomic analysis of serum from patients with acute decompensation of cirrhosis with and without ACLF and reported novel lipid fingerprints associated with liver dysfunction and infection [129]. In a metabolomic analysis of urine and serum samples from 211 participants, Bajaj et al reported alteration in the bioenergetics, lipid, and protein metabolism in outpatients with cirrhosis [130]. The use of proteomics in alcohol-related liver disease and viral hepatitis has also shown promising pathogenetic insights as well as prognostic values in various other studies [131-133]. Put together, as the pathophysiology of decompensated cirrhosis unravels, the extra-hepatic involvement of the disease is beginning to become more appreciated. As this happens, researchers and clinicians need a broader more holistic outlook that could help make sense of the complex shift in physiological dynamics and coupling that drives prognosis and treatment response.

## Physiological signals variability in liver diseases

Classical physiological interpretation relies on the Cannon principle that human physiology is maintained in a "fairly constant or steady state" [134]. This principle, however, has been debunked due to the continuing discovery that organ systems vary and interact in complex and nonlinear ways across time to achieve and maintain homeostasis in direct response to an ever-changing environment (stressors) or even in the absence of any environmental challenges for example during the wake-sleep cycle [1, 135]. Therefore, higher variability and complexity of the time series of physiological variables have been interpreted as an outcome of increased organ systems engagement (coupling) with the appearance of regularity linked with isolation of a system and reduction in adaptability and survival [136]. Thus, various measures including short and long-term indexes of heart rate variability (HRV), represent the complex interplay between various spatiotemporal signals from nervous, respiratory, and circulatory systems as well as the regulators of circadian oscillation of core body temperature, among others [137].

HRV can be computed from an electrocardiogram and measures the variation in the time intervals between consecutive heartbeats (R-R interval duration, RRI) [138, 139]. Higher HRV is linked with health and is interpreted as a higher influence of various organ systems on the heart rhythm. For instance, the short-term term-variation of heart rate indexed by the high-frequency distribution of power in an ECG recording may be attributed to the coupling of respiration (vagally controlled respiratory sinus arrhythmia) via the autonomic nervous systems with the cardiac cycle [140, 141]. Further, the long-term (24-hour) influence on HRV is linked with the cardiac coupling with the baroreflex loop, renin-angiotensin pathway, core body temperature, and circadian rhythms [137]. Indeed, recent works have shown a reduction in HRV indices in patients with cirrhosis which is significantly linked with survival [78, 142]. Interestingly, long-term nonlinear indices of HRV (i.e., standard deviation parallel to Poincare's line of identity, SD2) which is strongly influenced by baroreflex loop and thermoregulation significantly predicted survival in a study by Bhogal et al [143].

Mechanistically, the HRV reduction observed in cirrhosis has been linked with systemic inflammation [90]. Although decompensated cirrhosis is linked with cardiomyopathy, it is unclear whether this is associated with HRV change [144]. Alternatively, HRV reduction

in cirrhosis has been strongly linked with hepatic encephalopathy and was reported by Mani et al to correlate with systemic levels of inflammatory biomarkers such as interleukins 6 (IL-6). Thus, inflammation remains the main driver of HRV reduction in cirrhosis possibly because of the associated organ system network uncoupling (See [90, 144, 145]; *Figure 1.3*).

While HRV provides a simple, non-invasive measure of cardiac connectivity to other organ systems, it is limited by the availability of clean ECG data with a high signal-to-noise ratio. This may be impossible in cirrhosis patients with abnormal heart cycles (e.g. due to premature ventricular beats) or those that are active. In such patients, heart rate turbulence (HRT), which indexes the autonomic and baroreflex regulation of heart rhythm following a premature ventricular contraction [146, 147], is a viable alternative. Indeed, the prognostic value of heart rate turbulence was recently investigated. Specifically, the turbulence onset was found to predict 12-month survival in patients with cirrhosis independent of MELD and CTP scores [148].

In addition, variation in the body (skin or core) temperature is a complex process regulated by the nervous system (the hypothalamic thermoregulatory centre based on stimuli from thermoreceptors) in response to the circadian rhythm and the environment. Body temperature variability is driven by the interplay between hormonal, autonomic, and metabolic systems as well as systemic inflammatory response [149, 150]. Thus, body temperature variability may reflect the influence of disease state on the connectivity of the various systems involved in the thermoregulatory pathway [151]. Indeed, body temperature analysis have been assessed in both patients with cirrhosis and animal models [4, 151, 152]. For instance, short-term variability but not the absolute values of skin temperature in patients admitted with cirrhosis predicted 12-month survival independent of MELD and CTP scores [7, 144].

Put together, time series of physiological variables such as heart rate and skin temperature vary nonlinearly in response to various cues from various sources (via feedback loops) and remain reliable indices of the complex interplay between various organ systems coupling (or lack of).

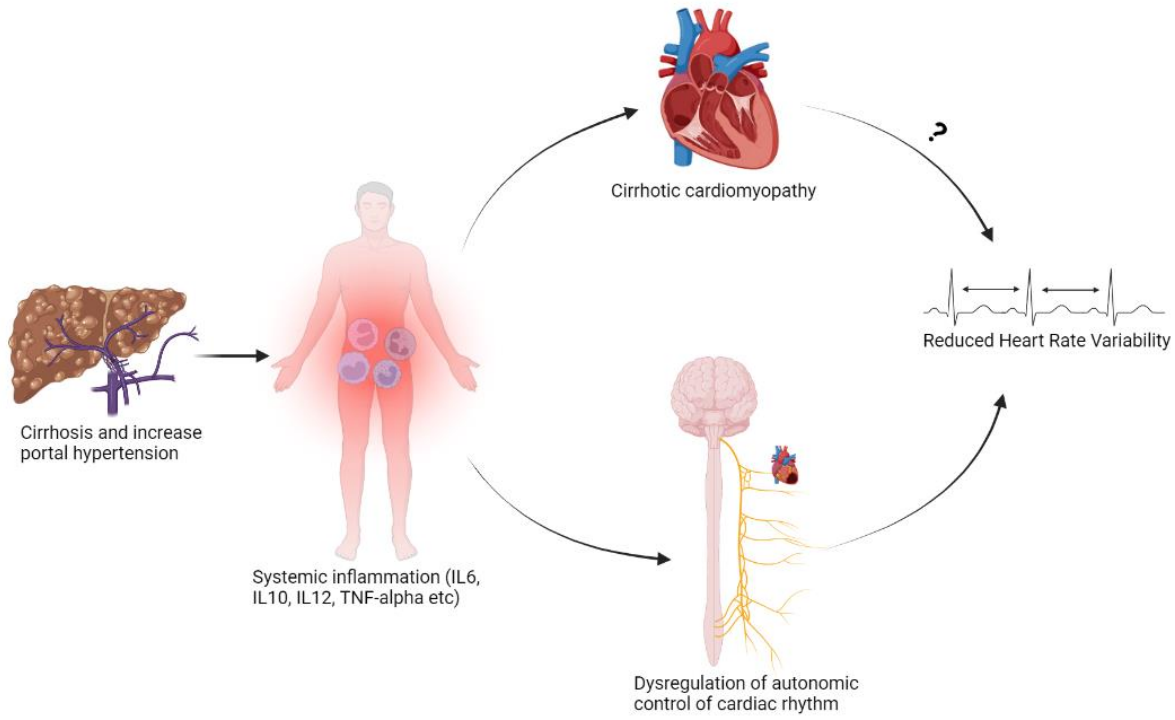


***Figure 1.3.*** *Pathogenesis of reduced heart rate variability (HRV) in cirrhosis is linked with systemic inflammation resulting in disruption to the autonomic (vagal) nervous control of cardiac rhythm. Image created using Biorender.*

## Impaired physiological network in other systemic diseases

The involvement of multiple organ systems in decompensated cirrhosis makes the disease a prime candidate for a network physiologic approach. However, this approach has been assessed in other disease types including sepsis. Sepsis is often an important cause of deterioration and mortality in patients with cirrhosis. Assessment of organ systems connectedness has been applied in patients with sepsis as well as critically ill patients admitted to the ICU. For instance, to validate a previous study that showed comparable results [153], Norris et al assessed HRV and cardiac isolation in over 2000 patients admitted to the ICU and reported that reduced HRV and cardiac uncoupling is a strong predictor of all-cause mortality. Indeed, cardiac isolation was also reported to be linked with systemic inflammation and multiple organ failure in these patients [154]. Various HRV indices were used in patients admitted to the ICU specifically for sepsis and have been reported to predict septic shock [155-157] as well as mortality [158-160]. In a systematic review by de Castilho et al., HRV was reported to be reduced in sepsis and predictive of mortality [161].

Further, variability (entropy) in oxygen saturation ($SpO_2$) was recently assessed by Gheorghita et al in critically ill patients with sepsis showing that $SpO_2$ entropy can predict mortality independent of Age, SOFA score, and mean $SpO_2$ [5]. This work corroborated a previous work by Bhogal et al, which showed that variability in oxygen saturation carries information about organ systems uncoupling that drives aging [162]. Other authors have assessed variability in core body and skin surface temperature in patients with sepsis. Indeed, wavelets and multiscale entropy analysis of body surface temperature were reported to discriminate patients with systemic inflammatory response syndrome (SIRS), sepsis, and septic shock [163].

Asada et al also performed a correlation network analysis of clinical variables representing various organ systems to assess their connectivity and show that lack of correlation of the cardiovascular system with hepatic and coagulation systems is linked with significantly poorer survival in critically ill patients admitted to intensive care unit (ICU) [12]. In another study involving 570 ICU patients, the stability of organ systems network clusters was analysed based on principle component analysis and showed a high organ

system disconnection and systemic instability in critically ill patients that did not survive ICU stay [11].

In general, assessment of organ system uncoupling via variability measures of physiological variables or network mapping provides valuable insights regarding the course of complex diseases with multiple organ involvement. Of note, these insights are usually not available using traditional statistical methods or machine learning and artificial intelligence. Importantly, Before the start of this research (2019), a preliminary database (Medline) search was performed to assess whether the overarching aim of this thesis (application of organ systems network connectivity for prognosis in cirrhosis) has been previously addressed. The resulting studies (specifically 16 studies) were not related to the aim of this study (see Appendix 1 for the search strategy used).

### *Hypothesis*

In this thesis, I investigated the hypothesis that the disruption in the network connectivity of the organ system drives the clinical course and prognosis of cirrhosis. Thus, the application of network physiology analysis may improve the prognostic value of current severity scoring systems in decompensated cirrhosis.

### *Aims of research*

1. To systematically appraise the literature on changes in heart rate variability (HRV) due to cirrhosis and whether HRV can predict survival in cirrhosis.
2. To evaluate the prognostic value of heart rate turbulence in patients with cirrhosis.
3. To develop a method for physiological network mapping in patients with cirrhosis based on routine clinical/biochemical biomarkers.
4. To assess the prognostic value of physiological network mapping for the prediction of survival in cirrhosis.
5. To assess the value of physiological network mapping in prediction of response to targeted albumin therapy in cirrhosis.

6. To assess that application of physiological network mapping in critically ill patients with paracetamol-induced acute liver failure.

7. To assess if a dynamic causal network approach can improve current prognostic models in patients with cirrhosis admitted to the ICU.

A Schematic outline of thesis chapters and research questions is presented in Figure 1.4.

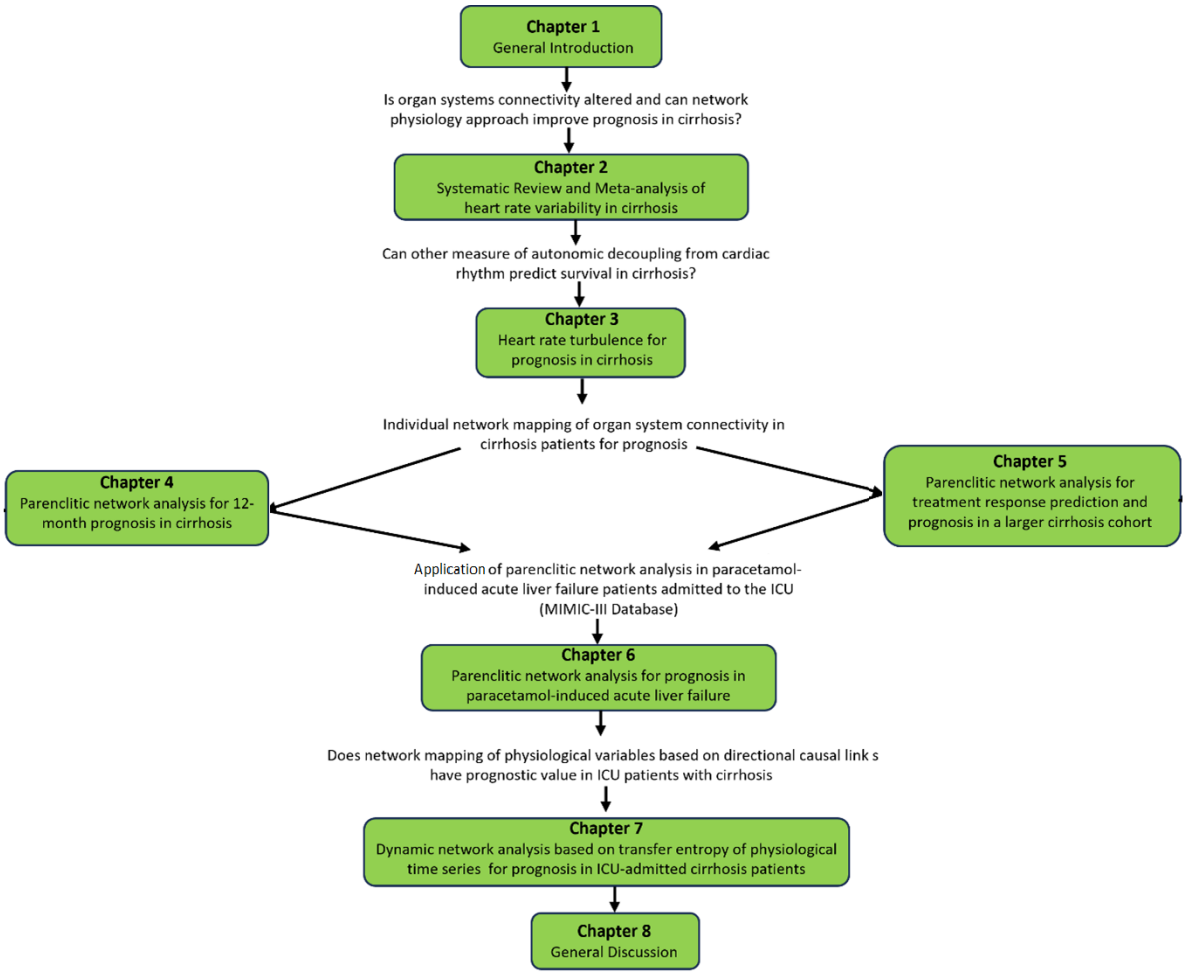


*Figure 1.4. Schematic outline of thesis chapters and research questions. ICU; Intensive Care Unit, MIMIC-III; Medical Information Mart for Intensive Care 3.*

# Chapter 2 : Heart rate variability in patients with cirrhosis: a systematic review and meta-analysis

## Introduction

Liver cirrhosis accounts for more than one million deaths annually worldwide, with numbers increasing year on year [164-166]. However, patients with cirrhosis have a range of conditions from early uncomplicated cirrhosis which is asymptomatic, to decompensated cirrhosis where organ systems start to fail and patients present with many complications such as ascites, hepatic encephalopathy, and variceal bleeding [167, 168]. Once a patient starts to develop complications, various scoring systems including MELD, MELD-Na, or UKELD are used to calculate the prognosis and the need for a liver transplant at the bedside or in the clinic. These scoring systems are widely available using Apps on smartphones or web-based calculators. However, the scoring systems do not consider the alteration in the autonomic nervous system (ANS) observed in cirrhosis [169].

A simple and especially useful tool to assess the state of the ANS is heart rate variability (HRV). HRV is the variation over time of the intervals between consecutive normal heartbeats (NN). Physiologically, instantaneous heart rate variation represents the capacity to adapt the heart rate (HR) to different internal and environmental circumstances and is modulated by the ANS [170]. Tsuji et al. for the first time demonstrated the prognostic value of HRV analysis in the Framingham cohort study and reported that individuals with reduced HRV had increased risk for all-cause mortality [171]. HRV provides a non-invasive evaluation of autonomic regulation of the cardiac rhythm and indexes the interplay between the intrinsic cardiac rhythm and external regulatory controls [172]. Importantly, with medical advances, it is becoming increasingly recognized that the calculation of HRV from continuous ECG tracing provides additional and clinically useful information to clinicians on both the severity and prognosis of patients with cirrhosis [173].

Although autonomic dysfunction and cirrhotic cardiomyopathy are well-established complications of cirrhosis [174, 175], these are not assessed by MELD, UKELD, or Child-Pugh. This is particularly important since recent reports suggest that HRV predicts survival in cirrhosis independently of MELD and Child-Pugh and therefore may provide additive information currently lacking in existing scoring systems [7, 90, 176]. However, for HRV to be of value in research and clinical practice, there needs to be standardization

of processes that enable simple and accurate assessment of HRV. This includes standardization of the ECG recording techniques, duration of recording, methods, and clinical interpretation of HRV and the availability of these at the point of care via mobile or web-based apps.

### *Aim of study*

to analyse the methods used to record and report HRV in the literature, assess HRV differences between patients with cirrhosis and controls, and finally propose the standardization of HRV measurement techniques to improve interpretation and clinical and research applicability.

### *Hypothesis*

HRV indices are different in patients with cirrhosis compared with healthy controls and the reduction is linked with survival.

## Methods

This systematic review was performed following the guidelines of the Preferred Reporting in Systematic Reviews and Meta-Analysis (PRISMA) [177]. Embase, Medline, and PubMed databases were searched on the 8th of July 2020 using wide-ranging search strategies combining several Medical Subject Headings (MeSH) terms (*Appendix 2*). Studies recovered from the search were uploaded to Endnote and duplicate studies were identified and removed. Studies were then uploaded to Rayyan software for screening based on the studies' title/abstract. Furthermore, a mini systematic review was performed to assess the prognosis value of HRV indices in patients with cirrhosis. This was carried out by screening the included papers for the performance of survival analysis.

### *Inclusion and exclusion criteria*

Only observational studies were included in the systematic review. Studies that assessed any of the HRV time, frequency, and non-linear indices in assessing autonomic cardiac control in cirrhosis were considered eligible. Studies that did not include a control group; studies involving non-cirrhotic liver disease; studies involving pharmacological or non-pharmacological interventions known to affect HRV indices; and studies involving orthostatic tilting as the sole method of HRV assessment were all excluded from this review. Further, studies where HRV indices of surviving and non-surviving patients were statistically compared irrespective of whether risk analysis was performed were included in the mini-systematic review.

### *Data collection*

Initially, titles and abstracts were thoroughly screened for potentially eligible studies. Articles found to be eligible were then evaluated to detect which studies meet the inclusion criteria for this review. Based on the criteria mentioned above, the following data were obtained: the name of the first author and year of publication, the aims of studies; the summaries of findings; sample and group sizes; study setting and country; aetiologies of Liver disease/cirrhosis; time length/duration of and the equipment used for recording; methods of HRV analysis such as whether data cleaning were performed, analysed time length (5, 10 minutes or hours), software used for HRV analysis and HRV indices assessed. The aetiology of cirrhosis including alcohol, fatty, primary biliary cholangitis, viral, and cryptogenic were also extracted (*Table 2.1* and *Table 2.2*). For the mini-

systematic review for prognosis, studies that used HRV indices for survival analysis in cirrhosis were included. Accordingly, sample size, follow-up time, mortality, HRV indices analysed, HRV indices that predict mortality independently of MELD and Child-Pugh scores, HRV indices of survivors and non-survivors, and hazard or odds ratios were extracted from eligible studies (*Table 2.3*).

### *Quality assessment*

The quality of the methods used in the included studies was rigorously assessed using a modified version of the Newcastle Ottawa Scale (NOS) [178]. The modified NOS scale had 3 major domains that evaluated the quality of patients' selection, comparability of patients' groups, and methods used for evaluating the outcomes in the included studies. These 3 domains comprised 6 assessment questions each of which were scored with a star if satisfied. Studies with scores ≤ average of total (≤ 3 stars) were considered highly biased studies (*Appendix 3*).

### *Data synthesis*

A meta-analysis of the extracted data was performed to evaluate the difference in indices of HRV between patients with cirrhosis and healthy controls. Forest plots of effect sizes of HRV indices between the groups were generated using the "m*etan*" procedure in Stata/SE15. For studies where HRV indices were reported as median and interquartile ranges, mathematical transformation to mean and standard deviation was performed according to [179, 180]. Further, HRV indices presented as natural logarithms in studies were transformed accordingly by finding mathematical exponents ($e^x$) of such. Finally, where day and night indices were reported in studies, the day's data were computed as day recording is more amenable in research settings.

The effect sizes were measured as standardized mean difference (SMD) of each of the reported indices using the Hedges' criteria [181]. The random-effect or fixed-effect model was used according to the computed between-studies heterogeneities. Effect sizes were computed as SMD with 95% confidence intervals (CI) for all HRV indices between the groups (i.e., patients and healthy controls). Forest plots were generated for visualization of the effect sizes and included the weights and between-studies heterogeneity. The

heterogeneity was computed as $I^2$ statistic and is interpreted as low, moderate, or high when values are ≤25%, 26% - 74%, or 75%, respectively [182]. The SMDs (Standardized Mean Differences) were translated according to the suggestions of Hopkins et al whereby values of 4.0 show extremely large effect sizes [183].

For the survival analysis, a meta-analysis was not performed due to the low quantity of studies included. Also, some of the studies where survival analysis was reported came from the same groups with overlapping data. Further, HRV indices computed as well as patient follow-up times in most of these studies differ significantly, making mathematical pooling infeasible.

## Results

A total of 247 studies were generated from the search of databases including 67 duplicate reports. The 180 studies remaining contained 30 studies that were regarded as hypothetically pertinent based on the inclusion criteria. Screening of the full texts resulted in the further exclusion of 16 papers to yield eligible 14 studies (*Figure 2.1*). For the mini systematic review, 7 studies performed survival analysis based on the HRV indices of patients with cirrhosis that survived and did not survive over a specific follow-up period.

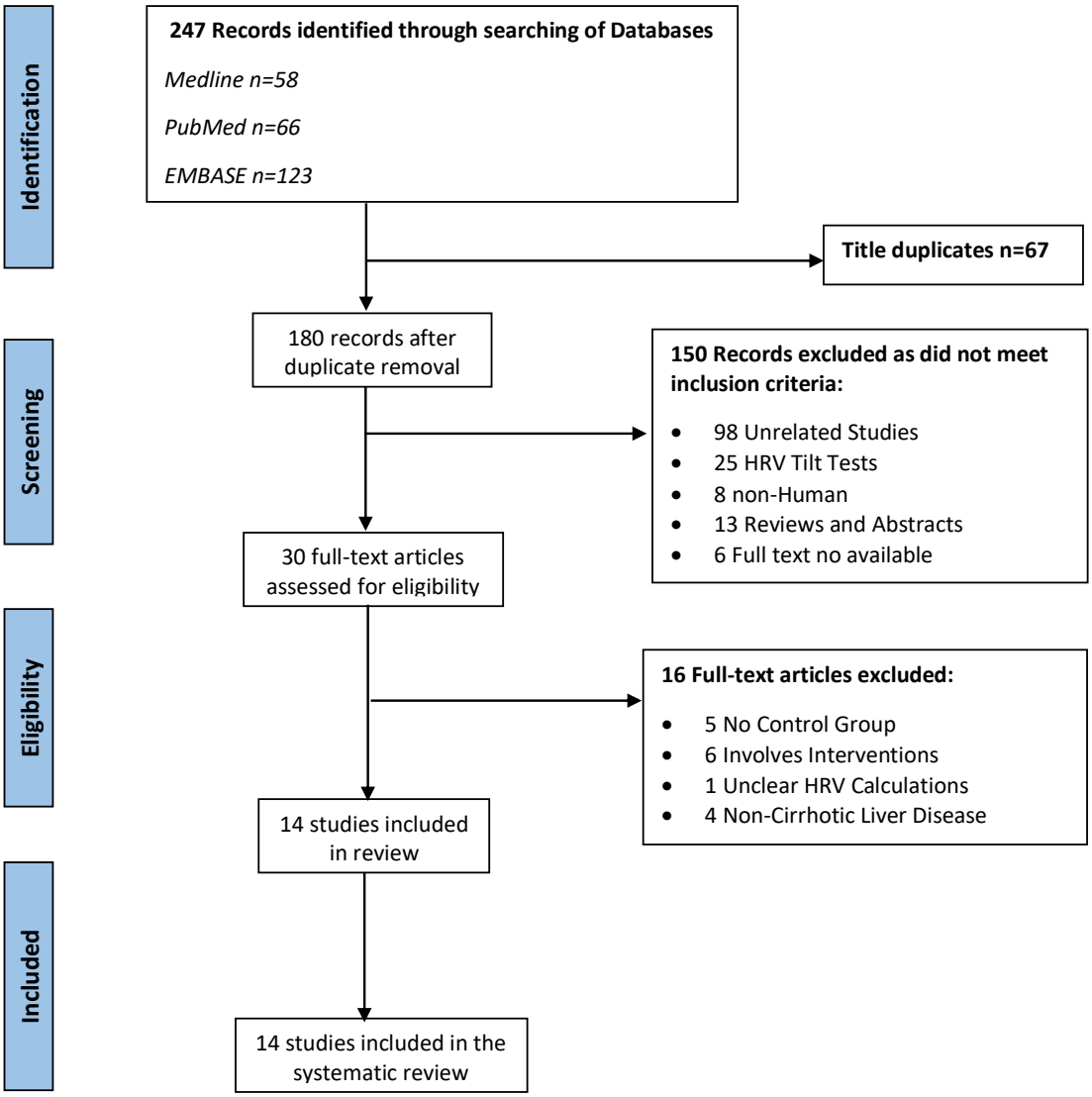


*Figure 2.1. Heart rate variability in patients with cirrhosis, systematic review according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses diagram.*

## Description of eligible studies

The general characteristics of included studies and the techniques used for ECG recording and HRV analysis and significantly different indices are presented respectively in *Table 2.1* and *Table 2.2*. The 14 eligible studies comprise a total of 583 cirrhosis patients and 349 healthy, matched controls. Overall, sample sizes ranged between 20 and 180 patients with all studies designed as observational, prospective, and conducted across 10 different countries. The NOS scale for the risk of bias assessment showed that most studies have low risk, with none scoring ≤ 3 (*Appendix 3*). Overall, most studies

reported a reduction in HRV indices except LF–HF ratio (LF: HF) which was reported to be increased in two studies [168, 184] while one study [174] reported a decrease in cirrhosis compared with healthy controls. The definitions and units of the indices of HRV are presented in *Appendix 4*.

Seven studies were included in the mini-systematic review involving 437 patients in total, of which, 104 (24%) did not survive at the end of the corresponding follow-up period (3–24 months). All (7) studies included observed significant differences in HRV indices between the survivors and non-survivors which were predictive of survival (*Table 2.3*).

*Table 2.1. General characteristics of included studies.*

| Author | Aim | Conclusion | Country | Setting | Liver Disease (Male) | Control (male) | Aetiology | Child-Pugh /Histological Classification |
|---|---|---|---|---|---|---|---|---|
| Ates F. et al. 2006 [185] | To assess using HRV autonomic dysfunction and its correlation with severity and 2-year survival in cirrhotic patients. | (1) HRV time-domain indices were significantly reduced in cirrhotic patients compared to healthy subjects. (2) HRV indices were also significantly reduced in nonsurvivors vs survivors after 2 years of follow-up. | Türkiye | NS | 30(19) | 28(16) | HBV = 22<br>HCV = 8 | A = 5<br>B = 11<br>C = 14 |
| Baratta L. et al. 2010 [186] | To assess the effect on liver transplantation (LT)) on autonomic control of cardiac function in cirrhotic patients using HRV. | (1) HRV indices (SDNN and RMSSD) were reduced in cirrhotic patients compared with healthy subjects. (2) LT corrected the reduced SDNN, but RMSSD and LF/HF remained unchanged. | Italy | outpatient | 30(20) | 27(14) | HBV = 4<br>HCV = 14<br>Other (NASH, Ethanol, Mixed) = 12 | A = 5<br>B = 18<br>C = 7 |
| Coelho, L. et al. 2001 [187] | To evaluate autonomic function in patients with liver cirrhosis using HRV and to evaluate the relationship with severity | (1) HRV is significantly reduced in chronic liver disease. (2) Reduced HRV is correlated with the severity of liver disease. (3) Autonomic dysfunction is not related to the aetiology of liver disease. (4) Markers of hepatocellular dysfunction are more accurate predictors of autonomic dysfunction compared to markers of cholestasis where SDNN correlated significantly with prothrombin (r=0.64, p=0.001) and serum albumin (r=0.40, p=0.05) but not total bilirubin. | Portugal | NS | 22 (11) | 20 | Alcohol = 12<br>HBV + HCV = 6<br>Autoimmune = 2<br>Others = 2 | A = 6<br>B = 9<br>C = 7 |
| Frokjaer V. G. et al. 2006 [188] | To determine if autonomic dysfunction is related to cerebral blood flow | (1) Cerebral autoregulation of blood flow is impaired in severe cases of liver cirrhosis. (2) Impairment of cerebral autoregulation correlated with autonomic dysfunction (3) severity of cirrhosis | Denmark | outpatients | 14 (9) | 11 (5) | Alcohol = 8<br>PBC = 4<br>Cryptogenic = 2 | A = 5<br>B = 6<br>C = 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | autoregulation in cirrhotic patients. | correlated with degree of autonomic dysfunction (4) loss of sympathetic innervation of cerebral vessels linked with cerebral autoregulation dysfunction in cirrhotic patients. | | | | | | |
| Iga A. et al. 2003 [189] | To assess autonomic abnormalities in patients with Liver cirrhosis using $^{123}$I-metaiodobenzylguanidine (MIBG) myocardial scintigraphy and HRV. | Autonomic dysfunction is present in cirrhotic patients and can be assessed by MIBG myocardial scintigraphy and HRV. | Japan | NS | 50 (27) | 50 (33) | HBV = 4<br>HCV = 40<br>HBV + HCV = 2<br>PBC = 4 | A = 20<br>B = 12<br>C = 18 |
| Ko F.Y. et al. 2013 [190] | To assess the relationship between psychological distress and clinical presentations of cirrhosis using biochemical and physiological (HRV) markers. | (1) Psychological distress was correlated with increased serum aspartate aminotransferase (AST) and reduced autonomic control of the heart (HRV). (2) Inflammation may have a role to play in cirrhotic psychological distress. | China | outpatients | 125(73) | 55(29) | Alcohol = 14<br>HBV + HCV = 83<br>Autoimmune = 5<br>Parasites = 5<br>Others = 18 | A = 43<br>B = 58<br>C = 24 |
| Lazzeri C. et al. 1997 [191] | To assess autonomic neuropathy in patients with non-alcoholic cirrhosis with ascites. | Ascitic patients with non-alcoholic cirrhosis have autonomic neuropathy compared with healthy controls and this is associated with significant differences in HRV indices between the groups. | Italy | outpatients | 12 (7) | 12 | HBV = 2<br>HCV = 8<br>Cryptogenic = 2 | A = 0<br>B = 5<br>C = 7 |
| Mani A.R. et al. 2008 [90] | To assess the relationship between indices of HRV, hepatic encephalopathy, and systemic inflammation in cirrhotic patients | (1) Reduced long-term HRV in cirrhotic patients compared with healthy subjects. (2) HRV negatively correlated with the degree of neuropsychiatric impairment. (3) 8% increased relative risk of death for every 1ms drop in HRV index (4) Indices of HRV and neuropsychiatric performance significantly correlated with the level of plasma IL6. | UK | inpatients (14)<br>outpatients (66) | 80(53) | 11 (5) | Alcohol = 65<br>HBV + HCV = 7<br>Mixed (Various) = 9 | A = 51<br>B = 13<br>C = 16 |

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|---|---|---|---|---|---|---|---|---|
| Milovanovic B. et al. 2009 [192] | To analyse risk predictors of sudden cardiac death (SCD) related to autonomic dysfunction in alcohol-related cirrhotic patients. | (1) Patients with ARLD are susceptible to autonomic dysfunction (56%). (2) ARLD patients also have lower HRV indices (SDNN, SDANN, TINN, LF, and HF), serious arrhythmia, prolonged QTc, and abnormal Poincare plot. (3) QTc inversely correlated with lnLF, lnHF (r= -0.53, r= -0.47; p<0.05) while Lown class correlated with autonomic function (r= -0.64; p=0.05). | Serbia | inpatients | 25 (20) | 19 (15) | Alcohol = 25 | NS |
| Miyajima H. et al. 2001 [193] | To assess the effect of portal blood flow volume and autonomic nervous function on abnormal gastric motility in cirrhotic patients. | (1) Autonomic dysfunction correlated with abnormal gastric motility in cirrhotic patients (2) Decreased gastric motility may result from abnormalities in autonomic function in cirrhotic patients | Japan | NS | 27(19) | 20(13) | Alcohol = 1<br>HBV = 5<br>HCV = 21 | A = 7<br>B + C = 20 |
| Moller S. et al. 2012 [194] | To assess sympathetic control of cardiac function in cirrhosis using mIBG scintigraphy and relate this to cardiovascular functions | (1) Cirrhotic patients have significantly reduced HRV and baroreflex activity. (2) Reduction in HRV and baroreflex correlated significantly with abnormal cardiac sympathetic nervous activity measured by catecholamine uptake by mIBG. | Denmark | outpatients | 10 (5) | 10 (5) | Alcohol = 10 | NS |
| Nagasako C.K. et al. 2009 [195] | To assess autonomic dysfunction in non-alcoholic cirrhosis and the relationship with disturbed intestinal transit time, as well as severity and prognosis using HRV. | (1) HRV indices (HF, lnHF, LF, lnLF, pNN50) were lower in cirrhotic patients (Child B) patients compared with child A and control subjects. (2) HRV indices correlated significantly with the risk of hepatic encephalopathy in cirrhotic patients. | Brazil | NS | 32(12) | 21(12) | HCB = 16<br>PBC = 6<br>Cryptogenic = 6<br>Others = 4 | A = 13<br>B = 19<br>C = 0 |
| Negru R.D. et al. 2015 [178] | To assess the use of HRV as a marker of autonomic dysfunction and severity in cirrhotic patients. | (1) HRV detected autonomic dysfunction in cirrhotic patients. (2) Aetiology of liver cirrhosis is linked with distinct types of autonomic dysfunctions. (3) HRV parameters correlated significantly with the severity of liver cirrhosis as assessed by Child-Pugh scores. | Romania | inpatients | 52 (27) | 30 (15) | Alcohol = 25<br>HBV = 6<br>HCV = 17<br>Mixed = 4 | A = 30<br>B = 8<br>C = 14 |

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| Satti R. et al. 2019 [176] | To extend the Poincare plot by introducing a sequential lag in the correlation computation and to evaluate the relationship with the severity and survival of cirrhotic patients. | (1) Traditional SD1 and SD2 correlated strongly with severity of liver cirrhosis. (2) Lagged SD1 and SD2 correlated significantly with liver disease severity. However, extended SD1 did not predict mortality independently of MELD. (3) SD2 predicted survival of cirrhotic patients independent of MELD. | Italy | Outpatients | 74 | 35 | NS | NS |

*NS, Not Shown; vs, versus; HRV, Heart Rate Variability; ARLD, Alcohol-Related Liver Disease; NALD, Non-Alcoholic Liver Disease (including virus-linked liver diseases); PBC, Primary Biliary Cholangitis; IL6, Interleukin-6; LT, Liver Transplantation; ln, Natural Logarithm; QTc, Q-T complex describing time Interval between the start of Q-wave and T-wave of an ECG recording; HCV, Hepatitis C Virus; MELD, Model for End-Stage Liver Disease; NN Interval, time lapse between consecutive QRS complexes of ECG recording; SDNN, Standard deviation of NN intervals; SDANN, Standard deviation of the average NN intervals for each 5-minute segments deduced from a 24-hour ECG recoding; pNN50, Percentage of successive RR intervals that vary by more than 50ms; RMSSD, Root mean square of differences in successive NN interval; TINN, Triangular Interpolation of the NN intervals' histogram; LF, Low Frequency; HF, High Frequency; SD1, Poincare plot Standard Deviation perpendicular to the line of identity; SD2, Poincare plot Standard Deviation along the line of identity; ApEn, Approximate Entropy.*

*Table 2.2.* ECG recording and HRV analysis techniques of included studies.

| Author | Sampling Time/length | Analysis time/length | Equipment (Manufacturer) | Sampling Rate (Hz) | Analysis Software | Data processed (filtering/ correction/ Selection/manual editing etc.) | HRV Indices Measures | Significant HRV indices ($p < 0.05$) |
|---|---|---|---|---|---|---|---|---|
| Ates F. et al. 2006 [185] | 24 hours | 24 hours | HolterWin P-V Version 5.40 Plus; Diagnostic Monitoring, SantaAna, CA, USA. | NS | HolterWin P-V Version 5.40 Plus; Diagnostic Monitoring, SantaAna, CA. (Commercial). | Yes | mean NN, SDNN, SDANN, RMSSD, pNN50 | mean NN, SDNN, SDANN, RMSSD, pNN50 |
| Baratta L. et al. 2010 [186] | 24 hours | NS | Accuplus 363 (Del Mar Medical System, Irvine, CA, USA) | NS | NS | Yes | SDNN, RMMSD, HF, LF, LF/HF | SDNN. RMSSD, HF (night) |
| Coelho, L. et al. 2001 [187] | 24 hours | 24 hours | NS | NS | NS | No | SDNN, pNN50, VLF, LF, HF, LF/HF | Reduced SDNN, pNN50, VLF, LF, HF |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Frokjaer V. G. et al. 2006 [188] | 24 hours | 24 hours | Portable Cardio-recorder (Spacelab 90208, USA | NS | Spacelab Medical FT3000. (Commercial). | Yes | SDNN, RMSSD, VLF, LF, HF, TP. | SDNN, VLF, LF, HF, TP |
| Iga A. et al. 2003 [189] | 24 hours | 512 heartbeats | SM-50 (Fukuda Denshi Corpo-ration, Tokyo, Japan) + DWM-9000H Workstation (Fukuda Denshi Corporation) | NS | MemCalc Ver2.5 (Suwa Trust, Tokyo, Japan). (Commercial). | No | LF, HF, LF/HF | LF, HF, LF/HF |
| Ko F.Y. et al. 2013 [190] | 5 minutes | 5 minutes | Ad hoc/customized | 256 | Open-Source Software (Physionet). (Commercial). | NS | SDNN, RMMSD, LnHF, LnLF, lnVLF, LF/HF, DFA α1, DFA α2 | SDNN, RMSSD, lnHF, lnLF, DFA α1 |
| Lazzeri C. et al. 1997 [191] | 24 hours | 512 RRI (Frequency Domain), 24 hours (Time Domain). | ELATEC 3.0 (ELA Medical, Segrate. Italy) | NS | HRV module for ELATEC 1.0. (ELA Medical, Segrate. Italy). (Commercial). | Yes | Mean RR, SDNN, SDANN, RMSSD, pNN50, TP, LF, HF, LF/HF. | SDNN, SDANN, RMSSD, pNN50, LF, HF |
| Mani A.R. et al. 2008 [90] | 10 minutes | 5 minutes | NS | 256 | Lab-built (Ad hoc) | Yes | SDNN, LF, HF, LF/HF, SD1, SD2, SampEn | SDNN, LF, HF, SD1, SD2, SampEn |
| Milovanovic B. et al. 2009 [192] | 24 hours | 24 hours | 3 Leads ECG equipment (Biosensor, USA) | 1000 | Biosensor, USA. (Commercial). | Yes | Mean RR, SDNN, SDANN, RMSSD, TP, HF, LF, LF/HF, lnHF, lnLF, TINN | SDNN, SDANN, TINN, lnHF, lnLF. |
| Miyajima H. et al. 2001 [193] | NS | 512 heartbeats | SM-50 (Fukuda Denshi Corporation, Tokyo, Japan) | NS | DWM-9000H (Fukuda Denshi Corporation, Tokyo, Japan). (Commercial). | NS | LF, HF, LF/HF | HF, LF/HF |
| Moller S. et al. 2012 [194] | 50 minutes | 50 minutes | Task Force Monitor (TFM) (CNSystems, Graz, Austria) | NS | Task Force Monitor (TFM) (CNSystems, Graz, Austria) | NS | VLF, LF, HF, LF/HF | LF, HF, LF/HF |
| Nagasako C.K. et al. 2009 [195] | 24 hours | Frequency Domain = 5 minutes (X72) Time Domain = 24 hours | Dynamics R300 (Cardios Sistemas,Sa˜o Paulo, Brazil) | NS | commercial software (DMI CardiosSistemas, Sa˜o Paulo, Brazil). (Commercial). | NS | Mean NN, SDNN, RMSSD, pNN50, TP, LF, lnLF, HF, lnHF, LF/HF | SDNN, pNN50, TP, HF, lnHF, LF, lnLF, LF/HF |
| Negru R.D. et al. 2015 [178] | 24 hours | 24 hours | 300-3A recorders (DMS - USA) | 128 | CARDIOSCAN 12 software (DMS-USA). (Commercial). | Yes | SDNN, SDANN, SDNN index, RMSSD, pNN50, TP, LF, HF, VLF | SDNN, SDNN index, SDANN, TP, VLF |
| Satti R. et al. 2019 [176] | 10 minutes | 8 minutes | Chart 5 (AD-Instrument, Australia) | 256 | Lab-built (Ad hoc) | Yes | Traditional SD1 and SD2 and r (Pearson Correlation); lagged SD1 and SD2 and r (Pearson Correlation) | SD1, SD2, r (Pearson Correlation) |

*ECG, Electrocardiograph; RRI, RR interval describing the time lapse between consecutive R-waves of an ECG recording; NN Interval, the time lapse between consecutive QRS complexes of ECG recording; SDNN, Standard deviation of NN intervals; SDANN, Standard deviation of the average NN intervals for each 5-minute segments deduced from a 24-hour ECG recoding; pNN50, Percentage of successive RR intervals that vary by more than 50ms; RMSSD, Root mean square of differences in successive NN interval; TINN, Triangular Interpolation of the NN intervals' histogram; TP, Total Power; VLF Very Low Frequency; LF, Low Frequency; HF, High Frequency; LF/HF, Ratio of LF to HF; SD1, Poincare plot Standard Deviation perpendicular to the line of identity; SD2, Poincare plot Standard Deviation along the line of identity; ApEn, Approximate Entropy; SampEn, Sample Entropy; DFA α1, Short-term fluctuation of Detrended Fluctuation Analysis; DFA α2, Long-term fluctuation of Detrended Fluctuation Analysis.*

***Table 2.3.*** *Characteristics of studies that reported the HRV indices as predictors of survival in patients with cirrhosis.*

| Author, Year | Conclusion | Sample Size | Follow-up (Months) | Analysis time /length | Non-Survivor (%) | HRV analysed | HRV of Non-survivors (Mean ± SD) | HRV of Survivors (Mean ± SD) | Hazard/Odds Ratio (95% CI) of the HRV index | HRV indices that are predictors of survivors independently of MELD or Child-Pugh scores |
|---|---|---|---|---|---|---|---|---|---|---|
| Ates F. et al. 2006 [185] | HRV indices were also significantly reduced in non-survivors vs survivors after 2 years of follow-up. | 30 | 24 | 24 hours | 13 (43%) | mean NN, SDNN, SDANN, RMSSD, pNN50 | mean NN (542±127), SDNN (51±13), SDANN (44±7), RMSSD (10±9), pNN50 (2.3±0.9) | mean NN (796±143), SDNN (84±15), SDANN (62±13), RMSSD (17±11), pNN50 (5.3±1.2) | NS | NS |
| Baratta L. et al. 2010 [186] | HRV long-term fractal-like exponent () predicts mortality in cirrhotic patients independent of MELD and Child-Pugh scores. | 38 | 12 | 24 hours | 15 (40%) | SDNN, cSDNN, SD1, SD2, VLF, LF, HF, DFA α1, DFA α2 | SDNN (67.35±6.46), cSDNN (236±23.8), SD2(90.25±8.62), VLF (3315±591), DFA α2(0.989±0.032) | SDNN (86.79±5.23), cSDNN (316.8±22.6), SD2(121.01±8.37), VLF (6,219±852), DFA α2(1.142±0.036) | SDNN [Hazard Ratio (95% CI) =0.979 (0.969-0.989), cSDNN [Hazard Ratio (95% CI) =0.993 (0.990-0.996), SD2 [Hazard Ratio (95% CI) =0.983 (0.976-0.990), VLF [Hazard Ratio (95% CI) =0.999 (0.998-0.999), HF [Hazard Ratio (95% CI) =1.001 (1.000=1.002), | DFA α2 |

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| | | | | | | | | | DFA- α2 [Hazard Ratio (95% CI) =0.011 (0.002-0.052) | |
| Bhogal A.S. et al. 2019 [184] | HRV indices (SD2 and cSDNN) predicted survival in cirrhotic patients independent of MELD and Child-Pugh scores. | 74 | 18 | 8 min | 24 (32%) | mean HR, SDNN, cSDNN, SD1, SD2, VLF, LF, HF, LF/HF, SampEn, DFA α1, DFA α2, Kurtosis, Skewness | SDNN (18.9±2.0), cSDNN (68.1±5.4), SD1 (9.5±1.3), SD2 (24.8±2.5), VLF (205±38), LF (89±33), HF (80±30) | SDNN (29.1±2.1), cSDNN (81.9±5.0), SD1 (15.1±1.4), SD2 (37.7±2.7), VLF (483±73), LF (212±44), HF (239±46) | SDNN [Hazard Ratio (95% CI): 0.935 (0.895-0.977), cSDNN [Hazard Ratio (95% CI): 0.975 (0.959-0.991), SD1 [Hazard Ratio (95% CI): 0.919 (0.861-0.980), SD2 [Hazard Ratio (95% CI): 0.950 (0.918-0.982), VLF [Hazard Ratio (95% CI): 0.997 (0.995-0.999), HF [Hazard Ratio (95% CI): 0.995 (0.991-0.999) | cSDNN, SD2 |
| Chan K.C. et al. 2016 [196] | Heart rate complexity and deceleration capacity increased the accuracy of MELD in predicting survival in patients with end-stage liver diseases | 30 | 12 | 30 min | 5 (17%) | SDNN, RMSSD, pNN50, pNN20, DC, AC, Complexity | pNN50 (0.006±0.003), pNN20 (0.079±0.058), DC (3.60±0.71), AC (3.51±0.81), Complexity (22.08±4.64) | pNN50 (0.065±0.070), pNN20 (0.269±0.180), DC (6.01±2.36), AC (6.06±2.77), Complexity (28.60±5.06) | NS | NS |
| Jansen C. et al. 2019 [197] | Baseline SDNN predicts 90-day survival in cirrhotic patients independent of actual indices of severity. | 111 | 3 | 5 min | 12 (11%) | SDNN | SDNN (11 (10-12)) | SDNN (26 (17-38)) | SDNN [Odds Ratio (95%) = 0.79 (0.65 - 0.97)] | SDNN |
| Mani A.R. et | There is an 8% increase in the | 80 | 20.3 | 5 min | 11 (14%) | SD1, SD2 | NS | NS | SD2 [Hazard Ratio (95% CI) = 0.923 (0.864-0.982)] | NS |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| al. 2009 [90] | relative risk of death for every 1ms drop in SD2 | | | | | | | | | |
| Satti R. et al. 2019 [176] | While SD1 and SD2 significantly predicted survival, only SD2 predicted survival of cirrhotic patients independently of MELD | 74 | 18 | 8 min | 24 (32%) | SD1 (lagged), SD2 (lagged), and Pearson's r | NS | NS | SD2 [Hazard Ratio (95% CI) = 0.950 (0.918–0.982)] | SD2 |

*HRV, Heart rate Variability; NN Interval, time lapse between consecutive QRS complexes of ECG recording; SDNN, Standard deviation of NN intervals; cSDNN, corrected SDNN; ms, millisecond; SDANN, Standard deviation of the average NN intervals for each 5-minute segments deduced from a 24-hour ECG recoding; pNN50, Percentage of successive RR intervals that vary by more than 50ms; RMSSD, Root mean square of differences in successive NN interval; TINN, Triangular Interpolation of the NN intervals' histogram; TP, Total Power; VLF Very Low Frequency; LF, Low Frequency; HF, High Frequency; LF/HF, Ratio of LF to HF; SD1, Poincare plot Standard Deviation perpendicular to the line of identity; SD2, Poincare plot Standard Deviation along the line of identity; ApEn, Approximate Entropy; SampEn, Sample Entropy; DFA α1, Short-term fluctuation of Detrended Fluctuation Analysis; DFA α2, Long-term fluctuation of Detrended Fluctuation Analysis; DC, Deceleration Capacity; MELD, Model for End-stage Liver Disease; CI, Confidence Interval; SD, Standard Deviation.*

*HRV time domains*

Thirteen studies reported significant differences in HRV time domains between patients with cirrhosis and healthy control groups. The standard deviation of NN intervals (SDNN), SDNN Index, standard deviation of the average NN intervals for each 5 min segment of a 24 h ECG recording (SDANN), root mean square of successive NN interval (RMSSD), and percentage of NN intervals that differ by 50% (pNN50) were all different between the groups. There were significantly higher time domain HRV indices (i.e., SDNN, SDANN, RMSSD, and pNN50%) in healthy controls compared with patients with cirrhosis. Further, the computed between-studies heterogeneities for all time domain indices were significantly high (***Figure 2.2****a-d*).

*SDNN*

SDNN is defined as the standard deviation of normal/non-ectopic RR intervals (NN). It is translated as the measure of the global influence of the autonomic nervous system on the cardiac rhythm [198, 199]. Nine studies reported a significant reduction in SDNN attributed to cirrhosis [90, 178, 185, 187, 188, 190-192, 195]. One of the 9 studies [195] presented the median SDNN with no interquartile range and was excluded from the effect size computation (***Appendix 5***). A "v*ery large"* effect size was seen whereby healthy controls have significantly higher SDNN compared with patients with cirrhosis [SMD (95%CI) = 3.41 (2.24, 4.58); ***Figure 2.2****a*]. This can be interpreted as a noticeable dysregulation of the autonomic control of the cardiac rhythm due to cirrhosis.

*SDNN Index*

SDNN Index is the average of all 5 minutes-SDNNs of a 24-hour ECG recording (i.e., 288). SDNN index has been physiologically associated with the overall autonomic control of heart rhythm [198, 199]. A significant difference in the SDNN index was reported in only one study whereby patients with cirrhosis had a lower index and dysregulated autonomic cardiac control compared with healthy controls (mean ± SD of 43.83 ± 15.66 vs 56.50 ± 17.04; ***Appendix 6***) [178].

*SDANN*

SDANN is the standard deviation of all computed averages of 5-minute NN intervals of a 24-hour ECG recording (i.e., 288). SDANN is physiologically comparable to SDNN as it provides a measure of the overall autonomic influence on the heart rhythm [198, 199]. A total of 3 studies reported significant reduction in SDANN attributed to cirrhosis (***Appendix 7***) [178, 185, 191]. A significantly lower SDANN

was observed in patients with cirrhosis compared with healthy controls with a "*very large"* standardized mean difference [SMD (95%CI) = 2.54 (0.81, 4.27); ***Figure 2.2****b*].

*RMSSD*

The root mean square of the NN intervals is defined as the square root of the average of the square of NN intervals and is often measured over 5 minutes. RMSSD is linked with vagal influence on the heart rhythm and indexes the respiratory sinus arrhythmia (RSA) [198, 199]. Three studies reported differences in RMSSD between patients with cirrhosis and healthy controls (***Appendix 8***) [185, 190, 191], with patients with cirrhosis showing significantly lower RMSSD compared with healthy controls. A "*large"* pooled standardized mean difference between the groups was also observed [SMD (95%CI) = 1.60 (0.73, 2.47); ***Figure 2.2****c*] representing a marked reduction in vagal control of the heart rhythm due to cirrhosis.

*pNN50*

Percentage of the NN intervals that differ from each other by more than 50ms. The pNN50 indicates the parasympathetic influence on cardiac rhythm, providing a less accurate assessment of RSA when compared with RMSSD [198, 199]. A total of 4 eligible studies reported a significant reduction in pNN50 in the cirrhosis group compared with healthy controls [185, 187, 191, 195]. However, one study reported median pNN50 without the interquartile range and was not included in the analysis (***Appendix 9***) [195]. Further, lower RMSSD was reported in the cirrhosis patients compared with the control groups with a "*very large"* effect size observed between the group [SMD (95%CI) = 2.54 (1.21, 3.87); ***Figure 2.2****d*].

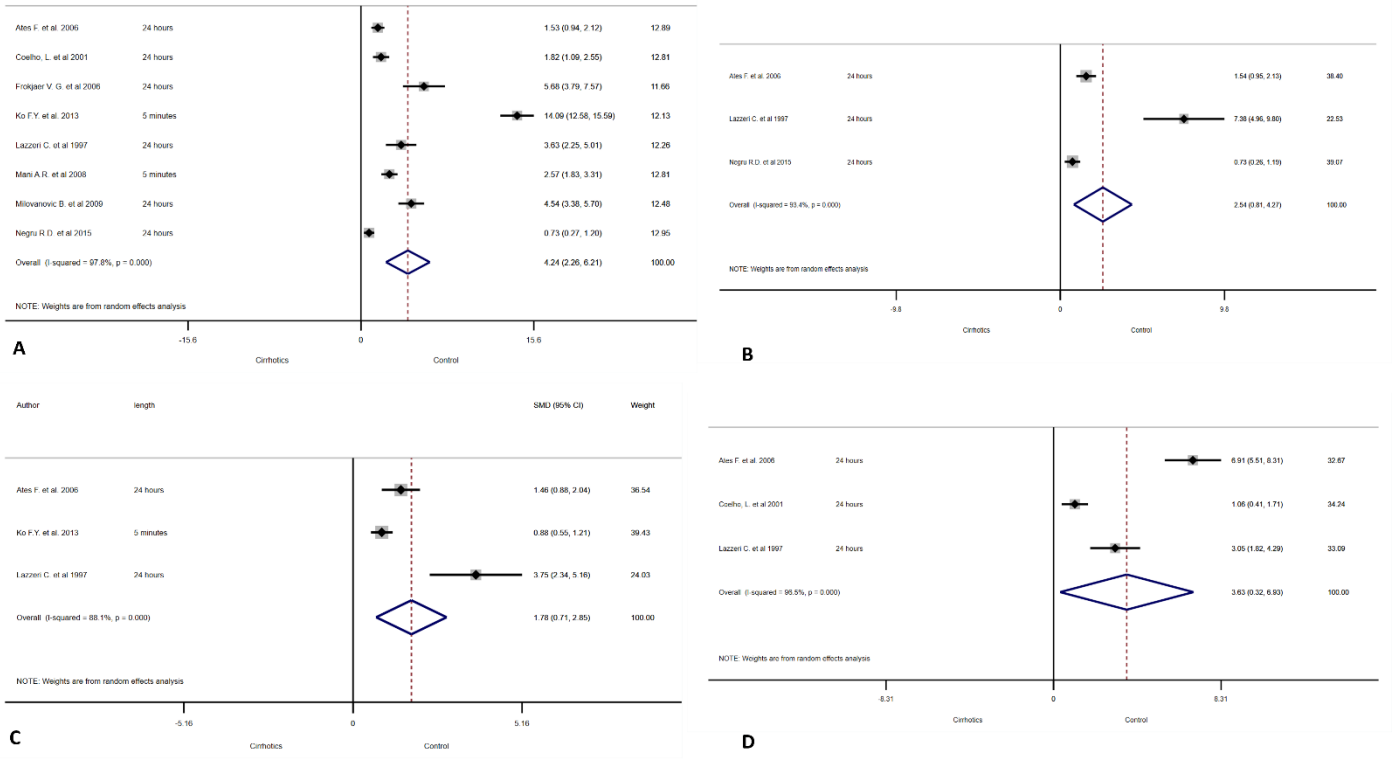



***Figure 2.2(a, b, c & d)***. *Forest plot for the standardized mean differences (SMD) in HRV time domain indices: SDNN (2a), SDANN (2b), RMSSD (2c), and pNN50 (2d) between patients with liver diseases and matched healthy controls. Hedges' G effect size estimates were calculated with a 95% confidence interval and computed using a random effect model. Continuous horizontal lines and diamond width represent a 95% confidence interval, and the diamond center and vertical red dotted line indicate the pooled random effect sizes*

*HRV Frequency Domain*

HRV Frequency domains define the signal and associated relative or absolute power distribution across various frequency bands of ECG recording. It involves complex analysis techniques such as autoregression (AR) or Fast Fourier Transformation (FFT) of NN variations. A total of 10 included studies reported significant differences in the various HRV frequency domain indices between healthy controls and patients with cirrhosis. Total Power (TP), High Frequency (HF), Low Frequency (LF), Very Low Frequency (VLF), and the ratio of HF to LF (HF: LF). A random effect model was used for pooling HF and LF due to significantly high between-studies heterogeneity as measured by $I^2$ statistics (**Figure *2.3***b and **Figure *2.3***c). Conversely, the between-studies heterogeneities for studies pooled for the SMD of TP and VLF were significantly low. Thus, a fixed effect model was used (**Figure *2.3***a and **Figure *2.3***d).

*Total Power (TP)*

power corresponds to the cumulative energy in all the frequency bands (ULF, VLF, LF, and HF) of an ECG recording [198, 199]. Two of the included studies reported significantly lower TP in patients with cirrhosis [178, 188]. TP was analysed from 24-hour ECG recordings in both studies and reported as a natural log in one study [188] (**Appendix 10**). Significantly higher TP was observed in controls compared with the patient with cirrhosis with a *moderate* standardized mean difference between the groups [SMD (95%CI) = 0.62 (0.23, 1.02); **Figure 2.3**a].

*High Frequency (HF)*

High frequency describes the absolute power within the 0.15Hz – 0.4Hz frequency band of the N-N time series. The HF is driven by the overall parasympathetic autonomic influence on the heart rhythm and correlates with respiratory sinus arrhythmia (RSA) [198, 199]. Nine of the included studies reported significant differences in HRV high-frequency power between the patients and controls [90, 186, 188-194]. Of which 8 studies reported reduced HF in cirrhosis compared with healthy control [191] (**Appendix 11**). The single study that reported higher HF in cirrhosis [191] was not included in the data analysis because the model used for pooling the data is not robust to differences in the direction of effect sizes [200]. An “extremely large” difference between the groups

was observed [SMD (95%CI) = 4.36 (1.94, 6.77); Figure *2.3*b] showing and easily discernible dysregulation in vagal control of the heart rhythm linked with cirrhosis.

*Low Frequency (LF)*

The HRV low-frequency measure defines the absolute power within the 0.04–0.15 Hz frequency band of an ECG recording. The LF relates significantly with baroreflex feedback and regulation of the heart rhythm. LF is correlated with both arms (sympathetic and parasympathetic) of the autonomic nervous controls [198, 199]. A total of 8 studies reported significant differences in LF between the cirrhosis and healthy control groups (Appendix 12) [90, 188-192, 194]. LF was observed to be significantly lower in cirrhosis compared with control with an *extremely large* effect size [SMD (95%CI) = 5.49 (2.32, 8.67); Figure *2.3*c], representing a significant decline in the response of the heart rhythm to the baroreflex loop in cirrhosis.

*Very Low frequency (VLF)*

HRV very low frequency represents absolute power distributed within the 0.0033–0.04 Hz frequency band of an ECG recording. Albeit the physiological factors responsible for the VLF are ambiguous, it has been associated with the activities of the renin-angiotensin system, endothelial factors, and thermoregulation [198, 199]. A total of 2 studies reported significant differences in 24-hour VLF between the groups (Appendix 13) [178, 188], and patients with cirrhosis were observed to have significantly moderately lower VLF compared with healthy control group [SMD (95%CI) = 0.73 (0.32, 1.13); Figure *2.3*d]. This shows that the heart rhythm of patients with cirrhosis is characterised by an attenuated response to renin-angiotensin, endothelial factors activities, and thermoregulation compared with healthy controls.

*Low Frequency – High-Frequency Ratio (LF: HF)*

The LF: HF is traditionally interpreted as the interplay and balance between the sympathetic and parasympathetic arm of the autonomic nervous system because LF is associated with sympathetic control while HF indexes parasympathetic cardiac controls [198, 199]. However, this concept was questioned based on the observation that both arms of the autonomic nervous system drive the HF power [201]. Three of the included

studies reported significant differences in LF: HF between the groups [189, 193, 194] of which, 2 studies reported a decrease in LF: HF in healthy controls [189, 193] (**Appendix 14**). The difference in LF: HF between the groups was not significant, however.

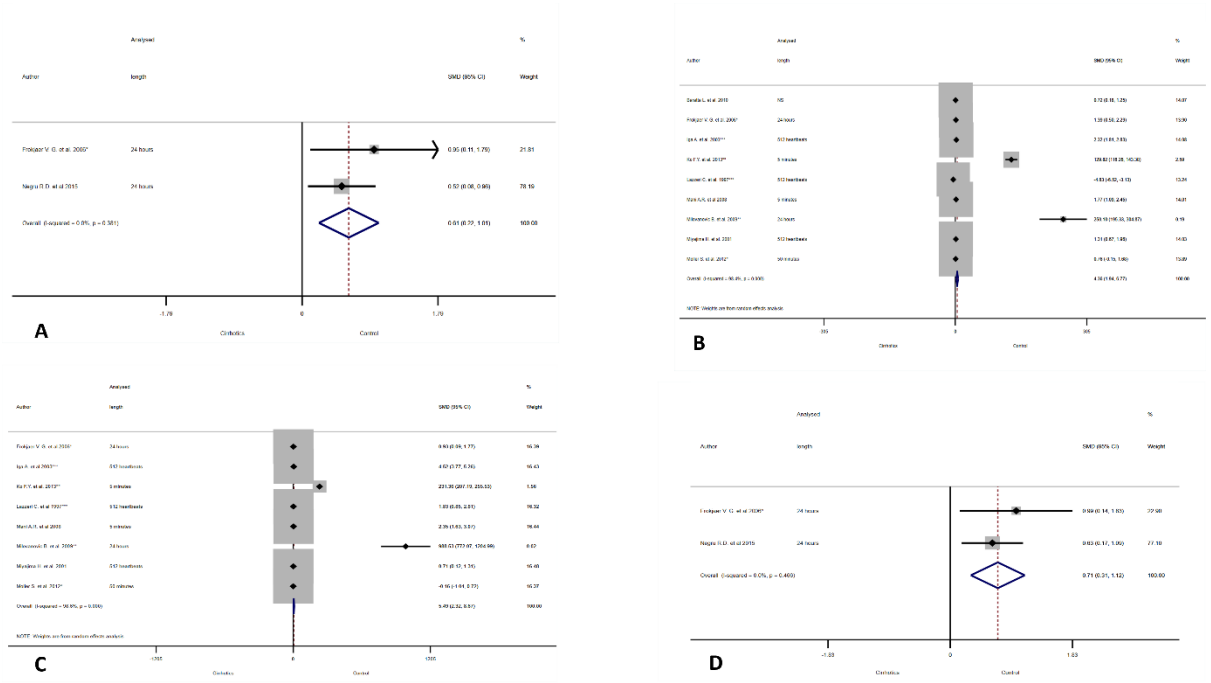



***Figure 2.3(a, b, c, & d).*** *Forest plot for the standardized mean differences (SMD) in HRV frequency domain indices: TP (a), HF (b), LF (c), and VLF (d) between patients with liver diseases and matched healthy controls. Hedges' G effect size estimates were calculated with a 95% confidence interval and computed using a random effect model. The width of the solid black diamonds represents a 95% confidence interval of the effect sizes of each of the pooled studies and the blue diamond and vertical red dotted line indicate the pooled random or fixed effect sizes.*

### *HRV Non-linear Indices*

A statistically significant difference in HRV non-linear indices between the cirrhosis and healthy control groups was reported in two of the included studies [90, 190] and included sample entropy, SD2 (short-term variation) (SD1) and SD2 (long-term variation) of the Poincare' plot, and scaling exponent (α) of the detrended fluctuation analysis (DFA). SD1, SD2, and Sample Entropy were all reported to be significantly reduced due to cirrhosis in one study [90] (**Appendix 15**) while DFA α1 (short-term scaling exponent), which corresponds to a fractal-like pattern of cardiac rhythm was also reported to be altered in cirrhosis compared with healthy controls in one study [6].

*HRV in Survival Analysis*

Seven studies evaluated the prognostic value of HRV in patients with cirrhosis with follow-up periods ranging between 3 to 24 months [7, 90, 176, 184, 185, 196, 197]. All studies established significantly different HRV indices between survivors and non-survivors. Further, according to the reported hazard or odds ratios, differences in DFA α2, SD2, cSDNN, SDNN, and VLF correlated significantly positively with survival (Table 2.3). Four of the 7 studies analysed for survival reported that DFA α2, cSDNN (corrected SDNN), SD2, and SDNN have prognostic values in cirrhosis which is independent of MELD and/or Child-Pugh scores.

## Discussion

This report shows the impact of cirrhosis on the autonomic nervous system's regulation of the cardiac rhythm indexed by heart rate variability indices. A significant difference was observed in HRV of patients with cirrhosis compared with healthy controls. However, the heterogeneity of included studies was found to be significantly high. To control for the observed heterogeneity, the random-effect model was used while the effect size was computed as standardized mean difference (SMD). The HRV indices are usually utilized as indexes of dysfunction in the two arms of the autonomic nervous system [202]. Further, HRV indices such as SDNN, cSDNN, DFA α2, and SD2 correlated significantly with the severity of cirrhosis and the survival of patients. Specifically, HRV time and frequency domain indices including SDNN, SDNN index, SDANN, RMSSD, pNN50 as well as TP, HF, LF, and VLF were significantly reduced and showed negative correlation with the severity of cirrhosis. The relationship between LF: HF and cirrhosis, which had been interpreted as a measure of sympathovagal cardiac regulation remains unclear with one study reporting an increase [194] and two studies reporting a decrease [189, 193].

Essentially, the systematic review for survival shows that HRV predicts survival in patients independently of the MELD score, a current measure of the severity of cirrhosis. This corroborates a recent observation in which SDNN was reported as an independent predictor of survival in patients with decompensated cirrhosis. Indeed, Jansen et al. also reported a significant reduction of SDNN which correlated negatively with systemic inflammatory response and the severity of cirrhosis [197]. Indeed, this correlated dysregulated systemic inflammatory response and cardiac autonomic dysfunction has been previously observed in cirrhosis [90] as well as in other diseases [203, 204]. Also, dysregulated inflammation has been linked with various other complications of decompensated cirrhosis including the development of acute on chronic liver failure (ACLF) [205-207]. Consequently, suggestions have been put forward that sudden reduction in HRV may allow for early detection of global systemic shifts that predispose acute decompensation in cirrhosis [197]. However, the mechanism of cardiac autonomic dysfunction due to systemic inflammation has only been reported in animal models of cirrhosis with no research in humans [86, 208-210]. Similarly, emerging data and reports continue to show that HRV changes correlates significantly with covert hepatic

encephalopathy [90], and may provide an indirect diagnostic tool to identify patients with sub-clinical hepatic encephalopathy without the need for an EEG and associated analytical limitations [211]. Conceivably, HRV may in future provide a comparatively easier and compact, yet effective assessment of covert encephalopathy.

A decrease in HRV has also been reported in non-cirrhotic liver patients. For instance, a significant reduction in HRV time and frequency domains in patients with primary biliary cholangitis compared with age-matched healthy controls was reported by Keresztes et al. Further, autonomic dysfunction combined with cardiovascular reflex abnormality was reported in 58% of the PBC patients studied [212]. Autonomic function evaluated by time and frequency HRV indices was also reported in patients with hepatitis C infection to be impaired, with the impairment correlating significantly with serum alanine aminotransferase levels, a measure of liver injury [213].

This systematic review also shows cirrhosis is associated with impaired non-linear HRV indices. For instance, sample entropy, SD1, and SD2 were higher in healthy controls compared with patients with cirrhosis. This finding validates the other studies in which SD2 as well as cSDNN were reported to predict mortality independent of MELD in patients with cirrhosis [184]. DFA α2, the long-term fractal-like scaling exponent, was also decreased in cirrhosis and shown as an independent predictor of mortality when compared with MELD [90]. Non-linear HRV non-linear indices measure the randomness of heart rhythm and index the complexity of consecutive N-N intervals. Decreased complexity may be inferred physiologically as decreased flexibility and reduced tendency of the cardiac rhythm to respond to sudden environmental shifts and autonomic nervous regulations. Indeed, increased memory length of cardiac rhythm, which translates to reduced functional malleability, has been reported in patients with cirrhosis compared to healthy controls [214]. Similarly, a recent study by our group showed that turbulence onset (TO) of heart rate turbulence (HRT) measure, a physiological activity regulated by the autonomic nervous system is significantly reduced and predicts survival in cirrhosis [215]. Put together, albeit the mechanistic connection is still unclear, dysregulated autonomic control of the cardiac rhythm is a hallmark of cirrhosis measured with changes in HRV indices. Thus, HRV measures may be used in combination with the current scoring systems to improve the clinical diagnosis and prognosis of patients with cirrhosis. Lastly,

while lower Sample Entropy (i.e., HRV complexity) has been reported in cirrhosis, the value as a prognostic physical marker has not been reported and warrants further investigation.

There are several limitations associated with this study. Firstly, the difference in the time of ECG recording in the studies is different and since HRV is influenced by circadian changes in physiological function [216], this is a source of weakness of this review. Secondly, non-linear indices of HRV were reported by only a few studies. Thus, data could not be pooled to assess the differences in these indices between the healthy control group and patients with cirrhosis. Finally, pooled survival analysis could not be computed because of the variability in the follow-up times of the studies included.

In conclusion, HRV has the potential for application in medical and research settings to assess dysfunction of the autonomic nervous system associated with cirrhosis and may be valuable for early detection of early, subclinical decompensation as in covert hepatic encephalopathy. Further, as an independent predictor of the outcome of cirrhosis, HRV may also be combined with MELD and Child-Pugh in appreciation of the multi-organ involvement of cirrhosis and may improve the prognostic values of these scores. Lastly, despite these various potentials, the inconsistency that exists in the techniques used for HRV measurement remains a major impediment to practical interpretation and application. Indeed, to generate data that is robust, the standardisation of techniques including ECG recording and HRV measurement needs to be improved. Hopefully, this review will provide a drive to standardize the HRV analysis methods to foster the development of this expanding field.

# Chapter 3 : Heart Rate Turbulence predicts survival independent of MELD in patients with cirrhosis

# Introduction

Patients with cirrhosis exhibit systemic manifestations such as autonomic dysfunction which affects adaptation to physiologic and pathologic challenges. The mechanism of autonomic dysfunction in cirrhosis is unknown but several longitudinal studies have shown that autonomic dysfunction is linked with poor prognosis in this patient population [184, 196]. Autonomic dysfunction can be assessed non-invasively using a variety of methods based on computational analysis of heart rate fluctuations [184, 196, 217].

Heart rate variability (HRV) reflects the dynamic modulation of heart rate by the autonomic nervous system. Reduced HRV is widely reported in cirrhotic patients [90, 184, 185, 197, 218, 219], particularly, by Bhogal et al. who demonstrated that the HRV indices can predict mortality in cirrhotic patients, independent of their MELD (Model of End-Stage Liver Dysfunction) score - one of the most used grading systems used to predict short term mortality [184]. However, these indices were only linked to mortality in cirrhotic patients with normal sinus rhythm. In cirrhotic patients with abnormal sinus rhythm with recurrent premature ventricular complexes (PVCs) observed on a 24-Hour ECG recording, the predictive capacity of HRV indices may be challenging.

Heart Rate Turbulence (HRT) is the variation in the length of the cardiac inter-beat intervals after PVCs. In normal conditions, HRT is characterized by a defined pattern: an initial acceleration followed by a deceleration of heart rate [220]. HRT was first described by Schmidt et al. in a study that linked the absence of this phenomenon with a higher risk of mortality in post-myocardial infarction patients, independently of other risk factors [221]. From a physiological point of view, vagus nerve activity plays a significant role in HRT. Blood pressure drop after the PVC leads to a vagal withdrawal and subsequent heart rate acceleration. In reverse, vagal recruitment seems to provoke the heart rate deceleration leading to the pre-PVC values [222]. Sympathetic excitation during the first phase of post PVC period and sympathetic inhibition during the late phase have also been observed [223]. This evidence supports the theory that the blood pressure drop after the PVC triggers the baroreflex, which leads both to the vagal modulation of the sinus cycle and to the increase of sympathetic activity in the peripheral tissues. For all these reasons, the physiological background of HRT looks to be strongly determined by autonomic system regulation.

We have recently investigated the prognostic value of physio-markers (e.g., conventional HRV indices and body temperature variability) in a cohort of patients with cirrhosis [7]. However, it rapidly became apparent to us that most patients exhibit PVCs in their 24-hour ECG recording. In these patients, HRT could be considered for assessment of autonomic function. Jansen et al. demonstrated the relationship between two parameters of HRT (Turbulence Onset and Turbulence Slope) and cirrhosis. Whereby, the severity of cirrhosis correlated positively and negatively with TO and TS respectively [217]. Nevertheless, a correlation between HRT indices and mortality in cirrhotic patients was not investigated in that study or any other.

The present study reports our investigations to define whether HRT parameters could predict mortality in cirrhotic patients. We also aimed to understand if HRT parameters correlate with mortality independent of Child-Pugh's and MELD scores.

### *Hypothesis*

Heart rate turbulence indices measure the autonomic control of heart rhythm following premature ventricular contraction and can predict survival in patients with cirrhosis independent of the severity of the disease as measured by MELD.

### *Aims of study*

- To define whether HRT indices could predict mortality in cirrhotic patients.
- To understand if HRT indices correlate with mortality independent of Child-Child-Pugh's and MELD scores.

# Materials and Methods

### *Ethics*

Ethics approval for the main research [224] was obtained from the University of Padova Ethics Committee (4169/AO/17). Written informed consent was provided by all patients involved and data was collected and stored appropriately. Patients also gave their consent regarding the future use of the collected data.

### *Study Population*

#### *Sample size calculation*

The sample size was calculated to demonstrate an area under the curve of 75% in the ROC curve for predicting mortality using a physiological marker. With the assumption of 45% mortality during 12 months of follow-up, 40 participants were required to reach a significance level of 0.05 with a power of 0.80.

### *Participants*

Forty patients who had been diagnosed with liver cirrhosis were randomly approached, consented, and enrolled between the 6$^{th}$ of April 2017 and the 2$^{nd}$ of February 2019 at the Clinica Medica 5, Padova University Hospital. Recruited patients were classified by the aetiology of their liver diseases based on clinical, laboratory, radiological, and histological results. The severity of liver failure was staged using the Child-Pugh and the Model for End-Stage Liver Disease (MELD) score. Patients were excluded if they were under 16 years of age; had cirrhosis on a transplanted liver, had atrial fibrillation or implanted pacemaker, had severe co-morbidity with short prognosis such as sepsis, a history of neurological or psychiatric disease other than hepatic encephalopathy, active alcohol misuse, or were on psychoactive medication. The mean age of the eligible patients was 64.62 ± 10.4 years.

### *Data Collection*

24-hour electrocardiograph (ECG) recordings suitable for HRT analysis were obtained for research purposes using a wireless Holter recorder (Actiwave Cardio, CamNtech, Cambridge, UK). Patients were then followed up for 12 months and information was

collected on the occurrence of death/liver transplantation. Patients who were transplanted due to liver failure were classed as non-survivors as they were in immediate need of a new liver and wouldn't survive without transplantation [184]. Patients who could not be followed up for the 12 months were censored on the date contact was lost. Patients who underwent liver transplantation due to hepatocellular carcinoma were censored on the date of transplantation as the main reason for transplantation was treatment of malignancy and not complications of liver failure.

### *Heart Rate Turbulence*

Patients having at least one PVC during the 24-hour ECG recording were eligible for HRT analysis [222]. Indices of HRT were calculated for each patient using the HRV analysis software (version 1.2, June 2019 release) [225]. PVCs were detected using an algorithm developed by Pichot et al. [225]. In brief, PVCs were detected by calculating the prematurity of each beat and its compensatory pause with the mean of the five previous beats as the reference. If the prematurity was > 20% and the compensatory pause > 120%, then, the beat was considered as a PVC. The indices of HRT measured were Turbulence Onset (TO) and Turbulence Slope (TS). Standard HRT calculation involved the presence of PVCs usually advanced by early 2-3 reduced R-R intervals followed by 10-20 increased R-R intervals [225]. TO is the difference between the mean of the two R-R intervals after a PVC and the two R-R interval preceding the PVC divided by the mean of the two RR intervals precceding the PVC $[(R\text{-}R_1 + R\text{-}R_2) – (R\text{-}R_{-2} + R\text{-}R_{-1})] / (R\text{-}R_{-2} + R\text{-}R_{-1})$ where $R\text{-}R_{-2}$, $R\text{-}R_{-1}$ are the two R-R intervals before/preceeding the PVCs and $R\text{-}R_2$, $R\text{-}R_1$ are the two R-R intervals immediately after/proceding the PVCs. Total TO is measured in percentage (%) and is calculated as the mean of the TOs of all the sampled PVCs within a 24-hour ECG recording. Physiologically, TO should be negative as $RR_{-2} + RR_{-1}$ is expected to be higher than $RR_2 + RR_1$.

TS is the maximum positive regression slope of any consecutive five points up to the 20th R-R intervals proceeding/following a PVC. The total TS is measured in ms/RR and is the overall average of all the TS computed over a 24-hour Holter ECG recording. By convention, physiologically normal TS should be positive with a higher TS associated with both significant early reduced and late increase in R-R interval after a PVC [220].

To investigate the correlation between HRT and HRV in this cohort of patients, HRV indices were measured by calculating SDNN (standard deviation of inter-beat intervals) in the PVC-free sections of the 24-hour ECG using a computational filter [225]. Since SDNN is heavily affected by basal heart rate, corrected SDNN (cSDNN) for basal heart rate was calculated using the following formula as descried by Monfredi et al. [226]:

$$\mathrm{cSDNN} = \frac{\mathrm{SDNN}}{e^{-\frac{\mathrm{Heart\ rate}}{58.8}}}$$

Other conventional measures of HRV including RMSSD (root mean square of the successive differences of R-R intervals), SDANN (standard deviation of the average R-R intervals calculated over 5 minutes), pNN50 (the proportion of pairs of successive R-R intervals that differ by more than 50 ms divided by total number of R-R intervals), Ultra-Low Frequency (ULF), Very-Low Frequency (VLF), Low-Frequency (LF) and High-Frequency (HF) powers were also calculated using HRV analysis software (version 1.2, June 2019 release) [225].

### *Statistical and Survival Analysis*

To assess the relationship between the indexes of HRT (i.e. TO and TS) and the survival outcome of patients, we performed an independent t-test for normally distributed data and the Man-Whitney U Test for data not normally distributed. A significant level was determined with a p-value less than 0.05. We performed Cox regression to analyse the effect of HRT parameters on patient survival. We calculated the Cox regression coefficient ($\beta$) and the Hazard Ratio ($e^{\beta}$). The null hypothesis ($\beta=0$, $e^{\beta}=1$) was tested against a p-value of <0.05 calculated by the Wald test. The ROC curve was used to decide the best HRT indices cut-off points with combined optimum sensitivity and specificity for the prediction of survival. For survival analysis, we used the Kaplan-Meier graph, and log-rank (Mantel-Cox) test to determine whether the cut-off generated can distinguish the two groups. To estimate the magnitude of differences in HRT indices between the two groups (survivors vs non-survivors) we used Hedges' g estimation of effect size to compare the TO and TS of the survivors and the non-survivors. SPSS Statistics 20 (IBM Corp, Armonk, New York) was used for statistical analysis.

# Results

### *Study Populations*

The recruitment and followup of patients was initially for a different investigation to assess if temperature variability can predict survival in cirrhotic patients. Thus, this is a secondary analysis of the data which was collected previously by the authors specified in [227]. Overall 40 patients were followed up for up to 12 months post-recruitment. Of these, 30 (75%) were males. As expected, the mean (±SD) of the MELD score between survivors (17.82±1.76) and non-survivors (23.76±1.98) was significantly different (p=0.031). The mean (±SD) Child-Pugh scores of the survivor (8.90±0.43) and the non-survivor (10.54±0.58) were also significantly different (p=0.022). The mean age of all patients was 64 (range 45 to 84) years with no significant difference in the mean (±SD) age of the survivor (64.0±2.3) and non-survivor (65.0±2.5) groups (p=0.768). There was also no significant difference in gender proportions between the groups (P=0.637). The demographics and general clinical features of the study population are presented in Table *3.1*.

*Table 3.1. Demographic and clinical variables in the study population. The data are expressed as mean ± SD. MELD, Model for End-stage Liver Disease, INR, International Normalized Ratio.*

| | |
|---|---|
| Age (years) | 64.62 ± 10.4 |
| Gender (male/female) | 30/10 |
| Etiology of cirrhosis (number patients) | Alcoholic (17), Viral (10), Metabolic (5), Viral + Alcoholic (6), Viral + metabolic (1), Cryptogenic (1) |
| Main reason for hospital admission (number of patients) | Hepatic encephalopathy (15), Tense ascites (11), Hepatorenal syndrome (6), Bleeding esophageal varices (1), combination of reasons (7) |
| MELD | 20.4 ± 8.6 |
| Child-Pugh Score | 9.6 ± 2.3 |
| Child class (number of patients) | A (3), B (15), C (22) |
| On admission sodium level (mEq/L) [normal range] | 136 ± 6 [135-145] |
| On admission creatinine level (μmol/L) [normal range] | 133 ± 133 [female: 44-97, male: 53-106] |
| On admission albumin level (g/L) [normal range] | 29.0 ± 8.4 [35-55] |
| On admission bilirubin level (μmol/L) [normal range] | 105 ± 128 [5.1-17] |
| On admission INR [normal range] | 1.74 ± 0.65 [0.8-1.1] |
| On admission erythrocyte sedimentation rate (mm/hr) [normal range] | 34 ± 28 [Female: ≤ 20 mm/hr, Male: ≤15 mm/hr] |
| On admission SpO2 (%) [normal range] | 98 ± 2 [95-100] |
| On admission body temperature (ºC) | 36.7 ± 0.8 |
| On admission hear rate (bpm) | 75 ± 14 |
| On admission systolic arterial pressure (mmHg) | 128 ± 21 |
| On admission diastolic arterial pressure (mmHg) | 70 ± 11 |
| History of hypertension (+/-) | 5/35 |
| History of diabetes (+/-) | 6/34 |
| Beta-blocker (+/-) | 15/25 |

During the follow-up period, 12 (30%) deaths were recorded and 21 (52.5%) survived. The causes of death were sepsis (n=4), hepatorenal syndrome (n=2), HCC (n=2), myocardial infarction (n=2), secondary bacterial peritonitis (n=1) and acute alcoholic hepatitis (n=1). Five (12.5%) of the patients had liver transplantation for liver failure while 2 (5%) had liver transplantation due to hepatocellular carcinoma (HCC) (Figure *3.1*). Patients who underwent transplantation for hepatocellular carcinoma were censored on the date of transplantation. Patients who were transplanted due to liver failure were classed as non-survivors as they were in immediate need of a new liver and wouldn't survive without transplantation. Therefore, the hazard ratio was calculated based on 17 mortality events.

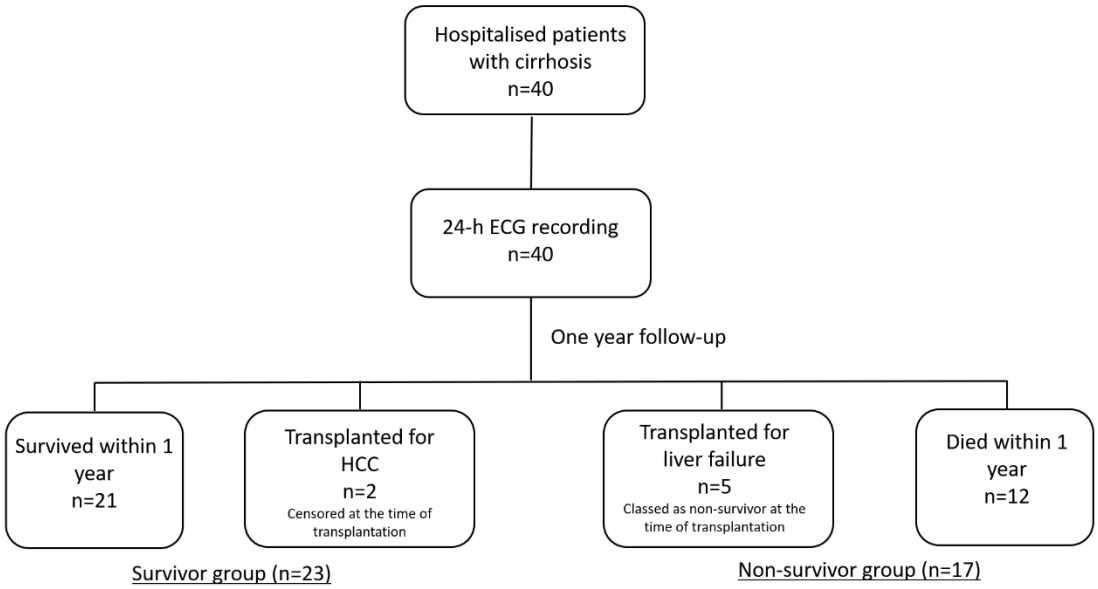



***Figure 3.1.*** *A flowchart of the study protocol. 40 patients with cirrhosis were followed up for one year. Patients who were transplanted due to liver failure were classed as non-survivors as they were in immediate need of a new liver and wouldn't survive without transplantation. Patients who underwent liver transplantation due to hepatocellular carcinoma were censored on the date of transplantation as the main reason for transplantation was treatment of malignancy and not complications of liver failure.*

### *HRT Indices between Survivor and Non-survivor*

The mean (±SD) number of PVCs during 24-hour recording was 27.87±35.47 and 31.88±40.76 in survivors and non-survivors respectively (p=0.7542). There was no

significant difference in the means of TS between the survivor and non-survivor. However, TO between the groups showed a significance difference (p=0.03, Table *3.2*). The effect size between the two groups was observed to be small in TS (Hedges' g = 0.2) while medium to large in TO (Hedges' g = 0.7).

*Table 3.2. The mean Heart Rate Turbulence indices of the study population.*

| | Survivors | Non-Survivors | p-value |
|---|---|---|---|
| Study Size | 23 | 17 | - |
| TO (%) | -0.01±2.6 | 1.42±1.3 | 0.03 |
| TS (ms/R-R) | 3.83±4.5 | 2.86±5.4 | 0.54 |
| No of PVCs in 24 hours: Median (range) | 7 (1-110) | 13 (1-155) | 0.742 |

*TS and TO data are expressed as mean ± SD. The level of significance is set at p<0.05. TO: Turbulence Onset, TS: Turbulence Slope, PVC: Premature Ventricular Contraction.*

*HRT and Survival*

To determine the relationship between HRT and mortality, Cox regression analysis was performed. Of the three indices (TO and TS and PVC number) analysed, only TO was significantly linked with mortality (Hazard Ratio = 1.351, $p<0.05$, Table *3.3*). With a hazard ratio of 1.351, translating into a 35% increase in mortality for every unit increase in TO.

*Table 3.3. The predictive effect of age, hepatic dysfunction, and indices of Heart Rate Turbulence on 1-year mortality. Univariate Cox regression analysis is used for the calculation of the hazard ratio.*

| | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| Age | 0.007 | 0.024 | 1.007 | 0.778 |
| MELD | 0.069 | 0.027 | 1.072 | **0.009** |
| Child-Pugh | 0.303 | 0.122 | 1.345 | **0.013** |
| TO | 0.301 | 0.122 | 1.351 | **0.014** |
| TS | -0.030 | 0.069 | 0.971 | 0.670 |
| No of PVCs in 24 hours | 0.000 | 0.006 | 1.000 | 0.987 |

*β is the coefficient of Cox regression analysis. SEM is the standard error of the mean of β, Hazard ratio =* $Exp(\beta) = e^{\beta}$*. TO: Turbulence Onset, TS: Turbulence Slope, PVC: Premature Ventricular Contraction.*

*HRT is independent of indices of liver failure in predicting survival*

Moving forward, we tested whether the predictive power of TO was independent of MELD and Child-Pugh scores. Cox regression analysis showed that TO significantly predicted mortality independently of MELD and Child-Pugh score (Hazard Ratio TO adjusted for Child-Pugh Score = 1.342, $p<0.05$ and Hazard Ratio TO adjusted for MELD Score = 1.290, $p<0.05$). As expected, MELD and Child-Pugh scores also showed a positive association with mortality, with higher scores correlating with poorer prognosis independently of the HRT score (Table *3.4* A and B).

*Table 3.4. The independence of Turbulence Onset from the MELD score (A) and Child-Pugh score (B) in predicting mortality in bivariate Cox regression analysis, TO: Turbulence Onset.*

| (A) | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| TO | 0.254 | 0.120 | 1.290 | 0.034 |
| MELD | 0.067 | 0.029 | 1.069 | 0.020 |

| (B) | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| TO | 0.295 | 0.138 | 1.342 | 0.033 |
| Child-Pugh | 0.297 | 0.128 | 1.346 | 0.020 |

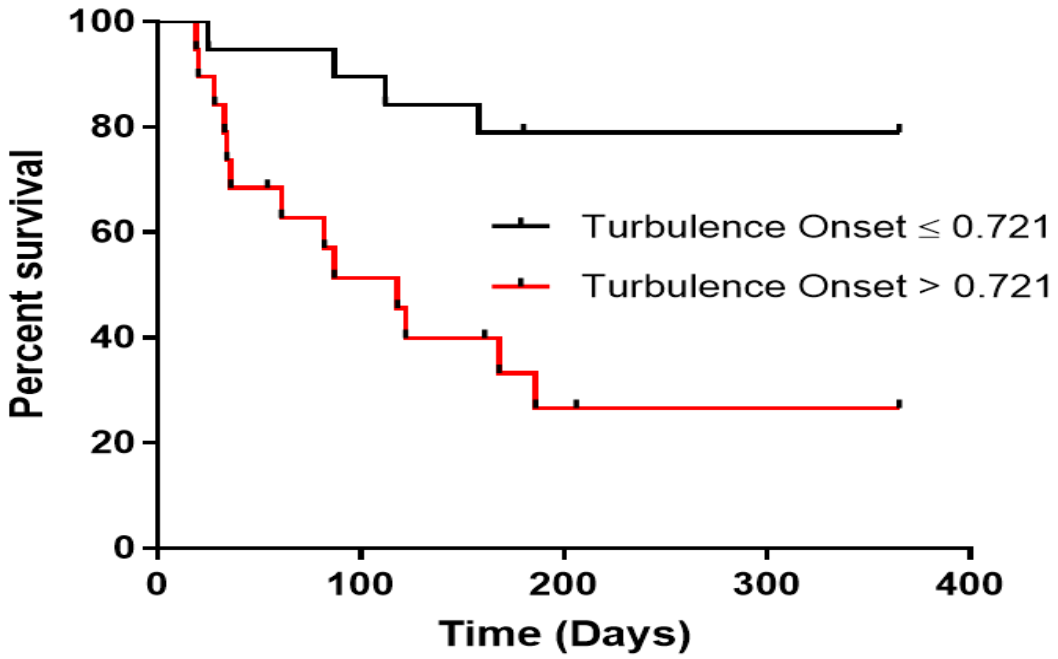
*Effect of Beta-Blocker on HRT*

Fifteen patients had received a beta blocker for the management of portal hypertension. We wondered if taking beta-blockers would affect HRT indices or survival rates in patients with cirrhosis. Basal heart rate was lower in cirrhotic patients who had received a beta-receptor blocking agent (81.8 ± 2.8 versus 64.4 ± 2.1 beats/min, $p<0.000$). TO was higher in patients with beta blocker medication (0.03 ± 0.41 versus 1.54 ± 0.57, $p<0.036$). However, there was no difference in TS between beta blocker-positive and beta blocker-negative groups (4.19 ± 1.15 versus 2.11 ± 0.64 ms/beat, $p=0.194$). Besides, receiving beta-blockers was not associated with an increase in mortality rate after 12 months of follow-up ($p=0.426$). Bivariate Cox regression analysis also showed that TO predicts mortality independently from beta-blocker treatment (Hazard ratio of TO adjusted for beta-blocker = 1.347, $p=0.02$). Bivariate Cox regression analysis also showed that taking beta-blocker is not a predictor of mortality in our cohort ($p=0.942$).

*Kaplan-Meier graph for Turbulence Onset*

Having shown that TO is significantly predictive of survival in cirrhotic patients independently of MELD, Child-Pugh, and beta-blocker treatment, Kaplan-Meier graphs were then obtained to further study this relationship. To determine the TO cut-off value which presents a balance between sensitivity and specificity in predicting survival, ROC curve analysis was performed. According to the ROC curve, the area under the curve for TO was 72.0 ± 8.1% ($p=0.019$) with a cut-off value of 0.721. This value distinguished cirrhotic patients with higher risk from patients with a lower risk of mortality with a sensitivity of 76.5% and a specificity of 65.2% (Appendix 15). This cut-off value significantly discriminated between patients with poor prognosis (TO>0.721%) and those with higher survival rate (TO≤0.721%) using a Kaplan-Meier survival analysis (Chi-

squared=7.5, p=0.0062, Log-rank Mantel-Cox test; Figure *3.2*).




| **Number of subjects at risk** | | | | | |
|---|---|---|---|---|---|
| Time (months) | 0 | 3 | 6 | 9 | 12 |
| **Turbulence Onset ≤ 0.721** | 19 | 18 | 15 | 11 | 11 |
| **Turbulence Onset > 0.721** | 19 | 10 | 6 | 3 | 3 |

***Figure 3.2.*** *Kaplan-Meier graphs illustrate how Turbulence Onset (TO) can predict survival in patients with cirrhosis. The survival graph depicts the overall survival of cirrhotic patients above and below the cut-off value for Turbulence Onset of 0.721% [Log-rank (Mantel-Cox) test, Chi-square = 7.500, p<0.01].*

### *Correlation between HRT and HRV indices*

As shown in ***Table 3.5***, both TS and TO were markedly correlated with SDNN and cSDNN (measures of total HRV). TS was also correlated with measures of short-term HRV such as pNN50, RMSSD, and HF. In contrast, TO showed significant correlations with measures of long-term HRV including SDANN, ULF, and VLF. Among HRV parameters only SDNN, cSDNN, SDANN and ULF were predictors of mortality as assessed by univariate Cox regression analysis (***Appendix 16***). As shown in ***Appendix 16***, none of the short-term HRV indices could significantly predict mortality.

*Table 3.5. Correlation between heart rate turbulence indices and heart rate variability indices in the study population.*

| Variables | TO | TS |
|---|---|---|
| SDNN | -0.382* | 0.322* |
| cSDNN | -0.413* | 0.410* |
| RMSSD | -0.085 | 0.522** |
| SDANN | -0.386* | 0.197 |
| pNN50 | -0.087 | 0.568** |
| ULF | -0.376* | 0.110 |
| VLF | -0.343* | 0.180 |
| LF | -0.156 | 0.255 |
| HF | -0.076 | 0.462** |

*TO: Turbulence Onset, TS: Turbulence slope, SDNN: Standard Deviation of inter-beat intervals, cSDNN: SDNN corrected for heart rate (cSDNN=SDNN/(e^- (Heart rate)/(58.8))). RMSSD: Root mean square of the successive differences of R-R intervals (a measure of short-term HRV). SDANN: Standard deviation of the average R-R intervals calculated over 5 minutes (a measure of long-term HRV). pNN50: The proportion of the number of pairs of successive RR intervals that differ by more than 50 ms divided by the total number of RR intervals. Ultra-Low Frequency (ULF), Very-Low Frequency (VLF), Low-Frequency (LF), and High-Frequency (HF) powers were calculated based on spectral analysis of HRV as described (14). Data are expressed as Pearson's correlation coefficient. * P<0.05, ** P<0.0001 to test the null hypothesis that there is no correlation between the indices (r=0).*

## Discussion

In this study, we report that an index of heart rate turbulence is a strong predictor of survival in patients with liver cirrhosis. Turbulence onset (TO) was discovered among other indices to be significantly correlated with mortality over a 12-month follow-up period. This predictive power was independent of prognostic markers of liver disease severity such as MELD and Child-Pugh scores. Our analysis also showed that although TO was higher in patients who took beta blockers, the prognostic value of TO did not depend on beta-blocker treatment.

The use of physiological biomarkers (physio-markers) for clinical assessment and prognosis in patients with chronic liver disease has been a topic of interest in recent years [90, 184, 185, 187, 196, 197, 217-219, 228, 229]. Much interest has been shown lately, especially in the use of indices of HRV as a prognostic tool in various diseases [230-235]. This is due to the ease of use and non-invasive nature of these methods. However, the presence of PVCs in ECG time-series compromises the viability of HRV indices as a physio-marker. HRT following PVCs is a common physiological feature characterized by proceeding increased and then reduced heart rates followed by a return to pre-ectopic rate (Figure *3.3*) [218]. Previously, HRT has been proposed to be controlled by the coordination of both arms of the autonomic nervous system, thus, indices of HRT have been purported as a good marker to assess autonomic neuropathy in disease settings [217, 221]. This is interesting as some indices of HRV, such as the short-term and long-term HRV have also been linked to the autonomic dysfunction in cirrhosis [90, 197, 236]. However, because of the dependence of conventional HRV methods on normal sinus rhythm, HRT turbulence can serve as a suitable alternative option especially since PVCs are expected in recorded time series [237]. Interestingly, we observed that TO was significantly correlated with the measures of long-term HRV while TS was associated with short-term HRV indices. Short-term HRV measures are mechanistically linked with the modulation of heart rate by the respiratory cycles; a physiological phenomenon mediated by the vagus nerve. Thus, it is not surprising to observe that short-term HRV indices are correlated with TS (a measure of vagus-mediated recovery of heart rate following a PVC). Only TO was an independent predictor of mortality in our cohort. This finding corroborates

with previous reports showing that only long-term measures of HRV are independent predictors of mortality in patients with cirrhosis [184, 219].

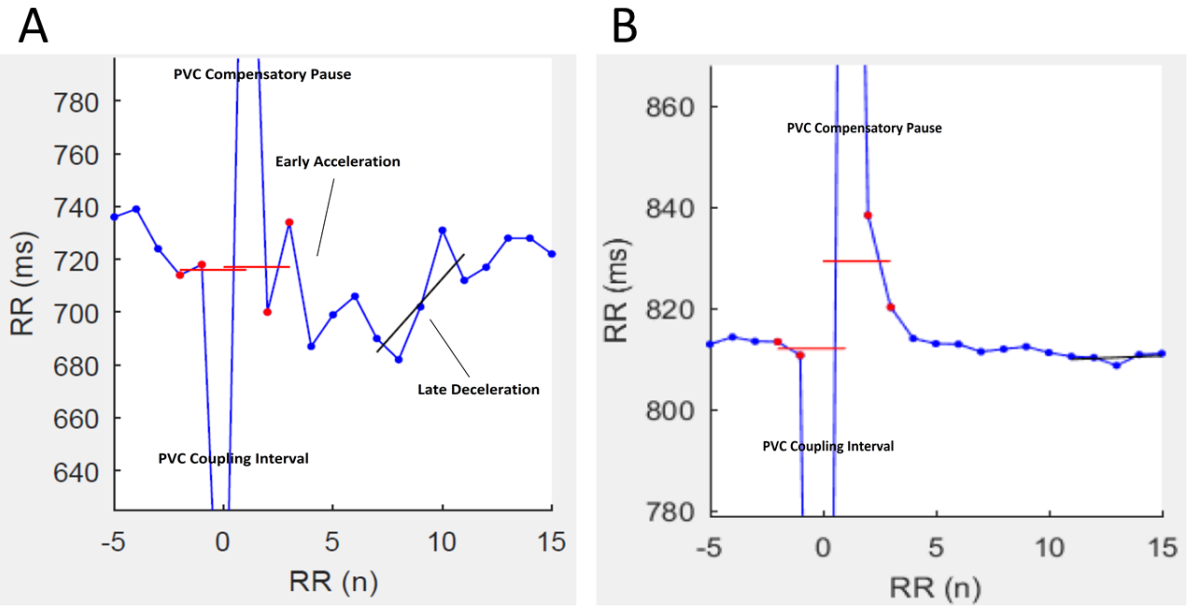



*Figure 3.3. Representative tachograms of heart rate turbulence (HRT) in two patients with cirrhosis. In patients (A) HRT is characterized by a post-PVC heart rate acceleration followed rapidly by a deceleration and then a return to the pre-PVC rate. A post-PVC heart rate fade-off in patient B is characteristic of autonomic dysfunction.*

Although the present study indicates that impaired HRT following PVCs is a predictor of mortality in patients with cirrhosis, the reason for this observation is not well understood. It can be speculated that impaired HRT or reduced HRV in cirrhosis may reflect the presence of cirrhotic cardiomyopathy. Indeed, cirrhotic patients often present with subclinical cardiomyopathy [238, 239]. However, there has been no report to show a significant correlation between indices of autonomic cardiac control (i.e., HRT, HRV) and clinical measures of cirrhotic cardiomyopathy in cirrhotic patients. Therefore, whether changes in HRT reported in cirrhosis reflect the presence of cirrhotic cardiomyopathy remains unclear and awaits further investigations. According to studies on animal models of cirrhosis, pharmacological interventions (e.g., nitric oxide synthase inhibitors and low molecular thiols) that improve cardiac function in cirrhotic rats do not improve cardiac autonomic dysfunction in these animal models [240]. This suggests that cirrhotic

cardiomyopathy and autonomic dysfunction in cirrhosis probably have different mechanisms and should not be considered as the same phenomenon.

Cardiac autonomic dysfunction is a hallmark of cirrhosis [184, 196, 217] and might be helpful for patients' prognostication for liver transplant allocation procedures. However, before suggesting the use of HRT as a prognostic factor for organ allocation, it is crucial to know whether autonomic dysfunction responds positively to liver transplant. It appears that autonomic dysfunction is improved only partially following transplantation in patients with cirrhosis [196, 241]. Thus, measures of autonomic dysfunction (e.g., impaired HRT) might be considered as a co-morbidity factor in the process of organ allocation [241]. In our study, among 17 non-survivors, 2 patients died of myocardial infarction which accounts for 11.7% of non-survivors. When we looked at their HRT indices, both patients had a TO higher than the cut-off value (0.734 and 1.016). This suggests that HRT may also identify subgroups of patients who suffer from co-morbidities (e.g., ischemic heart disease) not directly associated with liver failure. To test whether our results have been confounded by this co-morbidity, we retrospectively excluded those two cases who died of myocardial infarction and only included patients who died because of complications directly associated with cirrhosis (Appendix 18). The results showed that TO remains a significant predictor of survival independently of MELD and Child-Pugh's scores even after excluding patients whose cause of death was myocardial infarction (Appendix 19 and Appendix 20). Although this result shows that HRT predicts survival independently of liver dysfunction in cirrhosis, further studies are required to elucidate potential clinical applications of the link between impaired HRT and survival in cirrhosis.

In the present study, we determined a cut-off value for TO for the prediction of mortality in cirrhosis and did not report our results based on previously reported cut-off values (e.g., 0% for TO and 2.5 ms/R-R for TS) and categories of HRT (0-1-2) which were originally shown to be predictive for mortality in patients with acute myocardial infarction (MI) [220-222]. Although both acute MI and cirrhosis exhibit autonomic dysfunction, distinctive components of autonomic function appear to be involved in these two illnesses [7, 242]. Following acute MI, both TO and TS can predict mortality [221, 222]. However, in patients with cirrhosis TS is not significantly different between survivors and non-survivors and only TO is a predictor of mortality. Such a difference between acute MI and cirrhosis has

also been shown in studies where non-linear measures of HRV were used for the determination of prognosis. It appears that short-term fractal scaling (α1) of HRV is a powerful predictor of mortality among patients surviving an acute myocardial infarction while only long-term fractal scaling (α2) predicts mortality in patients with cirrhosis [7, 242]. Based on these reports, it is not surprising to observe that prognostic models that have been developed for acute MI may not apply to cirrhosis. As shown in Appendix 21, we applied categories HRT (0-1-2) for patients with cirrhosis and observed that although there is a significant difference in mortality between HRT=0 and HRT 1 or 2, cirrhotic patients with HRT=1 exhibit a higher rate of mortality in comparison with HRT=2 in the first 6 months of their follow up (Appendix 21). TS is not a predictor or death in patients with cirrhosis and this may explain why HRT categories that are based on a combination of TO and TS are not suitable for survival analysis in cirrhosis.

*Limitations*

Because data were collected from patients admitted to the hospital for decompensated liver disease, the result cannot be extrapolated to outpatients with less severe (compensated) cases of cirrhosis. Also, the sample size, although sufficiently powerful, represents a single Centre, random sub-population and may not represent the full spectrum of patients with liver cirrhosis. For further validation of the use of HRT as a prognostic physio-marker, we recommend the use of a larger and more diverse patient population validated with a healthy control group probably involving multiple and varied clinical settings.

The complexity of the clinical environment in hospitalized patients did not allow us to study circadian variations in HRT parameters in our study. It is well-established that cirrhosis is associated with circadian abnormalities (34, 35). Previous reports have also shown that there are circadian oscillations in HRT indices particularly in the TS (36). Future studies in controlled laboratory settings may indicate a difference in circadian variations of HRV/HRT indices in survivor and non-survivor groups.

Although we initially aimed to assess cardiac autonomic function in the presence of cardiac arrhythmia in patients with cirrhosis, our results can only be applied when PVCs disrupt normal sinus rhythms. The main challenge for the assessment of autonomic

function from R-R intervals is the presence of atrial fibrillation. Atrial fibrillation limits the interpretability of both HRV and HRT methods and requires further attention in future investigations.

### *Conclusion*

In conclusion, we report in this pilot study that TO, an index of heart rate turbulence, as a physio-marker may predict survival in cirrhotic patients. We also report a cut-off of TO that significantly distinguishes patients at higher risk of mortality within 12 months.

# Chapter 4 : Parenclitic Networks analysis for prognosis and survival modelling in cirrhosis

# Introduction

The liver is a physiological hub for multiple homeostatic, metabolic, synthetic, and immune functions. Thus, patients with liver failure exhibit various neural, renal, cardiovascular, endocrine, and metabolic manifestations. Cirrhosis (chronic fibrosis of the liver) is a complex disease of global significance [243] involving multiple organ systems and functions. Thus, the interpretation of organ dysfunction without consideration of the entire system has presented paradoxical and often insufficient insight into cirrhosis. This is most often evident in the relative difficulty in the management of complications of cirrhosis whereby targeting a single organ dysfunction may lead to the dysregulation of other tightly balanced pathways [244]. This predicts treatment response and prognosis especially challenging and further complicates the prioritization of liver transplantation [245, 246]. Indeed, the introduction of several prognostic scores and models such as Child-Pugh, MELD, and UKELD amongst others is in direct response to the complexity of decompensated cirrhosis and while these models have been useful, various limitations continue to surface [247].

The future of disease diagnosis, management, and prognosis may benefit from a network physiology approach providing a holistic view of the changes in the physiological interactome leading to disrupted states. Network physiology focuses on complex interactions among diverse organ systems in health and disease [14] and may provide a viable alternative to the conventional scoring methods and facilitate the evaluation of organ system interactions in complex disorders such as cirrhosis. Early work by Asada et al., on critically ill patients in intensive care showed a disrupted network of organ systems interaction in non-survivors [11]. In a recent report, we showed that functional connectivity of organ systems is significantly disrupted in patients with cirrhosis who did not survive during 12-month follow-up [15]. However, the methodology of these studies is based on correlation analysis of a population of patients and cannot be used for mapping the network connectivity at the level of individual patients. Hence, these reports provide insight into the pathophysiology in general but don't satisfy clinical application on individual subjects [11].

The parenclitic network analysis was proposed by Zanin et al., in 2014 to create a network from the perspective of an individual subject in a population [248]. Instead of looking at

the network of connections in a population, this approach provides a method for mapping a network for each subject, where nodes represent features and links are weighted according to the *deviation* between a subject's features and their corresponding typical relationship within a studied population ("Parenclitic" mean "deviation" in Greek) [248, 249]. In its simplest form, the model can be a simple linear regression between all possible pairs of features in the population, followed by the calculation of deviations between values of a particular subject and pre-constructed reference models (Figure 5.1). A network map is then constructed for individual subjects whereby each feature represents a node and deviation from the reference model is defined as edges between the nodes. The topological characteristics of the resulting network of individual subjects can be used to extract valuable information about the relationships of the system. Since its first description, parenclitic network analysis has been used in genetic mapping of cancer [250-252], Down syndrome [253], aging [254], and even criminology [255] and continues to open new insights into complex systems.

### *Hypothesis*

Could network analysis then be performed on standard clinical/laboratory data of individual patients with cirrhosis and could this provide insight into the changes in "interactome" associated with adverse outcomes of cirrhosis? If yes, can this change in the network of organ systems connectivity predict survival independent of current measures of severity such as MELD?

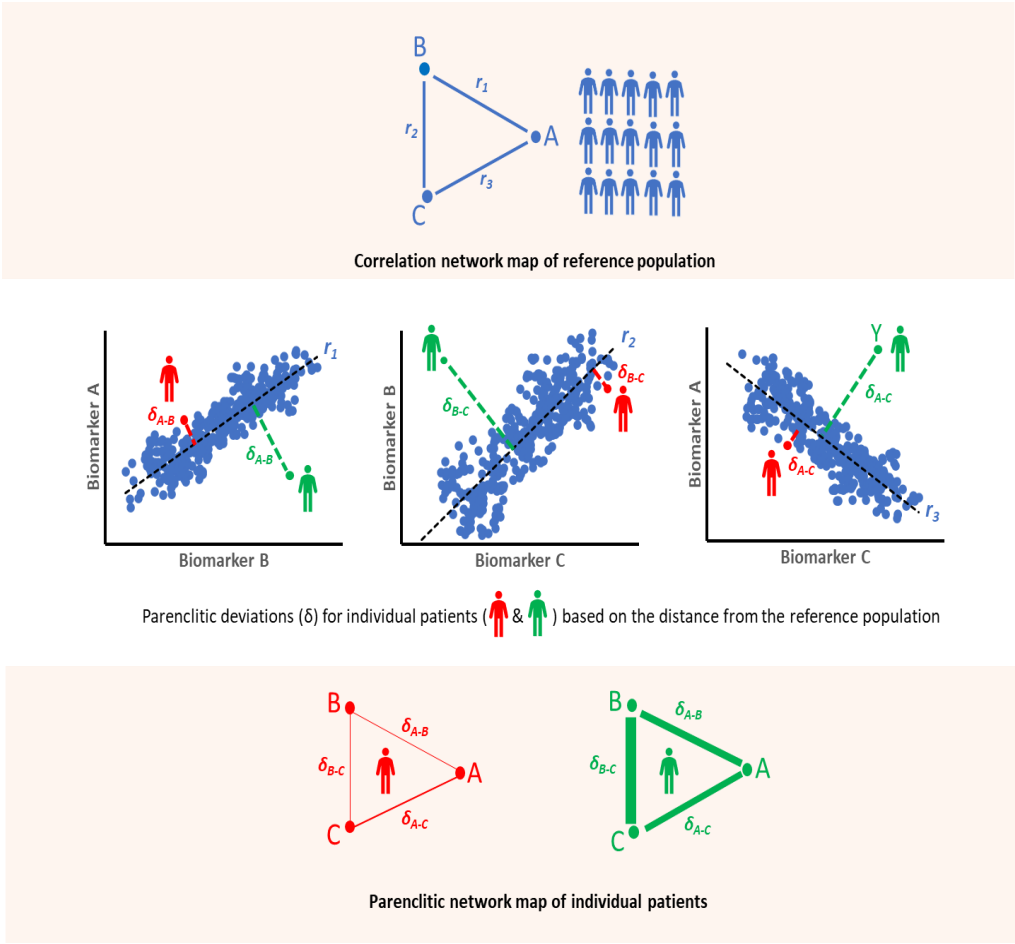



*Figure 4.1. A schematic representation of the network mapping method used in this study for the reference population (top panel) as well as individual patients (lower panel).*

*Top panel: The correlation between a pair of biomarkers (e.g., A-B, A-C, or B-C) was used for network mapping of the reference population (i.e., survivors in the standard care group). The blue dots represent individual reference data, the black regression lines represent the expected relationship models ($r_1$, $r_2$, and $r_3$ represent statistically significant correlation coefficients).*

*Middle panel: To map the network of individual patients a parenclitic approach was used. Parenclitic analysis measures the deviations of an individual patient from the expected relationship between variables in the reference population. In other words, parenclitic deviation indicates how far an individual biomarker level is from the expected model. In this example, the patient represented in red is closer to the reference population than the patient represented in green, in terms of the correlation between the biomarkers. Hence, the green patient has a higher parenclitic deviation (δ) than the red patient.*

*Lower panel: The resulting parenclitic network map of nodes A, B, and C is presented with edges weighted (in terms of thickness) according to the magnitude of deviations from the models for two individual patients (red and green). Higher thickness in edges in the green patient shows higher parenclitic deviation and thus, less functional connectivity between biomarkers. The red patient has less parenclitic deviation which means closer association with the reference model and higher functional connectivity between biomarkers.*

*Aims of study*

- To apply a parenclitic approach on standard clinical and laboratory variables for mapping and quantifying the physiological network of organ systems of individual patients with cirrhosis.
- To assess whether parenclitic network analysis could predict survival independently of MELD and Child-Pugh scores.

# Method

### *Ethics*

Ethical approval for this work was ratified by the Padova Hospital Ethics Committee. Written informed consent was provided by all participants included. The protocol for this study aligns with Good Clinical Practice (European) guidelines and was conducted following the Declaration of Helsinki (Hong Kong Amendment).

### *Patients Cohorts*

A total of 106 patients diagnosed with cirrhosis met the inclusion criteria for this study. Specifically, patients referred to the tertiary referral liver centre of the Clinica Medica V, University Hospital of Padova for formal hepatic encephalopathy assessment were recruited and enrolled between 2009 and 2018. This study involves a secondary analysis of the data collected by Formentin et al to assess the prognostic value of neurophysiological and neuropsychological indices in patients with cirrhosis [256].

### *Inclusion criteria*

Only patients with a confirmed diagnosis of cirrhosis based on clinical manifestations and/or liver imaging were included. Patients below the age of 16 years or above 80 years; or diagnosed with hepatocellular carcinoma; any severe co-morbidity with short prognosis; who had previously transplanted liver; significant head injury, neurological or psychiatric disease not classified as hepatic encephalopathy; active misuse of alcohol or acute infection were excluded from this study.

### *Follow up*

Patients with cirrhosis that meet the inclusion criteria were studied in retrospect and separated based on 12-month follow-up periods. Indeed, patients who underwent liver transplantation during follow-up were classified as non-survivors as their survival depended on the transplanted organ [215].

*Clinical laboratory Variables*

A total of 7 standard clinical variables representing various pathways, organ systems or clinical features were compiled (i.e., serum sodium, Na; hepatic encephalopathy, HE; total bilirubin, Bil; serum albumin, ALB; prothrombin time, PT; serum creatinine, Cr; ammonia, $NH_4$) based on a previous report [15]. Hepatic encephalopathy was classified into 3 stages based on a previously described model by Montagnese et al [257, 258] i.e., unimpaired, minimal, or overt.

*Network generation in the population*

Recruited patients were grouped according to their 12-month survival status into two classes and a network map based on significant linear regression was computed for each one of the classes [15]. This was to allow for the visual inspection of the correlation network map of the classes whereby edges were drawn between clinical variables (nodes) if the correlation was significant. Thus, pair-matched Spearman's correlation was computed to correct for missing data and significance was based on Bonferroni-corrected p-value (i.e. $p \leq 0.0024$) [259]. Pairs of biomarkers' p-values above the threshold for significant correlation were excluded from the downstream analysis. The correlation network maps for groups (survivors and non-survivors after a 12-month follow-up time) were then visually compared (Figure *4.2*).

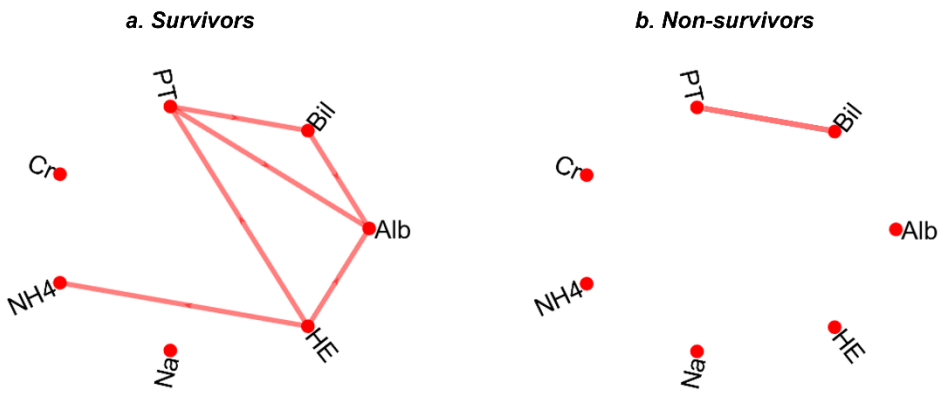



*Figure 4.2. Correlation network map of survivors (a) and non-survivors (b) following a 12-month follow-up period. The map is based on a pairwise Spearman's correlation's correlation based on a Bonferroni-corrected significant level (p = 0.0024). serum albumin, Alb; total bilirubin, Bil; prothrombin time, PT; serum creatinine, Cr; ammonia, NH4; serum sodium, Na; and hepatic encephalopathy, HE.*

### *Parenclitic network*

#### *Deviation values calculation (δ)*

Twelve-month survivors were used as the reference population to construct a regression model based on Bonferroni-corrected significantly correlated pairs of biomarkers listed above. A significant correlation was found between 6 of the 21 computed pairs of correlations in the survivor group. There is only one significant correlation between biomarkers in the non-survivor group (***Figure 4.2***b). The deviations between the data of all individual patients (survived and non-survived) from the pre-constructed reference model were then calculated as orthogonal residuals of the regression lines of each pair of correlated variables (Figure *4.1*a-d)

#### *Network topology analysis*

Network topology analysis describes the underlying dynamics of a connected system. The network topology analysis of physiological functions has been shown to provide information on the adaptability and dynamic flexibility of organ systems to changes in

environmental conditions [14]. Several network topology metrics weighed by parenclitic deviations were used to assess the changes in physiological connectedness in patients with cirrhosis. These include network in-degree centrality, shortest path length, global diameter, and efficiency. Appendix 22 presents the definitions and mathematical formulae of these indices.

### *Software development*

The software for computing the parenclitic network outputs was written in-house using MATLAB build R2021a [260] according to the originally described technique [248]. In summary, the software extracts and uses the data of the survivors to compute a Bonferroni-corrected regression model for all pairs of physiological variables (e.g., Na-Alb, Alb-Bil). The parameters (slope and intercepts) of significantly correlated pairs were used to compute the vertical and horizontal residuals (y and x respectively) which were then used to find the orthogonal residuals (delta, $\partial$) for all patients (survivors and non-survivors) as follows:

$$\partial = \frac{x.y}{\sqrt{x^2+y^2}}$$

The computed $\partial$'s were used as the weight of the connections between all correlated pairs of variables for all patients. Further, the individual $\partial$-weighted parenclitic network graphs are then used to perform the global network topology analyses. All computed results were combined into an output table which is labelled with the combined names of the variable pairs for the $\partial$'s and the computed network topology indices. The table is then written into a named, dated output saved in the workspace as a single excel file for further statistical analysis. The software is available in the GitHub repositor; https://github.com/topeoyelade.

### *Statistical analysis*

Statistical analysis was performed using both MATLAB build R2021a [260] and SPSS Statistics 26 (IBM Corp., Armonk, New York) [261]. Initially, a Receiver Operating Curve (ROC) analysis was performed and the Area Under the Curve (AUC) was used to generate cut-off values that combine optimum sensitivity and selectivity in differentiation

between the survivors and non-survivors for all computed output variables. Mann-Whitney U-test was used to compare the means of all output variables (∂'s and computed network topology indices) between the survivors and non-survivors. We performed Kaplan-Meier and log-rank (Mantel-Cox) tests to assess whether the cut-offs from the ROC analysis can distinguish the groups. Further, bivariate Cox regression was computed to assess whether the significantly different variables with survival prediction can predict mortality independent of MELD and Child-Pugh scores. The combined prognostic index (e.g., MELD-∂) was calculated using the regression coefficients according to the following equation: $MELD - \partial\ \text{index} = \beta_1\ MELD\ +\ \beta_2\ \partial$ where $\beta_1$ and $\beta_2$ are the regression coefficient of MELD and ∂ in bivariate Cox model respectively. Data are presented as median and interquartile range (IQR) and the significant level was defined as a two-tailed p-value < 0.05 in all analyses.

*Measurement of the performance of predictive models*

Assessment of improvement in prognostic value resulting from the addition of new variables to an existing one is computed using Brier scores, Integrated Discrimination Improvement (IDI), and Net Reclassification Indices (NRI). The brier score was first described in 1950 by Glenn W. Brier and measures the squared mean contrast between the reported risk of an event and the risk predicted by a probabilistic model [262]. Generally, models with higher predictive accuracies are associated with lower Brier scores compared with those with lower predictive accuracies. IDI and NRI are forms of risk reclassification methods aimed at showing the improvement in risk classification by a model following the addition of a new variable or marker [263]. The difference between NRI and IDI is mainly that NRI is based strictly on categorical risk stratification and is not robust to intermediate-risk groups. Thus, for the subpopulation with intermediate risk, the use of NRI may lead to an increase in the risk of type I error (false positive predictions) [264]. The IDI on the other hand is a category-free model performance calibrator and estimates the performance of a model across all risk levels [263]. Brier score, IDI, and NRI were computed for MELD and the composite variables including MELD and the parenclitic indices using standard statistical software Stata/MP version 17.0 (Stata Corp LP, College Station, Texas, USA)

# Results

## *Study Population*

Overall, 106 patients diagnosed with cirrhosis were followed up for 12 months. During the follow-up periods, 17 deaths were recorded; 14 patients underwent transplantation due to liver failure or associated complications and were recorded as dead as they were considered to need a new liver to survive. The demography and clinical characteristics of the studied population are described in Table 4.1. Baseline biomarkers as well as MELD and Child-Pugh scores are presented in Appendix 23 which shows a significant difference in most baseline biomarkers, MELD, and Child-Pugh scores between survivors and non-survivors.

*Table 4.1. Demographic and clinical variables in the study population.*

| | All patients (n=106) |
|---|---|
| Age [Median (min-max)] (years) | 58 (24-80) |
| Gender (male/female) | 82/24 |
| Aetiology of cirrhosis (alcohol/viral/others) (%) | 42/34/24 |
| MELD score [Median (min-max)] | 12 (6-38) |
| Child-Pugh score [Median (min-max)] | 8 (5-14) |
| Child class A/B/C | 21/55/30 |

*Min; minimum value, max; maximum value, MELD; Model for end-stage liver disease.*

## *Parenclitic deviation (∂'s) of survivors and non-survivors*

Parenclitic deviations were compared between survivors and non-survivors and the results are shown in Table 4.2. Based on the Mann-Whitney U-test, there were increased parenclitic deviations in Alb-Bil ($p < 0.001$) and Alb-PT ($p=0.004$), and Alb-HE ($p=0.034$) axes compared with the non-survivors (Table 4.2).

*Table 4.2: Comparison of parenclitic deviations of the studied population.*

| ∂ of variable pairs | Survivors; median (IQR) | Non-Survivors median (IQR) | p-value |
|---|---|---|---|
| Albumin-Bilirubin | 2.08 (1.07 – 2.83) | 5.09 (2.79 – 10.05) | **< 0.001** |
| Albumin-Prothrombin Time | 2.48 (1.14 – 4.12) | 4.83 (2.34 – 6.39) | **0.004** |
| Albumin-Hepatic Encephalopathy | 0.50 (0.28 – 0.76) | 0.63 (0.38 – 0.98) | **0.034** |
| Ammonia-Hepatic Encephalopathy | 0.59 (0.25 – 0.80) | 0.94 (0.30 – 1.28) | 0.121 |
| Bilirubin-Prothrombin Time | 5.73 (3.58 – 8.90) | 5.60 (2.31 – 11.49) | 0.481 |
| Hepatic Encephalopathy-Prothrombin Time | 0.60 (0.16 – 0.88) | 0.58 (0.10 – 0.98) | 0.827 |

*∂, parenclitic deviation; IQR, interquartile range.*

### *Parenclitic deviations in predicting survival*

Univariate Cox regression showed a significant link between a higher risk of mortality and parenclitic deviations along the Alb-Bil, Bil-PT, and Ammonia-HE axes (Table 4.3). Higher deviation in the Alb-PT axis resulted in a 20% increased risk of 12-month mortality (95% CI, 6% - 35%, $p < 0.001$). Finally, deviation in the Alb-HE axis was linked with a 3-fold increased risk of mortality after a 12-month follow-up period (95% CI, 5% - 7-fold, $p = 0.004$: Table 4.3).

*Table 4.3. Univariate Cox regression analysis of the parenclitic deviations.*

| ∂ of variable pairs | β | SEM | Hazard Ratio (95% CI) | p-value |
|---|---|---|---|---|
| Albumin-Bilirubin | 0.128 | 0.024 | 1.137 (1.084 – 1.192) | **< 0.001** |
| Albumin-Prothrombin Time | 0.179 | 0.062 | 1.195 (1.059 – 1.349) | **0.004** |
| Albumin-Hepatic Encephalopathy | 1.005 | 0.487 | 2.732 (1.052 – 7.099) | **0.039** |
| Bilirubin- Prothrombin Time | 0.030 | 0.006 | 1.030 (1.018 – 1.043) | **<0.001** |
| Hepatic Encephalopathy-Prothrombin Time | 0.324 | 0.467 | 1.383 (0.554 – 3.451) | 0.487 |
| Ammonia-Hepatic Encephalopathy | 1.369 | 0.606 | 3.933 (1.200 – 12.887) | **0.024** |

*∂, parenclitic deviation; β, coefficient of Cox regression analysis; SEM, standard error of the mean of β, CI, confidence interval.*

### *Independence of parenclitic deviations in predicting survival.*

To assess whether the ability of the parenclitic deviations to significantly predict survival is independent of the index of liver disease severity (MELD), we performed bivariate Cox regressions for parenclitic deviations with MELD as a covariate. The parenclitic deviation along the Alb-Bil (Hazard Ratio, 95% CI = 1.063, 1.000 -1.129; p = 0.048) and Alb-PT (Hazard Ratio, 95% CI = 1.138, 1.012 – 1.280; p = 0.031) axes predicted 12-month survival independent of MELD (**Table *4.4***). To study this further, we looked at the independence of parenclitic deviations from the Child-Pugh score, a classic measure for the severity of hepatic dysfunction. Our results showed that parenclitic deviation of the Alb-Bil, Alb-PT, and Bil-PT predicted 12-month survival independent of Child-Pugh scores (***Appendix 24***).

*Table 4.4. The prognosis effects of parenclitic deviations independent of MELD using bivariate Cox regression analysis.*

| ∂ with MELD | β | SEM | Hazard Ratio (95.0% CI) | p-value |
|---|---|---|---|---|
| Albumin-Bilirubin | 0.061 | 0.031 | 1.063 (1.000 – 1.129) | 0.048 |
| MELD | 0.119 | 0.038 | 1.126 (1.047 – 1.213) | 0.002 |
| Albumin-Prothrombin Time | 0.129 | 0.060 | 1.138 (1.012 – 1.280) | 0.031 |
| MELD | 0.435 | 0.092 | 1.166 (1.109 – 1.251) | <0.001 |
| Albumin-Hepatic Encephalopathy | 0.702 | 0.501 | 2.017 (0.756 – 5.383) | 0.161 |
| MELD | 0.152 | 0.030 | 1.164 (1.099 – 1.229) | <0.001 |
| Bilirubin-Prothrombin Time | 0.013 | 0.008 | 1.014 (0.997 – 1.030) | 0.101 |
| MELD | 0.143 | 0.033 | 1.153 (1.082 – 1.229) | <0.001 |
| Ammonia-Hepatic Encephalopathy | 1.093 | 0.628 | 2.983 (0.870 – 10.219) | 0.082 |
| MELD | 0.138 | 0.032 | 1.148 (1.078 – 1.223) | <0.001 |

*∂, parenclitic deviation; β, coefficient of Cox regression analysis; SEM, standard error of the mean of β, Ci, confidence interval; MELD, Model for End-stage Liver Disease.*

### *Receiver Operating Characteristics (ROC) curves of parenclitic deviations.*

ROC curves were computed for the parenclitic deviations that predicted 12-month survival independent of MELD. The deviation along the Alb-Bil axis showed a similar AUC in comparison with MELD (0.762 versus 0.792). As shown in Figure *4.3* and Table *4.5*, the addition of parenclitic deviation of Alb-Bil and Alb-PT axes increased the AUC for MELD from 0.792 to 0.835 and 0.824 respectively ($p<0.001$). Further, the Brier score shows that the addition of parenclitic deviation of Alb-Bil improves the predictive value of MELD Table *4.5*.

*Table 4.5. The area under the ROC curves (AUC) of parenclitic deviations (∂), MELD, and combined MELD-∂ during 12-month follow-up periods.*

| Prognostic index | AUC (95% CI) | p-value | Brier Score |
|---|---|---|---|
| Albumin-Bilirubin | 0.762 (0.652 – 0.872) | < 0.001 | 0.539 |
| Albumin-Prothrombin Time | 0.696 (0.569 – 0.824) | 0.004 | 0.335 |
| MELD | 0.792 (0.696 – 0.888) | < 0.001 | 0.107 |
| MELD-$\partial_{\text{Albumin-Bilirubin}}$ | 0.835 (0.747 – 0.924) | < 0.001 | 0.106 |
| MELD-$\partial_{\text{Albumin-Prothrombin Time}}$ | 0.824 (0.730 – 0.918) | < 0.001 | 0.195 |

*CI, confidence interval; AUC, area on the receiver operating curve.*

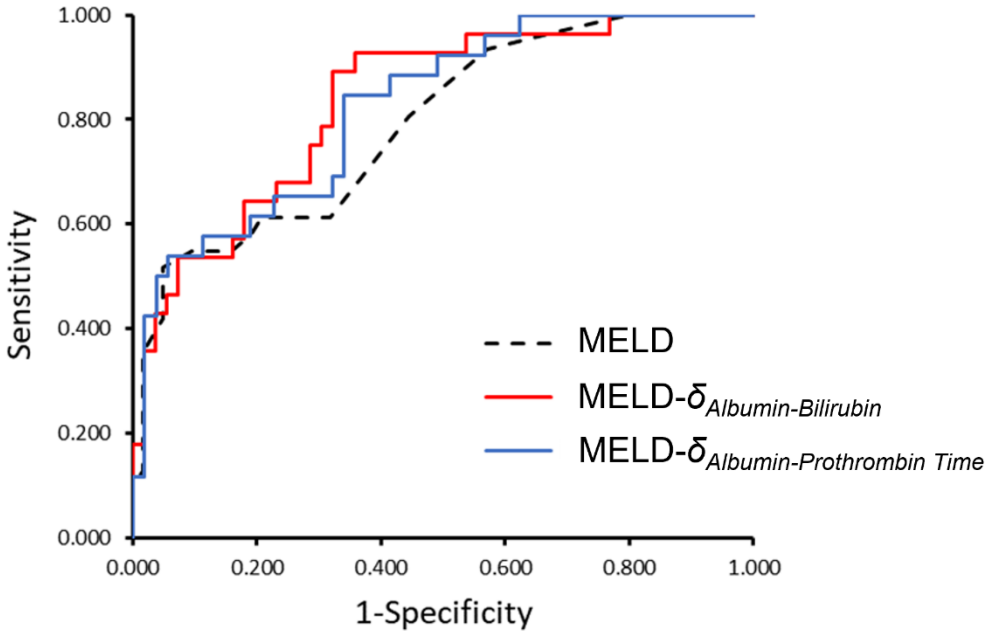



*Figure 4.3. The ROC curves comparing MELD alone with MELD-δAlb-Bil and MELD- δAlb-PT in classifying patients as survivors or non-survivors. Addition of parenclitic deviation of Alb-Bil and Alb-PT axes could increase the AUC for MELD from 0.792 (95% CI, 0.696 – 0.888) to 0.835 (0.747 – 0.924) and 0.824 (0.730 – 0.918) respectively (p<0.001 for all curves).*

### *Kaplan-Meier graphs of parenclitic deviations.*

For the parenclitic deviations that were significantly predictive of survival independent of MELD, cut-offs with the optimum sensitivity and specificity were generated from their ROC curves (i.e., optimum sensitivity and specificity for prediction of survival). The deduced cut-offs were then used to group the patients into group "predicted non-survivor" if the patients' parenclitic deviations were higher than or equal to the corresponding cut-off values or "predicted survivor" if otherwise. The binary output was then used to generate Kaplan-Meier graphs to assess the prognostic value. Figure *4.4* indicates that both Alb-Bil and Alb-PT deviations can predict 12-month survival with a statistically significant log-ranked test (Chi-square 19.03 and 7.81 respectively). Furthermore, the addition of Alb-Bil or Alb-PT deviations to MELD in a bivariate Cox regression model enhances the prognostic value of MELD alone (Figure *4.5*).

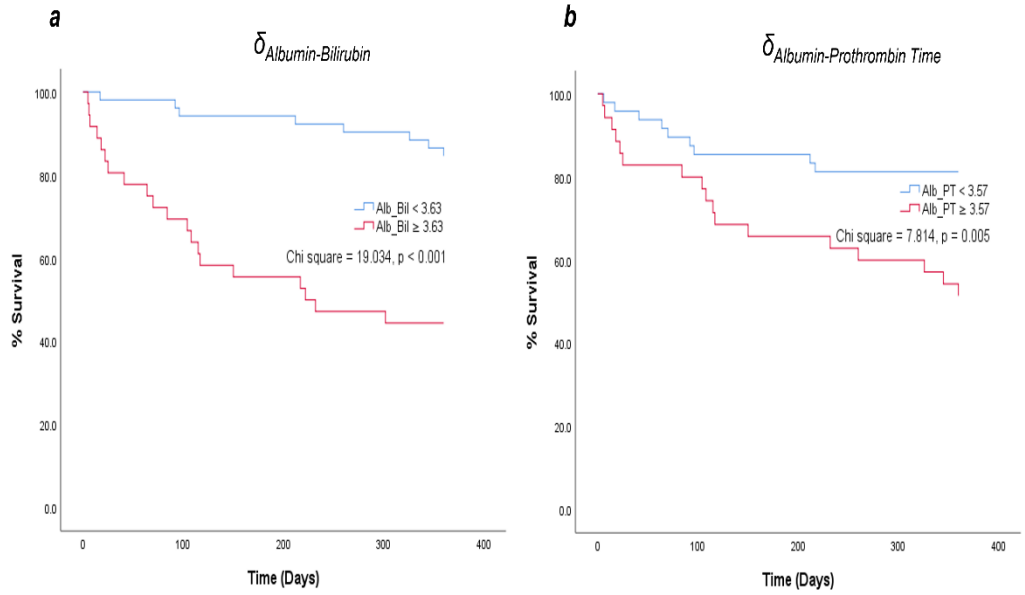



*Figure 4.4. Kaplan-Meier graphs showing 12-month survival predictions of parenclitic deviations along the (a) Albumin-Bilirubin (Alb_Bil) and (b) Albumin-Prothrombin Time (Alb_PT) axes based on the cut-off values of 3.63 and 3.57 respectively [Log-rank (Mantel-Cox) test, Chi-square = 19.034, p < 0.001 and 7.814, p = 0.005 respectively].*

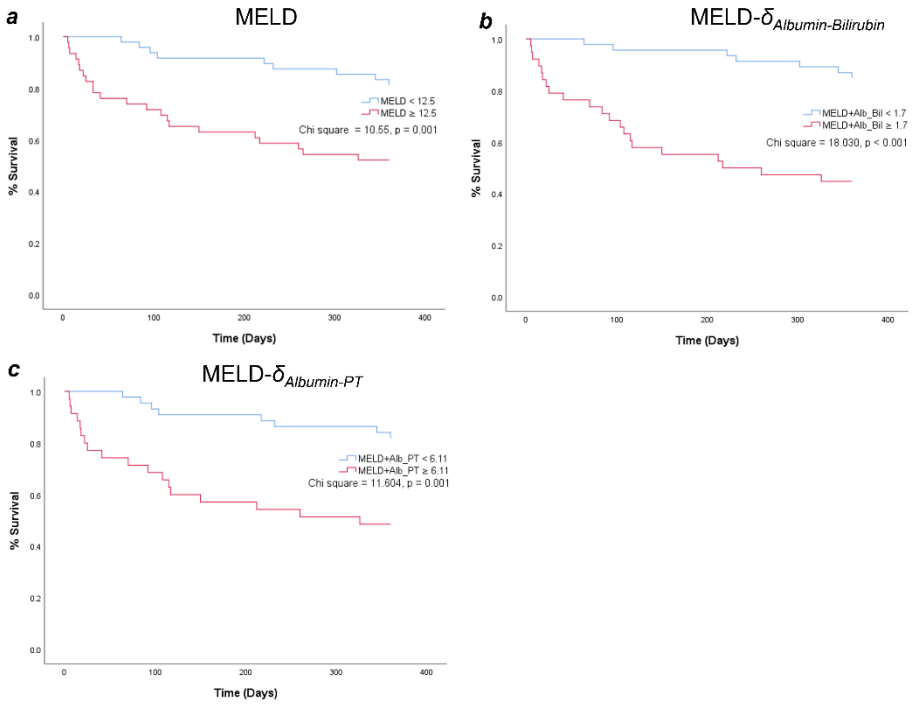



*Figure 4.5. Kaplan-Meier graphs showing 12-month survival predictions of MELD (a) and two combined indices: MELD-$\delta_{Albumin\text{-}Bilirubin}$ (MELD-Alb_Bil) (b) and MELD--$\delta_{Albumin\text{-}PT}$ (MELD-Alb_PT).*

*Network topology indices and prediction of survival in patients with cirrhosis.*

As shown in Appendix 25, there was a significant increase in the standard deviation of centrality between the survivors and non-survivor group ($p = 0.038$). Other topology indices did not exhibit statistically significant differences. Cox regression analysis was performed to determine the relationship between network topology indices and survival. Higher standard deviation of centrality increased the risk of mortality with a hazard ratio of 1.054 (95% CI, 1.026 – 1.083, $p<0.001$). Furthermore, the standard deviation of centrality was able to predict survival independent of the Child-Pugh score (Appendix 26).

## Discussion

In this study, a parenclitic approach was used to map the physiological network of patients with cirrhosis from routine clinical/laboratory data. By using the data of survivors to construct a reference model, deviations for each patient's pairs of variables from the reference model were calculated and used for prognosis calculation. We found that increased parenclitic deviations and reduced connectedness in the ammonia-HE axis are associated with a ~4-fold increase in the risk of mortality. Reduced connectedness along the Alb-Bil, Alb-PT, and Bil-PT axes were also linked with increased risk of mortality independent of routine prognostic indices such as MELD and Child-Pugh. Higher parenclitic deviations shown by non-survivors suggest a digression from the expected connection along various physiological axes and can be interpreted as significant network disruption between organ systems (i.e., more parenclitic deviation = less organ systems connectivity).

Furthermore, we analysed the network topology indices characterising the parenclitic networks defined by weighted deviations. This gives a quantitative measure of the network and evaluates the deviations and their collective relationships. From a set of topological indices, the standard deviation of centrality was significantly higher in the non-survivors than in survivors and showed a significant association with 12-month survival. This global index was also observed to predict survival independent of Child-Pugh. Put together, these results show that parenclitic network analysis can detect certain functional dynamics not picked up by the current models used for prognostication in cirrhosis. These results highlight the significance of interrelationships between clinical variables such as Alb-Bil, Alb-PT, and Ammonia-HE in reflecting the pathological stage of cirrhosis and provide insight into complex interactions between extrahepatic complications manifested in multiple organ systems and how they may exacerbate the prognosis of patients with cirrhosis.

A network approach to complex diseases such as liver failure has the potential to transform the landscape of assessing prognosis. The present study indicates that a parenclitic approach with routine laboratory tests (e.g., albumin, bilirubin, PT) may increase the accuracy of current prognostic factors and be used in conjunction with MELD to ultimately increase the number of lives saved. This is in line with previous

research revealing other physio-markers such as EEG or heart rate variability (HRV) in conjunction with MELD to increase the accuracy of prognostication [143, 265-267]. However, while analysis of EEG or HRV requires suitable recording equipment and analytical expertise, the parenclitic approach introduced in this study uses routine laboratory tests that are available in all clinical settings. This is an advantage of this approach and can be extended in future multi-centre prospective clinical investigations. Such a network approach also has the potential to be used in other complex illnesses such as sepsis and multiple organ failure for survival modelling as well as providing novel insight about the pathophysiology. If organ systems network disruption plays a significant role in critically ill patients [7], novel treatments may target enhanced levels of connectivity of organ systems rather than inducing functional systems isolation using pharmacological antagonists.

The results of this thesis indicate that a parenclitic deviation from albumin-bilirubin, albumin-PT, and ammonia-HE axes provides useful information for prognostication. Hepatic encephalopathy is a spectrum of neurophysiological disturbances that occurs in the background of acute or chronic liver failure [268]. Although classically linked with hyperammonaemia, systemic inflammation is known to precipitate or cause exacerbation of HE [269, 270]. While the exact link between systemic inflammation, ammonia, and HE remains unclear, systemic inflammation (due to endotoxemia, or bacterial translocation) may increase the susceptibility of the brain to hyperammonaemia thereby derailing the correlation between increased serum ammonia and HE. While there was a positive correlation between ammonia and HE in survivors ($r = 0.469$, $p = 0.002$), the severity of HE was not significantly associated with ammonia in non-survivors ($r = -0.027$, $p = 0.911$). This shows that factor(s) other than ammonia may be contributing to HE in non-survivors. Indeed, numerous studies have linked systemic inflammation with increased severity and poorer prognosis of HE [271-275]. Thus, the increased parenclitic deviation along the Ammonia-HE axis may reflect the contribution of a secondary physiological factor that predisposes to increased mortality from cirrhosis. This can be easily analysed using a parenclitic approach as described here or more traditional statistical methods such as analysis of covariance.

The analysis showed that the correlation between albumin and bilirubin is lost in non-survivors. There was a sharp reduction in serum albumin with increased bilirubin in survivors compared to non-survivors (Appendix 27). The reason for this disruption is not well clear. However, we hypothesis that; (i) The relatively high albumin observed even at significantly elevated bilirubin levels in non-survival may be due to clinical infusion which may not improve the effective systemic albumin or prognosis [276, 277] but may be associated with increased serious adverse events as was recently reported in the ATTIRE study [28]; (ii) The half-life of albumin is comparatively higher at about 3 weeks [278] compared to bilirubin which remains in circulation for about 6 minutes [279]. In addition, the half-life of albumin might be altered in critically ill patients due to impaired microcirculation compared with healthier patients [280] a factor that may contribute to differences in albumin-bilirubin correlation or survivors and non-survivors.

Albumin-PT was another axis that differentiated survivors from non-survivors in our study. The liver produces most procoagulant and anticoagulant proteins, responsible for maintaining haemostasis. In cirrhotic patients, the production of clotting factors and their inhibitors decreases, resulting in either a 'rebalanced' haemostatic equilibrium or a prothrombotic state due to systemic inflammation [281]. Increased bleeding risk has traditionally been regarded as the most significant haemostatic complication in patients with liver dysfunction, especially in the context of an elevated international normalized ratio (INR) [282]. However, the predictive value of INR in indicating the risk of haemorrhagic events has been contradicted in literature and remains unclear [283, 284]. On the contrary, there is an increasing recognition of hypercoagulability in some patients with cirrhosis where the risk of thrombotic events (e.g., portal vein thrombosis) might be higher than haemorrhage [285-287]. Portal vein thromboses and clotting of extracorporeal circuits are common in cirrhosis despite elevated INR values, while elevated bleeding tendency has been suggested to be associated with sepsis, hepatorenal syndrome, hypotension, and endothelial dysfunction instead of isolated liver dysfunction [244]. Indeed, venous thromboembolism (VTE) is an underdiagnosed and serious medical condition that occurs at a relative risk of >2% in cirrhotic patients and is associated with greater mortality in higher Child-Pugh stages [288, 289]. Also, low serum albumin has been found to be strongly predictive of increased risk of VTE, independent of INR or platelet account [290]. It is hypothesized that lower serum albumin

concentration is a surrogate for decreased protein synthesis by the liver and therefore correlated with decreased production of endogenous anti-coagulant factors such as Protein C and S. The results of this study show similar findings (Appendix 28), that the albumin levels are generally lower in non-survivors and remain low despite increase in PT while in survivors, albumin levels present a positive linear increase with PT. In cirrhosis, coagulopathy involves a complicated network of haemostatic factors, with the risks of thrombotic and haemorrhagic events reported to be independent of current markers or scores [244]. Therefore, a parenclitic approach to relationship between albumin and PT might pave the way for assessment of this relationship in routine clinical practice.

This study validates the feasibility of a parenclitic network-based approach for predicting the survival status of patients with liver cirrhosis, and its independence from Child-Pugh and MELD scores, indicating that including the correlation between biomarkers improves current prognostic indices and may help more accurate prognostication. This suggests that a parenclitic approach has the potential to complement current prognostic scoring systems for liver cirrhosis. However, there are some limitations. Firstly, the data of survivors used as a reference for measurement of deviations is limited in size and from a single referral medical centre. Future studies need to look at a more diverse multicentre cohort of patients with cirrhosis. Full applicability of the parenclitic method in survival modelling in cirrhosis requires further validation by applying the reference parameters developed in the current study to an external dataset of patients with cirrhosis. Alternatively, constructing parenclitic networks in a bootstrap replica of the data may provide further information on the reliability of this approach. Further studies can investigate the validation of such a network approach in a larger and more clinically diverse patient population. Another limitation of this study is that the relationship models of different clinical variables were based on linear regression, which assumes a correlative linear relationship between all pairs of variables. More sophisticated methods such as the 2-dimensional kernel density estimation [251] could potentially serve as a better approach, as it provides compatibility of categorical and continuous data. Further, various markers such as inflammatory biomarkers (e.g. IL-6) and physiological markers e.g., heart rate variability, heart rate turbulence, and temperature variability indices, all indices of autonomic function that were shown to predict mortality in cirrhosis patients [7, 291] could be included in the analysis to widen the scope and improve the prognostic value of

the parenclitic method. In addition, the results of this study might not be extendable to all subgroups of patients with cirrhosis as data were selected from patients referred to a tertiary referral clinic for evaluation of HE. For example, the parenclitic network may exhibit a different pattern in patients with acute-on-chronic liver failure (ACLF) than other forms of decompensation. This may give insight into the mechanism of decompensation and organ failure in cirrhosis. Future studies can focus on more diverse, and clinically relevant subgroups of patients with cirrhosis to provide a more comprehensive picture of organ systems network disruption in individual patients with cirrhosis. The present study also lacks a time-dependent approach to predicting outcomes using the parenclitic networks. Assessment of network structure over time can provide useful information on the trajectory of alterations in physiological processes involved in decompensation and might be of significant value for prognosis evaluation.

In conclusion, this study is the first to use the parenclitic network analysis of routine clinical data to assess organ system disruption and predict survival in individual patients with cirrhosis. Potential application of this method includes the prediction of treatment responses or patients likely to develop serious adverse events due to certain treatments. For example, patients with decompensated cirrhosis indicated for vasoconstrictors and/or albumin treatment who may not respond [292-294] or those likely to develop respiratory failure [295] or other side effects [296-299]. The integrated approach of the parenclitic network analysis may prove to be a better prognostic method and can provide novel pathophysiologic insight for understanding complex diseases such as chronic liver failure.

# Chapter 5 : Parenclitic network analysis identifies response to targeted albumin therapy in patients hospitalized with decompensated cirrhosis

# Introduction

There has been an epidemic of liver disease during the past 50 years in the UK with a four-fold increase in liver-related deaths [300]. This is now the leading cause of death in people aged 35 to 49 years, and the second leading cause of 'working years' lost in Europe [301]. Many patients present late in the disease course with advanced cirrhosis which confers a grim mortality [302, 303]. At this stage, the only life-prolonging treatment is liver transplantation, which is a limited and costly intervention. Albumin infusions have long been used for the management of complications of cirrhosis to improve plasma oncotic pressure and alleviate ascites and peripheral oedema [304, 305]. Current international guidelines recommend use after large-volume paracentesis, in patients with spontaneous bacterial peritonitis and hepatorenal syndrome [306, 307] and several studies have demonstrated potential beneficial immune-mediated/ anti-inflammatory properties [308-311]. Further, Bajaj et al reported that low serum albumin level was significantly linked with an increased risk of death among hospitalized patients with cirrhosis and infection [312]. However, albumin use outside of recommended indications remains a contentious clinical concept [313-318]. Indeed, the ATTIRE clinical trial of targeted albumin therapy did not show benefit over standard care, and patients in the albumin group, who received 10 times (median of 200g during hospitalization) as much albumin as those in standard-care (20g), had more severe or life-threatening serious adverse events, especially pulmonary oedema, or fluid overload [28, 319].

Other clinical trials of albumin infusion have shown conflicting results. For instance, a meta-analysis of albumin uses in patients with spontaneous bacterial peritonitis reported significant benefit [320] that was not seen in patients with other infections [321] and this latter trial was terminated because of lethal pulmonary oedema associated with albumin [321]. Taken together these data suggest that certain subgroups of advanced cirrhosis patients may benefit from targeted albumin therapy, but in others, this may cause harm. Yet extensive conventional subgroup analyses of the ATTIRE dataset did not identify patients that benefited and there are no current biomarkers to guide albumin therapy. We therefore undertook an unsupervised analytic approach.

Healthy individuals show a high degree of functional connectivity between physiological organ systems. Disruption of organ system coupling is a hallmark of complex diseases

and recent studies showed that reduced network connectivity was linked with poor survival in patients with sepsis [322] and poor survival regardless of the severity of liver disease in patients with cirrhosis [323]. This approach is novel as most prognostic indicators consider individual organ systems as separate units and do not reference their complex and non-linear interactions.

Targeted albumin infusion has the potential to challenge the physiological network through alteration of oncotic pressure, plasma volume, glomerular filtration rate (GFR), and transportation of various physiological molecules. Therefore, the assessment of physiological interaction as a network may allow for a more precise prediction of patients' responses or outcomes. The parenclitic network mapping allows network analysis of static, baseline clinical variables on the individual patient level [249]. Our group recently applied this method to a cohort of patients with cirrhosis using routine baseline clinical data and found that physiological network mapping can predict survival independent of the severity of liver disease as measured by the Model for End-stage Liver Disease (MELD) [324]. We hypothesised that parenclitic network analysis of routine clinical variables may provide valuable insight into the organ-system disconnections associated with albumin treatment response and mortality in patients hospitalised with decompensated cirrhosis. We therefore used network analysis to assess organ systems connectivity of individual patients based on their routinely available laboratory and clinical variables at trial entry, comparing treatment groups to identify baseline characteristics that precited a good or poor survival outcome to targeted albumin therapy.

# Method

### *Study population*

This is a sub-study (and extension) of the ATTIRE trial, which was a randomized control trial of targeted albumin infusions versus standard care involving 777 hospitalized decompensated cirrhosis patients from 35 hospitals across England, Wales, and Scotland (2016-2019) [28]. Thus, this is a secondary analysis of the data initially collected for the aim stated above. There were no differences in baseline characteristics and outcomes between treatment groups. Parenclitic network analysis was performed using routine clinical variables of patients in the standard care group alone to exclude any influences of targeted albumin treatment. Clinical variables analyzed include serum albumin (Alb), total bilirubin (Bil), international normalized ratio (INR), serum creatinine (Cr), serum sodium (Na), white cell count (WCC), C-reactive protein (CRP), mean arterial pressure and heart rate (HR). These values were collected at trial entry which was on average day 2 of hospitalization. Also, the variables were used for network analysis because they represent various physiological pathways directly and indirectly linked with liver function and are standard variables often measured in patients with liver cirrhosis. Further, a previous report using Random Forest machine learning algorithms indicated that the chosen variables can predict mortality [323]. Patients were grouped as survivors and non-survivors based on survival status after a 6-month follow-up period. The original trial (ATTIRE) was conducted following both the Declarations of Helsinki and was approved by the London-Brent Research Ethics Committee and the Medicines and Healthcare Products Regulatory Agency [28].

### *Parenclitic network analysis*

Parenclitic network analysis is a novel static network analytical method [248] that allows network mapping of individual datapoints from models built from a reference population with expected conditions (healthy, survivors, etc). The deviation of individuals' characteristics from expectations is used to weigh the connection between variables for that individual (***Figure*** 5.1). For a comprehensive description of the parenclitic network analysis in cirrhosis please see Zhang et al [324].

The ATTIRE Survivors' population at 6 months was used as the reference population for the development of the parenclitic model and deviations from this model for individual patients (survivors and non-survivors) were computed and used to weigh the correlation network map of clinical variables. Further, the parenclitic indices, including deviations along variable pairs as well as a global network topology of all patients (treatment and standard care) were computed using an in-house code in MATLAB (MathWorks, California, USA). For the measurement of global network topology, indices such as network diameter, mean centrality, and shortest path length were calculated. Please see Appendix 22 for definitions of network indices used in this study. In general, higher global parenclitic network topology indices such as diameter, mean centrality and shortest path length indicate lower connectivity among all components of the network (*Figure* 5.1).

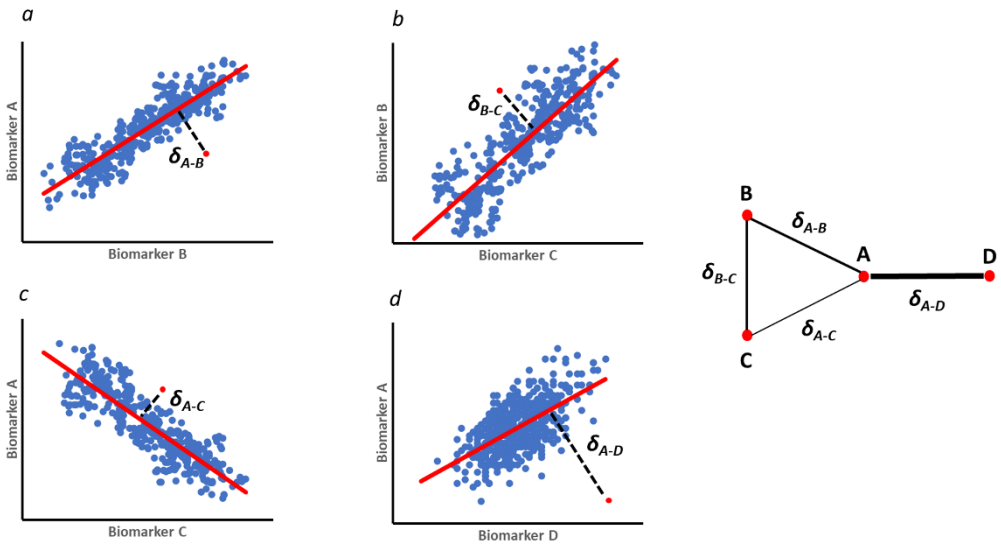



*Figure 5.1. A schematic representation of orthogonal residuals (δ) calculation and translation into parenclitic network. (a-d) First regression models are built for pairs of variables (A-B; B-C; A-C and A-D) from a reference population (e.g., survivors, treatment responders, etc.). The blue dots represent individual reference data, the red regression lines represent the expected relationship models, and the red dots are individual data of patients being studied. The black lines represent the deviation values (δ). The resulting parenclitic network map of nodes A, B, C, and D is presented with edges weighted (in terms of thickness) according to the magnitude of deviations from the models*

To validate the prognostic value of the parenclitic indices, a split technique was used whereby ~50% of patients in the standard treatment arm were randomly selected (training sample, n=194) and the remainder were used as the validation sample (n=203). Survivors

in the training group were used as a model for the calculation of the coefficients that were used for the calculation of parenclitic deviations in the validation group. Statistical analysis was performed to test whether the prognostic values persisted following the random split in the validation sample. This is to confirm whether the result generated was independent of the population studied.

### *Statistical analysis*

Statistical analysis was performed using Stata statistical software (Stata/MP, Version 17.0) and SPSS Statistics 26 (IBM Corp., Armonk, New York) with data presented as median and interquartile range (IQR) or mean ± standard deviation. A two-tailed p-value of $<0.05$ was defined as statistical significance in all analysis.

Initially, the Mann-Whitney U-test was performed to compare the median of computed parenclitic variables including the deviations along physiological axes (denoted as δ in this report) and global network topology indices, for the survivor and non-survivor group. Significantly different parenclitic variables were then tested for prognostic value by computing univariate and multivariate Cox regression controlling for MELD and age. Parenclitic variables with independent predictive values were combined with MELD to produce a combinatory prognostic index based on coefficients of bivariate Cox regressions as follows, Composite index= $\beta_1 \times$MELD + $\beta_2 \times \delta$; where $\beta_1$ and $\beta_2$ are respectively the regression coefficient of MELD and parenclitic indices in bivariate Cox model.

Receiver Operating Curve (ROC) analyses were performed and the Area Under the Curves (AUC) was computed for individual and combinatory indices to generate cut-offs. The specificity and sensitivity of resulting cut-offs were then used to generate the positive and negative predictive values based on Bayesian priors (% mortality) [325].

To test whether parenclitic indices may differentiate between survivors and non-survivors at 6 months following targeted albumin treatment, we performed a multivariate Cox regression including patients' treatment arm (albumin or standard care) and each of the parenclitic indices as interacting variables. The cut-off of parenclitic indices that showed significant interaction with treatment in predicting survival was then used to categorize

each patient into “1”, if ≥ cut-off and “0” if otherwise. The treatment arm of patients was then used to plot ROC curves to assess the 6-month survival grouped by the cut-offs of the significant parenclitic indices.

To assess possible improvement in the prognostic performance of MELD due to the addition of parenclitic indices Brier scores, Integrated Discrimination Improvement (IDI), and Net Reclassification Indices (NRI) were computed using standard statistical software Stata version 17.0 (Stata Corp LP, College Station, Texas, USA). Brier score provides the mean of the squared distance between observed and predicted risks of an event (mortality) for individual patients. Generally, the lower the Brier score the better the predictive model[262]. IDI and NRI measure the improvement of a binary predictive model due to the addition of new variables[263] (more details in chapter 4).

# Results

### *Patient characteristics at ATTIRE trial entry*

A total of 397 out of 777 (51%) patients received standard care and were subjected to parenclitic analysis. During the 6-month follow-up period 119 (30%) of these patients died. Table 5.1 presents the demographics and clinical characteristics of the study population.

*Table 5.1. Demographic and baseline clinical variables in the study population.*

| Characteristics | Survivors (n=278) | Non-survivors (n=119) | p-value |
|---|---|---|---|
| Mean age – years (SD) | 52.3 (10.5) | 57.1 (10.0) | < 0.001 |
| Male sex – no. (%) | 205 (74) | 89 (75) | 0.973 |
| | | | |
| Cause of cirrhosis – no. (%) | | | |
| Alcohol | 247 (88.9) | 104 (87.4) | |
| Hepatitis C | 26 (9.4) | 9 (7.6) | |
| Non-alcoholic fatty liver disease | 19 (6.8) | 10 (8.4) | |
| | | | |
| MELD score (median, IQR) | 17.96 (14.6 – 22.4) | 21.917(17.690-26.328) | <0.001 |
| | | | |
| Physiological variables | | | |
| Creatinine level — µmol/L | 67 (56 – 85) | 81 (58 – 123.5) | 0.001 |
| Bilirubin level — µmol/L | 90 (40.5 – 144.5) | 109.5 (59.5 – 222.5) | 0.002 |
| Serum Albumin — g/L | 24 (21 – 26) | 24 (21 – 25.8) | 0.768 |
| International normalized ratio | 1.6 (1.4 – 1.9) | 1.8 (1.5 – 2.1) | <0.001 |
| White Cell Count – $10^9$/L | 6.8 (4.9 – 9.7) | 8.8 (6.4 – 13.8) | <0.001 |
| Serum Sodium – mmol/L | 134 (130 – 137) | 131 (127 – 135) | <0.001 |
| C-Reactive Protein – mg/L | 21 (10 – 42) | 36.5 (16 – 66) | <0.001 |
| Heart Rate – beats/minute | 89 (79 – 100) | 92 (81 – 105) | 0.044 |
| Mean arterial pressure – mmHg | 83.3 (75.5 – 91.5) | 81.7 (74.3 – 90.4) | 0.241 |

*NAFLD; Non-alcoholic fatty liver disease, HCV; Hepatitis C virus, MELD; Model for End-stage Liver Disease, SD; Standard Deviation, IQR; Interquartile Range.*

### *Parenclitic indices at baseline predict 6-month survival.*

The correlation network maps show that survivors had a significantly higher association between the baseline clinical variables compared with non-survivors (Figure 5.2). Overall, patients who survived for at least 6 months showed significantly lower parenclitic deviation between baseline clinical variables compared with non-survivors in all indices of network topology (centrality, shortest path length, efficiency, and diameter) Specifically, there was significantly lower parenclitic deviation along the WCC-CRP axis in survivors (Table 5.2).

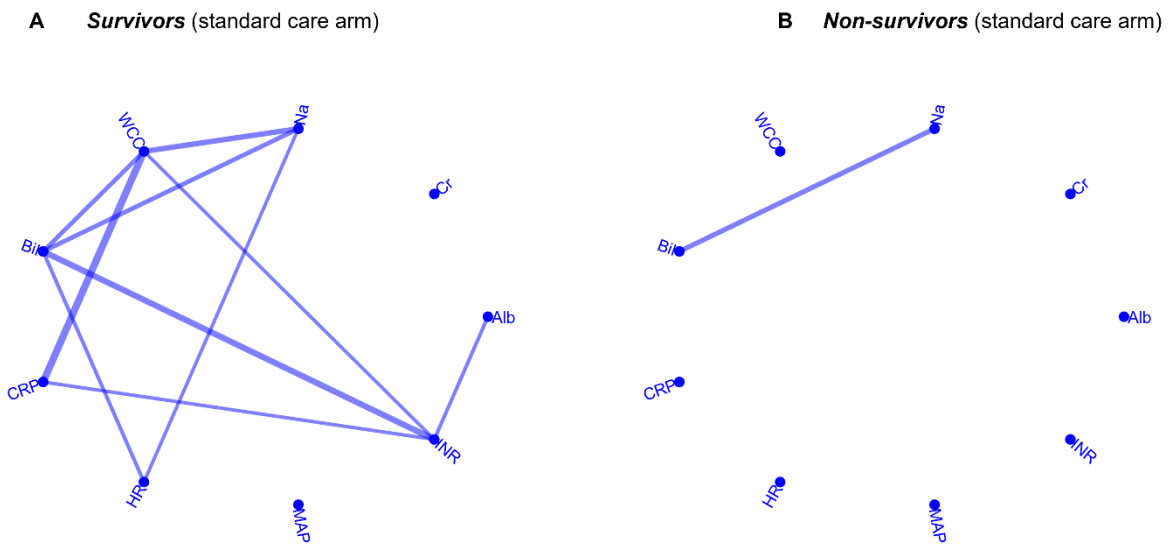



*Figure 5.2. Correlation network map of survivors (A) and non-survivors (B) after 6-month follow-up period of patients with decompensated cirrhosis under standard treatment. Each link shows a statistically significant correlation between two biomarkers after Bonferroni correction for multiple comparisons.*

*Table 5.2. Differences in parenclitic indices between survivors and non-survivors that received standard care in the studied population.*

| Variables | Survivors | Non-survivors | p-value |
|---|---|---|---|
| δ (WCC-Na) | 1.492 (0.784-2.548) | 0.254 (0.110-0.472) | 0.063 |
| δ (Bil-Na) | 3.381 (1.797-6.244) | 1.968 (0.809-3.162) | 0.604 |
| δ (INR-Alb) | 0.276 (0.139-0.420) | 3.563 (2.086-6.494) | 0.981 |
| δ (HR-Na) | 2.736 (1.157-4.832) | 2.710 (1.594-5.236) | 0.315 |
| δ (Bil-WCC) | 2.500 (1.279-4.219) | 3.155 (1.235-5.268) | 0.089 |
| δ (WCC-CRP) | **2.050 (0.980-3.810)** | **3.070 (1.330-5.320)** | **0.001** |
| δ (INR-WCC) | 0.253 (0.120-0.430) | 0.305 (0.144-0.506) | 0.082 |
| δ (HR-Bil) | 9.102 (4.887-15.052) | 10.451 (4.847-17.969) | 0.138 |
| δ (INR-Bil) | 0.251 (0.125-0.433) | 0.299 (0.129-0.498) | 0.097 |
| Mean Centrality | **6.440 (4.660-8.320)** | **7.670 (4.890-10.610)** | **0.001** |
| Mean Shortest path | **3.450 (2.520-4.670)** | **4.160 (2.800-6.000)** | **<0.001** |
| Diameter | **12.320 (8.550-18.990)** | **15.280 (9.760-22.270)** | **0.005** |
| Age | **51.858 (45.398-58.888)** | **56.320 (50.360-64.556)** | **<0.001** |
| MELD | **17.955 (14.467-22.385)** | **21.917 (17.690-26.328)** | **<0.001** |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, MELD; Model for End-stage Liver Disease.*

### *Prognostic values of baseline parenclitic indices to predict 6-month outcome.*

According to our univariate Cox regression analysis, greater parenclitic deviations along WCC-Na, Bil-WCC, WCC-CRP, HR-Bil, and INR-Bil axes were all associated with an

increased risk of 6-month mortality (Table 5.3). Likewise, higher measures of parenclitic network topology (i.e., mean centrality, mean shortest path length, and diameter) were associated with an increased risk of mortality up to 6 months (Table 5.3). Expectedly, a unit increase in MELD resulted in a 7.7% increase in the risk of 6-month mortality (hazard ratio: 1.077 (1.051-1.104), p<0.001).

*Table 5.3. Result of univariate COX regression analysis for parenclitic indices.*

| Variables | β | SE | p-value | Hazard Ratio (95%CI) |
|---|---|---|---|---|
| δ (INR-Alb) | 0.376 | 0.235 | 0.11 | 1.456 (0.919-2.307) |
| δ (WCC-Na) | 0.128 | 0.046 | 0.005 | 1.137 (1.039-1.243) |
| δ (Bil-Na) | 0.022 | 0.023 | 0.351 | 1.022 (0.976-1.07) |
| δ (HR-Na) | 0.052 | 0.029 | 0.071 | 1.054 (0.995-1.115) |
| δ (Bil-WCC) | 0.075 | 0.021 | <0.001 | 1.077 (1.033-1.124) |
| δ (WCC-CRP) | 0.12 | 0.026 | <0.001 | 1.128 (1.072-1.186) |
| δ (INR-WCC) | 0.476 | 0.214 | 0.026 | 1.609 (1.058-2.448) |
| δ (HR-Bil) | 0.024 | 0.009 | 0.008 | 1.024 (1.006-1.043) |
| δ (INR-Bil) | 0.41 | 0.192 | 0.033 | 1.507 (1.034-2.197) |
| Mean Centrality | 0.137 | 0.029 | <0.001 | 1.146 (1.083-1.214) |
| Mean Shortest path | 0.213 | 0.044 | <0.001 | 1.237 (1.134-1.349) |
| Diameter | 0.032 | 0.008 | <0.001 | 1.033 (1.016-1.05) |
| MELD | 0.074 | 0.013 | <0.001 | 1.077 (1.051-1.104) |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, MELD; Model for End-stage Liver Disease.*

*Independent prognostic values of baseline parenclitic indices to predict 6-month outcome.*

Multivariate Cox regression was performed to assess whether parenclitic indices that individually predicted 6-month mortality had prognostic value independent of age and MELD. Parenclitic deviations along the WCC-CRP axis (Hazard Ratio, 95% CI = 1.112, 1.053-1.174) and Bil-WCC axis (Hazard Ratio, 95% CI = 1.062, 1.017-1.108) significantly predicted outcome independent of age and MELD of patients at trial entry. Mean centrality (Hazard Ratio, 95% CI = 1.094, 1.030-1.162), mean shortest path length (Hazard Ratio, 95% CI = 1.163, 1.059-1.276), and network diameter (Hazard Ratio, 95% CI = 1.025, 1.007-1.043) also predicted survival independent of age and MELD (**Table 5.4**).

**Table 5.4.** Prognostic values of parenclitic indices independent of age and MELD at admission.

| Variables | β | SE | p-value | HR (95%CI) |
|---|---|---|---|---|
| **δ (WCC-CRP)** | **0.106** | **0.028** | **<0.001** | **1.112 (1.053-1.174)** |
| MELD | 0.091 | 0.014 | <0.001 | 1.095 (1.066-1.125) |
| Age | 0.050 | 0.009 | <0.001 | 1.051 (1.032-1.070) |
| | | | | |
| δ (WCC-Na) | 0.067 | 0.047 | 0.151 | 1.07 (0.976-1.173) |
| MELD | 0.083 | 0.013 | <0.001 | 1.086 (1.059-1.114) |
| Age | 0.046 | 0.009 | <0.001 | 1.047 (1.029-1.066) |
| | | | | |
| **δ (Bil-WCC)** | **0.06** | **0.022** | **0.006** | **1.062 (1.017-1.108)** |
| MELD | 0.083 | 0.013 | <0.001 | 1.087 (1.06-1.114) |
| Age | 0.047 | 0.009 | <0.001 | 1.048 (1.03-1.066) |
| | | | | |
| δ (HR-Bil) | 0.014 | 0.009 | 0.131 | 1.014 (0.996-1.033) |
| MELD | 0.084 | 0.013 | <0.001 | 1.087 (1.061-1.115) |
| Age | 0.046 | 0.009 | <0.001 | 1.047 (1.029-1.065) |
| | | | | |
| δ (INR-Bil) | -0.327 | 0.219 | 0.135 | 0.721 (0.469-1.107) |
| MELD | 0.1 | 0.015 | <0.001 | 1.106 (1.073-1.139) |
| Age | 0.05 | 0.009 | <0.001 | 1.051 (1.033-1.069) |
| | | | | |
| **Mean Centrality** | **0.090** | **0.031** | **0.004** | **1.094 (1.030-1.162)** |
| MELD | 0.084 | 0.014 | <0.001 | 1.088 (1.059-1.119) |
| Age | 0.046 | 0.009 | <0.001 | 1.047 (1.028-1.066) |
| | | | | |
| **Mean Shortest path** | **0.151** | **0.048** | **0.002** | **1.163 (1.059-1.276)** |
| MELD | 0.085 | 0.014 | <0.001 | 1.089 (1.059-1.119) |
| Age | 0.046 | 0.009 | <0.001 | 1.047 (1.028-1.066) |
| | | | | |
| **Diameter** | **0.024** | **0.009** | **0.006** | **1.025 (1.007-1.043)** |
| MELD | 0.091 | 0.014 | <0.001 | 1.096 (1.066-1.126) |
| Age | 0.047 | 0.009 | <0.001 | 1.048 (1.029-1.068) |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, MELD; Model for End-stage Liver Disease.*

The results of a parenclitic network analysis of a split sample (randomly selected patients) extracted from the study population showed similar correlation network maps of biomarkers (Appendix 32 and Appendix 33) and parenclitic deviations of the validation sample (203 random patients) calculated from the training sample (194 random patients), demonstrated comparable results to the original findings using the overall sample (Appendix 30). Specifically, parenclitic deviation along the WCC-CRP axis as well as the mean shortest path length and diameter predicted 6-month survival independent of MELD and age of patients in this validation subset (Appendix 30).

*Receiver operating characteristic (ROC) curve values of baseline parenclitic indices.*

The area under the ROC curves (AUC), cut-offs, and the sensitivity, specificity, positive (PPV), negative (NPV) predictive values, and Brier scores of these cut-offs are presented in *Table 5.5*. Combining parenclitic indices with MELD (composite indices) consistently improved the AUC by up to 7% (0.709 vs 0.664) and approximately increased the positive predictive value of the optimum cut-off by 20% (40.59% vs 48.23%). Further, Brier scores show that adding indices of the parenclitic network improves the predictive performance of MELD (*Table 5.5*). Results from IDI and NRI analysis also show an increased prognostic value by adding parenclitic network indices to MELD (*Table 5.6*).

*Table 5.5. Area on the ROC curves of parenclitic indices in combination with MELD compares with MELD alone.*

| Variables | AUC | p-value | Cut-off | Sensitivity | Specificity | % Increase in AUC vs MELD | PPV | NPV | Brier Score |
|---|---|---|---|---|---|---|---|---|---|
| MELD | 0.664 | <0.001 | 20.49 | 0.613 | 0.616 | - | 0.406 | 0.788 | 0.131 |
| Composite index: MELD-δ (WCC-CRP) | 0.707 | <0.001 | 1.89 | 0.639 | 0.649 | 6.48 | 0.438 | 0.808 | 0.124 |
| Composite index: MELD-δ (Bil-WCC) | 0.686 | <0.001 | 1.67 | 0.636 | 0.638 | 3.31 | 0.429 | 0.804 | 0.128 |
| Composite index: MELD-Centrality | 0.701 | <0.001 | 2.30 | 0.642 | 0.705 | 5.57 | 0.482 | 0.821 | 0.123 |
| Composite index: MELD-Shortest path | 0.709 | <0.001 | 2.23 | 0.642 | 0.684 | 6.78 | 0.465 | 0.817 | 0.122 |
| Composite index: MELD-Diameter | 0.706 | <0.001 | 2.07 | 0.632 | 0.662 | 6.33 | 0.445 | 0.809 | 0.125 |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, MELD; Model for End-stage Liver Disease.*

*Table 5.6. Measure of prognostic improvement of MELD due to the addition of parenclitic indices.*

| | IDI | p-value | NRI | p-value |
|---|---|---|---|---|
| MELD + WCC-CRP | 0.0359 | **0.003** | 0.3384 | **0.004** |
| MELD + Bil-WCC | 0.0157 | **0.040** | 0.2146 | 0.051 |
| MELD + Centrality | 0.0396 | **0.002** | 0.2991 | **0.012** |
| MELD + Shortest Path | 0.0419 | **0.001** | 0.3795 | **0.001** |
| MELD + Diameter | 0.0284 | **0.007** | 0.3040 | **0.009** |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, MELD; Model for End-stage Liver Disease.*

*Assessment of the prognostic value of baseline parenclitic indices to differentiate between 6-month outcomes comparing standard care to targeted albumin therapy.*

The result of multivariate Cox regression for interaction of targeted albumin treatment with baseline parenclitic variables to predict 6-month survival showed that measures of global parenclitic network characteristics (i.e., diameter and mean shortest path length), WCC-CRP parenclitic deviation, baseline serum albumin, and white cell count significantly interacted with targeted albumin treatment to predict survival (Appendix 31). Further, the Kaplan-Meier survival curves for patients grouped by the parenclitic indices demonstrated significant differences when targeted albumin and standard care groups were compared (Figure 5.3, Figure 5.4, and Figure 5.5). Specifically, patients with lower deviation along the WCC-CRP axis (<2.42) showed consistently lower survival (%) over the whole of the 6-month follow-up period in the targeted albumin arm compared to standard care, not just for the trial treatment period (p=0.023, Figure 5.5a). The same pattern applied when we considered disruption/deviation in the global parenclitic network, with patients with lower network shortest path length and lower network diameter in standard care showing significantly higher survival rates compared with the targeted albumin group (p=0.013 and 0.008 respectively, Figure 5.3a and Figure 5.4a). Again, the difference in survival persisted throughout the 6-month follow-up period. This difference in 6-month survival between treatment groups could not be detected when stratifying by the baseline MELD score and there was no difference between survival between treatment groups in the overall study population. In patients with higher deviation along the WCC-CRP axis (>2.42), network shortest path length (>3.86), and network diameter (>13.75) there were no differences in survival between treatment groups (Figure 5.3, Figure 5.4, and Figure 5.5).

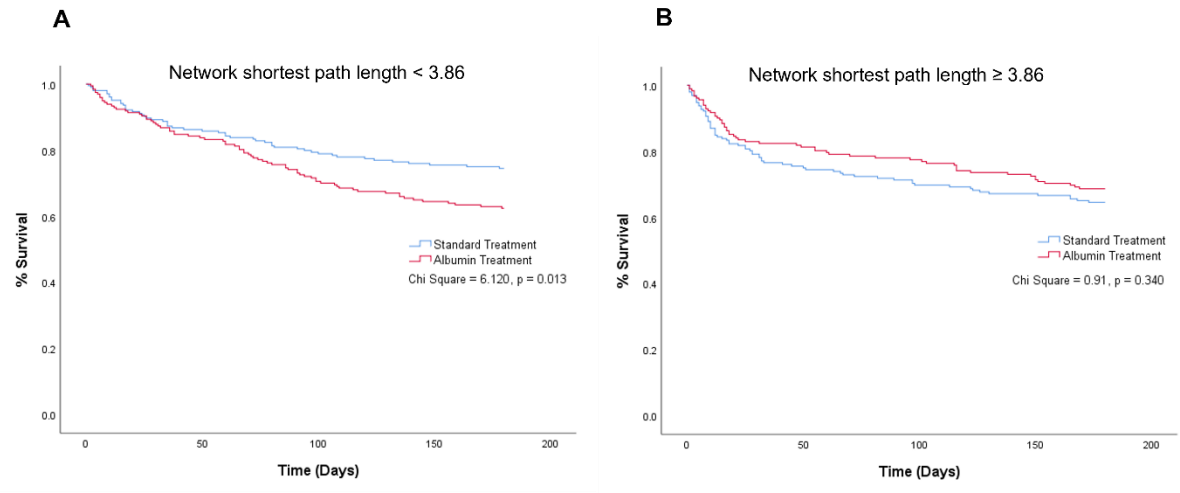



*Figure 5.3. Kaplan Meier graph representing 6-month survival prediction of patients based on Network Shortest path length cut-off (A versus B) and treatment (standard care versus targeted albumin therapy)*

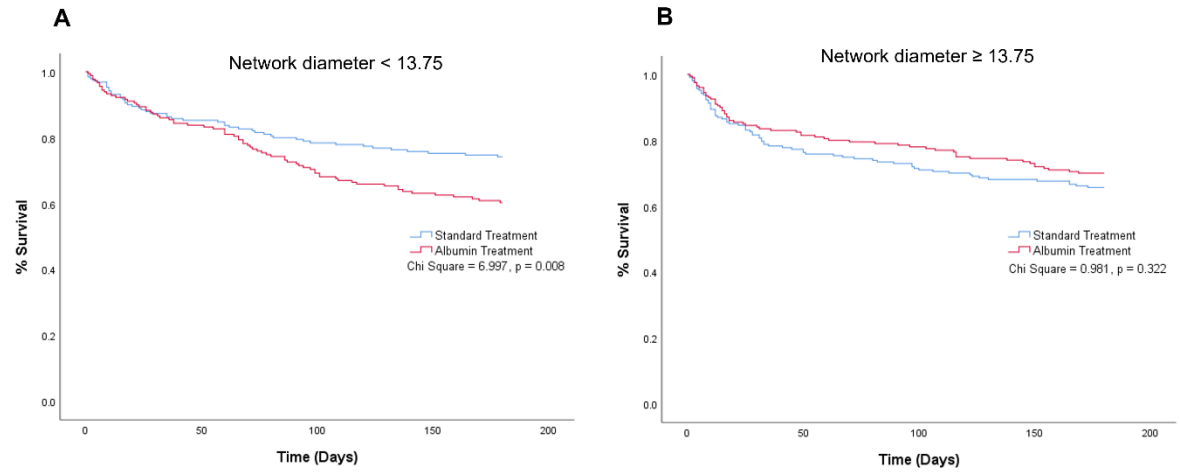



*Figure 5.4. Kaplan Meier graph representing 6-month survival prediction of patients based on Network diameter cut-off (A versus B) and treatment (standard care versus targeted albumin therapy).*

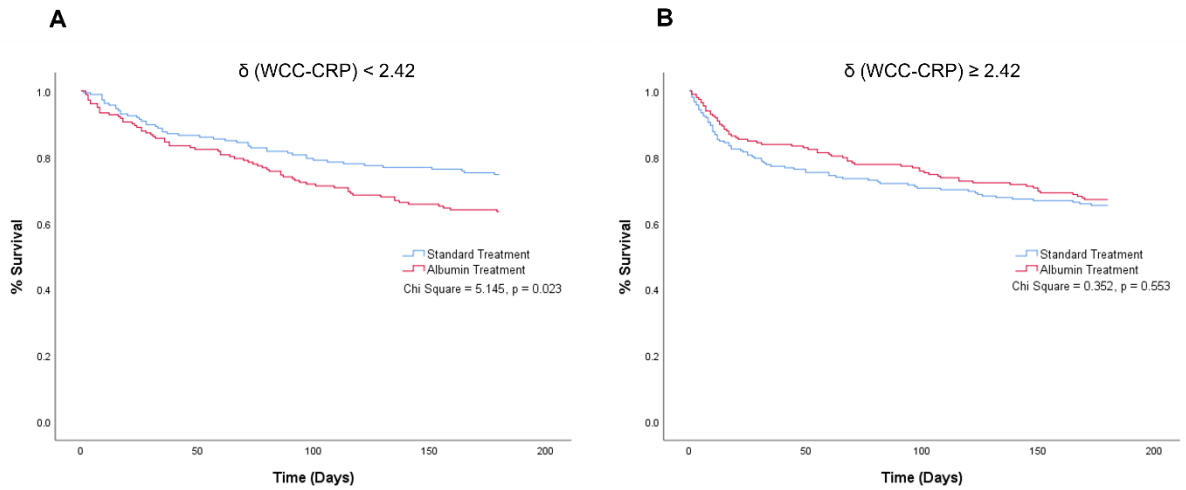



***Figure 5.5.*** *Kaplan Meier graph representing 6-month survival prediction of patients based on δ (WCC-CRP) cut-off (A versus B) and treatment (standard care versus targeted albumin therapy). δ; Deviation along an axis. WCC; White Cell Count, CRP; C-Reactive Protein.*

# Discussion

Our parenclitic network analyses using baseline clinical data accurately predict outcomes in patients with decompensated cirrhosis hospitalized for acute complications, based on disruption of organ systems coupling. These analyses also identified that patients with preserved organ system coupling had significantly poorer outcomes following increased albumin treatment.

Reduced organ systems correlation was associated with poorer prognosis in hospitalised patients with cirrhosis independent of the severity of the disease and age. The dyscoordination in the crosstalk between the key markers of systemic inflammatory response, CRP and WCC, provides a novel pathophysiological insight into the dysregulated inflammatory response in decompensated cirrhosis not captured by the MELD severity scoring system. Specifically, we found that reduced coordination between CRP and WCC predicted poorer 6-month survival in patients receiving standard clinical treatment. This was in line with our previous validation study in decompensated cirrhosis [324]. Further, the parenclitic network approach used here is based on data that is routinely available anywhere in the world and cost-effective while also providing interpretable physiological insights compared with the machine learning or artificial intelligence approach which requires a larger sample size or involves an uninterpretable "Blackbox" [326]. Compared to Zhang et al [324], our study involves a larger group of patients hospitalized for cirrhotic decompensation who were prospectively recruited and followed up across various hospitals in the United Kingdom. Also, to assess the integrity of the method employed, we used a split validation method which confirmed that the result of the analyses was robust.

Prognostic modelling in cirrhosis from Child-Turcotte-Pugh to MELD-plus and acute-on-chronic liver failure continues to move towards greater recognition of the impact on survival of the extra-hepatic involvements of cirrhosis [33, 327, 328]. A major trigger for this organ system disconnection is inflammation, either pathogen- (PAMP) or damage-associated molecular pattern (DAMP) [329, 330] with the presence of infection linked to a 4-fold increase in the risk of mortality [331, 332]. CRP is an acute-phase protein that increases in plasma following systemic inflammation and tissue or cell death [333-335]. CRP transcription and synthesis are primarily induced by IL-6 and result in the activation

of the classical complement pathways and the recruitment of phagocytic cells to the site of infection [336, 337]. Thus, under physiological conditions, the activities of WCC and CRP are closely coordinated toward achieving an effective response to infections. However, because CRP is produced by the hepatocytes in response to inflammation, the use of serum CRP level as a biomarker of inflammation or infection in cirrhosis has been contested [338-342].

Leukopenia is associated with a significantly higher risk of decompensation and mortality in cirrhosis patients [342, 343]. However, there are no established standardized thresholds suitable to accurately distinguish survivors and non-survivors in cirrhosis [344]. Therefore, both CRP and WCC, although useful markers of infection and inflammation in cirrhosis, have weak prognostic values when considered individually. However, when we considered both biomarkers as a coordinated axis in a network, this significantly predicted survival independent of MELD. The combination of these biomarkers of inflammatory response into a single physiological axis appears to provide a more sophisticated picture of the pathophysiological disturbance in inflammasomes due to cirrhosis.

Unexpectedly patients with lower parenclitic deviation and by extension higher organ systems connectivity showed significantly lower survival after targeted albumin therapy for a maximum of 2 weeks that persisted over the 6-month follow-up. We hypothesise that patients with preserved organ system connectivity may achieve this by the diversion of energies/resources needed for normal physiological functions toward maintaining an effective inflammatory response [345]. Targeted albumin therapy for a brief period may represent an adverse biological challenge to an already delicately balanced physiological state which may underlie their significantly poorer prognosis. Conversely, patients with significant disturbances in organ system connectivity appeared to respond slightly better to targeted albumin therapy over the 6-month follow-up period. and this raises the possibility that targeted albumin infusions in these patients improve homeostasis. Perhaps continuation of targeted albumin infusions beyond two weeks which had been shown to have beneficial effects in the ANSWER trial [346] could be further investigated in patients with decompensated cirrhosis especially those with higher organ system disconnection.

The main limitation of this study includes the inherent inability of the parenclitic network to capture the time course of organ systems connectivity due to the cross-sectional, static feature of the data analysed. For instance, our analysis is not robust to immediate and temporal changes to the network of organ systems that may follow albumin infusion or result from major clinical events that occur after the baseline data were gathered. Finally, although this study is multicentre in design, it applies to patients with decompensated cirrhosis admitted into hospitals in the United Kingdom where 'standard care' may differ in definition from other countries and regions of the world. Thus, the interpretation of our findings should be contextualized. However, the clinical management of patients is still strongly informed by baseline clinical variables and the ATTIRE study represents one of the largest clinical trials of hospitalized patients with cirrhosis, prospectively recruited and carefully followed up. Calculation of parenclitic deviation along the WCC-CRP axis is feasible at the bedside. However, further validation of the WCC-CRP axis is required in a larger, globally representative reference group to allow clinical incorporation as a bedside scoring system.

In summary, network analysis using routine clinical data collected at baseline provides novel insights into the pathophysiology of cirrhosis independent of the MELD score and significantly improves MELD's prognostic value. Further, we showed that unsupervised network analysis has potential value and may predict a poor response to targeted albumin therapy, which was not observed in conventional analyses. Future studies should further investigate the value of WCC-CRP and network mapping to predict outcomes in decompensated cirrhosis and improve the selection of patients for further trials of albumin or other immune/inflammation-modulating therapies.

# Chapter 6 : The application of physiological network mapping in the prediction of survival in critically ill patients with acute liver failure

# Introduction

The human body is a complex system composed of an intertwined network of physiological components that interact with each other to maintain homeostasis. The study of robustness in physiological complex networks is important, as it can help understand disease processes and the compensatory mechanisms that lead to survival after critical illnesses such as multiple-organ failure. Our knowledge about predictors of survival in critically ill patients is limited to clinical and epidemiological studies that have identified risk factors for mortality, such as a higher number of failing organs assessed by the SOFA score (Sequential Organ Failure Assessment) [347, 348]. Recent studies using various machine learning approaches have also indicated that the aggregation of previous disease history and acute physiology measures can predict in-hospital mortality in critically ill patients [349]. While all these studies are useful for prognostication in intensive care units, they rarely provide hypotheses that may lead to the development of interventions to alter the progression of the disease.

Recently, the network of physiological organ interactions has been studied within the context of critical illness [11, 12, 350]. The emerging field of Network Physiology has established the groundwork for understanding and quantifying global physiological behaviours arising from networked interactions across systems in health and disease [351, 352]. At least in theory, mapping the physiological network during an acute pathological insult in survivors may reveal compensatory mechanisms deployed to regain homeostasis with the potential for the development of new therapies. Indeed, physiological network mapping can also improve our understanding of the pathophysiology of the disease.

The liver plays a pivotal role in physiological processes within the body, positioning it as a central hub in the control of various physiological mechanisms. Clinicians readily acknowledge the functional connectivity of the liver with other organs, particularly evident in patients with liver failure who manifest involvement of multiple organs such as neural, cardiovascular, renal, and metabolic dysfunction, as well as acid/base and electrolyte imbalance [353]. In fact, it is well documented that chronic liver failure (cirrhosis) is associated with impaired cardiovascular control [90, 354, 355] and thermoregulatory dynamics [227, 356, 357]. The application of a network approach in patients with

cirrhosis has also revealed that organ system network disruption is associated with a poor prognosis in patients with chronic liver failure [323, 358]. Specifically, survivors of liver cirrhosis were found to exhibit a higher correlation between physiological biomarkers than non-survivors, indicating more connected organ systems in the survivors [323, 358]. Furthermore, physiological network connectivity indices could predict 6-month survival in patients with cirrhosis independent of age and the severity of liver disease [358, 359]. Such a network approach in liver cirrhosis is helpful, as it can provide prognostic information (e.g., for application in liver transplant allocation) as well as aid in predicting response to therapy (e.g., targeted albumin therapy) [359].

The process of cirrhosis is a slow process that takes more than a decade to affect the physiological networks significantly. On the other hand, Acute Liver Failure (ALF) is an acute process defined as the presence of severely worsening acute liver injury (<26 weeks) in patients with no history of chronic liver disease [360]. ALF can be caused by acute exposure to high doses of hepatotoxins such as paracetamol. Paracetamol-induced ALF is the most common cause of ALF in Western countries [361]. The rapid deterioration of liver function in ALF is strongly associated with a high risk of mortality, which may be prevented by timely liver transplantation. However, a subpopulation of ALF patients is known to recover fully without transplantation. Due to its accidental occurrence (e.g., drug overdose), paracetamol-induced ALF often occurs in otherwise healthy individuals. These patients need to rapidly adapt and employ suitable compensatory mechanisms to survive. While ALF is a multi-organ systemic disease and involves other organ systems (e.g., hepatic encephalopathy), a network approach has not yet been applied to understand the differences in organ-system interaction between survivors and non-survivors. In this chapter, a network mapping approach to investigate the interaction between multiple variables representing various organ systems in a cohort of critically ill patients with ALF was assessed. The prognostic value of the network mapping approach was also compared with the current clinical prognostic indicators used to assess critically ill patients with ALF (e.g., the King's College Criteria score and SOFA).

*Hypothesis*

Because of the multi-organ involvement of ALF, I hypothesize that understanding the complex interaction between multiple variables representing various organ systems may provide better insight as well as improve the prognostic value of the current prognostic models.

*Aim of study*

To assess the prognostic value of parenclitic network analysis in patients with p-ALF admitted to the ICU.

# Method

### *Database Description and Extraction*

The data analysed in this study was sourced from the third version of the Medical Information Mart for Intensive Care (MIMIC-III) following training, application, and obtention of required permissions (Record ID: 48067739). The MIMIC-III dataset includes over 53,000 unique hospital admissions. Initially, the complete MIMIC-III clinical dataset was downloaded to a secured cloud storage of the University College London (UCL) and structure query language (SQL) code was used to extract the required data based on the inclusion criteria.

Thus, the inclusion criteria include being an adult (aged 18 years and above) and being diagnosed with ALF linked with paracetamol/acetaminophen overdose at the time of ICU admission. Patients with less than 50% of clinical data records or those with missing follow-up and hospital mortality records we excluded from this study.

Specifically, the SQL code extracted data of patients aged 16 years and above of any gender who have been diagnosed (ICD_DIAGNOSIS) with acute liver failure based on the International Classification of Diseases 9$^{th}$ revision (ICD9) Code (570) [362] and who have a combination of the strings 'acetaminophen' or 'paracetamol' or other known commercial names of acetaminophen-containing combination drugs as well as the string 'overdose' in their clinical notes (NOTEEVENTS). The combination of the clinical note details with the ICD9 code was performed to reduce the error inherently associated with the now obsolete ICD9 diagnostic code used in the MIMIC-III data, especially for acetaminophen/paracetamol-induced ALF [363]. The list of patients was then used to extract the laboratory variables (LABEVENTS), and vitals (CHARTEVENTS). Other clinical variables of identified patients including age, sex, ICU length of stay, and in-hospital mortality, were also extracted for analysis.

Further, the minimum Glasgow Coma Score (GCS) and King's College Criteria (KCC) which index patients' level of consciousness [364] and severity of ALF [365] respectively were computed based on the available data recorded during the first day of ICU admission and included in the analysis. The GCS was calculated based on the patient's verbal, and motor responses and eye-opening [366]. For KCC, patients were scored

based on the level of acidosis (arterial pH < 7.30), coagulopathy (International Normalized Ratio of > 6.5), kidney function (serum creatinine > 3.4 mg/dL, and presence of hepatic encephalopathy of grade 3 or 4 according to the West Haven grading system [365]. However, because the MIMIC-III dataset does not contain the West Haven grades (or any grading system) of hepatic encephalopathy, patients' GCS score of ≤ 8 was classed as West Haven grade III or IV according to clinical guidelines see [367, 368], for more).

The sequential organ failure assessment (SOFA) score, which is often used to assess the severity (morbidity) of critically ill patients was also calculated since the study population is primarily those admitted to the ICU [369]. Finally, mortality was defined as patients who died within 28 days of hospitalisation and those that underwent liver transplantation (within 28 days) because these patients probably would not have survived if they had not been transplanted.

*Parenclitic Network Analyses*

Once the extracted data were cleaned and sorted, parenclitic analysis was performed as previously described [358, 359]. The software to perform the parenclitic analysis was previously developed on MATLAB as described in Chapter 4. Briefly, parenclitic network analysis based on patients' clinical or biochemical biomarkers was performed. Parenclitic network mapping is a novel approach for network analysis [248], facilitating the mapping of individual data points within models constructed from a reference population (i.e., patients who survive ALF). Initially, the correlation between clinical/biochemical biomarkers is assessed in the reference population to find out the expected relationship between the pair of biomarkers. To map the network of individual patients, a parenclitic approach was used. This analysis measures the deviations of an individual patient from the expected relationship between variables in the reference population. For a detailed exploration of parenclitic network analysis in the context of cirrhosis, refer to Chapter 5 and Chapter 6. In this chapter, the variables of patients who survived p-ALF 28 days post-ICU admission were used as the reference population. Regression analysis was performed on pairs of clinical variables based on a p-value that is corrected for the number of comparisons (Bonferroni correction). A population network was then created based on statistically significant regressions. The parenclitic deviations from the

significantly correlated models along each axis (pairs of variables) were computed and used to weigh the network map of individual patients. The deviation along each axis is reported as δ-A/B (e.g., δ-chloride/bicarbonate), where A or B represents a biochemical variable (e.g., chloride and bicarbonate), and δ denotes deviation from the regression line between A and B in survivors with ALF (i.e., the reference population). Network mapping was carried out using an in-house code developed in MATLAB (MathWorks, CA, USA).

*Detection of local clusters within the network*

For a complex physiological network containing a high number of nodes, understanding the cliques/communities of variables could give insight into the behaviour of nodes inside the network. The k_clique percolation method is a community detection technique that is robust to the overlap of shared characteristics between communities in a network. The technique defines all the cliques (a subgraph of a network where all member nodes are adjacent to each other) within a network that shares k-1 (at least one) node [370]. Clique in this instance is defined as a complete sub-network within the overall correlation network comprised of physiological variables with a higher likelihood of being correlated compared with variables from other communities [371]. Thus, the physiological communities are defined as organ systems more closely aligned in functionality compared with other nodes within the overall network. In this study, the k-clique percolation method was employed using a MATLAB function originally written by Ahn-Dung Nguyen [372]. The nodes and edges of the detected community (cliques) are color-coded for clarity and show which variable belongs to the same clique.

*Principal Component Analyses*

Since parenclitic network mapping is based on the correlation between physiological variables in a reference population, it shares some statistical insight with the principal component analysis commonly used for dimension reduction. Thus, principal component analysis was also performed on the patients' variables to assess which combination of variables (principal components, PC) can predict survival in patients with paracetamol-induced ALF. This analytic method can identify clusters of variables that are highly correlated before dimension reduction. Therefore, it has been used for the identification

of clusters during network analysis within the context of critical care [12]. Briefly, the principal component analysis (PCA) reduces the dimension of a dataset by computing the best combination of variables (PC's) that explains most of the variability in a dataset [373]. For PCA, Bartlett's test of Sphericity (Chi-squared) and KMO-MSA (Kaiser, Meyer, Olkin's Measure of Sampling Adequacy) was used to assess whether the dataset is suitable for the Factor Analysis (PCA) while Kaiser's rule based on eigenvalues > 1.0 was used to determine relevant PC's that explains a significant portion of the variability in the data. To extract variables that could be interpreted easily, the normalized varimax rotation method was used [374].

*Statistical analysis*

The characteristics of patients who survived and those who did not survive were compared with continuous and categorical variables presented respectively as mean ± standard deviation (SD) and median and interquartile range (IDR). For comparison of continuous variables between the patients' groups, independent samples t-test or Mann-Whitney U tests were used depending on whether the data was normally distributed. Chi-squared test was used for comparing categorical variables. Significantly different variables (parenclitic and principal components) were subjected to univariate and then multivariate COX regression analysis. To compare the hazard ratios of the network indices, the scales of parenclitic deviations were normalised before Cox regression analysis using Z transformation. Receiver operating curve (ROC) analyses were performed for variables that were independently predictive of 28-day ICU mortality and the area under the curve (AUC) computed. Positive and negative predictive values of the ROC cut-offs were computed using the sensitivities and 1-specificities. Most statistical analyses were performed on SPSS Statistics 26 (IBM Corp., Armonk, NY). For the assessment of prognostic improvement from the combination of parenclitic indices and principal components with SOFA, Brier score, integrated discrimination improvement (IDI), and the net reclassification indices (NRI) were computed on Stata statistical software (Stata/MP, Version 17.0). Lower Brier scores translate into a better predictive model while IDI and NRI give the percentage decrease in misclassification due to the addition of a new variable to a predictive model [262, 263]. For the interpretation of all statistical analysis results, a 2-tailed p-value less than 0.05 was used as the significant

cut-off. For the combination of SOFA with the independently predictive indices, composite scores were created using the formula β1 ×SOFA + β2 × δ or PC's, where β is the multivariate Cox regression coefficients of the variables.

# Results

### *Patients' characteristics*

A total of 640 patients with ALF due to paracetamol overdose were included in this chapter of which 249 (38.9%) either did not survive ICU stay or underwent liver transplantation. Overall, there was no significant difference in age (years), ICU length of stay (LOS), serum bicarbonate, serum sodium (Na), International Normalized Ratio (INR), white blood cell count (WBC), heart rate (HR), respiratory rate (RespRate), and blood pH (pH). However, there was significantly higher ALT (alanine aminotransferase) and AST (aspartate transaminase), serum albumin, chloride, platelet count, body temperature, mean blood pressure, oxygen saturation, haemoglobin, and Glasgow Coma Score (GCS) in the survival compared with non-survivors (Table 6.1).

*Table 6.1. Significantly different clinical and laboratory variables between ICU survivors and non-survivors based on T-test or Mann-Whitney U Test.*

| Variables | Survivors (391)<br>Mean ± SD/ Median (IQR) | Non-survivors (249)<br>Mean ± SD/ Median (IQR) | p-value |
|---|---|---|---|
| Age (years) | 57 (43 – 69) | 53 (43 – 68) | 0.38 |
| Male Sex, n (%) | 204 (52.17) | 148 (59.44) | 0.072 |
| ICU Length of stay (days) | 12.19 ± 15.99 | 7.15 ± 6.30 | <0.001 |
| SOFA | 7.05 ± 3.75 | 8.70 ± 4.04 | <0.001 |
| King's College Criteria (KCC) | 1.00 (0.00 – 1.00) | 1.00 (0.00 – 1.50) | <0.001 |
| Alanine Aminotransferase (U/L) | 2082 ± 3187 | 1344 ± 2031 | 0.003 |
| Aspartate Transaminase (U/L) | 2741 ± 4081 | 2293 ± 3237 | 0.180 |
| Alkaline Phosphatase (U/L) | 104 (71 – 161) | 124 (82 – 178) | 0.007 |
| Bilirubin (mg/dL) | 2.00 (0.90 – 5.45) | 2.80 (1.00 – 8.90) | 0.016 |
| Albumin (g/dL) | 3.04 ± 0.65 | 2.80 ± 0.54 | <0.001 |
| INR | 2.66 ± 2.47 | 2.83 ± 2.47 | 0.411 |
| Urea (mg/dL) | 37.80 ± 28.94 | 43.12 ± 27.76 | 0.022 |
| Creatinine (mg/dL) | 2.21 ± 1.83 | 2.46 ± 1.91 | 0.093 |
| Sodium (mEq/L) | 139.99 ± 5.43 | 140.10 ± 5.94 | 0.811 |
| Chloride (mEq/L) | 107.28 ± 7.04 | 106.07 ± 7.29 | 0.038 |
| Phosphate (mg/dL) | 4.37 ± 2.44 | 5.33 ± 2.24 | <0.001 |
| Bicarbonate (mEq/L) | 22.55 ± 4.70 | 22.36 ± 5.18 | 0.984 |
| Lactate (mmol/L) | 3.10 (1.90 - 5.55) | 5.05 (2.80 - 8.80) | <0.001 |
| Arterial blood pH | 7.42 ± 0.07 | 7.39 ± 0.11 | 0.004 |
| Glucose (mg/dL) | 177.36 ± 80.81 | 202.65 ± 105.80 | 0.001 |
| Haemoglobin (mg/dL) | 11.65 ± 2.15 | 11.32 ± 2.13 | 0.058 |
| Platelet count (x 1000/µl) | 198 ± 118 | 180 ± 108 | 0.061 |
| White Blood Count (x 1000/µl) | 14.31 ± 9.33 | 14.86 ± 8.10 | 0.453 |
| Temperature (°C) | 36.88 ± 1.22 | 36.69 ± 1.63 | 0.089 |
| Heart Rate (beat/min) | 94 ± 18 | 95 ± 18 | 0.522 |
| Mean Blood Pressure (mmHg) | 81.07 ± 12.00 | 78.02 ± 11.95 | 0.002 |
| Respiratory Rate (breath/min) | 20.55 ± 4.97 | 21.24 ± 4.92 | 0.084 |
| $SpO_2$ (%) | 96.74 ± 2.97 | 95.84 ± 4.71 | 0.003 |
| Glasgow Coma Score (GCS) | 9.27 ± 5.11 | 7.62 ± 4.81 | <0.001 |

*Data is expressed as Mean ± Standard Deviation or Median (Interquartile Range) depending on the type of the variable. SOFA; Sequential Organ Failure Assessment score, INR; International Normalized Ratio, SpO2; Oxygen Saturation, KCC; King's College Criteria. Either the t-test or the Mann-Whitney U Test was used for statistical analysis based on the normality of data distribution.*

*The difference in network indices between ICU survivors and non-survivors*

There was an overall more correlated variables showing higher organ systems connectivity in the survivors compared with the non-survivors. Detection of clusters within the networks using the k-clique percolation method showed observable differences in the pattern of network clusters between the groups with optimum k of 5. Specifically, a cluster of liver function-related biomarkers was only found in survivors. Also, the arterial blood pH shows a higher correlation with kidney function markers (serum creatinine) in survivors (*Figure 6.1*), while in the non-survivors, the pH forms a community with oxygen saturation and respiratory rate (*Figure 6.2*). Further to the correlation map, we mapped the parenclitic network of individual patients based on the deviation of pairs of physiological variables from the reference model. In general, the statistically different parenclitic deviations along all physiological axes were significantly higher in non-survivors compared with survivors except along the chloride-bicarbonate and creatinine-alkaline phosphatase axes (*Table 6.2*).

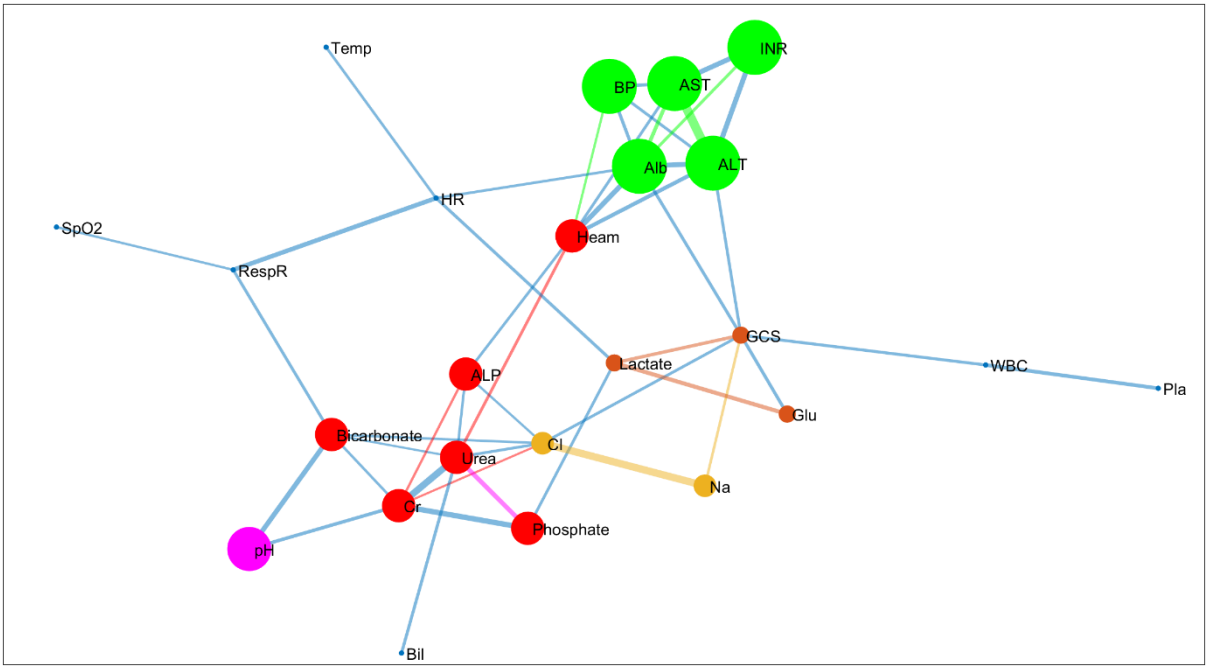



*Figure 6.1. Network map of clinical and laboratory variables showing correlation and K-Clique percolation communities of patients with acute liver failure that survived ICU stay (Optimized k-clique size = 3). Cl; chloride, AST; aspartate transaminase, ALT; alanine aminotransferase, GCS; Glasgow Coma Score, Bil; Total Bilirubin, ALP; Alkaline Phosphatase, Cr; Serum Creatinine, Na; Serum Sodium, Glu; Blood Glucose, HR; Heart Rate, Temp; Temperature.*

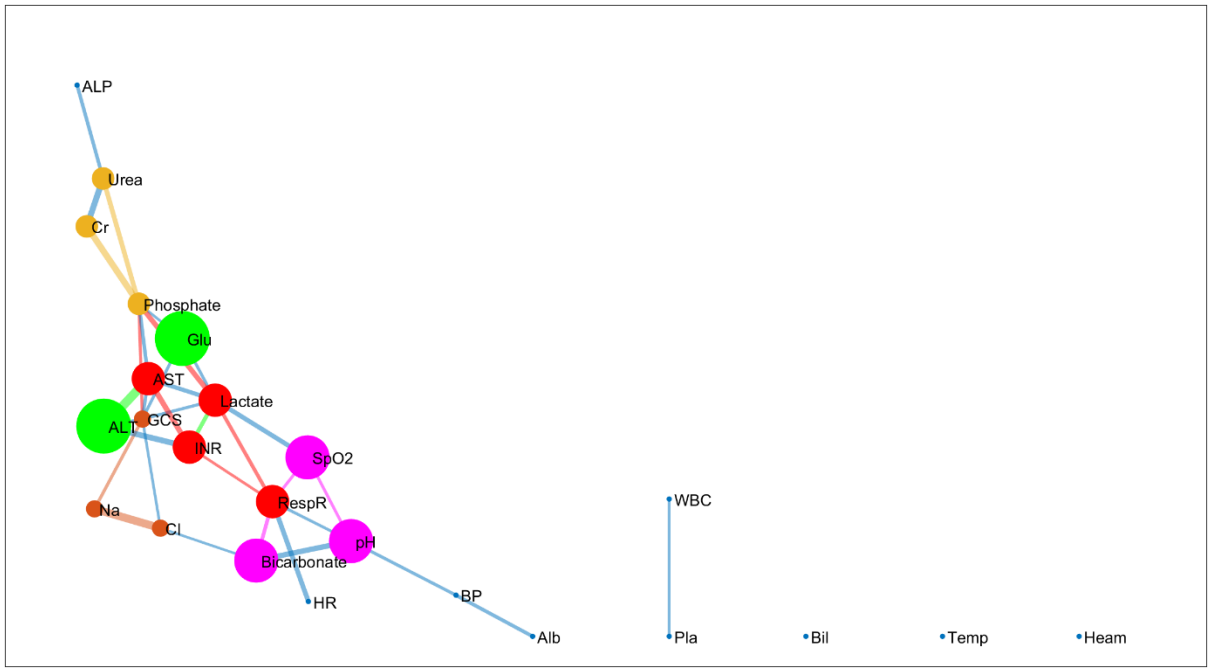



*Figure 6.2. Network map of clinical and laboratory variables showing correlation and K-Clique percolation communities of patients with acute liver failure that did not survive ICU stay (Optimized k-clique size = 3). Cl; chloride, AST; aspartate transaminase, ALT; alanine aminotransferase, GCS; Glasgow Coma Score, Bil; Total Bilirubin, ALP; Alkaline Phosphatase, Cr; Serum Creatinine, Na; Serum Sodium, Glu; Blood Glucose, HR; Heart Rate, Temp; Temperature.*

*Table 6.2. Significantly different parenclitic deviations between ICU survivors and non-survivors based on the T-test or Mann-Whitney U Test according to the distribution of data (normality test).*

| Variables | Survivors | Non-survivors | p-value |
|---|---|---|---|
| δ-Chloride/bicarbonate | 0.83 (0.44 - 1.24) | 0.72 (0.39 - 1.23) | **0.021** |
| δ-pH/bicarbonate | 0.04 (0.02 - 0.07) | 0.05 (0.02 - 0.09) | **0.001** |
| δ-GCS/ALT | 5.34 (2.92 - 5.94) | 5.65 (3.35 - 6.25) | **0.013** |
| δ-Cr/ALP | 1.20 (0.69 - 1.62) | 0.93 (0.56 - 1.48) | **0.043** |
| δ-pH/Cr | 0.04 (0.02 - 0.07) | 0.06 (0.03 - 0.09) | **<0.001** |
| δ-GCS/Na | 2.18 (1.15 - 3.27) | 2.42 (1.35 - 3.37) | **0.043** |
| δ-Lactate/Glu | 1.60 (0.79 - 2.46) | 2.00 (1.00 - 4.08) | **0.001** |
| δ-Urea/Bil | 2.66 (1.55 - 4.69) | 2.99 (1.69 - 6.09) | **0.045** |
| δ-Lactate/HR | 1.53 (0.84 - 2.57) | 1.82 (0.95 - 3.46) | **0.015** |
| δ-$SpO_2$/RespR | 0.97 (0.46 - 1.6) | 1.03 (0.57 - 1.85) | **0.043** |

*Cl; chloride, AST; aspartate transaminase, ALT; alanine aminotransferase, GCS; Glasgow Coma Score, Bil; Total Bilirubin, ALP; Alkaline Phosphatase, Cr; Serum Creatinine, Na; Serum Sodium, Glu; Blood Glucose, HR; Heart Rate, Temp; Temperature.*

*Parenclitic deviations (δ's) predict survival*

Parenclitic deviations along pH-bicarbonate, pH-creatinine, lactate-glucose, lactate-heart rate, and $SpO_2$-respiratory rate axes were significantly linked with increased risk of 28-day ICU mortality according to univariate Cox regression analysis. Specifically, each unit respective deviations along the blood pH-bicarbonate and pH-creatinine axes were both associated with over 37% and 36% increase in the risk of ICU mortality. Also, unit deviations along the lactate-glucose, lactate-heart rate, and $SpO_2$-respiratory rate axes were respectively linked with approximately 40%, 36%, and 21% increase in the risk of 28-day mortality in the ICU (Table 6.3).

*Table 6.3. Univariate Cox regression analysis of parenclitic indices based on ICU survival and follow-up.*

| Variables | β | SEM | Hazard Ratio (95% CI) | p-value |
|---|---|---|---|---|
| δ-Chloride/bicarbonate | 0.079 | 0.094 | 1.08 (0.90 – 1.30) | 0.402 |
| **δ-pH/bicarbonate** | **0.317** | **0.082** | **1.37 (1.17 – 1.61)** | **<0.001** |
| δ-GCS/ALT | 0.078 | 0.127 | 1.08 (0.84 – 1.39) | 0.539 |
| δ-Cr/ALP | 0.119 | 0.085 | 1.13 (0.95 – 1.33) | 0.162 |
| **δ-pH/Cr** | **0.308** | **0.078** | **1.36 (1.17 – 1.59)** | **<0.001** |
| δ-GCS/Na | - 0.066 | 0.107 | 0.94 (0.76 – 1.15) | 0.536 |
| **δ-Lactate/Glu** | **0.334** | **0.076** | **1.40 (1.20 – 1.62)** | **<0.001** |
| δ-Urea/Bil | 0.088 | 0.073 | 1.09 (0.95 – 1.26) | 0.228 |
| **δ-Lactate/HR** | **0.307** | **0.077** | **1.36 (1.17 – 1.58)** | **<0.001** |
| **δ-$SpO_2$/RespR** | **0.190** | **0.070** | **1.21 (1.05 – 1.39)** | **0.007** |

*Cl; chloride, AST; aspartate transaminase, ALT; alanine aminotransferase, GCS; Glasgow Coma Score, Bil; Total Bilirubin, ALP; Alkaline Phosphatase, Cr; Serum Creatinine, Na; Serum Sodium, Glu; Blood Glucose, HR; Heart Rate, Temp; Temperature, SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval.*

*Parenclitic deviations predict survival independent of SOFA*

Multivariate Cox regression analyses were performed to assess whether the significantly predictive network indices are independent of the severity of ALF measured by SOFA and KCC. SOFA and KCC were initially assessed for their predictive values and were both found to be individual predictors of 28-day mortality in this study population (HR, 95% CI = 1.04, 1.01 – 1.08, p = 0.014 and 1.18, 1.02 – 1.34, p = 0.034 respectively). Accordingly, multivariate analysis showed that physiological deviations along blood pH-bicarbonate (hazard ratio, 95% CI = 1.32, 1.11 – 1.56), blood pH-serum creatinine (hazard ratio, 95% CI = 1.30, 1.11 – 1.52), lactate-glucose (hazard ratio, 95% CI = 1.34, 1.15 – 1.57), lactate-heart rate (hazard ratio, 95% CI = 1.32, 1.14 – 1.54) and $SpO_2$-respiratory rate (hazard ratio, 95% CI = 1.19, 1.03 – 1.36) axes predicts 28-day mortality independent of SOFA score and King's College Criteria (Table 6.4).

*Table 6.4. Multivariate Cox regression of parenclitic indices vs SOFA in predicting survival in ALF patients admitted to the ICU.*

| Variables | β | SEM | Hazard Ratio (95% CI) | p-value |
|---|---|---|---|---|
| **δ-pH/bicarbonate** | **0.275** | **0.086** | **1.32(1.11 – 1.56)** | **0.001** |
| **SOFA** | **0.053** | **0.022** | **1.06(1.01 – 1.10)** | **0.017** |
| KCC | 0.092 | 0.107 | 1.10(0.89 – 1.35) | 0.391 |
| | | | | |
| **δ-pH/Cr** | **0.259** | **0.080** | **1.30(1.11 – 1.52)** | **0.001** |
| **SOFA** | **0.050** | **0.023** | **1.05(1.01 - 1.10)** | **0.027** |
| KCC | 0.098 | 0.107 | 1.02(0.82 - 1.26) | 0.362 |
| | | | | |
| **δ-Lactate/Glu** | **0.295** | **0.079** | **1.34(1.15 – 1.57)** | **<0.001** |
| SOFA | 0.032 | 0.023 | 1.03(0.99 - 1.08) | 0.164 |
| KCC | 0.113 | 0.107 | 1.10(0.89 – 1.36) | 0.289 |
| | | | | |
| **δ-Lactate/HR** | **0.281** | **0.078** | **1.32(1.14 – 1.54)** | **<0.001** |
| SOFA | 0.033 | 0.023 | 1.03(0.99 – 1.08) | 0.159 |
| KCC | 0.133 | 0.106 | 1.14(0.93 – 1.41) | 0.211 |
| | | | | |
| **δ-$SpO_2$/RespR** | **0.171** | **0.071** | **1.19(1.03 – 1.36)** | **0.016** |
| SOFA | 0.027 | 0.021 | 1.03(0.99 – 1.07) | 0.188 |
| KCC | 0.082 | 0.098 | 1.09(0.9 – 1.31) | 0.402 |

*SOFA; Sequential Organ Failure Assessment score, Cr; Serum Creatinine, Glu; Blood Glucose, HR; Heart Rate, SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval.*

*Principal component analysis*

A total of 9 principal components had eigenvalues >1 and were included in further analysis (Appendix 34). Of these, only principal components 1, 3, and 7 were significantly linked with mortality according to univariate Cox regression analysis (Appendix 35). Further, multivariate Cox regression analysis showed that each unit increase in Factors 1 and 6 are respectively associated with a 27% and 24% increase in the risk of mortality (Table 6.5). PC-1 identified *ALT, AST,* and *INR* while PC-2 identified *albumin*, *mean blood pressure,* and *haemoglobin* as clusters for dimension reduction as shown in Appendix 34.

***Table 6.5.*** *Multivariate Cox regression of Principal Components vs SOFA in predicting survival in ALF patients admitted to the ICU.*

| Variables | β | SEM | Hazard Ratio (95% CI) | p-value |
|---|---|---|---|---|
| **PCA-1** | **0.248** | **0.091** | **1.28 (1.07 - 1.53)** | **0.006** |
| SOFA | 0.059 | 0.033 | 1.06 (1.00 - 1.13) | 0.068 |
| KCC | 0.180 | 0.145 | 1.20 (0.90 - 1.59) | 0.213 |
| **PCA-3** | **-0.300** | **0.098** | **0.74 (0.61 - 0.90)** | **0.002** |
| SOFA | 0.042 | 0.034 | 1.04 (0.98 - 1.11) | 0.209 |
| **KCC** | **0.297** | **0.149** | **1.35 (1.01 - 1.80)** | **0.047** |
| PCA-7 | 0.164 | 0.105 | 1.18 (0.96 - 1.45) | 0.117 |
| SOFA | 0.054 | 0.033 | 1.06 (0.99 - 1.13) | 0.103 |
| KCC | 0.171 | 0.152 | 1.19 (0.88 - 1.60) | 0.261 |

*SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval, SOFA; Sequential Organ Failure Assessment score.*

*Parenclitic network indices improve the predictive value of SOFA*

Based on the area under the ROC curve, the addition of both parenclitic indices and principal components significantly improves its predictive value as shown by the lower Brier scores of some of the composite scores compared with that of SOFA alone (*Table 6.6*). Results from IDI and NRI analysis show that the addition of the parenclitic indices and principal components significantly improves the prognostic performance of the SOFA score. With regards to the IDI and NRI, the addition of principal component 1 showed the highest reduction (IDI = 15.1% and NRI = 73.2%) in overall predictive error from using SOFA alone (*Table 6.7*).

Also, the Kaplan-Meier survival curve (with the Chi-Square test) shows that compared with SOFA alone, the ROC cut-off of the composite scores significantly discriminates between ICU-admitted ALF patients who survived and those who did not. Specifically, cut-offs of composite scores including SOFA and principal components 1 ($p = 0.005$) and 6 ($p = 0.01$) as well as the independently predictive parenclitic deviations (pH-Bicarbonate, $p < 0.001$; pH-Creatinine, $p = 0.002$; Lactate-Glucose, $p = 0.001$; Lactate-Heart Rate, $p = 0.002$) significantly classified survivors and non-survivors (*Figure 6.3* & Figure 6.4).

*Table 6.6. Area on the ROC curves, sensitivity, specificity, PPV, NPV, and Brier score of parenclitic indices and principal components in combination with SOFA compared with SOFA alone.*

| Variables | AUC | p-value | Cut-Off | Sensitivity | Specificity | % AUC increase | PPV | NPV | Brier Score |
|---|---|---|---|---|---|---|---|---|---|
| SOFA | 0.617 | <0.001 | 6.5 | 0.683 | 0.56 | - | 0.497 | 0.735 | 0.1294 |
| δ-pH/Carbonate + SOFA | 0.658 | <0.001 | 0.725 | 0.606 | 0.614 | 6.65 | 0.5 | 0.71 | 0.1268 |
| δ-pH/Creatinine + SOFA | 0.633 | <0.001 | 0.695 | 0.601 | 0.592 | 2.59 | 0.484 | 0.7 | 0.1276 |
| δ-Lactate/HR + SOFA | 0.646 | <0.001 | 0.4954 | 0.657 | 0.626 | 4.7 | 0.528 | 0.741 | 0.1231 |
| δ-Lactate/Glu + SOFA | 0.652 | <0.001 | 0.4902 | 0.684 | 0.641 | 5.67 | 0.548 | 0.761 | 0.1232 |
| δ-$SpO_2$/RespR + SOFA | 0.654 | <0.001 | 0.6461 | 0.623 | 0.619 | 6 | 0.51 | 0.721 | 0.1226 |
| Principal component 1 + SOFA | 0.754 | <0.001 | 0.6422 | 0.715 | 0.714 | 22.2 | 0.614 | 0.797 | 0.0519 |
| Principal component 3 + SOFA | 0.712 | <0.001 | 0.5692 | 0.692 | 0.667 | 15.4 | 0.57 | 0.773 | 0.0509 |

*SOFA; Sequential Organ Failure Assessment score, Cr; Serum Creatinine, Glu; Blood Glucose, HR; Heart Rate, SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval.*

*Table 6.7. Measures of prognostic improvement of SOFA due to the addition of parenclitic indices and principal components.*

| Variables | IDI | p-value | NRI | p-value |
|---|---|---|---|---|
| δ-pH/Bicarbonate + SOFA | **0.0539** | **<0.001** | **0.3928** | **<0.001** |
| δ-pH/Creatinine + SOFA | **0.0521** | **<0.001** | **0.3939** | **<0.001** |
| δ-Lactate/ Heart Rate + SOFA | **0.0464** | **<0.001** | **0.3790** | **<0.001** |
| δ-Lactate/Glucose + SOFA | **0.0401** | **<0.001** | **0.3641** | **<0.001** |
| δ-$SpO_2$/Respiratory Rate + SOFA | **0.0406** | **<0.001** | **0.3723** | **<0.001** |
| Principal component 1 + SOFA | 0.0062 | 0.2063 | 0.1542 | 0.234 |

*SOFA; Sequential Organ Failure Assessment score, IDI; Integrated discrimination improvement, and NRI; Net reclassification indices (NRI).*

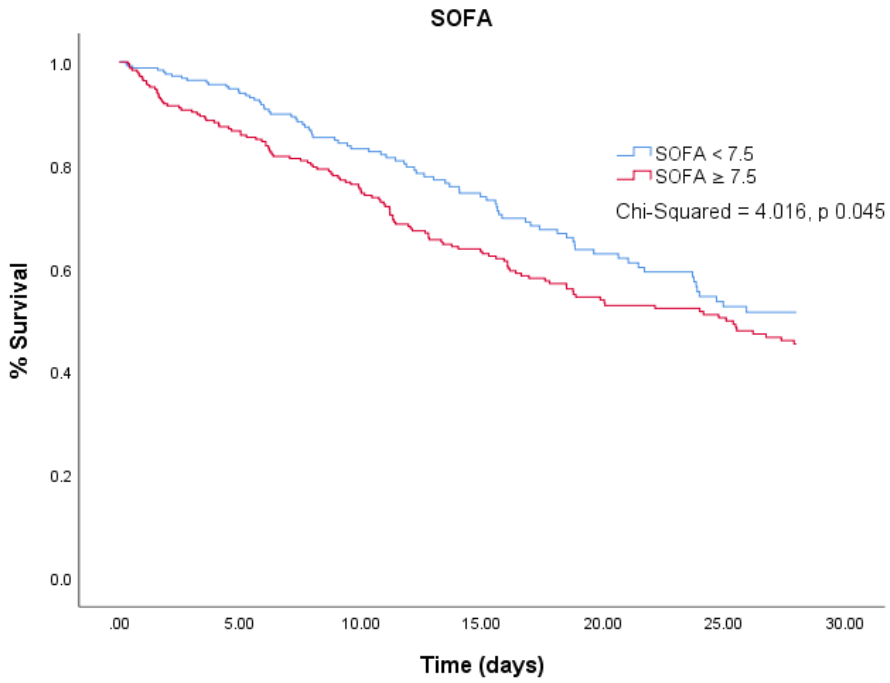
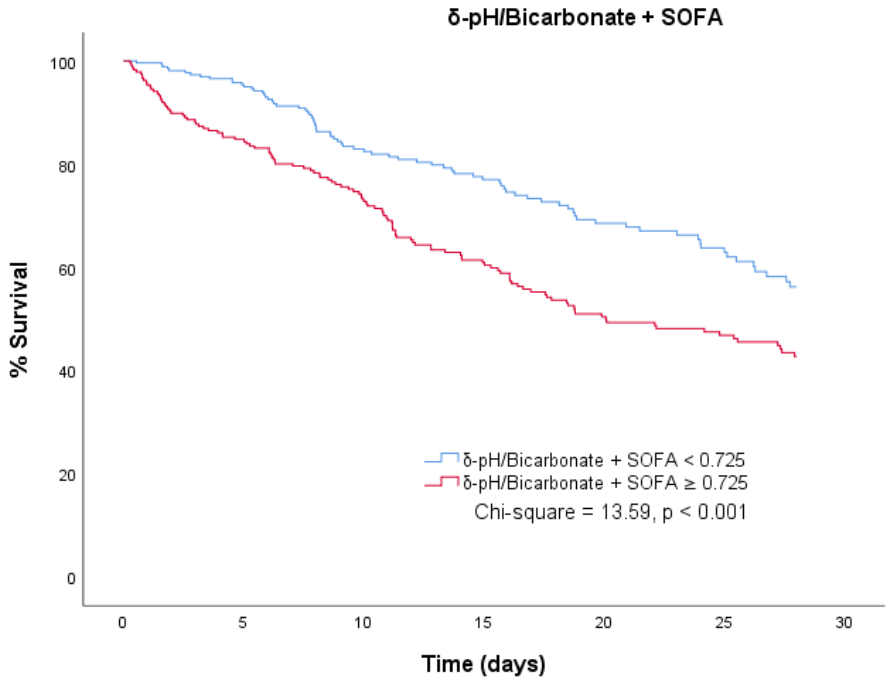
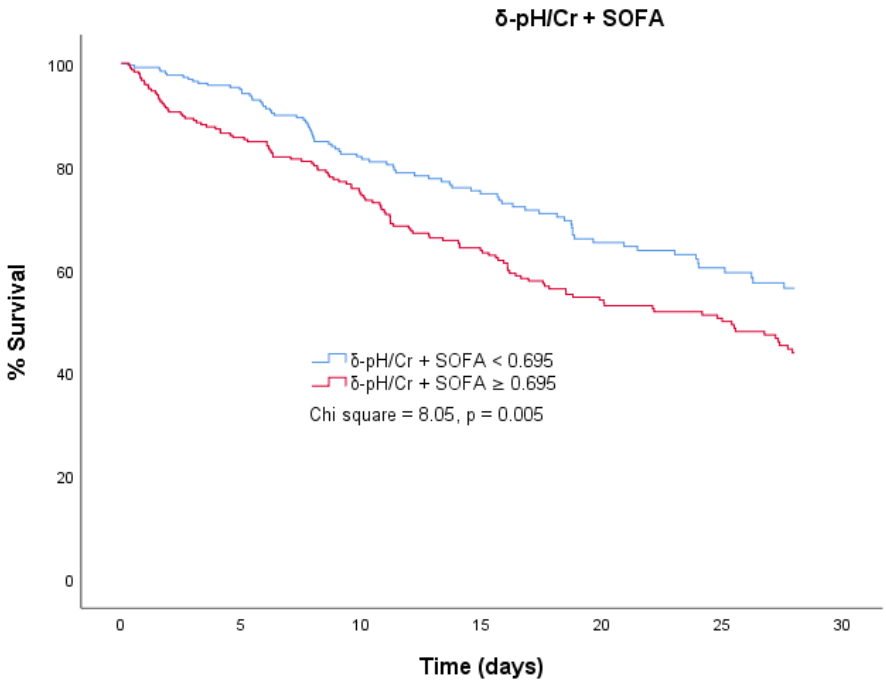
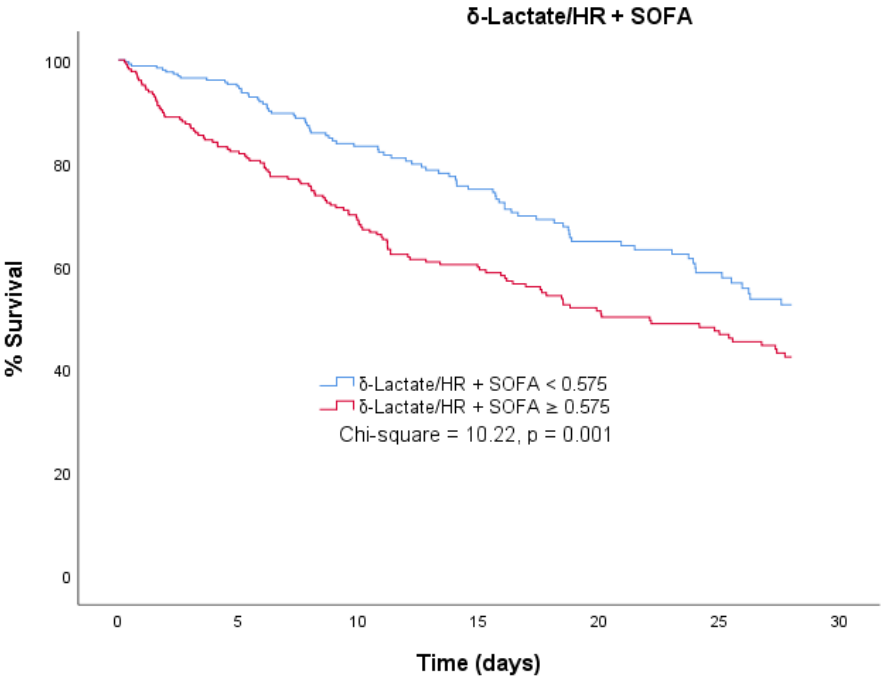



*Figure 6.3.* *Kaplan Meier graphs of patients with acute liver failure admitted to the intensive care unit that survived and those that did not survive as classified by the cut-off of SOFA.*




δ-pH/Cr + SOFA
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (days)
δ-pH/Cr + SOFA < 0.695
δ-pH/Cr + SOFA ≥ 0.695
Chi square = 8.05, p = 0.005


δ-Lactate/HR + SOFA
% Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
Time (days)
δ-Lactate/HR + SOFA < 0.575
δ-Lactate/HR + SOFA ≥ 0.575
Chi-square = 10.22, p = 0.001

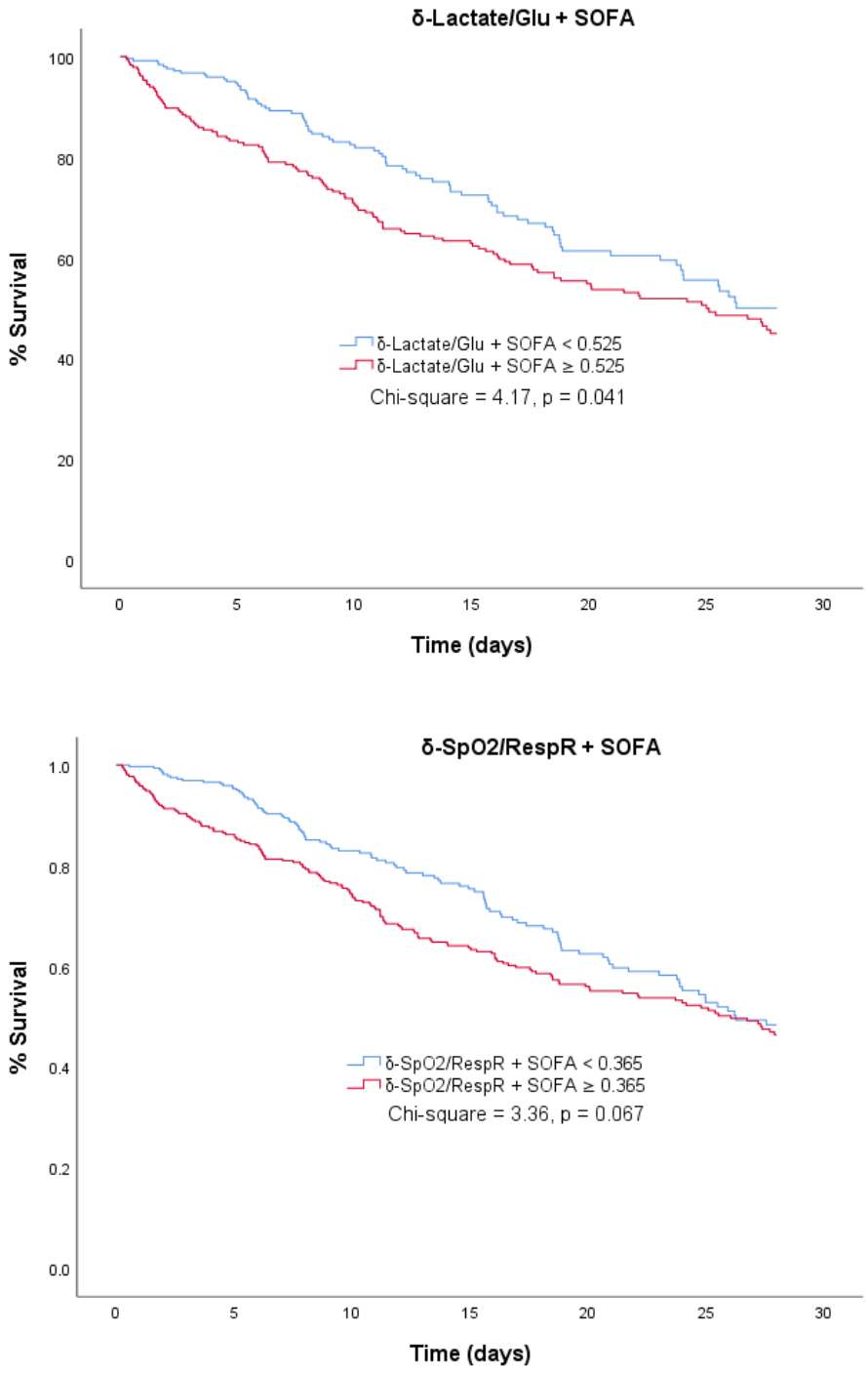



*Figure 6.4. Kaplan Meier graphs of patients with acute liver failure admitted to the intensive care unit that survived and those that did not survive after 28 days as classified by the cut-offs of the composite scores from the combination of SOFA with the parenclitic deviations along the pH-Bicarbonate (pH-$CO_3$), pH-Creatinine (pH-Cr), Lactate-Heart rate (Lactate-HR), Lactate-Glucose (Lactate-Glu), and Oxygen saturation-Respiratory rate axes.*

## Discussion

The liver serves as a major hub in the physiological network, and acute dysfunction is associated with mortality in many patients. Those who survive acute liver failure (ALF) may have adapted compensatory mechanisms that enhance survival. However, identifying these mechanisms necessitates a holistic or network approach. In this study, we employed parenclitic network mapping to demonstrate the prognostic value of this network approach in predicting survival among ICU-admitted patients with paracetamol-induced ALF (p-ALF). The results revealed that parenclitic deviations can predict survival in this patient population independently of the current clinical prognostic factors (SOFA and KCC). Also, the combination of the independent survival predictors with SOFA resulted in an over 30% reduction in the classification error compared to SOFA alone. This is the first study, to the best of our knowledge, to apply holistic network mapping in the prediction of transplant-free survival in ICU-admitted p-ALF patients.

In terms of correlation network, those p-ALF patients who did not survive in the ICU for at least 28 days were found to have an overall lower organ system connectivity compared with survivors. Further, network community detection also showed a marked difference in network structure between the survivors and non-survivors characterised by a significant difference in organ systems clustering (community formation). Generally, a community in a network defines a sub-population of nodes (organ systems) that are more closely linked (clustered) than other nodes outside of the community [375-377]. Expectedly, the variables associated with liver function (e.g., ALT, AST, INR, Alb) were relatively more clustered in survivors compared with the non-survivors (green nodes in **Figure 6.1** vs **Figure 6.2**). This finding is in line with the results of the principal component analysis as PC-1 in this analysis also found ALT, AST, and INR in the same cluster. According to survival analysis, this cluster (PC-1) along with its parenclitic deviations could predict mortality independently of SOFA and KCC.

In addition to the liver function community in the correlation network, arterial pH was also found to cluster with serum creatinine and bicarbonate in survivors, compared with non-survivors where it clustered with respiratory nodes ($SpO_2$, Respiratory rate) and bicarbonate (purple nodes in **Figure 6.1** and **Figure 6.2**). Thus, inferring a physiologically different compensatory mechanism for acid-base balance between the groups.

Essentially, it appears that the regulation of arterial pH was more closely linked with kidney function in survivors, while in non-survivors, this function was mainly controlled by respiratory compensation. The role of the liver in acid-base homeostasis via the regulation of systemic clearance of lactic acid and urea is generally dysregulated in liver disease [378]. This means that in liver disease, the role is generally shifted to the classical regulatory pathways involving the respiratory and renal systems. In the context of ALF, acid-base disequilibrium has been previously shown to be driven mainly by a systemic increase in lactic acid especially due to overproduction in the peripheral organs [379]. Generally, the kidney plays a protective role in lactic acidosis through a pH-dependent increase in the rate of clearance of systemic lactic acid [380, 381]. Indeed, lactic acidosis is significantly linked with poorer prognosis in ALF [382-387]. Interstitial, despite ALF patients typically exhibiting markedly increased lactate levels, frequently, there's no evident acid-base imbalance due to compensatory hypoalbuminaemic alkalosis [388]. In our study, arterial pH was only slightly reduced in non-survivors with ALF in comparison with the survivor group (*Table 6.1*). It appears that sufficient renal function may be associated with improved survival in ALF patients admitted to the ICU especially since non-survivors in this study showed significantly higher deviation in the pH-creatinine axis (δ-pH/Creatinine, *Table 6.2* - *Table 6.4*). In line with this observation, δ-pH/Creatinine was an independent prognostic factor in predicting mortality, indicating the importance of this axis in the survival of critically ill patients with ALF.

Aside from connectivity and organ systems community structure, overall parenclitic deviations of non-survivors were found to be significantly higher compared with survivors. For instance, non-survivors have higher deviation along the pH-bicarbonate, pH-creatinine, lactate-glucose, lactate-heart rate, and $SpO_2$-respiratory rate axes which are independently associated with survival. This translates to significantly reduced physiological coupling between these pairs of variables. Specifically, most of the physiological disconnections observed in non-survivors are associated with acid-base homeostasis (i.e., blood pH, serum bicarbonate level, and serum lactate). Thus, corroborating the significance of acid-base compensatory mechanisms in the prognosis of patients with ALF and critically ill patients [389]. Importantly, these network deviations predicted mortality even though there was only a slight difference in arterial pH between the patient groups. Thus, showing the importance of considering the physiological

network context rather than the individual isolated organ system in clinical management and prognosis. Also, deviation along the $SpO_2$-respiratory rate axes predicted survival independent of SOFA and KCC. $SpO_2$ estimates the percentage of oxyhaemoglobin relative to the total blood haemoglobin and reflects the cardiopulmonary efficiency in terms of systemic oxygen transportation. Although multiple cardiorespiratory factors contribute to oxygen saturation dynamics [352], $SpO_2$ and respiratory rate have been shown to be physiologically inversely correlated [390]. Interestingly, there was a significantly higher mean baseline $SpO_2$ and lower respiratory rate in p-ALF patients who survived compared with non-survivors (*Table 6.1*). Thus, loss of correlation and coordination between these two variables may be interpreted as patients' loss of adaptability and reduced ability to maintain systemic oxygen levels. Indeed, a previous study by Mower et al showed a poor correlation between oxygen saturation and respiratory rate in around 15,000 patients admitted to the ICU for various reasons. However, the authors did not assess the relationship with patients' survival [391].

Indeed, the results of this study corroborate previous research in the field, where changes in organ system connectivity measures using network analysis and other methods were shown to improve traditional scoring systems in predicting patient outcomes in cirrhosis or even detecting subgroups of patients that may respond to therapy (e.g., targeted albumin therapy)[15, 77, 358, 359]. Specifically, a previous study using parenclitic network analysis showed a reduced organ system connectivity (based on population-level correlation network mapping) in patients with cirrhosis who did not survive compared with survivors [359]. This corroborated a previous study in patients with cirrhosis referred for formal clinical assessment of hepatic encephalopathy with similar findings where organ system connectivity was relatively lower in patients that did not survive after 12-month follow-up [358]. However, the reduction observed in the patients with decompensated cirrhosis was more in magnitude compared with ALF in the non-survivors. This may be due to the significantly different time course of development or clinical history of these diseases whereby cirrhosis often develops over decades while ALF progresses more rapidly over a few weeks or days in otherwise healthy individuals [21, 392]. Thus, while chronic cirrhosis probably affects the overall physiology of patients, culminating in a general loss of organ systems coupling, ALF is likely linked with a shift in compensatory mechanisms to counter the abrupt and rapidly deteriorating liver function. Regardless of

the time course of liver failure, a physiological network approach appears to provide information not currently offered by existing clinical criteria (e.g., SOFA). This could open new avenues for improved prognostication and the discovery of novel personalized therapies based on individual networks in future investigations.

To further verify the results of the network analysis, especially the network community detections, we performed a principal component analysis to assess whether similar variables will be combined within each component and whether these components can also predict survival in p-ALF patients. While principal component analysis is useful for dimension reduction in multivariate datasets, it is limited in scope when compared with network mapping (correlation or parenclitic). Thus, while it could be used for validation, it does not suffice as a viable replacement for network analysis. This is due to several inherent limitations of principal component analysis. Firstly, principal component analysis is prone to loss of crucial information and oversimplicity resulting from the reduction of dimensions of the datasets [393]. Importantly, because principal component analysis is based on linear correlation between variables in the overall population, compared with parenclitic network analysis, it does not consider the possibility that correlation may be different between the subgroups (e.g., survivor and non-survivors, healthy and diseased, etc.) within the study population.

Indeed, the principal components related to liver function (component 1; AST, ALT, and INR), haemodynamic function (component 4; Serum albumin, mean blood pressure, and haemoglobin) as well as metabolic function (component 7; lactate, glucose e.g., liver-dependent Cori cycle for lactate-to-glucose recycling) were found to be significantly associated with patients' survival. However, only components 1 (liver function) and 3 (haemodynamic) were independent of SOFA score and the KCC (*Table 6.5*). Essentially, component 1 captures the ALF-related rapid deterioration of liver function due to paracetamol-induced, cytochrome P450-driven toxicity, as well as increased intra-hepatic glutathione depletion, oxidative stress, mitochondrial dysfunction resulting in hepatocyte necrosis [394, 395]. This finding is in line with a recent study by Yang et al which found that the addition of INR (and alkaline phosphatase) could significantly improve Hy's model for predicting patients with drug-induced liver injury (DILI) likely to develop ALF [396]. Hy's model is a risk-scoring model developed in 1968 by Hyman

Zimmerman for the prediction of the progression to ALF in patients with DILI and incorporates the patient's total bilirubin, AST, and ALT [397]. Indeed, due to the relatively lower specificity other variations to the original Hy's law and other prognostic models have been proposed and validated including the "new Hy's law" with higher specificity [398-400]. Also, the ratio of AST to ALT remains one of the popular models in diagnosis and prognosis of various aetiologies of liver disease including those of primary biliary, alcoholic, or viral origin [401-403].

Further, results of the study show that haemodynamic dysregulation represented by component 3 (serum albumin, mean blood pressure, and haemoglobin) is also significantly linked to poorer prognosis in patients with p-ALF. This finding is in line with previous works in the field showing the implication of haemodynamic dysregulation and instability in the prognosis of acute liver failure [404]. ALF is clinically linked with dysfunction in multiple organ systems including hyperdynamic circulation characterised by hypotension (reduced mean arterial pressure), hyperdynamic circulation, and reduced vascular resistance [405]. Thus, a reduction in renal perfusion pressure resulting from the haemodynamic dysregulation remains the key driver of renal failure, a complication observed in up to 82% of patients with ALF and linked with a significantly poor prognosis [406-409]. Indeed, the haemodynamic changes in ALF have been shown to correlate with the severity of liver disease and are a strong determinant of liver transplantation outcomes [410].

Finally, principal component 7 comprising glucose and lactate levels also predicted mortality albeit not independently of patients' severity score. The Cori cycle describes the conversion of the muscle-generated lactate, a by-product of anaerobic glycolysis to glucose in the liver. Physiologically, gluconeogenesis converts lactate to glucose in the liver usually in response to hypoglycaemia. Thus, it could be assumed that hypoglycaemia and hyperlactatemia should not coexist. However, in conditions such as paracetamol-induced ALF, characterised by severe hepatocyte necrosis and loss of liver function, and dysfunction in the Cori cycle, these conditions may coexist [411, 412]. Indeed, hyperlactatemia and hypoglycaemia have been shown to be individually associated with poorer prognosis in p-ALF [383].

*Limitations*

One limitation of this study is the retrospective nature of this study which is inherently linked with selection bias and lack of some relevant variables (e.g., West Haven HE score) since the record was not specifically designed for the assessment of ALF [413]. Another limitation of this study is in the characteristics of the patient population as patients are from a single centre in the USA which caters to specific regions and demographics. Thus, the result herein should be interpreted with this in mind. Finally, the MIMIC-III dataset is recorded using the ICD9 code for diagnosis which has been shown to be less specific compared with the later update to the ICD (e.g., version 10) [363]. Indeed, to minimize error in the selection the ICD9 code was combined with the use of the words "overdose" and any commercial names of paracetamol in the clinical notes (NOTEEVENTS) of patients. However, this study is based on one of the largest populations of p-ALF patients subjected to a rigorous mathematical and statistical analysis.

*Conclusion*

Reduced organ system connectivity and a shift from renal to respiratory compensatory mechanisms are associated with poorer prognosis in patients with ALF. This is further supported by physiological network disconnections along pH-associated axes which predict mortality independent of SOFA and KCC. Indeed, the strength of network analysis is the ability to assess the interactions of multiple organ systems in critically ill patients where the risk of multiple organ failure is high, and similar findings continue to show significant promise [5, 414]. Future studies could benefit from using a multicentre, multinational cohort of ALF patients to validate the findings herein.

# Chapter 7 : Dynamic network analysis for prognosis in intensive care patients with decompensated cirrhosis

## Introduction

Decompensated cirrhosis the end stage of cirrhosis characterized by multiple organ systems dysfunction and failure and is associated with significantly high short-term mortality [21, 415, 416]. Clinically, the definitive treatment for decompensated cirrhosis is liver transplantation which may increase survival by up to 8 years [417] and is offered according to the severity of decompensation and guided by prognostic models [415]. The MELD-Na is currently the gold standard for prognostication in patients with cirrhosis. However, it is associated with several weaknesses and is not especially accurate for the prediction of patients in ICU possibly with multiple organ systems failure (i.e., acute-on-chronic liver failure, ACLF) [418]. Recently, the European Association for the Study of Liver Disease proposed the CLIF-C (Chronic Liver Failure Consortium) score, which improves on the SOFA score to index the degree of extra-hepatic organ failure [419]. Generally, various improvements to the MELD system have been the addition of further clinical variables to create composite scores such as MELD-Na and the MELD-Plus [420]. Albeit, these additions have improved the prognostic values of the models, subgroup of patients continue to fall through the "prognostic crack".

Further, the prognostic models currently in use depend on mathematical algorithms that consider organ systems as isolated, independent units with no consideration for the physiological context within which they exist. The human body is a complex system comprised of various units interacting across varied spatiotemporal dimensions to maintain physiological states (stable states), especially in response to and to counteract changes in the environment [13, 421]. Indeed, a unique feature of a complex system is that the activities of such systems could not be accurately characterised by simply summing up the activities of the individual components [422, 423]. Thus, simply adding up the benchmarks of dysfunctional organ systems in decompensated cirrhosis may not tell the whole physiological story especially since the activities of one organ system could well regulate that of another which could itself regulate or be regulated by others, et cetera.

Recent research findings have shown the importance of context in the approach to medicine. For instance, the effect of the gut microbiome on the brain (termed gut-brain axis) has amounted to an in-depth understanding of previously unknown physiological

relationships and the implications in general well-being, disease onset, response to treatment, and even prognosis of various diseases (ref). Indeed, the new field of network physiology, which aims to define states of well-being based on the strength and dimension of organ systems connectivity is continuing to gain traction [424] and brave new insights are starting to come to the fore regarding not only the pathophysiology of diseases but their implication in terms of response to treatment as well as prognosis [425]. For instance, recent studies have shown that heart rate variability (HRV), a non-invasive index of various organ systems coupling with the heart rhythm, is generally reduced, and can predict survival in cirrhosis independent of MELD [4, 78, 143, 227, 426-428]. Also, heart rate turbulence following premature ventricular contractions was recently found to predict 1-year survival independent of MELD and Child-Turcotte-Pugh scores [77].

Regarding other methods for organ systems network mapping, Tan et al were the first to show that a reduction in organ system correlation network is significantly linked with poorer prognosis in patients with cirrhosis [15]. Recently, using parenclitic analysis, a static network algorithm that provides a cross-sectional physiological map at the individual patient's level from routine baseline clinical data, was applied to a population of patients hospitalised with decompensated cirrhosis. The result showed that reduced organ system connectivity as well as breakdown in physiological correlation between immune mediators (white blood cells and C-reactive protein) can predict 6-month survival and response to increased albumin therapy [425].

In previous chapters (2, 3), HRV and HRT are reported to be markedly reduced in cirrhosis and their long-term indices (DFA α2, SD2, TO) are significantly and independently linked with patients' survival. The hypothesis that decoupling of the cardiac rhythm (driven by the electrical firing within the sinoatrial node) from extrinsic regulatory influence (including the autonomic nervous system, thermoregulation, hormonal balance, circadian rhythm, etc.) and associated reduction in HRV and HRT indices may be driven by inflammatory dysregulation in cirrhosis has been previously tested and reported [291, 429]. Thus, reduced HRV indexes and increased systems' isolation probably drive prognosis in cirrhosis. This is in line with Pincus' proposal that a healthy system is a system constantly communicating with its parts and regularity/predictability in serial physiological trends is a measure of isolation in a complex system [430]. Coincidentally, randomness is a

function of information content as proposed by Claude Shannon [431]. Thus, if the fundamental measurement of information strength is irregularity, then the classic idea that regularity in physiology is associated with a healthy state does not hold. Indeed, this is corroborated by various studies that have linked various disease states and increased risk of all-cause mortality with reduced variability in physiological variables such as heart rate and oxygen saturation [5, 162, 432, 433]. Put together, reduced HRV and HRT are a measure of organ systems decoupling and provide useful surrogates for inferring short- and long-term regulation of the cardiac rhythm. However, HRV and HRT do not establish causal links. Thus, a major question is whether there are available methods for establishing causal links between organ systems. Transfer entropy, based on the conditional probability of two systems is one such method that provides a causal inference between two parallel time series and has been used extensively in various physiological contexts [434]

*Hypothesis*

Dynamic network analysis based on transfer entropy measure of information flow between heart rate, respiratory rate, and SpO2 time series can predict survival in patients with cirrhosis, admitted to the ICU.

*Aim of study*

This project aims to assess whether using dynamic network analysis to analyse time series from various variables in patients with cirrhosis can provide prognostic value. This is a progression from the static, classic network analysis using the parenclitic network algorithm and involves the use of transfer entropy to establish a direct causal relationship between organ systems. The dynamic network analysis based on transfer entropy assesses the causality link between two interacting systems and provides a superior measure of the relationship compared to the current paradigm whereby the relationship is based on statistical correlation. However, even Karl Pearson knows that “correlation is not causation”.

## Materials and methods

### *Data Source*

Data for this study was retrospectively collected from the MIMIC-III database containing comprehensively curated information of 46,520 ICU adult patients admitted between 2001 and 2012 to the Beth Israel Deaconess Medical Centre, Harvard Medical School, Boston, Massachusetts, United States. The MIMIC-III data contains demographics, vitals measurements, laboratory test results, medications and procedures, and clinical notes as well as ICD-9 (International Classification of Diseases, Ninth Revision) codes for diagnosed diseases. The database also contains a matched dataset of 22,317 waveform records and 22,247 numeric records of 10,282 distinct ICU patients. The recorded signals include electrocardiograph (ECG), arterial blood pressure (ABP), photoplethysmograph (PPG), respiratory rate, heart rate, blood pressure (systolic, diastolic, and mean), and oxygen saturation ($SpO_2$) simultaneously recorded by patients' bedside monitors. The numeric data contains physiological recordings with a sampling rate of 1Hz.

### *Cohort Selection. Data extraction and curation*

Data of ICU patients with a single admission for cirrhosis based on the ICD-9 code was extracted along with patients' vitals, clinical records, and laboratory data. The ICD-9 codes used are 5712, 5715, and 5716 for the diagnoses of alcoholic, non-alcoholic, and biliary cirrhosis respectively based on the MIMIC-III diagnosis dictionary (D_ICD_DIAGNOSES). The initial extraction was performed using a Structured Query Language (SQL) code designed in-house to query the MIMIC-III clinical database. Patients' identity codes from SQL data were used to extract the numeric datasets on MATLAB using the WFDB toolbox (https://archive.physionet.org/physiotools/matlab/wfdb-app-matlab/). Specifically, the earliest numeric time series within the first ICU admission for decompensated cirrhosis were downloaded for each patient using the "*rdsamp*" function. Where the first waveform recording could not be retrieved, an attempt was made to retrieve the second recording for the same ICU admission. The extracted data were then curated and aligned with header files containing the signal information, sampling frequency, and signal class (variable names) using the "*wfdbdesc*" function. Only patients

with a minimum of 20 minutes of continuous simultaneous waveform recording were included in the final analysis (*Figure 7.1*).

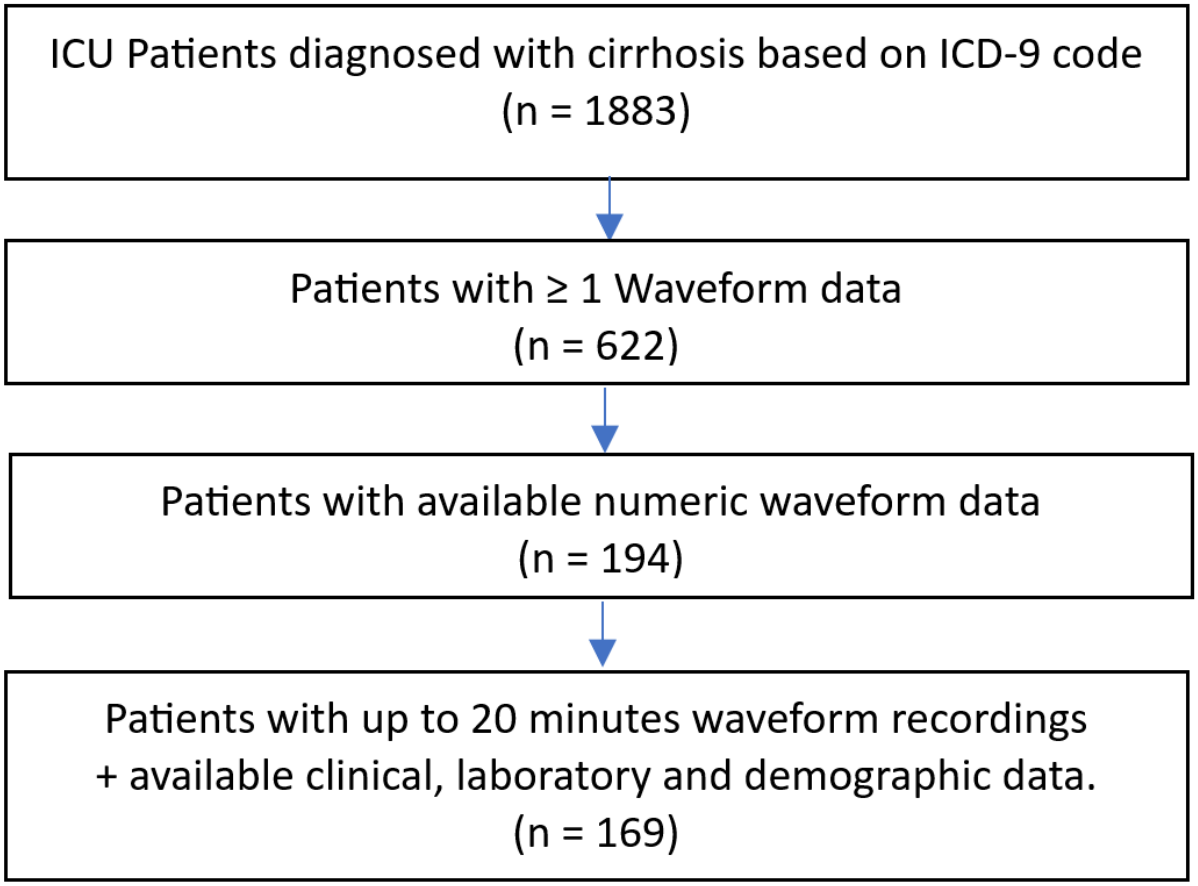



***Figure 7.1.*** *Flow diagram of patients included. ICD-9; International Classification of Diseases, Ninth Revision, ICU; Intensive Care Unit.*

For comparison, waveform data of sepsis patients were used. Data from the sepsis cohort have previously been extracted following a similar procedure as those described here (details available elsewhere [5]). In summary, TE analysis (with Monte Carlo correction) was performed for 179 ICU patients diagnosed with sepsis and a final cohort involved in the comparison analysis included 164 ICU patients with 30-day mortality data. The comparison of the organ systems information flow of cirrhosis with sepsis patients is to test the hypothesis that there is a difference in physiological network disruption in chronic and acute disease types in terms of tolerance to clinical and pathological insults. This is in line with previous findings regarding increased physiological tolerance (chronotropic responsiveness to cholinergic stimulation) to inflammatory insult in rat models of cirrhosis

compared with non-cirrhotic rats [435]. The sepsis cohort thus, serves as a control group with relatively similar clinical settings (i.e., ICU admission).

*Network Analysis*

Dynamic network mapping was based on causality measure as deduced by the pairwise analysis of transfer entropies (TE's) of individual patient's physiological time series. Transfer entropy is based on conditional probabilities of interacting systems derived by Thomas Schreiber from the theory of causality pioneered by Wiener [436] and refined by Granger [437]. TE provides details of how one time series influences a simultaneously recorded one [434]. Generally, the TE from Y to X is defined as the reduction in uncertainty in predicting the future of X based on the presence or past of X giving the knowledge of the present or past of Y. If knowledge about the present or past of Y improves the probability of predicting the future of X based on the present or past of X alone, then mutual information (I) is present between the systems [434, 438] (***Figure 7.2***).

$$T_{Y\to X} = H(X_{t+1}|X_t) - H(X_{t+1}|X_t, Y_t)$$

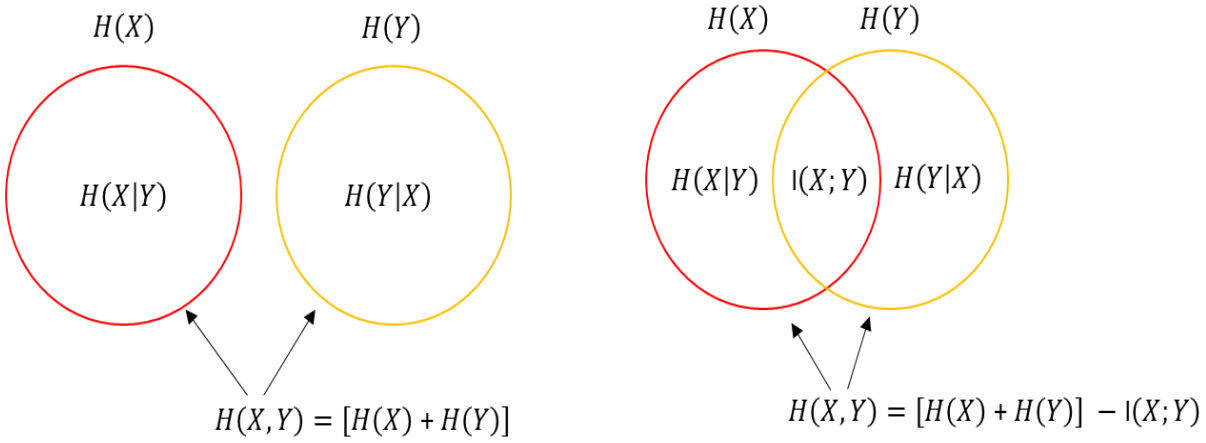



***Figure 7.2.*** *Description of transfer entropy computation based on the reduction of H (entropy) of the target. The entropy of the target equates to the computational work needed to predict its trends. I, mutual information; X and Y are two systems being studied for a causal link.*

Specifically, each patient's map includes 3 nodes (for HR, RespR, and SpO2) and the edges between these nodes are weighted based on the computed TEs (***Figure 7.5 A and B***) [352]. The directional information transfer between the variables as well as the global characteristics of the network was analysed for each patient using weighted network topology indices (centrality and shortest path length). The prognostic values of these indices were statistically tested.

*Computation of Transfer Entropy*

For the transfer entropy measure, a minimum of 20 minutes of simultaneous recordings of physiological variables were analysed. Transfer entropy was calculated in MATLAB using the "*transferEntropyPartition*.m" function developed by Lee et al [439] and available on Physionet ([440], https://www.physionet.org/content/tewp/1.0.0/). The function requires 4 inputs including two simultaneously recorded time series X and Y (e.g., HR, RR, or SpO2); and time lag for information transfer between the source and target time series (t and w). For this analysis, a time lag of 10 seconds was used based on preliminary analysis to detect the optimum transfer entropy between all the time series of the cirrhosis ICU patients. Specifically, lag time was varied between 1 and 20, and the mean TE's between HR, RR, and SpO2 were computed. A graph was drawn to visualize the changes in the mean TE's as the lag times vary to detect the lag time with the highest mean TE's (***Figure 7.3***). Based on the result of the optimization analysis, a lag of 10 seconds was used as it captures the optimum information flow in the patient's group.

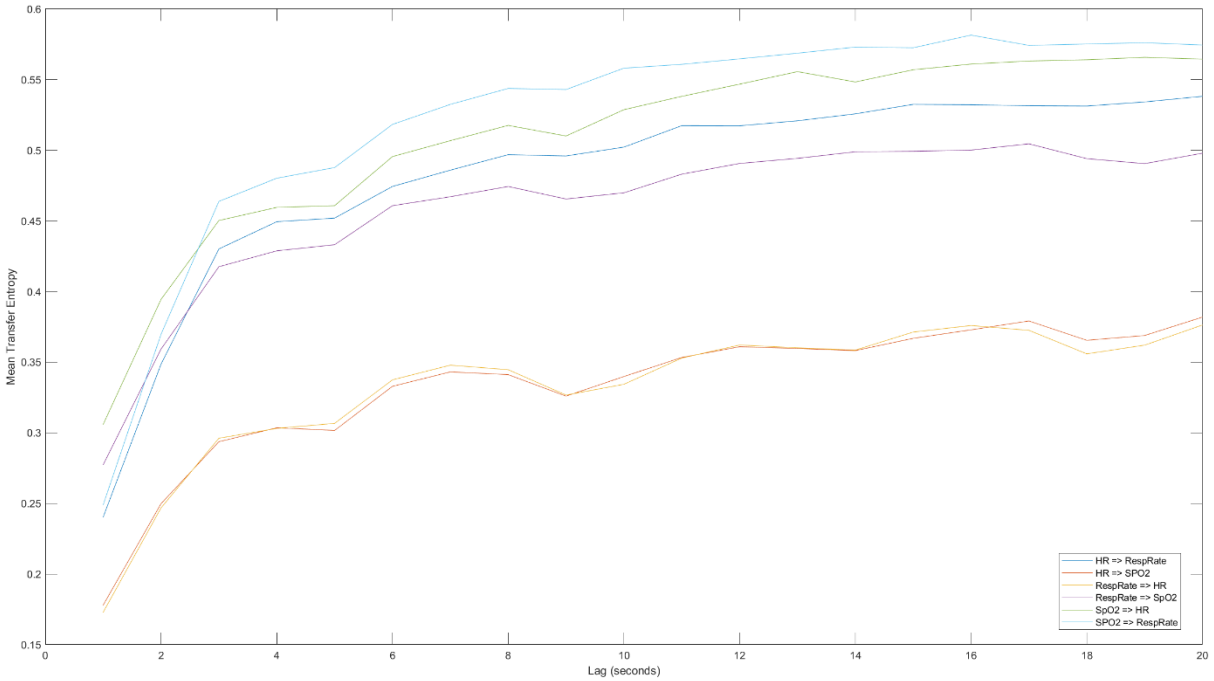



***Figure 7.3.*** *Changes in mean transfer entropy across varied lag times of 1-20 seconds. The legend presents the individual Transfer entropies along all directional axes between heart rate, respiratory rate, and oxygen saturation ($SpO_2$).*

The time lag translates to the highest time window from which the probability density is computed and assumes that information transfer to an organ system may occur with up to 10 seconds delay. The freedom to choose 10 seconds was adequate for 20-minute-long data points (1200 seconds) as shorter recordings would leave less degree of freedom. Accordingly, the probability density estimation follows the Darbellay-Vajda partitioning algorithm [439] and estimates the increase in the probability of predicting future iteration of a target time series or variable (e.g., respiratory rate), based on its past and present iteration conditioned on the past and present of a source time series (e.g., heart rate; ***Figure 7.4***)

To verify whether the computed transfer entropy is significant, Monte Carlo analysis was performed as originally described by Lee et al [439]. In summary, the target variable was randomised 200 times with the transfer entropy from the source time series estimated each time. The 200 iterations of the randomised TE's were pooled into a probability distribution with confidence intervals. The 95% confidence intervals of the probability distribution were then computed for each TE. Finally, the unrandomized, initial TE is deemed significant if it is outside the 95% confidence interval of the randomised TE's. The

assumption here is if the TE's, computed from timeline events are not based on mere chance, then TE's computed from 200 iterations of randomisation of target variables and the unrandomized TE should be statistically different with a 95% confidence interval (p = 0.05). Thus, if the computed TE is within the 95% probability distribution of the randomised TE, then it is merely based on chance and is zero.

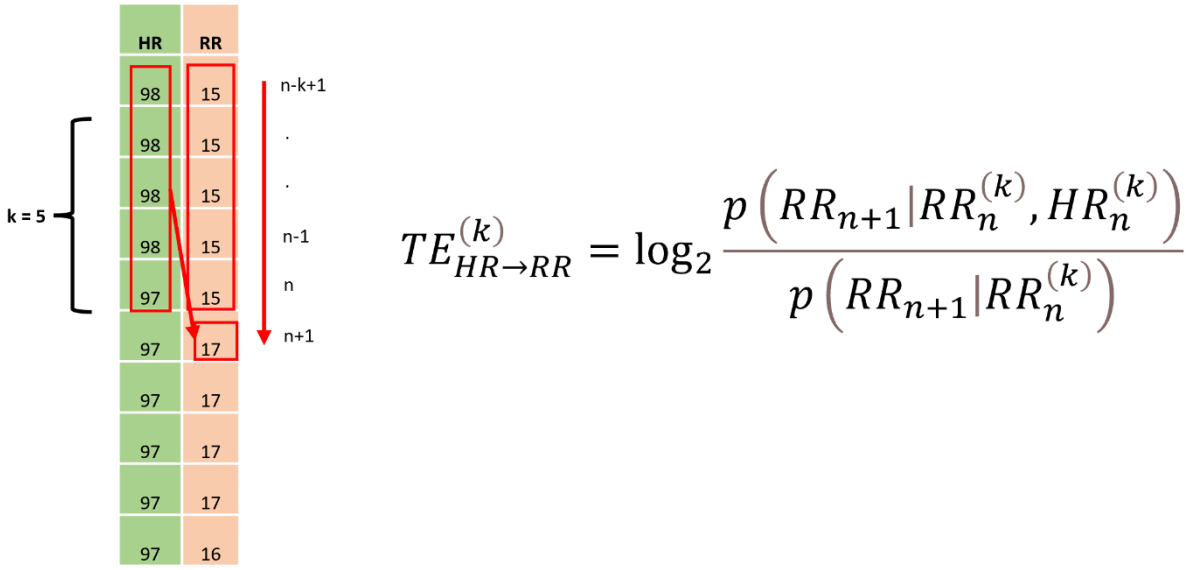



*Figure 7.4. The estimation of transfer entropy from heart rate (HR) to respiratory rate (RR) with source and target history/lag length of k = 5. In this instance, the transfer entropy describes the increase in certainty in predicting the future iteration of RR based on the 5 events prior, conditioned on the 5-prior iteration of HR. This lag time can be expanded depending on data record length and reported delay in information flow between organ systems (see [439] for more details).*

### *MELD-Na and SOFA Calculations*

The first-day MELD-Na of patients was calculated using the formula: MELD-Na=MELD + 1.32 x (137 - Na) - [0.033 x MELD*(137 - Na)] with MELD calculated as MELD=10* ((0.957* ln [Creatinine]) + (0.378* ln [Bilirubin]) + (1.12* ln [INR])) + 6.43) as described [441, 442]. For the calculation of MELD-Na, serum creatinine level was capped at 4.0 mg/dL to avoid bias in the model against patients with sarcopenia or malnutrition [443]. First-day SOFA was also calculated for patients based on patients' urine output; administration of norepinephrine, epinephrine, or dobutamine; Glasgow Coma Score (GCS), mean arterial pressure (MAP), the fraction of inspired oxygen ($FiO_2$), arterial oxygen pressure ($PaO_2$), and ventilation status; as well as the patient's serum creatinine, total bilirubin, and platelet count as described by Vincent et al [444].

*Dynamic Network Mapping*

The dynamic network was mapped for individual patients based on the computed transfer entropy (in bytes) between the organ systems. Specifically, each patient's map includes 3 nodes (for HR, RespR, and SpO2) and the edges between these nodes are weighted based on the computed TEs (***Figure 7.5** A and B*) [352]. The directional information transfer between the variables as well as the global characteristics of the network was analysed for each patient using weighted network topology indices (the maximum indegree centrality and shortest path length). The prognostic values of these indices were statistically tested.

*Statistical analysis*

All statistical analyses were performed using SPSS Statistics 26 (IBM Corp., Armonk, NY). Mann Whitney U test was used to compare the directional information transfer (transfer entropy) and network topology indices between survivors and non-survivors. To assess which variables are linked with survival, a univariate Cox regression analysis was used. Categorical data are presented as counts and percentages while continuous data are presented as median and interquartile range (IQR) or mean ± SD based on normality distribution. For this study, patients who received liver transplantation were recorded as non-survivors as they would not have survived without transplantation [78, 445]. For survival analysis, follow-up was censored at 28 days after ICU admission.

Also, to assess differences in information transfer linked with mortality and disease pathophysiology, further analysis was performed. For a measure of interaction between mortality and disease group on the information flown between physiological variables, a generalized linear model was performed following a factorial design of the Likelihood Ratio Chi-Squared test for the TE's between heart rate, respiratory rate, and SpO2. This analysis assesses whether mortality and disease type have a combined effect on the transfer entropies between the physiological variables and is an alternative to the two-way Analysis of Variance (ANOVA) that is more robust to non-normal data where the assumption of equal variances is not satisfied. For all statistical analyses, a two-tailed p-value of $< 0.05$ was considered statistically significant.

# Results

A total of 169 patients with 20 minutes or more simultaneous recordings of physiological variables (heart rate, respiratory rate, and blood oxygen saturation, SpO2) as well as corresponding demographic, laboratory, and clinical records were included in this study. The baseline clinical characteristics as well as the demography of the study population are presented in *Table 7.1*.

*Table 7.1.* Demography and baseline clinical variables of patients.

| Characteristic | Survivors (90) | Non-survivors (79) | p-value |
|---|---|---|---|
| Age (years) | 57.5 ± 13.7 | 58.1 ± 11.6 | 0.77 |
| Male Sex - Count (%) | 58 (64) | 47 (59.5) | 0.529 |
| | | | |
| *Aetiology of liver cirrhosis - Count (%)* | | | |
| Alcoholic | 47 (52.2) | 36 (45.6) | |
| Non-Alcoholic | 41 (45.6) | 40 (50.6) | |
| Biliary | 2 (2.2) | 3 (3.8) | |
| | | | |
| MELD-Na score | 18.38 ± 11.57 | 25.72 ± 12.1 | 0.001 |
| SOFA score | 5 (3 - 8) | 9 (6 - 12) | <0.001 |
| | | | |
| *Physiological variables* | | | |
| Serum Creatinine | 1 (0.7 - 1.96) | 1.35 (0.86 - 3.08) | 0.009 |
| Serum Albumin | 2.934 ± 0.54 | 2.89 ± 0.66 | 0.725 |
| Total Bilirubin | 1.83 (1.1 - 7.15) | 7.21 (2.88 - 22.58) | <0.001 |
| INR | 1.6 (1.3 - 1.86) | 1.79 (1.4 - 2.68) | 0.002 |
| WCC ($10^9$/L) | 8.11 (5.63 - 12.26) | 9.43 (5.4 - 13.7) | 0.277 |
| Serum Sodium | 136.49 ± 12.71 | 136.53 ± 5.69 | 0.963 |
| MAP | 81.25 (70.15 - 88.71) | 72.36 (67.84 - 81.67) | 0.003 |
| Heart Rate | 83.98 ± 14.41 | 87.57 ± 18.07 | 0.152 |
| $SpO_2$ | 97.24 (95.76 - 98.47) | 97.33 (95 - 98.5) | 0.58 |
| Respiratory Rate | 17.97 ± 3.61 | 18.65 ± 3.72 | 0.231 |

### *Dynamic network analysis*

Dynamic network mapping based on the directional transfer of information (in bytes) between organ systems in survivors versus non-survivors is represented in Figure 7.5A and Figure 7.5B. Further, Table 7.2, shows the result of Mann Whitney U test to assess whether any statistical differences exist in median information transfer amongst organ systems between survivors and non-survivors. Accordingly, while median information transfers were higher in patients who survived 28 days of ICU stay, this difference was not significant.

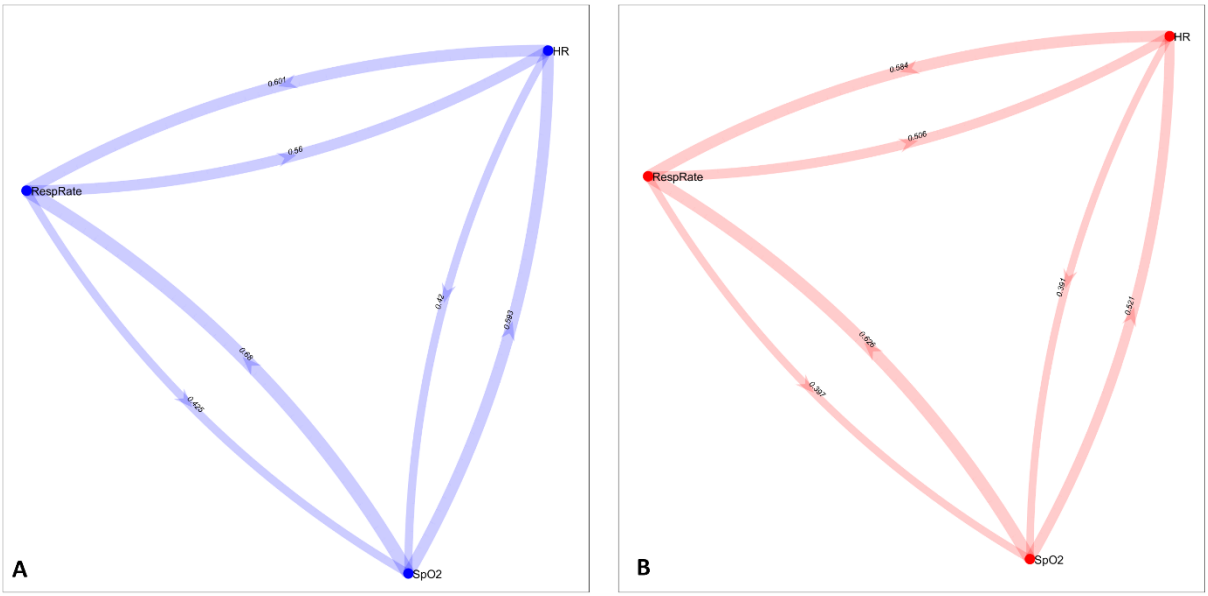



*Figure 7.5. Directional transfer of information in bytes between the 3 physiological parameters assessed (heart rate; HR, Respiratory rate; RespRate, and SpO2; Oxygen saturation) in cirrhosis patients that survived((A) and those that did not survive (B) intensive care unit (ICU) stay after 28 days. The information transfer was estimated based on a mean transfer entropy calculation of 20 minutes of simultaneous recordings of the included clinical variables (time series) and a 10-second lag time.*

*Table 7.2. Differences in directional information transfer between physiological variables of survivors and non-survivors based on Mann Whitney U test.*

| Transfer Entropy (bytes) | Survivors, median (IQR) | Non-survivors, median (IQR) | p-value |
|---|---|---|---|
| Heart Rate → Respiratory Rate | 0.6(0.4 - 0.69) | 0.58(0.33 - 0.71) | 0.857 |
| Heart Rate → SpO2 | 0.42(0.23 - 0.53) | 0.39(0.27 - 0.47) | 0.500 |
| Respiratory Rate → Heart Rate | 0.56(0.42 - 0.66) | 0.51(0.29 - 0.65) | 0.218 |
| Respiratory Rate → SpO2 | 0.43(0.18 - 0.57) | 0.4(0.18 - 0.51) | 0.354 |
| SpO2 → Heart Rate | 0.59(0.32 - 0.74) | 0.52(0.24 - 0.67) | 0.203 |
| SpO2 → Respiratory Rate | 0.68(0.2 - 0.79) | 0.63(0.19 - 0.76) | 0.467 |
| Centrality | 1.13(0.8 - 1.25) | 0.99(0.59 - 1.24) | 0.216 |
| Diameter | 0.74(0.34 - 0.81) | 0.67(0.36 - 0.79) | 0.398 |

*Results are presented as median and interquartile range (IQR). SpO2, Oxygen Saturation; IQR, Interquartile range.*

- ***Prognostic values of dynamic network***

Indeed, no significant difference was observed between the transfer entropies of survivors and non-survivors, and this was supported by univariate Cox regression analysis of all TE's which showed no prognostic value. Further, no significant prognostic values were detected for the mean centrality and diameter of patients' dynamic network. Also, while

measures of disease severity assessed by MELD-sodium predicted survival, SOFA scores were not associated with patients' 28-day survival. (Table 7.3).

*Table 7.3. Univariate Cox regression analysis of transfer entropy between physiological variables to assess and predict 28-day survival in cirrhosis patients.*

| Variables | Beta | SEM | p-value | Hazard ratio |
|---|---|---|---|---|
| Heart Rate → Respiratory Rate | 0.116 | 0.514 | 0.821 | 1.12(0.41 - 3.07) |
| Heart Rate → SpO2 | 0.509 | 0.538 | 0.344 | 1.66(0.58 - 4.78) |
| Respiratory Rate → Heart Rate | -0.673 | 0.502 | 0.18 | 0.51(0.19 - 1.36) |
| Respiratory Rate → SpO2 | 0.314 | 0.473 | 0.507 | 1.37(0.54 - 3.46) |
| SpO2 → Heart Rate | -0.344 | 0.398 | 0.387 | 0.71(0.33 - 1.55) |
| SpO2 → Respiratory Rate | 0.19 | 0.357 | 0.595 | 1.21(0.6 - 2.43) |
| Centrality | -0.122 | 0.323 | 0.705 | 0.89(0.47 - 1.67) |
| Diameter | 0.032 | 0.37 | 0.932 | 1.03(0.5 - 2.13) |
| MELD Sodium | 0.02 | 0.01 | 0.039 | 1.02(1 - 1.04) |
| SOFA | 0.028 | 0.029 | 0.329 | 1.03(0.97 - 1.09) |

*SpO2; Oxygen saturation, 95% CI; 95% Confidence Interval, SEM; Standard Error of Mean, MELD; Model for End-stage Liver Disease, SOFA; Sequential Organ Failure Assessment.*

*Comparison of organ systems information flow between patients with cirrhosis and sepsis admitted to the ICU*

While both are characterised by multiple organ involvement, cirrhosis is a chronic disease with a clinical course that may last over a decade while sepsis is associated with acute deterioration following inflammation [21, 24, 446, 447]. The difference in organ systems information transfer between these two patient groups as well as the influence of the mortality was assessed. The result of the Mann-Whitney U test used to assess the difference in TE's between survivors and non-survivors in the sepsis group is presented in *Table 7.4*. Also, Figure 7.6A and Figure 7.6B represent the dynamic network map of the survivors and non-survivors of sepsis. Generally, median information transfer was reduced in the sepsis group compared with the cirrhosis group. According to the generalized linear model, disease type and survival significantly interact to alter the information transfer from heart rate to respiratory rate ($p = 0.001$), respiratory rate to heart rate ($I^2 = 4.49$; $p = 0.034$), respiratory rate to SpO2 ($I^2 = 4.03$; $p = 0.045$), SpO2 to heart rate ($I^2 = 5.18$; $p = 0.023$) and SpO2 to respiratory rate ($I^2 = 8.40$; $p = 0.004$). This shows that reduction in physiological information transfer across these variables is influenced by both disease type and survival status whereby patients diagnosed with sepsis who did not survive approximately 4-week ICU stay have significantly reduced TE's along these axes compared with patients with cirrhosis. There was no interaction between mortality and disease group on the transfer entropy from heart rate to SpO2 ($I^2 = 3.76$; $p = 0.052$), with TE difference across both grouping variables (*Figure 7.7*). Further, univariate Cox regression analysis showed that TE's across all axes of the variables as well as the global network information flow (centrality and diameter) are associated with survival in the sepsis group (*Table 7.5*).

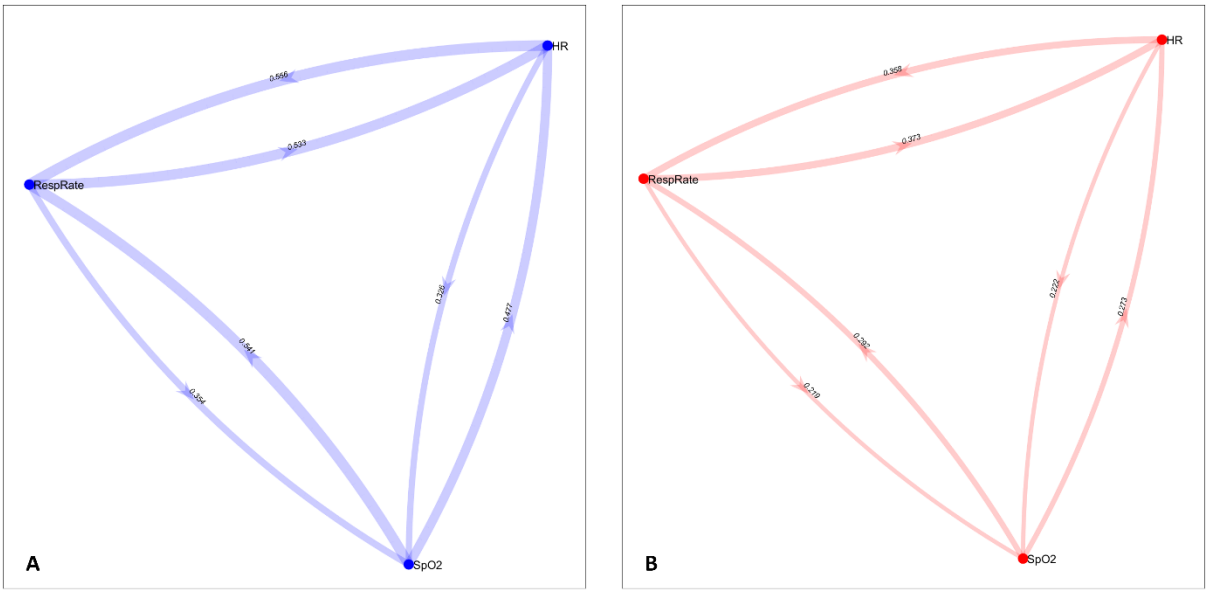



*Figure 7.6. Directional transfer of information in bytes between the 3 physiological parameters assessed (heart rate; HR, Respiratory rate; RespRate, and SpO2; Oxygen saturation) in sepsis patients that survived (A) and those that did not survive (B) intensive care unit (ICU) stay after 30 days. The information transfer was estimated based on the mean transfer entropy calculation of 20-minute simultaneous recordings of the included clinical variables (time series) and 5-second lag time.*

*Table 7.4. Differences in directional information transfer between physiological variables of survivors and non-survivors based on Mann Whitney U test of the sepsis patients' data.*

| Transfer Entropy (bytes) | Survivors, median (IQR) | Non-survivors, median (IQR) | p-value |
|---|---|---|---|
| Heart Rate → Respiratory Rate | 0.58(0.48 - 0.66) | 0.34(0.22 - 0.57) | < 0.001 |
| Heart Rate → SpO2 | 0.37(0.17 - 0.48) | 0.23(0.03 - 0.37) | 0.007 |
| Respiratory Rate → Heart Rate | 0.56(0.46 - 0.65) | 0.44(0.1 - 0.58) | 0.001 |
| Respiratory Rate → SpO2 | 0.41(0.2 - 0.51) | 0.16(0 - 0.41) | 0.002 |
| SpO2 → Heart Rate | 0.58(0.32 - 0.69) | 0.07(0 - 0.62) | 0.001 |
| SpO2 → Respiratory Rate | 0.67(0.47 - 0.76) | 0(0 - 0.63) | < 0.001 |
| Centrality | 1.33(1.22 - 1.45) | 0.98(0.67 - 1.31) | < 0.001 |
| Diameter | 0.7(0.51 - 0.77) | 0.34(0.03 - 0.64) | < 0.001 |

*IQR; Interquartile range, SpO2; Oxygen saturation.*

*Table 7.5. Univariate Cox regression analysis of transfer entropy between physiological variables to assess and predict 28-day survival in cirrhosis patients.*

| Transfer Entropy (bytes) | Beta | SEM | p-value | Hazard ratio (95% CI) |
|---|---|---|---|---|
| Heart Rate → Respiratory Rate | -3.742 | 0.725 | < 0.001 | 0.02(0.01 - 0.1) |
| Heart Rate → SpO2 | -2.129 | 0.867 | 0.014 | 0.12(0.02 - 0.65) |
| Respiratory Rate → Heart Rate | -2.822 | 0.702 | < 0.001 | 0.06(0.02 - 0.24) |
| Respiratory Rate → SpO2 | -2.516 | 0.822 | 0.002 | 0.08(0.02 - 0.41) |
| SpO2 → Heart Rate | -1.958 | 0.587 | 0.001 | 0.14(0.05 - 0.45) |
| SpO2 → Respiratory Rate | -1.96 | 0.521 | < 0.001 | 0.14(0.05 - 0.39) |
| Centrality | -2.595 | 0.521 | < 0.001 | 0.08(0.03 - 0.21) |
| Diameter | -1.665 | 0.508 | 0.001 | 0.19(0.07 - 0.51) |

*SpO2; Oxygen saturation, 95% CI; 95% Confidence Interval, SEM; Standard Error of Mean.*

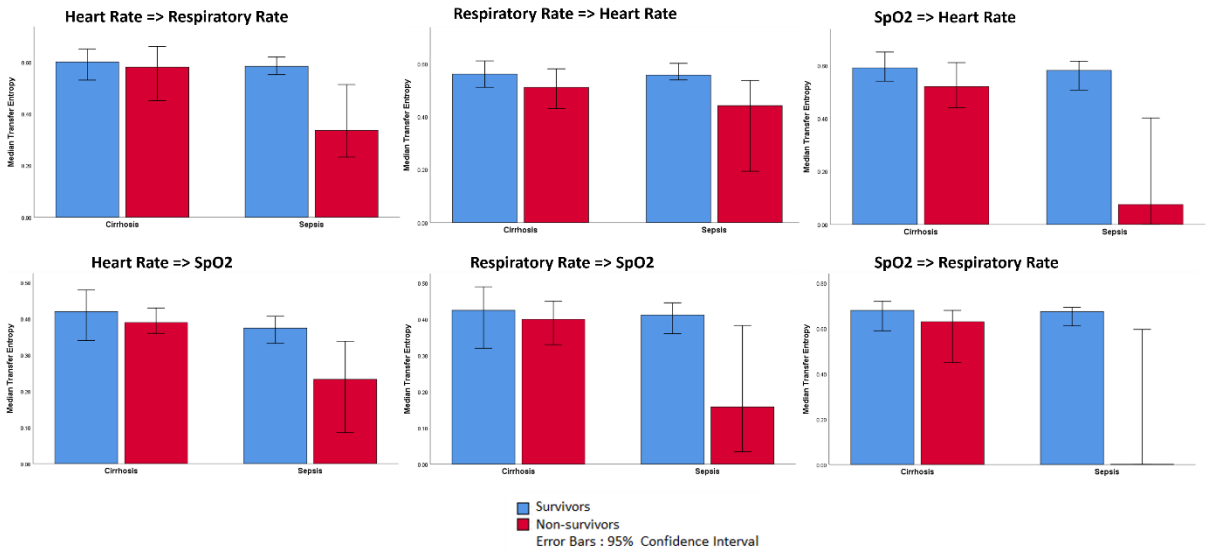



*Figure 7.7. Bar graph showing interactions between mortality and disease type on TE's between heart rate, respiratory rate, and $SpO_2$ of patients admitted to the intensive care unit. Comparison is based on a measure of interaction according to generalised linear model analysis with factorial design. Mann Whitney U test was then used to assess differences in the TE's within the disease. ns; Not significant based on Bonferroni-corrected p value, $SpO_2$; Blood oxygen level/saturation.*

## Discussion

To the best of our knowledge, this is the first study where the transfer entropy measure, an information algorithm used to assess causal links between time series, is assessed for prognostic value in cirrhosis. While various previous studies investigating organ systems connectivity in cirrhosis have shown valuable results regarding the prognosis and treatment response, the methods used to establish links were based on statistical correlations. However, while correlation may infer association, it is not a measure of causality [448]. Results of this study show that while there is a general reduction in organ systems information transfer in cirrhosis patients who did not survive up to 28 days of ICU stays compared with survivors, these differences were neither statistically significant nor linked with mortality. However, patients' MELD-Na, the standard prognostic and severity measure in cirrhosis was linked with 28-day mortality.

Several reasons may explain the lack of association between reduced physiological information transfer and survival in the study population. Firstly, some patients are critically ill with clinical complications that warrant ICU admission. Thus, these patients may possess various comorbidities or may be undergoing procedures and treatments that could influence organ systems coupling. Indeed, the influence of procedures like different ventilatory methods may interfere with normal organ system connectivity. For instance, Nataj et al. showed previously that the conventional mode of mechanical ventilation results in reduced heart rate variability compared with fractal-like ventilation in an experimental model of critically ill cirrhosis (i.e., bile duct ligated rats challenged with endotoxin) [67]. The severity and stage of cirrhosis is a major influencing factor in the clinical course of patients. For instance, Sundaram et al showed that for patients with acute-on-chronic liver failure, conventional severity scoring systems (e.g., MELD) do not capture the pathophysiology and clinical course (prognosis) of patients [449].

Another influencing factor may be how and when clinical data and time series were recorded for each patient. Because the MIMIC-III project was designed to observe the clinical course of critically ill patients with all possible underlying diseases there is heterogeneity in the patients' groups [450]. Thus, influences of factors like the circadian rhythm on organ systems connectivity could not be accounted for. Indeed, previous studies have shown that the sleep-wake cycle may influence organ systems connectivity

measured by the ratio of low-frequency to high-frequency power of HRV, an index of sympathovagal regulation of the heart rhythm [451, 452]. Importantly, information on possible influencing factors was not considered based on the retrospective design of this study and the general nature of the data source. Indeed, the heterogeneity of the studied population means patients' disease course is not uniform, and grouping them analytically may present reduced coherence due to inherent heteroscedasticity [453]. However, computation of transfer entropy requires specialised dataset; specifically, parallel recordings of physiological variables to assess temporal information transfer and the MIMIC-III dataset presents a unique opportunity to show the feasibility of causality measures calculation in patients with cirrhosis.

Also, a temporal information transfer delay of 10 seconds was used based on the optimisation step to assess the changes in the mean TE's across a lag time varied between 1 and 20 seconds. Indeed, the delayed information relay detected in the optimisation test result aligns with previous studies which showed that physiological memory length within the cardio-respiratory system is between 5 to 10 seconds in healthy adults and delayed in cirrhosis patients [92], as well as in patients with congestive heart failure [94]. The delayed information flow and longer memory length are hypothesised to be associated with organ systems disconnection whereby physiological perturbations are sustained longer in disease states compared with healthy controls. Similarly, results from Chapter 3 showed abnormal heart rate turbulence and associated reduction in baroreflex sensitivity. Specifically, indices of heart rate turbulence (i.e., TO and TS), computed between 1 to 15 heartbeat intervals following a premature ventricular contraction were shown to be altered in cirrhosis [215]. Indeed, diminished baroreflex sensitivity is a well-documented effect of cirrhosis [454-456] and may indicate a decoupling of the baroreceptor from the cardiac rhythm. Thus the 10-second delay resulting from the optimisation step agrees with previous results in this thesis. In line with this, other studies have also shown uncoupling of the autonomic modulation of cardiac rhythm based on heart rate variability measures [78, 144, 457]. Further, the dampening of the sympathetic tone of the splanchnic and peripheral vasculature resulting from hyperdynamic circulation due to liver failure and portal hypertension has been well documented [458, 459] and may explain the delay in physiological information transfer. Other factors such as inflammatory dysregulation (e.g., increased circulation of pro-inflammatory biomarkers

[90]), and the activation of the renin-angiotensin-aldosterone pathways [460, 461] associated with decompensated cirrhosis may alter information flow in unique ways which may further elongate the time required for efficient organ system information transfer (thus, decoupling). Indeed, the optimization steps described in the method of this chapter show a continuous increase in TE towards 20 seconds. A potential focus of further research in this field would be to extend the lag time to assess whether the severity of cirrhosis and survival is linked with even higher lag time and the part played by certain disease dynamics including treatment and inflammatory dysregulation.

Another limiting factor is the low sample size analysed which may explain the lack of statistical significance observed between survivors and non-survivors in terms of transfer entropies in cirrhosis patients in the ICU. Perhaps a higher sample size would have provided a better representation of the population. Also, a multicentred, prospectively designed study targeted mainly at cirrhosis patients possibly in the general ward who are followed up for longer periods would have provided more power, and more details about patients' characteristics with the opportunity to control for various confounding factors. It would be interesting to see if studies of such in the future could extract possible values of dynamic network analysis in cirrhosis or other chronic diseases.

Further, this study assessed the information flow between heart rate, respiratory rate, and SpO2 which represents the cardiopulmonary pathways. The lack of prognostic significance of the transfer entropies between these variables may be linked with inherently short information relays between them. For instance, previous findings from the investigation for HRV and HRT in cirrhosis (Chapter 2 and Chapter 3) have shown that indices of long-term effects (SD2, DFA-α, SDNN, and TO) mostly driven by physiological regulators that work over extended periods (such as metabolism, baroreflex, thermoregulation, endocrine systems, circadian rhythm) may offer better prognostic values compared with those that span shorter time. However, the source of the data used in this study (MIMIC-III database) is limited in terms of clean available data. Specifically, data on heart rate, respiratory rate, and SpO2 were the only available variables analysable and importantly provided a chance to assess the feasibility of transfer entropy-based dynamic network analysis of physiological time series in cirrhosis.

Finally, physiological information transfer between patients with cirrhosis and those diagnosed with sepsis was compared to understand how disease pathophysiology may affect organ systems connectivity. The result of the analysis shows that while there is no significant difference between the TE's of survivors and non-survivors in the cirrhosis group, the information flow to and from all variables was significantly different based on survival in the sepsis group. However, a generalised linear model with a factorial design showed that the disease group interacts with survivors to drive the changes in information transfer between the physiological variables except along the heart rate -> SpO2 axis (*Figure 7.7*). These interactions further support the observed difference between the groups whereby patients with sepsis show marked differences in cardiorespiratory TE's compared with cirrhosis patients. Put together, overall cardiorespiratory information flow was significantly more altered in sepsis patients who did not survive compared with those who survived even though this difference was not observed in cirrhosis patients irrespective of survival status. This finding corroborates a previous study where organ systems connectivity based on HRV measures was assessed in animal models of cirrhosis versus control. Specifically, Haddadian et al investigated the effect of endotoxin injection (to model systemic inflammatory response syndrome) on cardiac-autonomic nervous system connectivity and chronotropic responsiveness of their isolated atria to cholinergic stimulation [435]. Cirrhotic rats were found to exhibit significant but diminished sensitivity to endotoxin injection in terms of HRV changes and there were no significant changes in chronotropic responsiveness to cholinergic stimulation in cirrhosis compared to controls [435]. Thus, cirrhotic rats showed reduced coupling of the cardiac rhythm with autonomic regulation which may be linked with inflammation [291]. Indeed, the reduced disruption to the connectivity of the cardiorespiratory system in cirrhosis compared with sepsis patients in the ICU may be driven by a similar mechanism due to the relatively chronic clinical history and the development of cardiorespiratory tolerance to inflammatory dysregulation previously reported in a rat model of cirrhosis [462].

In conclusion, dynamic network analysis based on the transfer entropy of physiological variables can be assessed in cirrhosis and is not significantly linked with survival. Also, while sepsis patients who did not survive ICU stay showed significantly reduced cardiorespiratory information transfer, there was no significant difference in cirrhosis patients who survived and those who did not survive ICU stay. Future studies should

investigate changes in organ system coupling based on transfer entropy measures between physiological time series in a more representative cirrhosis population in a multicentred group possibly outside of the ICU.

# Chapter 8 : Discussion

# General Discussion

The ability to self-organize in response to stimuli is a universal characteristic of complex systems such as social networks, internet networks, networks of millions of ants that form ant colonies, networks of cells that form a functional tissue, networks of tissue that make up an organ and network within an organ system. Indeed, the human body represents such complex systems propped up through intricate communes of various functioning units evolving together in response to information received from the environment. Such informational stimuli could be physical in the form of touch, light, sound, or temperature; chemical in the form of food, or water; and of course, social in the feeling and response to various cues. Over the past decades, the implications of some of these stimuli have been shown to have initially subtle, but increasingly significant effects on various aspects of human functioning [463, 464]. Increasingly, a holistic, multisystemic approach is becoming evident as a better alternative to the current reductionistic methods in the management of various diseases [465-467].

The human body is a combination of diverse yet convergent organ systems that continuously communicate across varied time and space to regulate and optimize survival [13]. In this thesis, I have used various measures of organ system coordination and communications to assess the prognosis and response to treatment in liver diseases including cirrhosis and acute liver failure. The findings are as follows.

1. Following a systematic review of literature, heart rate variability (HRV) which measures the influence of various organ systems on heart rhythm is significantly reduced in cirrhosis and may predict survival independent of MELD. Specifically, long-term indices of HRV were significantly linked with survival in cirrhosis (*Chapter 2*).
2. Where HRV could not be accurately assessed due to artifacts in ECG measurement such as premature ventricular contractions (PVC's), heart rate turbulence (HRT), a measure of the physiological coordination aimed at normalizing the heart rhythm following PVC's is a viable alternative able to predict survival in cirrhosis independent of severity models (Child-Turcotte-Pugh and MELD scores) (*Chapter 3*).

3. Correlation network analysis in two independent cohorts of patients with cirrhosis (one from UK and other from Italy) indicated that cirrhotic patients with poor prognosis have less significant correlation between clinical/biochemical biomarkers.
4. Parenclitic network analysis which measures the physiological deviation of individual patients from expectation can predict survival in cirrhosis independent of MELD. The deviation was found to be linked with disruption in coordination or coupling along the albumin-bilirubin and albumin-prothrombin time axes (*Chapter 4*).
5. Parenclitic network analysis in a larger group of decompensated cirrhosis patients admitted to clinics (in England, Scotland, and Wales) for decompensation events also predicted survival independent of MELD with deviation specifically linked with inflammatory dysregulation along the WCC-CRP physiological axis. The parenclitic network analysis also identifies patients likely to not respond to short-term (two weeks) increased albumin treatments (*Chapter 5*).
6. Analysis of physiological variables using parenclitic network methods predict survival in ICU patients with paracetamol-induced acute live failure. In this retrospective analysis of clinical and laboratory data (from the MIMIC-III Database), regulation of pH was different between survivors and non-survivors although no significant difference was found between the mean pH of the groups. Also, variables linked with haemodynamic, and liver function were linked with survival following a principal component analysis (*Chapter 6*).
7. Finally, dynamic network analysis based on information transfer between the physiological waveform of heart rate, respiratory rate, and $SpO_2$, recorded in parallel did not predict survival in critically ill patients with cirrhosis admitted to the ICU. Comparison with patients admitted for sepsis shows that sepsis is accompanied by a significant reduction in cardiopulmonary information flow which is significantly associated with mortality (*Chapter 7*).

Overall, this thesis falls within various themes of prognosis research covered within the recently developed PROGRESS framework, aimed at streamlining prognostic studies for easier synthesis and clinical translation [468]. Specifically, the assessment of prognostic values of parenclitic network analysis in patients with cirrhosis admitted for

decompensated cirrhosis in different health settings (Italy in *Chapter 4* and the UK in *Chapter 5*) who are subjected to standard of care, falls within theme 1 of the PROGRESS framework (i.e., overall prognosis research). The second theme of the framework identifies prognostic factors research which covers various studies directed toward the identification of factors that drive the risks of specific clinical outcomes. *Chapter 3* to *Chapter 6* of this thesis identify various factors (HRT and parenclitic network indices) independently linked with mortality in cirrhosis [469]. Network physiologic analysis intrinsically involves the modelling of organ system coupling as a measure of a physiological stable state. In this thesis organ systems network models (based on HRT and parenclitic network analysis) have shown organ system decoupling as a predisposition to poor prognosis in cirrhosis. Thereby fulfilling the requirements of the third theme of the PROGRESS framework [470]. The last theme of the PROGRESS framework covers research aimed at investigating factors associated with treatment effects. Specifically, this theme describes studies aimed at factors that could define how individual patients respond to a particular treatment [471]. These types of predictive studies are the bedrock of targeted and precision medicine which has been repeatedly earmarked as the next big step in critical care as well as the management of complex diseases [472]. This is because they signal a move away from the classic "syndrome" and monochromatic approach to critical and complex illnesses, paving the way for a context-based method for interpretation, management, and treatment that appreciates the implication of varied contributory factors to the clinical dynamics of seemingly similar clinical conditions (please see for [473] more). In *Chapter 5*, parenclitic network indices were used to identify patients admitted to the clinic for decompensated cirrhosis who did not benefit from two weeks of increased and targeted albumin infusion. Thus, this study falls within the requirements of the fourth theme of the PROGRESS framework.

Cirrhosis is a multisystemic disease with effects on various extra-hepatic organs leading to dysfunctions and failures. Thus, decompensation is characterised by the dysregulation of various organ systems directly and indirectly related to the regulation of heart rhythm. Indeed, HRV in cirrhosis has been extensively studied and reported widely to be especially trended towards a significant reduction both in short-term and long-term indices [90, 178, 185, 191, 202]. These indices represent various functional associations between heart rhythm and extra-cardiac regulations. For instance, while indices such as HF (high-

frequency power) represent respiratory sinus arrhythmia, others such as the ratio of low to high-frequency power (LF: HF) and ultra-low frequency power (ULF) may respectively represent sympathovagal regulation as well as the influence of core-body temperature, metabolism, and hormones on the heart rhythm [199]. Conversely, HRV measures the interactions of various organ systems with the sinoatrial node, the main source of cardiac electric firing. Thus, HRV is a reliable surrogate measure of network interactions between various organ systems communicating together to regulate cardiac output. While HRV is generally reduced in cirrhosis, some indices have also been shown to independently predict the survival of patients regardless of disease severity. For instance, Bhogal et al showed that SD2 of the Poincare plot and corrected SDNN predicted 18-month survival in patients with cirrhosis independent of MELD [184]. Further, reduction in SDNN and detrended fluctuation analysis alpha 2 (DFA α2) were also reported to be linked with poorer prognosis in cirrhosis [7, 197]. These indices are mainly physiologically linked with influences from organ systems that exert their regulation over a lengthy period (≥ 2 minutes). For instance, the SD2 has been associated with the low-frequency power of HRV and may reflect baroreflex activity as well as both arms of the autonomic nervous system's influence on the heart rhythm [199].

Accordingly, this work found that while HRV has been intensely researched in cirrhosis, translation to the clinic is limited mainly by the lack of consistency in ECG sampling techniques (recording time, length, and sample frequency), and HRV computation (e.g., standardized length of recordings for calculation of indices) resulting in significant variability has shown by Chi-squared measure of between-studies heterogeneity when the studies were pooled in a meta-analysis (see *Chapter 2*). Aside from the variability in reporting, another significant limitation of HRV is that it depends on clean ECG recordings. While this may be possible in some, patients with cirrhosis have been reported to show abnormal sinus rhythm with artifacts that may result in exaggerated calculated variability in heart rhythm (e.g., LF: HF), and this may result in misinterpretation of research findings [474, 475]. Where ECG is constantly interspersed with artifacts such as PVC's, heart rate turbulence (HRT) indices provide a viable alternative to assess the state of autonomic nervous control of the sinus rhythm in cirrhosis. Heart rate turbulence indexes the interplay between various organ systems including both sympathetic and parasympathetic arms of the autonomic nervous system as well as baroreceptors

(baroreflex) activity, to return the heart rhythm to pre-PVC level. Mechanistically, transient blood pressure drop following a PVC is detected by baroreceptors resulting in vagal nerve inhibition and sympathetic nervous activation. This results in a sharp blood increase which is followed by a blood pressure fall up to approximately the pre-PVC level. Thus, HRT is mainly driven by baroreflex and sympathetic nervous control and can provide information about the state of these organ systems [220]. TO measures the average difference between the R-R intervals from before and after PVCs and is generally lower in healthy subjects while TS is the steepest regression line fitted over 5 consecutive post-PVC sinus rhythms and measures deceleration of the sinus rhythms following the post-PVC tachycardia [221].

Indeed, previous studies have shown the independent prognostic values both of turbulence onset (TO) and turbulence slope (TS) in patients who survived myocardial infarction [476]. In line with these previous studies, this thesis shows that TO is associated with mortality and predicted survival independent of the severity of cirrhosis measured by MELD and Child-Turcotte-Pugh scores (Chapter 3). Indeed, analysis in this study shows a relatively lower TO in survivors compared with non-survivors (*Table 3.2*). This corroborates the study by Ksela et al which reported significantly lower TO in heart failure patients with preserved ejection fraction that did not survive following 1 year follow-up period [477]. While the mechanistic link is not well defined, findings from this work and others further support dysregulation in baroreflex and autonomic nervous system in cirrhosis which pathologically increases the risk of mortality. Generally, the association between HRV and physiological network indexes autonomic cardio-respiratory coupling and may not directly reflect the influence of all other interacting components within the physiological network hub (e.g., coagulation, biliary excretion among others). A novel method was thus developed to visualize and map organ systems networks based on routine clinical data.

Coordination between organ systems is the basis for stability against external or internal perturbations and loss of correlation has been previously linked with poorer prognosis in patients with sepsis. Specifically, Asada et al reported that a population-based correlation network between organ systems can help assess systemic instability and is associated with a higher risk of mortality in critically ill patients admitted to the intensive care unit [11,

478]. However, the network analysis performed by Asada et al is population-based and does not cater to individual patients' variability limiting its potential use for personalised diagnosis, prognosis, and treatment. In this work, a novel network analysis method was used to resolve this limitation. Using parenclitic network analysis, which assesses the deviation of a single patient pair of variables from a modelled expectation, on cirrhosis patients, this research found lower correlation network connectivity in non-survivors compared with survivors (*Table 4.2*a vs *Table 4.2*b), supporting works by Asada et al [11, 478]. For visualization and context, directed acyclic graphs (DAGs), was used to infer causal links between the variables used in the construction of parenclitic models in this thesis. DAG is a useful tool that uses unidirectional arow arrows to indicate causal link between two variables such that the lack of direct causation is implied by the absence of an arrow [479, 480]. DAGs were constructed for the variables included in the parenclitic models used in Chapter 4, and Chapter 5 (i.e., Figure 8.1 and Figure 8.2 respectively). This is to provide biological context for the expected links between physiological variables included in the models used to predict survival in patients with decompensated cirrhosis.

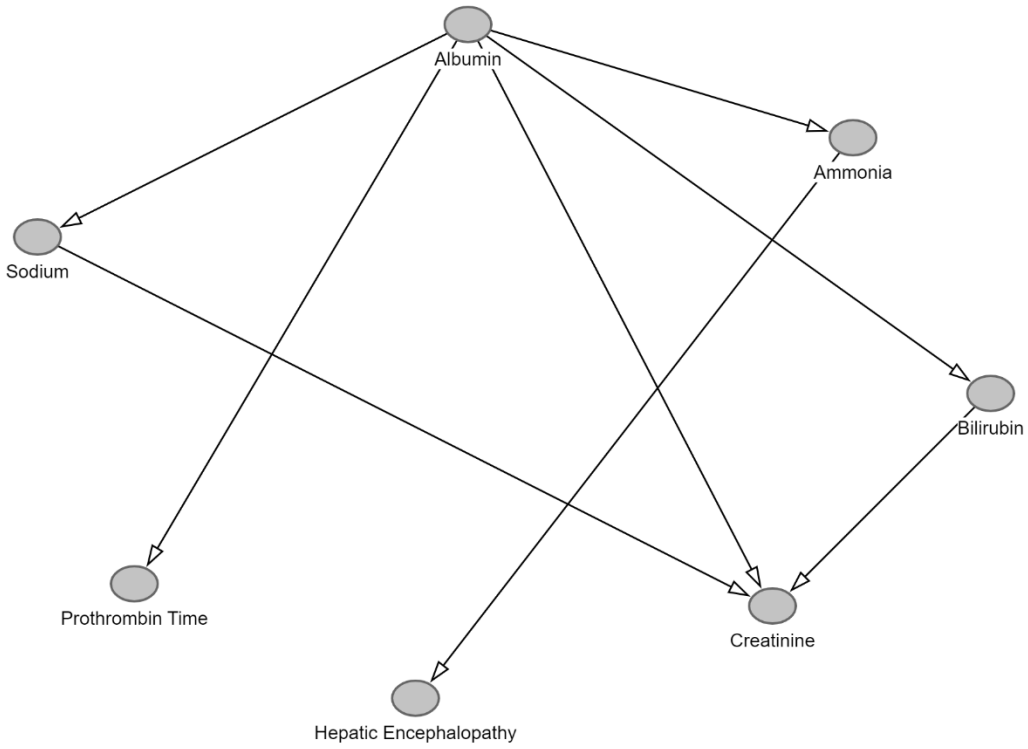



*Figure 8.1. Directed Acyclic Graph (DAG) showing the inferred causal relationship between serum albumin, ammonia, total bilirubin, serum creatinine, hepatic encephalopathy, prothrombin time and serum sodium.*

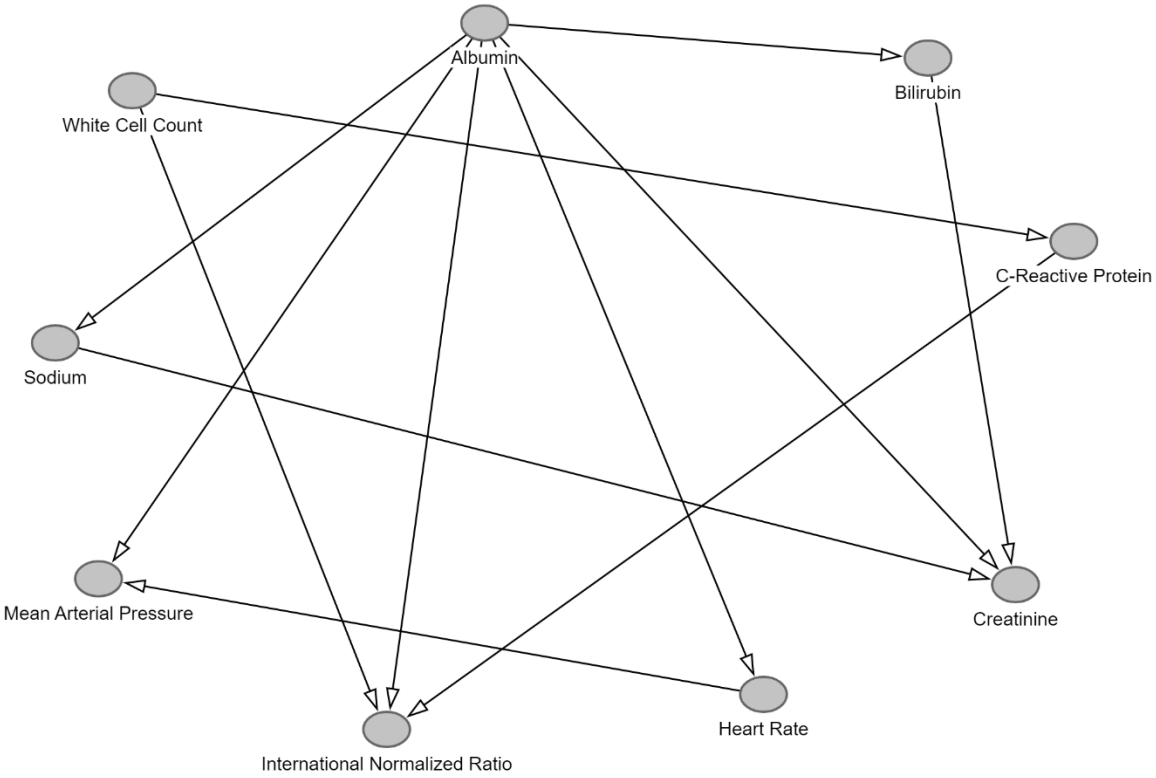



***Figure 8.2.*** *Directed Acyclic Graph (DAG) showing the inferred causal relationship between serum albumin, ammonia, total bilirubin, C-reactive protein, serum creatinine, heart rate, international normalized ratio, mean arterial (blood) pressure, serum sodium, and white cell count.*

Further, parenclitic deviations along the albumin-bilirubin and albumin-prothrombin time pathways were found to predict 12-month survival independent of MELD (Table 4.4). The combination of these parenclitic variables with MELD created a composite score which significantly improved the predictive value of MELD (***Figure 4.3***). The prognostic importance of albumin and bilirubin in combination shown in this work is also supported by results by Wang et al. Although combined as isolated variables, the ALBI (albumin-bilirubin) score was found to predict the prognosis and severity of hepatitis B-related cirrhosis [481]. Also, prothrombin time measures coagulopathy which is well well-established prognostic variable in cirrhosis [482]. However, how it links with albumin to drive prognosis remains to be investigated and clarified in future research.

In a further validation study using parenclitic network analysis on 777 patients admitted into hospitals for decompensated cirrhosis, this study shows that deviations along the CRP-WCC and Bil-WCC pathways, as well as global network measures such as mean

centrality, shortest path length, and network diameter, significantly predict survival independent of MELD and patients' age (*Table 5.4*). Also, deviations along CRP-WCC, mean centrality, shortest path length, and network diameter significantly improved the predictive values of MELD (*Table 5.6*) and most importantly predicted patients likely to benefit from albumin infusion (*Figure 5.3* – *Figure 5.5*). Importantly, the predictive value of the CRP-WCC was found to be independent of the individual components of the axis (i.e., CRP and WCC; **Error! Reference source not found.**), showing that the association b etween these variables provides physiological information not offered by the variables individually. Specifically, this work shows for the first time that patients with relatively better organ system connectivity do not benefit from short-term (14 days) increased albumin infusion compared with patients with poorer connectivity for which no significant difference was found in terms of survival (*Figure 8.3*). Indeed, previous studies have reported relatively higher adverse events such as pulmonary oedema and fluid overload in patients infused with albumin [28, 295, 483].

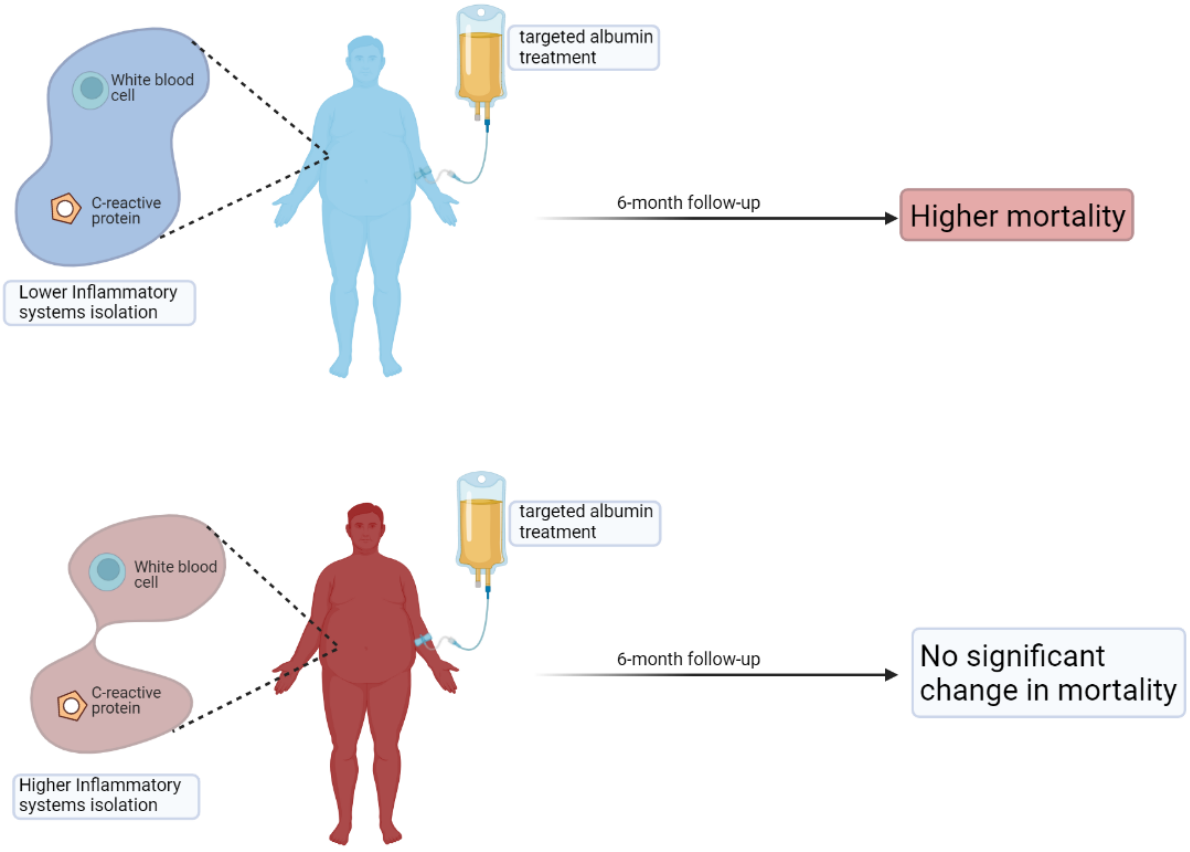


*Figure 8.3. According to the analysis of organ system connectivity using a parenclitic network, patients with lower network disconnection in the inflammatory pathways are more likely to be armed by increased albumin infusion compared with patients with higher inflammatory system isolation for which infused albumin did not result in a significant difference in mortality (see [9] for more). Image created using Biorender.*

The process of cirrhosis is a slow process that takes more than a decade to affect the physiological networks significantly. On the other hand, Acute Liver Failure is an acute process defined as the presence of severely worsening acute liver injury (<26 weeks) in patients with no history of chronic liver disease. I wondered if parenclitic deviation can be applied to acute conditions where liver function has deteriorated rapidly without any previous underlying chronic predisposition or insults. Thus, the prognostic value of parenclitic network analysis was further investigated in a group of ICU patients diagnosed with paracetamol-induced acute liver failure (p-ALF). The p-ALF population was extracted from the Medical Information Mart for Intensive Care (MIMIC) III database [484] and involved 640 patients. The result of k-clique percolation shows different physiological communities for survival compared with non-survivors related to pH regulation which is strongly linked with kidney function (Cr) in survivors and linked with respiratory function in non-survivors (***Figure 6.1*** *and* ***Figure 6.2***). Further, parenclitic deviation along pH-bicarbonate, pH-creatinine, lactate-glucose, lactate-heart rate, and SpO2-respiratory rate pathways predicted mortality independent of the patients' SOFA (sequential organ failure assessment) score and the Kings College Criteria (KCC) (table 6.4). Indeed, composite scores combining the parenclitic deviations with SOFA scores of p-ALF patients showed an increased prognostic value. The SOFA score is a prognostic model used for predicting mortality in patients admitted to the ICU and models severity based on the combination of the patient's platelet count, Glasgow Coma Scale, total bilirubin, creatinine/urine output, arterial partial pressure of oxygen ($PaO_2$) and the fraction of inspired oxygen ($FiO_2$) as independent units irrespective of the aetiology of critical illness [485]. This work shows that composite scores created from the combination of parenclitic network indices, especially those related to pH balance and lactate with SOFA resulted in increased prognostic performance (***Table 6.6*** and ***Table 6.7***). Lactic acidosis is a main effect

of paracetamol overdose which may predispose or occur in patients already showing signs of hepatoxicity [486, 487]. Irrespective of the temporal difference in the onset of arterial lactic acidosis, its diagnosis has been significantly linked with an increased risk of mortality in patients with p-ALF [488, 489]. Indeed, blood lactate level and arterial pH are included in the King's College Criteria [490] which was found to be significantly linked with mortality in this thesis. However, the parenclitic deviations were found to predict the 4-week survival of p-ALF patients independent of KCC and SOFA scores (table 6.4), showing that it can detect certain dimensions of the pathophysiology of the disease not captured by these models. Specifically, the cluster analysis identifies differences in the physiological communities (i.e., organ systems clustering) between survival and non-survival specifically linked with pH regulation. Specifically, survivors' pH clusters with kidney function which may imply better renal compensatory mechanisms to regulate acidosis in the group. Indeed, the kidney is a major player in the systemic regulation of pH [491] and this connection was not found in the non-survivors. Also, the parenclitic indices were combined only with SOFA because it is the most likely scoring model used for prognosis in the ICU, and KCC is most likely not used [492].

Overall, parenclitic network analysis, measuring the strength of the relationship between physiological variables provides a cross-section of organ system connectivity in patients and improves on the current prognostic paradigm of considering biomarkers and organ systems as isolated, independent units. To further assess the robustness of the parenclitic network analysis algorithm, the bidirectionality of the parenclitic deviations was tested. Specifically, the aim was to verify that the parenclitic deviation along the axis of variable X to Y is the same as the deviation along the axis of variable Y to X by flipping the variables around and computing the parenclitic deviation along both axes. Results confirmed that irrespective of the direction of correlation (either X-Y or Y-X) parenclitic deviations remain the same. Thus, the computation of parenclitic deviation is not affected by the arrangement of variables in the data analysed.

Despite the various strengths of the parenclitic network method, its dependency on correlation as the basis for establishing association remains a major limitation. Thus, a network method that could establish causal links as a basis for physiological coupling may provide a better measure of the health state of patients. In line with this hypothesis,

dynamic network analysis was performed on ICU patients diagnosed with cirrhosis based on causality measures between time series of physiological variables. Specifically, information flow between organ systems was computed based on transfer entropy between heart rate, respiratory rate, and oxygen saturation ($SpO_2$) and used to construct a physiological network map for individual patients. The resulting TE's as well as the global network map topology were planned to be assessed for prognostic value against the current models used both for cirrhosis and generally in ICU patients (MELD-Na and SOFA respectively). The TE's of patients diagnosed with sepsis were also computed to assess whether there are fundamental differences in information flow between patients with the chronic complex disease (decompensated cirrhosis) and acute deterioration (sepsis). Results show that information flow between the variables (mainly representing organ systems associated with cardiorespiratory functions) was not associated with mortality in cirrhosis patients. A possible reason for this may be due to the limited physiological scope covered by the analysed variables. Specifically, these variables are closely linked with the respiratory sinus arrhythmia related to the short-term indices of the HRV measures which although reduced in cirrhosis were not linked with survival. Indeed, as shown in ***Chapter 2*** and ***Chapter 3***, long-term indices of HRV and HRT offer better prognostic value compared with short-term indices. Thus, the inclusion of other physiological variables in the dynamic network especially those with long-term variation may provide prognostic insights into cirrhosis. Also, in terms of the comparison of severity level, the cirrhosis patients included in the dynamic network analysis of TE's are critically ill and need ICU admission. This means that the study population is physiologically more like the non-survivors in the ATTIRE cohort of patients admitted to the general Wald in ***Chapter 5***. Specifically, the calculated median MELD score for the overall MIMIC-III cirrhosis population included in the dynamic network analysis was found to be close to that of the non-survivors in the ATTIRE study population [i.e., median (IQR): 21.92(12.36 – 31.17) vs 21.92(17.69 – 26.33)] (see ***Table 5.1***). Providing a further potential reason for the lack of association between the TE's and patients' ICU survival. Despite the several limitations mostly related to the data source (MIMIC-III), this thesis shows that computation of TE's for causal linkage between physiological variables is feasible and lays the foundation for future prospective studies on the subject.

*Limitations*

Despite its usefulness, some universal limitations of the network approach to physiology have been highlighted. Firstly, quantification of the dimension and dynamics of network physiology relies heavily on the availability and quality of relevant data [493, 494]. Although some more data could be generated using the omics approach or specialized equipment, this will be relatively too expensive and may not be feasible in many clinical settings around the world. Thus, the current approach of using routine clinical data for parenclitic network analysis is a relatively simple, useful, less expensive, and highly accessible method. For the assessment of organ systems connectivity using HRV, entropy, and other measures of variability of physiological variables, the main limitation remains the heterogeneity of the methods used which limits meaningful interpretation, generalization, and clinical applicability [78].

Also, while static network approaches (e.g., correlation and parenclitic), which assess organ systems coupling based on correlation are feasible with routine data, the dynamic network approach, which provides the network map based on causal links between physiological time series requires relatively more sophisticated datasets not currently standard in most clinical settings. The assessment of dynamic networks is especially important and should be developed and tested further since interacting organ systems generate information at varying time scales (from milliseconds to hours) with associated differences in dynamic outputs (random, stochastic, oscillatory, etc.) sometimes with transient information sharing corresponding to internal and external challenges to the overall system [3, 495]. This 'fleeting', multiscale coupling may elucidate a crucial juncture in the dynamic network of the system which is important for in-depth understanding of the current physiological state as well as predicting future clinical events [3]. Indeed, the use of causal indices based on mathematically more sophisticated methods may provide further insights and should be the focus of future research in the field. However, for the computation of causal (dynamic) networks, the reliance on specialised, ICU-based equipment is a major hindrance that may be overcome by larger, carefully designed prospective, multicentre, case-control studies targeting the specific population of interest equipped with relevant tools for simultaneous physiological data gathering. Indeed, some of the required data may be partly generated using wearable technologies which are becoming more useful in clinical research [496, 497]. Further, the findings in this thesis

need external validation with a larger, multicentre, and possibly multinational patient population representing a diverse standard of care. Also, the parenclitic deviation is computed based on linear regression which can be improved in the future using non-parametric alternatives as well as orthogonal regression analysis for the computation of the deviation. For clinical translation, the parenclitic network mapping requires a standard reference population which needs to be investigated and validated.

## Conclusion and prospect

Despite current limitations associated with data availability and techniques, the clinical usefulness of network physiology in chronic diseases such as decompensated cirrhosis, sepsis, and critical illness is self-evident and provides new valuable insights to researchers. In fact, because of its ability to detect and quantify physiological connections (or lack of), network physiology provides quantitative evidence to support previously clinically reported but unexplained pathophysiological observations as shown recently for CRP and WCC in *Chapter 5* [9]. This thesis provides a springboard for future research aimed at investigating the value of a integrative network approach in not just prognosis but also personalized medicine through network-based prediction of patients' subpopulations likely to respond to treatment.

Potentially, network mapping algorithms such as the parenclitic analysis which has shown prognostic and predictive values in this thesis can be incorporated into clinical use as bedside mobile apps with the ability to assist clinicians in combination with other variables to stratify patients for targeted therapy as well more accurately prognosticate. The dependence of this approach on routine data enhances the applicability combined with the ability to identify specific pathophysiological pathways for targeted treatment. This ability to identify pathophysiological pathways for potential targeted treatment is a strong merit of the network approach which makes it superior to the current artificial intelligence approaches such as the neural network approach which requires larger sample sizes and is based on a "black box"/closed algorithms. Perhaps, the "holy grail" will be a future combination of network physiology with the artificial intelligence approach for a synergistic output with superior prognostic and predictive values.

Another potential application of the network approach especially in cirrhosis is for remote monitoring. Indeed, recent work has shown the clinical applicability of remote monitoring in cirrhosis especially in regions with poor transport connection. For instance, CirrhoCare, a remote monitoring, bidirectional system (and mobile application) is currently being trialled for monitoring and treatment of decompensated cirrhosis in non-clinical settings with the potential to bring care to patients while cutting healthcare costs. Indeed, initial evaluation has shown that this approach achieved good patient engagement while helping clinicians (hepatologists) detect early signs of new decompensation events [498]. Indeed,

network physiology may be incorporated into this and similar systems to improve remote detection of pathological shifts in organ system connectivity with prognostic and treatment implications in cirrhosis and other diseases.

Thus, as we march into the brave new world of big data, artificial intelligence, and personalised medicine, finding the pathophysiological needle in the complex haystack of dynamically interacting organ systems in decompensated cirrhosis and other complex diseases might be driven by a deep understanding of the network characteristics of the individual patients based on data from “all organs at all times”. Irrespective of the current limitations, the future of diagnosis and prognosis in cirrhosis may be ‘network physiologic’ in nature.

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# Chapter 10 Appendixes

*Appendix 1. Search strategy for application of network physiologic approach in cirrhosis.*

| Database: Ovid MEDLINE(R) ALL <1946 to March 25, 2024><br>Search Strategy: |
|---|
| 1 network physiology.mp. (117) |
| 2 Physiological network.mp. (106) |
| 3 organ system* network*.mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms, population supplementary concept word, anatomy supplementary concept word] (5) |
| 4 network connect*.mp. [mp=title, book title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms, population supplementary concept word, anatomy supplementary concept word] (6464) |
| 5 1 or 2 or 3 or 4 (6668) |
| 6 cirrhosis.mp. (154599) |
| 7 liver cirrhosis.mp. or exp Liver Cirrhosis/ (119374) |
| 8 liver disease.mp. or exp Liver Diseases/ (675993) |
| 9 6 or 7 or 8 (694251) |
| 10 5 and 9 (32) |
| 11 limit 10 to yr="1860 - 2019" (16) |

## *Appendix 2.* *Databases search strategies*

Database: Embase Classic+Embase <1947 to 2020 February 19>

Search Strategy:

---

1 heart rate variability/or autonomic nervous system/(95567)

2 Heart Rate Varia*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word](34850)

3 liver disease/or liver cirrhosis/or chronic liver disease/(254250)

4 1 or 2 (101756)

5 3 and 4 (250)

6 limit 5 to conference abstract status (58)

7 5 not 6 (192)

8 limit 7 to english language (154)

9 limit 8 to editorial (8)

10 8 not 9 (146)

11 limit 10 to "reviews (best balance of sensitivity and specificity)" (24)

12 10 not 11 (122)

Search strategy for Embase.

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to February 20, 2020>

Search Strategy:

---

1 Autonomic Nervous System/(26244)

2 Heart Rate Varia*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms](18846)

3 Liver Diseases/or Liver Cirrhosis/(133838)

4 1 or 2 (40775)

5 3 and 4 (78)

6 limit 5 to (abstracts and "reviews (best balance of sensitivity and specificity)") (5)

7 5 not 6 (73)

8 limit 7 to english language (57)

9 limit 8 to editorial (0)

10 8 not 9 (57)

Search strategy for Medline.

| Recent queries in pubmed | | | |
|---|---|---|---|
| Search | Query | Items found | Time |
| #6 | Search (((((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV))) NOT (((((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV)) AND Review[ptyp]) Filters: Humans Sort by: Author | 66 | 09:27:41 |
| #5 | Search (((((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV))) NOT (((((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV)) AND Review[ptyp]) | 79 | 09:22:50 |
| #4 | Search (((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV) Filters: Review Sort by: Author | 6 | 09:21:20 |
| #3 | Search (((((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic*)) AND ((heart rate variability) OR HRV) | 85 | 09:21:15 |
| #2 | Search (((Liver Disease*) OR Chronic Liver Disease*) OR Liver Cirrhosis) OR Cirrhotic* | 249446 | 09:19:20 |
| #1 | Search (heart rate variability) OR HRV | 27650 | 09:17:44 |

Search strategy for PubMed.

## *Appendix 3.* *Risk of bias assessment of included studies using modified Newcastle-Ottawa scale.*

| Studies | Selection | Comparability | Outcome | Total |
|---|---|---|---|---|

| | Random recruitment of patients and health control | Diagnosis of CLD | Age-matched | Sex-matched | Patients with diseases, treatments, or lifestyles that influence HRV excluded | MELD or Child-Pugh reported | (≤3 Stars = High Risk of Bias) |
|---|---|---|---|---|---|---|---|
| Ates F. et al. 2006 [185] | * | | * | * | * | * | ***** |
| Baratta L. et al. 2010 [186] | * | | * | * | * | * | ***** |
| Coelho, L. et al. 2001 [187] | * | | * | * | | * | **** |
| Frokjaer V. G. et al. 2006 [188] | * | * | * | | * | * | ***** |
| Iga A. et al. 2003 [189] | * | * | * | * | * | * | ****** |
| Ko F.Y. et al. 2013 [190] | * | * | * | | * | * | ***** |
| Lazzeri C. et al. 1997 [191] | * | * | * | * | * | * | ****** |
| Mani A.R. et al. 2008 [90] | * | * | * | | * | * | ***** |
| Milovanovic B. et al. 2009 [192] | * | * | * | * | * | | ***** |
| Miyajima H. et al. 2001 [193] | * | * | * | * | | * | ***** |
| Moller S. et al. 2012 [194] | * | * | * | * | * | * | ****** |
| Nagasako C.K. et al. 2009 [195] | * | * | * | | * | * | ***** |
| Negru R.D. et al. 2015 [178] | * | * | * | * | * | * | ****** |
| Satti R. et al. 2019 [176] | * | * | * | | | * | **** |

*CLD, Chronic Liver Disease; HRV, Heart Rate Variability; MELD, Model for End-stage Liver Disease*

*Appendix 4. Definitions and units of included HRV indices.*

| HRV Indices | Units | Descriptions |
|---|---|---|
| NN intervals. | ms | Time difference between consecutive normal QRS complexes of an ECG recording. |
| SDNN | ms | The standard deviation of NN intervals. |
| cSDNN | ms | Corrected SDNN is the standard deviation of the NN interval that has been corrected for heart rate. It is calculated as SDNN/$e^{-(HR/58.8)}$ [see (15)]. |
| SDANN | ms | The standard deviation of the average NN intervals for each 5-minute segment was deduced from a 24-hour ECG recording. |
| SDNN Index | ms | The mean of standard deviations of all NN intervals for each 5-minute segment was deduced from a 24-hour ECG recording. |
| pNN50 | % | Percentage of successive NN intervals that vary by more than 50ms. |
| RMSSD | ms | Root mean square of differences in successive NN interval. |
| TINN | ms | Width of the base of a computed RR interval histogram. |
| TP | | Variance of entire NN interval of either 5 minutes (short) or 24-hour (long) ECG recording. |
| VLF | $ms^2$ | Power of the frequency band between 0.0033-0.04 Hz. |
| LF | $ms^2$ | Power of the frequency band between 0.04-0.15 Hz. |
| HF | $ms^2$ | Power of the frequency band between 0.15-0.4 Hz. |
| LF/HF | | The ratio of LF power to HF power. |
| SD1 | ms | The length of the line or standard deviation perpendicular to the line of identity of a Poincare plot |
| SD2 | ms | The length of the line or standard deviation parallel to the line of the identity of a Poincare plot |
| ApEn (Approximate Entropy) | | Measure of irregularity and complexity of a series of NN intervals. |
| SampEn (Sample Entropy) | | Measure of irregularity and complexity of a series of NN intervals. |
| DFA α1 (Short-term scaling exponent) | | Measure of short-term fractal-like fluctuations of inter-beat intervals. |
| DFA α2 (Long-term scaling exponent) | | Measure of long-term fractal-like fluctuations of inter-beat intervals. |

*ECG, Electrocardiograph; NN Interval, the time lapse between consecutive QRS complexes of ECG recording; SDNN, Standard deviation of NN intervals; ms, millisecond; SDANN, Standard deviation of the average NN intervals for each 5-minute segment deduced from a 24-hour ECG recoding; pNN50, Percentage of successive RR intervals that vary by more than 50ms; RMSSD, Root mean square of differences in successive NN interval; TINN, Triangular Interpolation of the NN intervals' histogram; TP, Total Power; VLF Very Low Frequency; LF, Low Frequency; HF, High Frequency; LF/HF, Ratio of LF to HF; SD1, Poincare plot Standard Deviation perpendicular to the line of identity; SD2, Poincare plot Standard Deviation along the line of identity; ApEn, Approximate Entropy; SampEn, Sample Entropy; DFA α1, Short-term fluctuation of Detrended Fluctuation Analysis; DFA α2, Long-term fluctuation of Detrended Fluctuation Analysis; ms, millisecond.*

*Appendix 5. Reported SDNN of included studies.*

| Author | Normal SDNN (Mean±SD) (ms) | Abnormal SDNN (Mean±SD) (ms) |
|---|---|---|
| Ates F. et al. 2006 [185] | 134 ± 52 | 70 ± 28 |
| Coelho, L. et al. 2001 [187] | 148.9 ± 33.97 | 84.14 ± 35.78 |
| Frokjaer V. G. et al. 2006 [188] | 139 ± 13 | 72 ± 10 |
| Ko F.Y. et al. 2013 [190] | 42.9 ± 15.1 | 22.8 ± 13.8 |
| Lazzeri C. et al. 1997 [191] | 57.99 ± 7 | 36.55 ± 4 |
| Mani A.R. et al. 2008 [90] | 36.9 ± 14.2 | 14.4 ± 7.7 |
| Milovanovic B. et al. 2009** [192] | 463.15 ± 111.83 | 93.42 ± 42.69 |
| Nagasako C.K. et al. 2009*** [195] | 79 | 56 |
| Negru R.D. et al. 2015 [178] | 129.60 ± 45.70 | 101.88 ± 31.79 |

***=Reported as natural log; ***=Reported as median, no interquartile range. All other indices are reported as mean ± standard deviation. SDNN; Standard deviation of NN intervals; SD, Standard Deviation; ms, Millisecond.*

*Appendix 6. Reported SDNN Index of included studies.*

| Author | Normal SDNN index (Mean±SD) (ms) | Abnormal SDNN index (Mean±SD) (ms) |
|---|---|---|
| Negru R.D. et al. 2015 [178] | 56.50 ± 17.04 | 43.83 ± 15.66 |

*SDNN; Standard deviation of NN intervals; SD, Standard Deviation; ms, Millisecond.*

*Appendix 7. Reported SDANN of included studies.*

| Author | Normal SDANN (Mean±SD) (ms) | Abnormal SDANN (Mean±SD) (ms) |
|---|---|---|
| Ates F. et al. 2006 [185] | 118 ± 49 | 55 ± 30 |
| Lazzeri C. et al. 1997 [191] | 125.57 ± 8 | 68.14 + 7 |
| Negru R.D. et al. 2015 [178] | 114.80 ± 43.48 | 88.21 ± 31.48 |

*All indices are reported as mean ± standard deviation. SDNN; SDANN, Standard deviation of the average NN intervals for each 5-minute segment deduced from a 24-hour ECG recoding; SD, Standard Deviation; ms, Millisecond.*

*Appendix 8. Reported RMSSD of included studies.*

| Author | Normal RMSSD (Mean±SD) (ms) | Abnormal RMSSD (Mean±SD) (ms) |
|---|---|---|
| Ates F. et al. 2006 [185] | 39±22 | 14±10 |
| Ko F.Y. et al. 2013** [190] | 28.2 ± 10.1 | 18.3 ± 11.7 |
| Lazzeri C. et al. 1997 [191] | 41 ± I | 27 ± 5 |

***= Reported as a natural log. All other indices are reported as mean ± standard deviation. RMSSD, Root mean square of differences in successive NN interval; SD, Standard Deviation; ms, Millisecond.*

*Appendix 9.* *Reported pNN50 of included studies.*

| Author | Normal pNN50 (Mean±SD) (%) | Abnormal pNN50 (Mean±SD) (%) |
|---|---|---|
| Ates F. et al. 2006 [185] | 8.2 ± 0.6 | 4.0 ± 0.6 |
| Coelho, L. et al. 2001 [187] | 11.17 ± 9.88 | 3.54 ± 4.61 |
| Lazzeri C. et al. 1997 [191] | 13 ± 2 | 3 ± 4 |
| Nagasako C.K. et al. 2009*** [195] | 15.5 | 3.3 |

****= Reported as median, no interquartile range. All other indices are reported as mean ± standard deviation. pNN50, Percentage of successive RR intervals that vary by more than 50 ms; SD, Standard Deviation; ms, Millisecond.*

*Appendix 10.* *Reported Total Power (TP) of included studies.*

| Author | Normal TP (Mean±SD) ($ms^2$) | Abnormal TP(Mean±SD) ($ms^2$) |
|---|---|---|
| Frokjaer V. G. et al 2006** [188] | 2307 ± 3258.1 | 204 ± 144.2 |
| Negru R.D. et al. 2015 [178] | 2724.74 ± 1530.04 | 2007.70 ± 1247.49 |

***= Reported as a natural log. All other indices are reported as mean ± standard deviation. TP, Total Power; SD, Standard Deviation; ms, Millisecond.*

*Appendix 11.* *Reported High Frequency (HF) of included studies.*

| Author | Normal HF (Mean±SD) ($ms^2$) | Abnormal HF (Mean±SD) ($ms^2$) |
|---|---|---|
| Baratta L. et al. 2010 [186] | 51.8 ± 11 | 40.6 ± 18.5 |
| Frokjaer V. G. et al. 2006* [188] | 514.7 ± 961.9 | 25.5 ± 21.4 |
| Iga A. et al. 2003ɣ [189] | 141 ± 58 | 28 ± 36 |
| Ko F.Y. et al. 2013** [190] | 706.3 ± 2.4 | 135.6 ± 5 |
| Lazzeri C. et al. 1997 ɣ [191] | 15 ± 1 | 20 ± 1 |
| Mani A.R. et al. 2008 [90] | 188 ± 253 | 26 ± 34 |
| Milovanovic B. et al. 2009** [192] | 1096.6 ± 2.7 | 333.6±3.2 |
| Miyajima H. et al. 2001 [193] | 177.5 ± 94.0 | 77.2 ± 57.3 |
| Moller S. et al. 2012* [194] | 473.8 ± 514.3 | 151.3 ± 252.8 |

**= Reported as mean and interquartile range; **= Reported as natural log; ɣ= Reported as day and night [day recorded]. All other indices are reported as mean ± standard deviation. HF, High Frequency; SD, Standard Deviation; ms, Millisecond.*

*Appendix 12.* *Reported Low Frequency (LF) of included studies.*

| Author | Normal LF (Mean±SD) ($ms^2$) | Abnormal LF (Mean±SD) ($ms^2$) |
|---|---|---|
| Frokjaer V. G. et al. 2006* [188] | 1171.1 ± 1726.2 | 74.5 ± 51.9 |
| Iga A. et al. 2003ɣ [189] | 213 ± 51 | 33 ± 23 |
| Ko F.Y. et al. 2013** [190] | 1085.7 ± 2.3 | 183.1 ± 4.4 |
| Lazzeri C. et al. 1997 ɣ [191] | 82 ± 2 | 79 ± 1 |
| Mani A.R. et al. 2008 [90] | 191 ± 145 | 29 ± 51 |
| Milovanovic B. et al. 2009** [192] | 3604.7 ± 2.7 | 943.9 ± 2.6 |
| Moller S. et al. 2012* [194] | 647.5 ± 865.2 | 150.3 ± 174.6 |

*= Reported as mean and interquartile range; **= Reported as natural log; ɣ= Reported as day and night [day recorded]. LF, Low Frequency; SD, Standard Deviation; ms, Millisecond.*

***Appendix 13.*** *Reported Very Low Frequency (VLF) of included studies.*

| Author | Normal VLF (Mean±SD) ($ms^2$) | Abnormal VLF (Mean±SD) ($ms^2$) |
|---|---|---|
| Frokjaer V. G. et al. 2006* [188] | 734.6 ± 932.2* | 103.9 ± 71.7* |
| Negru R.D. et al. 2015 [178] | 2045.96 ± 1311.92 | 1385.86 ± 840.08 |

*= Reported as mean and interquartile range. VLF, Very Low Frequency; SD, Standard Deviation; ms, Millisecond.*

***Appendix 14.*** *Reported Low Frequency/High-Frequency Ration (LF: HF) of included studies.*

| Author | Normal LF:HF (Mean±SD) ($ms^2$) | Abnormal LF:HF (Mean±SD) ($ms^2$) |
|---|---|---|
| Iga A. et al. 2003ɣ [189] | 1.14 ± 0.85 | 1.27 ± 0.22 |
| Miyajima H. et al. 2001 [193] | 1.20 ± 1.01 | 3.56 ± 2.31 |
| Moller S. et al. 2012* [194] | 1.27 ± 0.32 | 0.62 ± 0.15 |

*=Reported as mean and interquartile range; ɣ=Reported as day and night (day recorded). LF:HF, Ratio of Low Frequency/High Frequency; SD, Standard Deviation; ms, Millisecond.*

***Appendix 15.*** *Reported HRV non-linear indices; SD1, SD2, Sample Entropy (SampEn), and Detrended Fluctuation Analysis- (DFA α1) of included studies.*

| Author | Normal SD1 (Mean± SD) | Abnormal SD1 (Mean± SD) | Normal SD2 (Mean± SD) | Abnormal SD2 (Mean± SD) | Normal SampEn (Mean± SD) | Abnormal SampEn (Mean± SD) | Normal DFA α1 (Mean± SD) | Abnormal DFA α1 (Mean± SD) |
|---|---|---|---|---|---|---|---|---|
| Ko F.Y. et al. 2013 [190] | NS | NS | NS | NS | NS | NS | 1.14 ± 0.15 | 0.89 ± 0.25 |
| Mani A.R. et al. 2008 [90] | 23.2 ± 14.0 | 8.8 ± 5.9 | 57.8 ± 18.4 | 23.3 ± 12.6 | 2.89 ± 0.52 | 2.05 ± 0.60 | NS | NS |

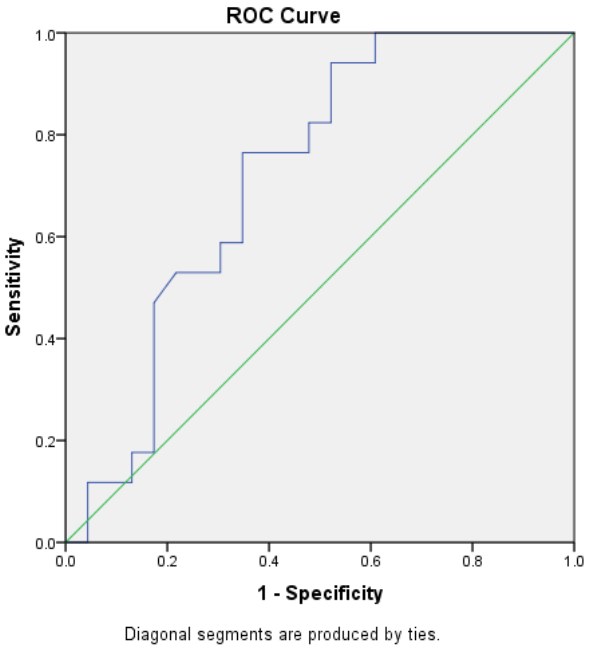
*SD1, Poincare plot Standard Deviation perpendicular to the line of identity; SD2, Poincare plot Standard Deviation along the line of identity; SampEn, Sample Entropy; DFA α1, Short-term fluctuation of Detrended Fluctuation Analysis; NS, Not Shown.*

*Appendix 16. The predictive effect of HRV indices calculated from 24-h ECG on 1-year mortality in hospitalised patients with cirrhosis. Univariate Cox regression analysis was used for the calculation of the hazard ratio.*

| | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| SDNN | -0.026 | 0.010 | 0.975 | **0.010** |
| cSDNN | -0.007 | 0.003 | 0.993 | **0.015** |
| RMSSD | 0.015 | 0.011 | 1.016 | 0.151 |
| SDANN | -0.037 | 0.012 | 0.964 | **0.003** |
| pNN50 | 0.024 | 0.016 | 1.024 | 0.129 |
| ULF | -0.000 | 0.000 | 1.000 | **0.044** |
| VLF | 0.000 | 0.000 | 1.000 | 0.241 |
| LF | 0.000 | 0.000 | 1.000 | 0.587 |
| HF | 0.001 | 0.001 | 1.001 | 0.134 |

*β is the coefficient of Cox regression analysis. SEM is the standard error of the mean of β, Hazard ratio =$Exp$ (β) = $e^{\beta}$. SDNN: Standard Deviation of inter-beat intervals, cSDNN: SDNN corrected for heart rate ($\boldsymbol{cSDNN = \frac{SDNN}{e^{-\frac{Heart\ rate}{58.8}}}}$). RMSSD: Root mean square of the successive differences of RR intervals (a measure of short-term HRV). SDANN: Standard deviation of the average NN intervals calculated over short periods, usually 5 minutes (a measure of long-term HRV). pNN50: The proportion of the number of pairs of successive RR intervals that differ by more than 50 ms divided by the total number of RR intervals. Ultra-low frequency (ULF), Very-Low Frequency (VLF), Low-Frequency (LF), and High-Frequency (HF) bands were calculated based on spectral analysis of HRV.*

*Appendix 17. ROC curve for prediction of mortality using Turbulence Onset (TO). Area under the curve for TO = 0.720 (p=0.019).*



*Appendix 18. The mean Heart Rate Turbulence indices of the study population after excluding two patients who died due to myocardial infarction. The data are expressed as mean ± SD. TO: Turbulence Onset, TS: Turbulence Slope. PVC: Premature Ventricular Complex.*

| | Survivors | Non-Survivors | p-value |
|---|---|---|---|
| Study Size | 23 | 15 | - |
| TO | -0.01±2.6 | 1.49±1.3 | **0.025** |
| TS | 3.83±4.5 | 3.15±5.6 | 0.685 |
| No of PVCs in 24 hours | 27.87±35.47 | 25.20±27.04 | 0.806 |

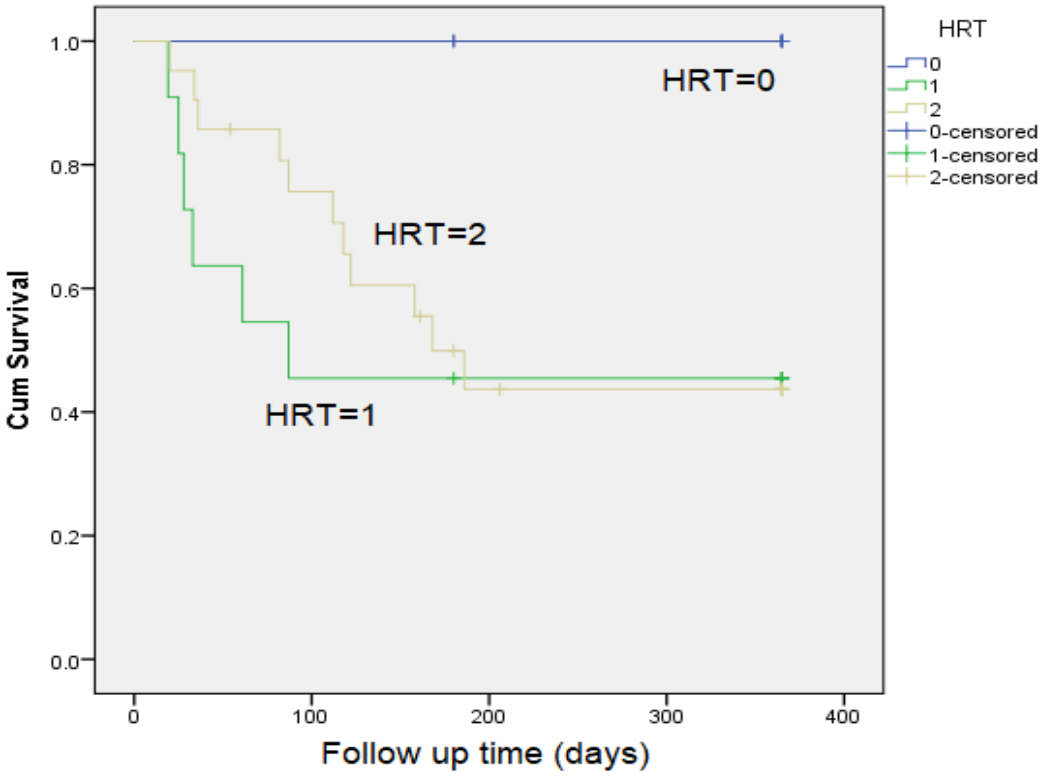
*Appendix 19. The independence of Turbulence Onset from the MELD score in predicting mortality excluding two patients who died due to myocardial infarction. Bivariate Cox regression analysis was used for the calculation of the hazard ratio. TO: Turbulence Onset. β is the coefficient of Cox regression analysis. SEM is the standard error of the mean of β, Hazard ratio =$Exp(\beta) = e^{\beta}$. TO: Turbulence Onset.*

| | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| TO | 0.267 | 0.127 | 1.306 | **0.036** |
| MELD | 0.063 | 0.031 | 1.065 | **0.042** |

*Appendix 20. The independence of Turbulence Onset from the Child-Pugh score in predicting mortality excludes two patients who died due to myocardial infarction. Bivariate Cox regression analysis was used for the calculation of the hazard ratio. TO: Turbulence Onset. β is the coefficient of Cox regression analysis. SEM is the standard error of the mean of β, Hazard ratio =$Exp(\beta) = e\beta$. TO: Turbulence Onset.*

| | β | SEM | Hazard Ratio | p-value |
|---|---|---|---|---|
| TO | 0.301 | 0.142 | 1.351 | **0.034** |
| Child-Pugh | 0.260 | 0.132 | 1.296 | **0.048** |

*Appendix 21. Kaplan-Meier graphs illustrate three categories of heart rate turbulence (HRT) based on criteria established for prediction of mortality in patients with acute myocardial infarction by Barthel et al. (Circulation 2003; 108:1221-1226). Within the context of acute myocardial infarction, the cut-off value for Turbulence Onset (TO) and Turbulence Slope (TS) is 0% and 2.5 ms/RR respectively. Patients were classified into the following HRT categories: category 0 if both TO and TS were normal (TO<0% and TS >2.5 ms/RR), category 1 if either TO or TS was abnormal, or category 2 if both TO and TS were abnormal (Chi-square = 6.26, p=0.044).*



*Appendix 22. Description and definitions of network topology measures.*

| Network Topology Measures | Definition | Mathematical Formula |
|---|---|---|
| Degree Centrality | The sum of incidental edges to the node. In a directed network, it is represented as the sum of weighted incoming or outcoming edges of each node. | $D(i) = \sum_{j=1}^{n} A_{ij}$<br>-$A_{ij}$ is the *ij*-th element of the adjacency matrix A of the graph<br>-n is the number of nodes in the graph [499]. |
| Shortest Path Length (SPL) | A path with the minimum number of edges between two nodes. In a directed and weighted network, it is a path with the minimum sum of edge weights starting at source node s and ending at target node t. | $d(i,j)$<br>-distance *d(i,j)* denotes the length of the shortest path between node i and node j |
| Efficiency | The efficiency between two nodes is defined as the reciprocal of their shortest path length. The global efficiency of a graph is the average efficiency over all pairs of nodes. | $E(G) = \frac{1}{n(n-1)} \sum_{i^1 j} \frac{1}{d(i,j)}$<br>[500] |
| Diameter | The shortest path length between the most distant nodes. | $d(G) = \max_{ij} \{d(i,j)\}$ |

*Appendix 23. Comparison of circulating biomarkers, MELD, and Child-Pugh scores in survival and non-survival patients with cirrhosis after one year follow up.*

| Variable | Survivors | Non-Survivors | p-value |
|---|---|---|---|
| Albumin (g/L) | 34.9 (30.8 – 38.1) | 30.0 (27.1 – 36.1) | **0.005** |
| Bilirubin (μmol/L) | 23.1 (15.4 – 38.2) | 73.9 (31.1 – 141.8) | **<0.001** |
| Prothrombin time (% activity) | 54 (48 - 67) | 46 (32 – 55) | **<0.001** |
| Ammonia (μmol/L) | 59.5 (35.7 – 115.0) | 60 (29.0 – 85.3) | 0.266 |
| Creatinine (μmol/L) | 74 (65 - 93) | 89 (70 – 115) | **0.039** |
| Sodium (mmol/L) | 138 (136 – 140) | 136 (134 – 138) | **0.008** |
| MELD score | 11 (9 – 14) | 19 (12 – 23) | **<0.001** |
| Child-Pugh score | 7 (6 – 9) | 10 (8 – 11) | **<0.001** |

*Data are shown as Median (interquartile range). P-values are calculated using the Mann-Whitney U test.*

*Appendix 24. The prognosis effects of parenclitic deviations independent of Child-Pugh score.*

| ∂ of variable pairs | β | SEM | Hazard Ratio (95.0% CI) | p-value |
|---|---|---|---|---|
| Albumin-Bilirubin | 0.095 | 0.025 | 1.100 (1.048 – 1.154) | **<0.001** |
| Child-Pugh | 0.430 | 0.098 | 1.537 (1.269 – 1.861) | **<0.001** |
| Albumin-Prothrombin Time | 0.106 | 0.063 | 1.162 (1.016 – 1.327) | **0.028** |
| Child-Pugh | 0.153 | 0.032 | 1.545 (1.291 – 1.849) | **<0.001** |
| Albumin-Hepatic Encephalopathy | 0.569 | 0.515 | 1.766 (0.644 – 4.844) | 0.269 |
| Child-Pugh | 0.431 | 0.090 | 1.539 (1.289 – 1.837) | **<0.001** |
| Bilirubin-Prothrombin Time | 0.024 | 0.007 | 1.024 (1.010 – 1.038) | **0.001** |
| Child-Pugh | 0.466 | 0.095 | 1.594 (1.324 – 1.091) | **<0.001** |
| Ammonia-Hepatic Encephalopathy | 0.651 | 0.673 | 1.918 (0.513 – 7.171) | 0.333 |
| Child-Pugh | 0.456 | 0.112 | 1.578 (1.268 – 1.963) | <0.001 |

*∂, parenclitic deviation; β, coefficient of Cox regression analysis; SEM, standard error of the mean of β, CI, confidence interval.*

*Appendix 25. Parenclitic network topology of the studied population.*

| Network topology | Survivors; median (IQR) | Non-Survivors; median (IQR) | p-value |
|---|---|---|---|
| Centrality Sum | 25.61 (15.98 – 31.58) | 32.20 (21.82 – 51.05) | 0.070 |
| Centrality Mean | 3.66 (2.28 – 4.51) | 4.60 (3.12 – 7.29) | 0.070 |
| Centrality Standard Deviation | 5.88 (3.64 – 8.61) | 8.71 (4.57 – 13.88) | **0.038** |
| Shortest Path Length Sum | 17.88 (12.28 – 24.45) | 21.86 (15.70 – 34.11) | 0.202 |
| Shortest Path Length Mean | 2.55 (1.75 – 3.49) | 3.12 (2.24 – 4.87) | 0.202 |
| Shortest Path Length Standard Deviation | 2.40 (1.61 – 3.91) | 2.99 (1.91 – 4.40) | 0.446 |
| Efficiency | 0.03 (0.02 – 0.05) | 0.03 (0.02 – 0.04) | 0.303 |
| Diameter | 6.11 (4.23 – 9.79) | 6.77 (5.05 – 11.35) | 0.303 |

*IQR, Interquartile range.*

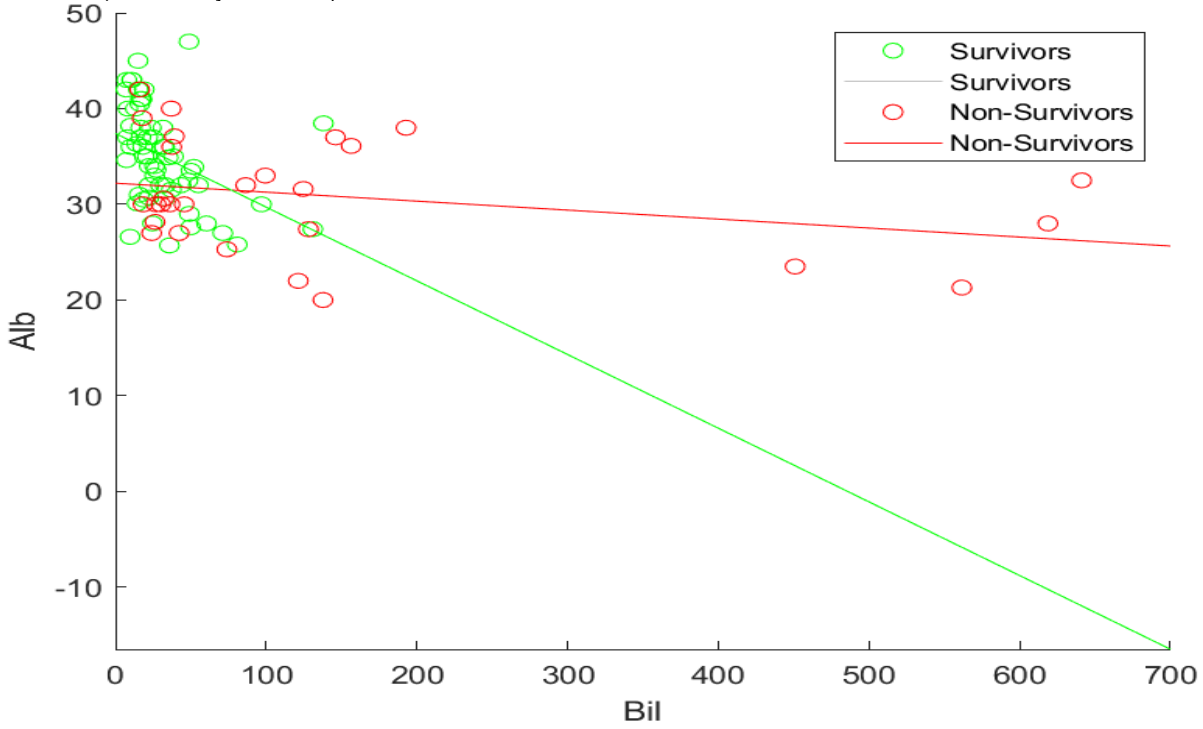
*Appendix 26. The prognosis effects of parenclitic network indices independent of MELD and Child-Pugh score.*

| Network topology | β | SEM | Hazard Ratio (95.0% CI) | p-value |
|---|---|---|---|---|
| Standard Deviation of Centrality | 0.021 | 0.018 | 1.022 (0 .986 – 1.058) | 0.231 |
| MELD | 0.112 | 0.043 | 1.119 (1.029 – 1.216) | **0.009** |
| Standard Deviation of Centrality | 0.037 | 0.014 | 1.037 (1.010 – 1.066) | **0.008** |
| Child-Pugh | 0.539 | 0.122 | 1.714 (1.350 – 2.176) | **<0.001** |

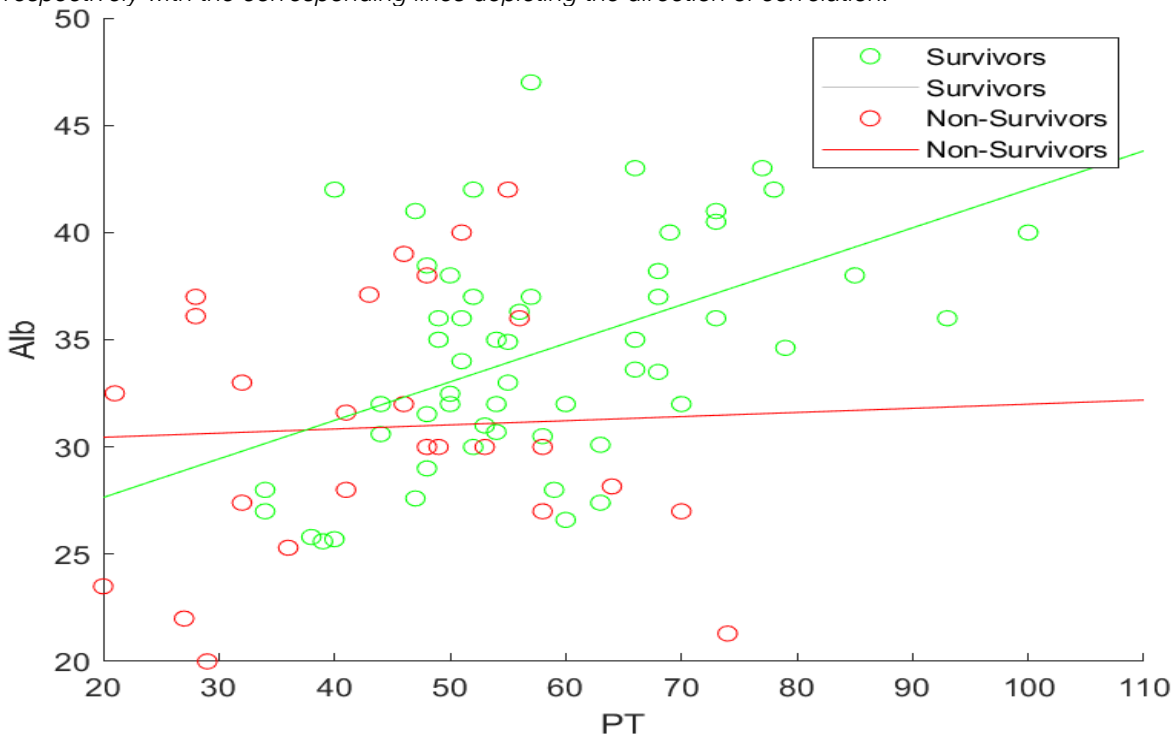
*β, coefficient of Cox regression analysis; SEM, standard error of the mean of β, CI, confidence interval.*

*Appendix 27. Graph showing the relationship between albumin and bilirubin in patients that were followed up for 12 months. The red and green dots represent the data of the non-survivors and survivors respectively with the corresponding lines depicting the direction of correlation. Analysis of covariance (ANCOA) was applied to assess the effect of the group (survivors versus non-survivors) in the relationship between albumin and bilirubin. ANCOVA showed F(group)= 4.215, P<0.05. This provides statistical evidence that*

*there are statistically significant differences in albumin levels between the groups (i.e., survivors and non-survivors) when adjusted for patients' bilirubin levels.*




***Appendix 28.*** *Graph showing the relationship between albumin and prothrombin time in patients that were followed up for 12 months. The red and green dots represent the data of the non-survivors and survivors respectively with the corresponding lines depicting the direction of correlation.*



*Appendix 29. Differences in parenclitic indices between survivors and non-survivors in the validation group. Survivors in the training group (n=194) were used as a model for the calculation of the coefficients that were used for the calculation of parenclitic deviations and network indices in the validation group (n=203).*

| Variables | Survivor | Non-survivors | p-value |
|---|---|---|---|
| δ (WCC-CRP) | 3.74 (1.17-5.78) | 2.23 (1.17-3.79) | 0.061 |
| δ (Bil-WCC) | 4.11 (1.33-7.84) | 2.66 (1.16-4.28) | 0.040 |
| Mean Centrality | 6.37 (4.09-9.15) | 5.12 (3.23-7.21) | 0.013 |
| Mean Shortest path length | 6.4 (4.39-10.03) | 5.18 (3.58-7.53) | 0.011 |
| Diameter | 17.9 (11.52-27.54) | 13.51 (8.49-20.29) | 0.017 |

δ*; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin.*

*Appendix 30. Prognostic values of parenclitic indices independent of age and MELD in the randomly split (~50%) standard treatment group. During validation, the correlation coefficients were recalculated in a training sample of 194 of randomly selected patients. The individual parenclitic deviations were calculated using these coefficients for the validation sample comprising the remainder of 203 patients. Cox regression was used to estimate hazard ratios based on 6-month follow-up survival data.*

| Variables | β | SEM | p-value | Hazard Ratio (95%CI) |
|---|---|---|---|---|
| δ (WCC-CRP) | 0.098 | 0.049 | 0.048 | 1.102 (1.001-1.214) |
| Age | 0.047 | 0.014 | 0.001 | 1.048 (1.02-1.077) |
| MELD | 0.088 | 0.019 | <0.001 | 1.092 (1.052-1.133) |
| | | | | |
| δ (Bil-WCC) | 0.074 | 0.029 | 0.011 | 1.077 (1.017-1.14) |
| Age | 0.043 | 0.013 | 0.001 | 1.044 (1.017-1.071) |
| MELD | 0.078 | 0.018 | <0.001 | 1.081 (1.044-1.119) |
| | | | | |
| Mean Centrality | 0.09 | 0.049 | 0.066 | 1.094 (0.994-1.205) |
| Age | 0.047 | 0.014 | <0.001 | 1.049 (1.021-1.077) |
| MELD | 0.082 | 0.02 | <0.001 | 1.085 (1.043-1.129) |
| | | | | |
| Mean Shortest path length | 0.086 | 0.042 | 0.042 | 1.09 (1.003-1.184) |
| Age | 0.047 | 0.014 | 0.001 | 1.048 (1.021-1.077) |
| MELD | 0.083 | 0.02 | <0.001 | 1.086 (1.045-1.129) |
| | | | | |
| Diameter | 0.03 | 0.014 | 0.034 | 1.03 (1.002-1.059) |
| Age | 0.047 | 0.013 | <0.001 | 1.049 (1.021-1.077) |
| MELD | 0.084 | 0.019 | <0.001 | 1.088 (1.048-1.13) |

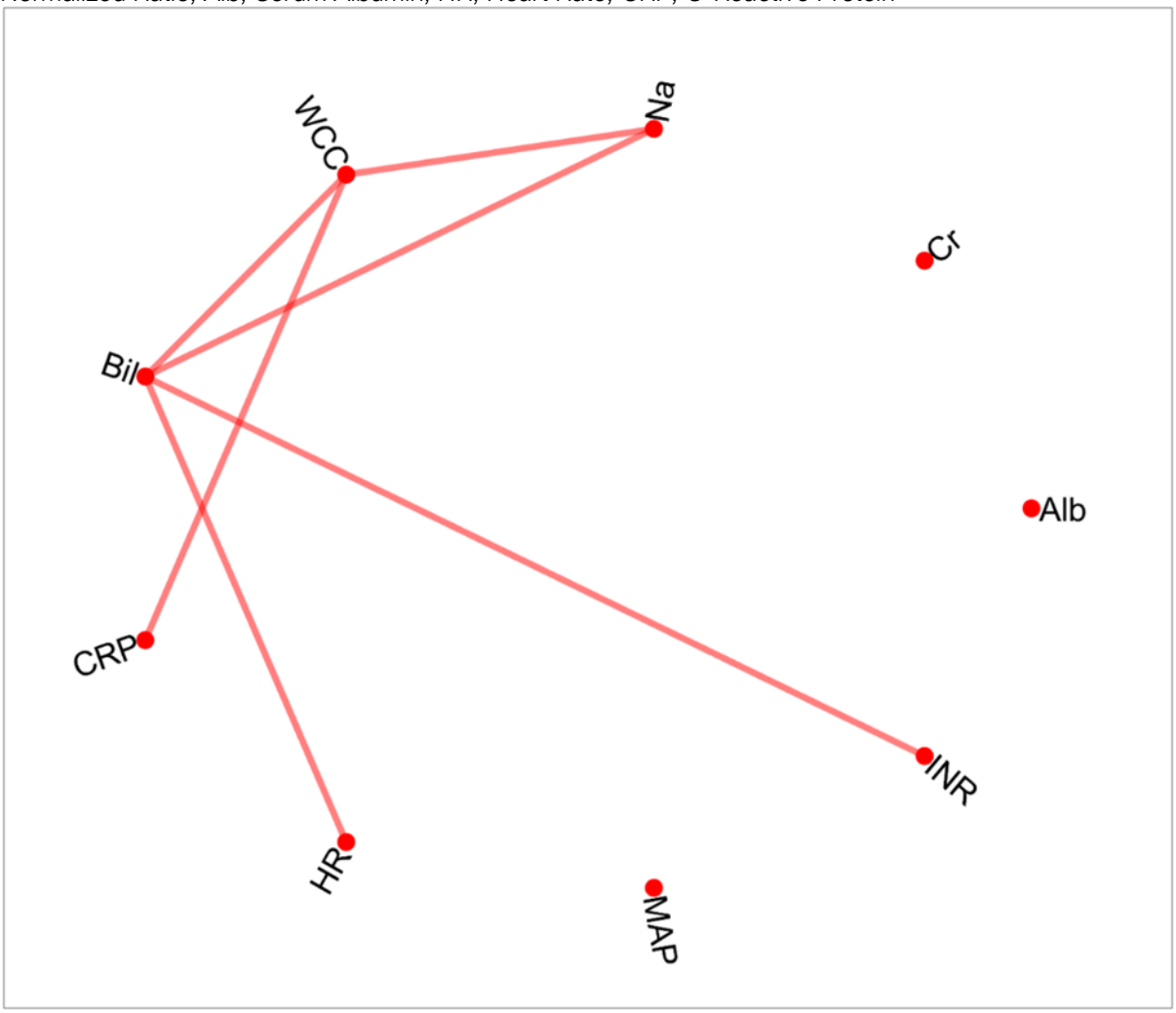
*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, Cent; Centrality, SPL; Shortest Path Length, MELD; Model for End-stage Liver Disease.*

*Appendix 31. Interaction between parenclitic indices and albumin administration in predicting 6-month survival.*

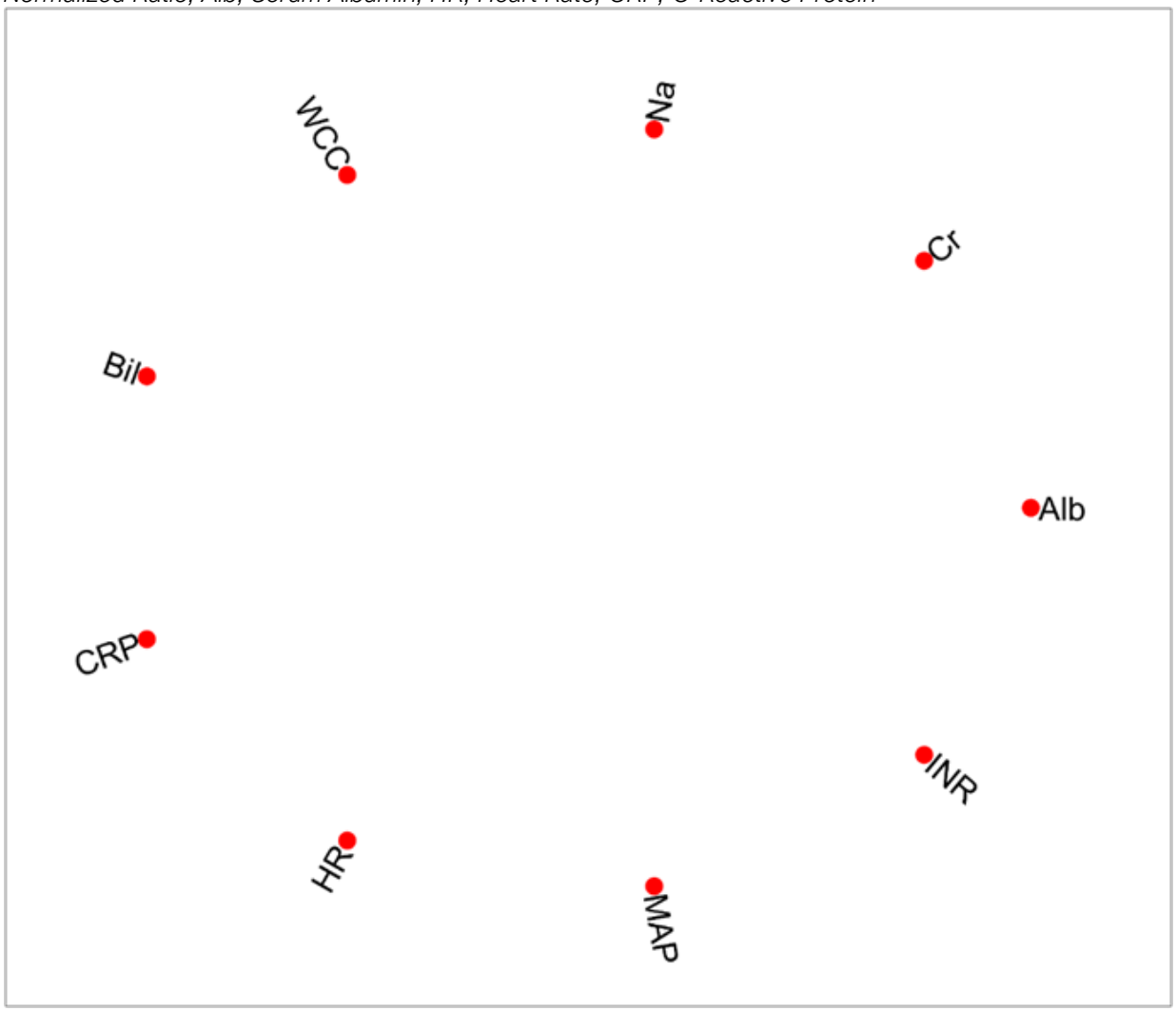
| Variables | β | SEM | Hazard Ratio (95%CI) | p-value |
|---|---|---|---|---|
| Age | 0.06 | 0.013 | 1.062 (1.037-1.089) | <0.001 |
| TX | -0.451 | 0.408 | 0.637 (0.286-1.418) | 0.269 |
| Age*TX | 0.015 | 0.018 | 1.015 (0.98-1.051) | 0.402 |
| | | | | |
| MELD | 0.026 | 0.008 | 1.026 (1.01-1.043) | 0.002 |
| TX | -0.738 | 0.68 | 0.478 (0.126-1.812) | 0.278 |
| MELD*TX | 0.011 | 0.012 | 1.011 (0.988-1.035) | 0.357 |
| | | | | |
| Gender | -0.203 | 0.194 | 0.816 (0.558-1.194) | 0.296 |
| TX | -0.38 | 0.383 | 0.684 (0.323-1.449) | 0.321 |
| Gender*TX | 0.187 | 0.285 | 1.205 (0.69-2.106) | 0.512 |
| | | | | |
| Diameter | 0.005 | 0.01 | 1.005 (0.985-1.025) | 0.64 |
| TX | -0.573 | 0.246 | 0.564 (0.348-0.913) | 0.02 |
| **Diameter*TX** | **0.026** | **0.013** | **1.026 (1.00-1.052)** | **0.049** |
| | | | | |
| Mean Shortest path | 0.058 | 0.042 | 1.06 (0.976-1.151) | 0.167 |
| TX | -0.657 | 0.273 | 0.518 (0.303-0.886) | 0.016 |
| **Mean Shortest path *TX** | **0.118** | **0.057** | **1.125 (1.005-1.259)** | **0.040** |
| | | | | |
| Mean Centrality | 0.057 | 0.026 | 1.059 (1.007-1.114) | 0.027 |
| TX | -0.552 | 0.288 | 0.576 (0.327-1.013) | 0.055 |
| Mean Centrality *TX | 0.053 | 0.036 | 1.054 (0.983-1.131) | 0.14 |
| | | | | |
| δ (Bil-WCC) | 0.017 | 0.022 | 1.017 (0.973-1.063) | 0.459 |
| TX | -0.369 | 0.175 | 0.691 (0.49-0.975) | 0.035 |
| δ (Bil-WCC)*TX | 0.06 | 0.031 | 1.062 (0.999-1.128) | 0.053 |
| | | | | |
| δ (WCC-CRP) | -0.001 | 0.021 | 0.999 (0.958-1.042) | 0.973 |
| TX | -0.572 | 0.181 | 0.565 (0.396-0.804) | 0.002 |
| **δ (WCC-CRP)*TX** | **0.122** | **0.033** | **1.13 (1.058-1.206)** | **<0.001** |
| | | | | |
| Alb | -0.078 | 0.023 | 0.925 (0.884-0.967) | 0.001 |
| TX | -1.909 | 0.776 | 0.148 (0.032-0.678) | 0.014 |
| **Alb*TX** | **0.078** | **0.034** | **1.081 (1.012-1.154)** | **0.021** |
| | | | | |
| WCC | 0.026 | 0.015 | 1.027 (0.997-1.057) | 0.081 |
| TX | -0.671 | 0.247 | 0.511 (0.315-0.829) | 0.006 |
| **TX*WCC** | **0.056** | **0.021** | **1.058 (1.015-1.103)** | **0.007** |
| | | | | |
| CRP | 0.002 | 0.001 | 1.002 (1-1.004) | 0.069 |
| TX | -0.37 | 0.161 | 0.691 (0.504-0.948) | 0.022 |
| **CRP*TX** | **0.006** | **0.002** | **1.006 (1.002-1.01)** | **0.003** |

*δ; Deviation along an axis, WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein, Cent; Centrality, SPL; Shortest Path Length, MELD; Model for End-stage Liver Disease, TX; Treatment.*

***Appendix 32.*** *Network map of the 6-month survivors in the randomly split standard treatment group (training sample). WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein*



*Appendix 33.* *Network map of the 6-month non-survivors in the randomly split standard treatment group (training sample). WCC; White Cell Count, Na; Serum Sodium, Bil; Total Bilirubin, INR; International Normalized Ratio, Alb; Serum Albumin, HR; Heart Rate, CRP; C-Reactive Protein*



*Appendix 34. Principal components after Varimax and rotation and Kaiser Normalization. The KMO (Kaiser-Meyer-Olkin) test that the sample is adequate for PCA showed p-values < 0.001 (Chi-Square = 1844.299, p-value = <0.001).*

| | Principal Components | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variables | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Alanine Aminotransferase | 0.828 | | | | | | | | |
| Aspartate Transaminase | 0.817 | | | | | | | | |
| International Normalized Ratio | 0.764 | | | | | | | | |
| Oxygen Saturation | | | | | | | | | |
| Serum Creatinine | | 0.845 | | | | | | | |
| Urea | | 0.807 | | | | | | | |
| Phosphate | | 0.69 | | | | | | | |
| Serum Albumin | | | 0.692 | | | | | | |
| Mean Blood Pressure | | | 0.649 | | | | | | |
| Haemoglobin | | | 0.607 | | | | | | |
| Alkaline Phosphatase | | | | | | | | | |
| Serum Sodium | | | | 0.924 | | | | | |
| Chloride | | | | 0.831 | | | | | |
| Bicarbonate | | | | | 0.801 | | | | |
| Blood pH | | | | | 0.765 | | | | |
| Heart Rate | | | | | | 0.815 | | | |
| Respiratory Rate | | | | | | 0.707 | | | |
| Blood Glucose | | | | | | | 0.809 | | |
| Lactate | | | | | | | 0.571 | | |
| Glasgow Coma Score | | | | | | | | | |
| Platelet Count | | | | | | | | 0.836 | |
| White Blood Count | | | | | | | | 0.757 | |
| Total Bilirubin | | | | | | | | | 0.744 |
| Body Temperature | | | | | | | | | -0.728 |

*Na; Serum Sodium, Cl; chloride, AST; aspartate transaminase, ALT; alanine aminotransferase, GCS; Glasgow Coma Score, Bil; Total Bilirubin, ALP; Alkaline Phosphatase, Cr; Serum Creatinine, Na; Serum Sodium, Glu; Blood Glucose, HR; Heart Rate, Temp; Temperature, SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval.*

*Appendix 35. Univariate Cox regression analysis of the Principal Components based on ICU survival of patients.*

| Variables | β | SEM | HR (95% CI) | p-value |
|---|---|---|---|---|
| 1 | 0.255 | 0.092 | 1.08(1.29 - 1.55) | 0.005 |
| 2 | 0.168 | 0.097 | 0.98(1.18 - 1.43) | 0.082 |
| 3 | -0.258 | 0.093 | 0.64(0.77 - 0.93) | 0.005 |
| 4 | 0.13 | 0.106 | 0.93(1.14 - 1.4) | 0.222 |
| 5 | -0.084 | 0.102 | 0.75(0.92 - 1.12) | 0.414 |
| 6 | 0.111 | 0.107 | 0.91(1.12 - 1.38) | 0.299 |
| 7 | 0.226 | 0.103 | 1.03(1.25 - 1.53) | 0.027 |
| 8 | -0.07 | 0.099 | 0.77(0.93 - 1.13) | 0.478 |
| 9 | -0.07 | 0.099 | 0.77(0.93 - 1.13) | 0.478 |

*SEM; Standard Error of Mean, HR; Hazard Ratio, CI; Confidence Interval.*

*Appendix 36. Multivariate Cox regression analysis of parenclitic deviation along the WCC-CRP axis independent of WCC and CRP*

| Covariate | B | SEM | Hazard ratio (95% CI) | p value |
|---|---|---|---|---|
| WCC_CRP | 0.025 | 0.037 | 1.025 (0.953 - 1.103) | 0.503 |
| WCC | 0.046 | 0.023 | 1.048 (1.002 - 1.095) | 0.040 |
| | | | | |
| WCC_CRP | 0.070 | 0.027 | 1.073 (1.018 - 1.131) | 0.008 |
| CRP | 0.006 | 0.002 | 1.006 (1.002 - 1.010) | 0.005 |

*WCC; White Cell Count, CRP; C-Reactive Protein, SEM; Standard Error of Mean, HR; Hazard Ratio, CI; confidence Interval.*