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VARIABILIDADE DA FREQUÊNCIA CARDÍACA COMO PREDITOR DE MORTALIDADE NA SEPSE

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VARIABILIDADE DA FREQUÊNCIA CARDÍACA COMO PREDITOR DE MORTALIDADE NA SEPSE

Tese apresentada ao Programa de Pós-Graduação em Saúde do Adulto da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do título de Doutor em Saúde do Adulto.

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“Muitas são as coisas prodigiosas sobre a terra, mas nenhuma mais prodigiosa do que o próprio homem. (...) Doma a fera agressiva acostumada à luta, coloca sela no cavalo bravo, e mete canga no pescoço do furioso touro da montanha. A palavra, o jogo fugaz do pensamento, as leis que regem o Estado, tudo ele aprendeu, a si próprio ensinou. Como aprendeu também a se defender do inverno insuportável e das chuvas malsãs. Vive o presente, recorda o passado, antevê o futuro. Tudo lhe é possível. Na criação que o cerca só dois mistérios terríveis, dois limites. Um, a morte, da qual em vão tenta escapar. Outro, seu próprio irmão e semelhante, o qual não vê e não entende. Se não resiste a ele, é esmagado. Se o vence, o orgulho o cega e vira um monstro que os deuses desamparam.”

Sófocles (apr. 496-406 A.C), Antígona / Tradução de Millôr Fernandes.

Resumo da Tese

Introdução: A sepse é uma síndrome com elevada mortalidade e cuja prevalência tem aumentado nas últimas décadas. O papel do sistema nervoso autônomo na fisiopatologia da sepse tem sido cada vez mais estudado. O objetivo deste trabalho é avaliar a Variabilidade de Frequência Cardíaca (VFC) como preditor de mortalidade na sepse através de um estudo de coorte e de uma revisão sistemática

Método: No estudo de coorte prospectivo com pacientes sépticos, o recrutamento de pacientes foi realizado em um CTI de um hospital terciário entre março de 2012 e fevereiro de 2014. Dados clínicos e exames laboratoriais foram coletados na admissão. Cada paciente foi submetido à realização de um Holter de 20-minutos, seguido de um Holter de 24-horas no primeiro dia de seguimento. O desfecho primário foi morte por qualquer causa até 28 dias de seguimento.

Na revisão sistemática de estudos sobre VFC como preditor de mortalidade na sepse, a busca foi feita por pesquisadores independentes nas bases de dados PubMed, LILACS e Cochrane, incluindo trabalhos em inglês, português ou espanhol, indexados antes de 20 de agosto de 2017 com pelo menos 10 pacientes. A qualidade dos estudos foi verificada através da escala Newcastle-Ottawa. Para analisar os resultados, os estudos foram divididos entre aqueles que fizeram gravação de curta duração (≤ 1 hora) e aqueles que realizaram gravações longas (≥ 24 horas).

Resultados: No estudo de coorte, um total de 63 pacientes foram incluídos. Os pacientes foram categorizados em grupo de não-sobreviventes (n=16) e grupo de sobreviventes (n=47), dependendo do desfecho primário. Os pacientes sobreviventes eram mais jovens (48,6 anos vs 63,0 anos), tinham melhor função renal e menores valores nos escores de gravidade (APACHE II e SOFA) comparado com os não-sobreviventes. No Holter de 20-minutos, os parâmetros SDNN, TP, VLF, LF e LF/H dos não-sobreviventes eram significativamente menores que os dos sobreviventes ($p \leq 0{,}001$, $p = 0{,}003$, $p = 0{,}002$, $p = 0{,}006$, $p = 0{,}009$, respectivamente). Construímos curva ROC para o SDNN, que mostrou área sob a curva de 0,772 (0,638±0,906) para mortalidade. O valor de 17ms foi escolhido como o melhor ponto de corte do SDNN para discriminar sobreviventes e não-sobreviventes. No modelo de COX, ajustada para SOFA e para o APACHE II, o SDNN ≤ 17ms estava associado com maior risco de morte, com hazard ratios de 6,3

(1,4±28,0; p = 0,015) e 5,5 (1,2±24,8; p = 0,027), respectivamente. A adição do SDNN dicotômico ao escore SOFA reduziu o Akaike Information Criterion (AIC) ou Critério de Informação de Akaike (um estimador de qualidade do modelo) e aumentou a concordância estatística e o coeficiente de determinação (R^2), indicando que o poder preditivo do modelo SDNN + SOFA é melhor do que o poder predito do modelo SOFA isolado.

Na revisão sistemática, nove estudos foram incluídos com um total de 536 pacientes. Todos os estudos eram observacionais. A qualidade dos estudos variou entre 4 e 7 estrelas da escala de Newcastle-Ottawa. A taxa de mortalidade dos estudos variou de 8 a 61%. Sete estudos realizaram análise da VFC em gravações de curto período. Com exceção de um estudo que não informava qual grupo tinha os menores valores, todos os outros estudos mostraram redução de parâmetros da VFC no grupo não-sobrevivente em relação ao grupo de pacientes sépticos sobreviventes. SDNN, TP, VLF, LF, LF/H, nLF, α1/α2 e r-MSSD do grupo não-sobrevivente eram reduzidos em relação ao grupo de sobreviventes em pelo menos um estudo. Dois estudos encontraram que o SDNN estava associado a mortalidade na sepse, mesmo após ajuste para possíveis fatores de confusão. Três estudos realizaram análise da VFC utilizando gravações de longa duração. Dentre estes, apenas um estudo encontrou diferença entre sobreviventes e não sobreviventes e, mesmo assim, em apenas um parâmetro da VFC: LogHF.

Conclusão: Vários parâmetros da VFC estão reduzidos em pacientes sépticos não-sobreviventes em comparação com os sobreviventes. Em nosso estudo de coorte, o SDNN $\leq$ 17ms mostrou-se um fator de risco para morte em pacientes sépticos, mesmo após ajuste para escores de gravidade. Na revisão sistemática, o SDNN apareceu associado de maneira independente à mortalidade na sepse em dois estudos. Esses achados sugerem que o SDNN seja um parâmetro útil como preditor de mortalidade na sepse.

Abstract

Introduction: Sepsis is a severe medical condition with increasing prevalence and high mortality. The role of the autonomic nervous system in the pathophysiology of sepsis has been increasingly researched. The objective of this study is to evaluate the heart rate variability (HRV) as a predictor of mortality in septic patients through a cohort study and a systematic review.

Methods: In the prospective cohort of patients diagnosed with sepsis, patient recruitment was carried out at ICU in a tertiary university hospital between March 2012 and February 2014. Clinical data and laboratory exams were collected at admission. Each patient underwent a 20-minute Holter and a 24-hour Holter on the first day of enrollment. The primary outcome was the 28-day all-cause mortality.

In the systematic review of studies evaluating HRV as a predictor of death in patients with sepsis, the search for articles was performed by independent researchers in PubMed, LILACS and Cochrane, including papers in English, Portuguese or Spanish, indexed until August 20th, 2017 with at least 10 patients. Study quality was assessed by the Newcastle-Ottawa Scale. To analyze the results, we divided the articles between those who measured HRV for short-term recordings ($\leq$ 1 hour), and those who did long-term recordings ($\geq$ 24 hours).

Results: In the cohort study, a total of 63 patients were included. Patients were categorized into nonsurvivor group (n = 16) or survivor group (n = 47) depending on this endpoint. Survivors were younger (48.6 years vs. 63.0 years), had better renal function and lower values in severity scores (APACHE II and SOFA) compared to nonsurvivors. In the 20-minute Holter, SDNN, TP, VLF, LF and LF/H of nonsurvivors were significantly lower than those of survivors ($p \leq 0.001$, $p = 0.003$, $p = 0.002$, $p = 0.006$, $p = 0.009$ respectively). ROC curve of SDNN was built, showing area under the curve of 0.772 (0.638±0.906) for mortality prediction. The value of 17ms was chosen as the best SDNN cutoff point to discriminate survivors and nonsurvivors. In the Cox proportional regression, adjusted for SOFA score and for APACHE II, a SDNN $\leq$ 17ms was associated with a greater risk of death, with hazard ratios of 6.3 (1.4± 28.0; $p = 0.015$) and 5.5 (1.2±24.8; $p = 0.027$), respectively. The addition of the dichotomized SDNN to the SOFA model reduced AIC and increased the concordance statistic and the

R^2, indicating that predictive power of the SDNN + SOFA model is better than predictive power of SOFA only.

In the systematic review, nine studies were included with a total of 536 patients. All of them were observational studies. Studies´ quality varied from 4 to 7 stars in Newcastle-Ottawa Scale. The mortality rate in studies ranged from 8 to 61%. Seven studies performed HRV analysis in short-term recordings. With the exception of one study that did not explain which group had the lowest results, all other studies showed reduction of several HRV parameters in the non-survivors in relation to the surviving septic patients. SDNN, TP, VLF, LF, LF/H, nLF, α1/α2 and r-MSSD of the non-survivor group were reduced in relation to the survivors in at least one study. Two studies found that SDNN is associated with mortality in sepsis, even after adjusting for possible confounding factors. Three studies performed HRV analysis using long-term recordings. Only one of these studies found difference between surviving and non-surviving groups, and even so, in only one HRV parameter: LogHF.

Conclusions: Several HRV parameters are reduced in nonsurviving septic patients. In our cohort study, SDNN ≤ 17 ms appeared as a risk factor for death in septic patients, even after adjusting for severity scores. In the systematic review, SDNN seems to be independently associated with mortality in sepsis in two studies. These findings make SDNN emerge as the most useful HRV parameter to predict mortality in septic patients.

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1 – ANTECEDENTES CIENTÍFICOS

1.1 – Sepse

1.1.1 – Conceitos

A sepse é definida como disfunção orgânica com risco de morte causada por uma resposta desregulada de um paciente à infecção(1). Essa definição revista em 2016 por duas das principais sociedades de terapia intensiva do mundo (Society of Critical Care Medicine e European Society of Intensive Care Medicine) visa destacar os três pontos mais importantes dessa doença: ser consequência de uma resposta descontrolada do hospedeiro, causar risco de morte e demandar reconhecimento médico imediato.

Não existe um exame padrão-ouro para o diagnóstico de sepse. O critério diagnóstico clássico criado na década de 1990 relaciona sepse à presença de infecção e Síndrome da Resposta Inflamatória Sistêmica (SIRS)(2). São considerados como portadores de SIRS pacientes com pelo menos dois dos critérios da tabela 1. Assim, eram considerados como portadores de sepse, os pacientes com infecção e SIRS. Entretanto, a presença de SIRS não significa necessariamente disfunção orgânica e pode ser apenas uma resposta adaptativa e adequada à infecção(1). Com o avanço do entendimento da fisiopatologia desta síndrome, esse critério diagnóstico passou a ser questionado e precisou ser revisto. O conceito atual de sepse envolve necessariamente a presença de disfunção orgânica, o que pode ser evidenciado por diferentes escores e exames laboratoriais. O aumento na pontuação do SOFA (Sequential [Sepsis-related] Organ Failure Assessment – Tabela 2)(3) de 2 ou mais pontos associada a infecção foi escolhido pelo Terceiro Consenso Internacional de Sepse e Choque Séptico como marcador de disfunção orgânica para o diagnóstico de sepse. Tal escolha deveu-se a relativa simplicidade do escore, ao fato de ele ser mundialmente conhecido e a sua elevada capacidade de descriminar mortalidade hospitalar(4).

Tabela 1 - Síndrome da Resposta Inflamatória Sistêmica (SIRS)

Pelo menos 2 dos critérios abaixo:
Temperatura > 38ºC ou < 36ºC
Frequência cardíaca > 90 batimentos/minuto
Frequência respiratória > 20 movimentos/minuto ou PaCO2< 32 mmHg (< 4,3 kPa)
Leucócitos > 12.000 células/mm3, ou < 4.000 célula/mm3, ou > 10% de formas jovens

Tabela 2 - Sequential [Sepsis-related] Organ Failure Assessment [a]

	Ponto				
Sistema	0	1	2	3	4
Respiratório PaO_2/FIO_2, mm Hg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) com suporte ventilatório	<100 (13.3) com suporte ventilatório
Coagulação Plaquetas ×103 /µL	≥150	<150	<100	<50	<20
Fígado Bilirrubina mg/dL (µmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular[b]	PAM ≥70 mm Hg	PAM <70 mm Hg	Dopamina <5 ou dobutamina (qualquer dose)	Dopamina 5.1-15 ou epinefrina ≤0.1 ou noraepinefrina ≤0.1	Dopamina >15 ou epinefrina >0.1 ou noraepinefrina >0.1
SNC ECG	15	13-14	10-12	6-9	<6
Renal Creatinina, mg/dL (µmol/L)	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)
Débito urinário, mL/d				<500	<200

FIO_2= Fração Inspirada de O_2; PAM= Pressão arterial média; SNC = Sistema Nervoso Central; ECG = Escala de Coma de Glasgow; [a] Tabela adaptada de Vincent et al.(3); [b] Catecolaminas administradas em µg/kg/min por pelo menos 1 hora

Choque séptico é definido como falência circulatória na qual existem alterações do metabolismo celular tão pronunciadas que acarretam elevação da mortalidade. Para receber esse diagnóstico o paciente séptico deve apresentar hipotensão requerendo uso de vasopressor para manter PAM >65mmHg com lactato >2 mmol/L (18 mg/dL), apesar de ressuscitação volêmica adequada(1).

1.1.2 – Epidemiologia

A incidência de sepse tem crescido nas últimas décadas. Dados dos EUA, por exemplo, mostram 164.072 casos em 1979 (82,7 por 100.000 habitantes) e 659.935 casos em 2000 (240,4 por 100.000 habitantes)(5). Outros estudos de base populacional mostram que a incidência desta doença continuou aumentando, em vários países, nas últimas duas décadas(6, 7), permitindo estimar a incidência mundial anual da doença em 31,5 milhões de casos com 5,3 milhões de mortes(6). Tal aumento reflete, entre outros fatores, o envelhecimento da população, que passa a ter mais comorbidades, além do

aumento do reconhecimento desta entidade. Entre adultos, há aumento progressivo da incidência com a idade e a maioria dos casos ocorre em pacientes idosos(8). Além do aumento da incidência global da sepse, há dados que sugerem maior proporção de casos graves(9), embora esse aumento possa refletir a melhora da acurácia diagnóstica de disfunção orgânica através de escores ao longo dos anos.

Com relação ao Brasil, os dados epidemiológicos são bastante escassos. Se formos extrapolar para a população brasileira (de cerca de 207 milhões de habitantes) os dados epidemiológicos dos EUA que mostram incidência de 3 casos de sepse grave para cada 1.000 habitantes(8), teríamos 621 mil casos anuais de sepse em nosso país. O estudo SPREAD (Sepsis Prevalence Assessment Database)(10) baseou-se na distribuição de UTI brasileiras para escolher uma amostra dos leitos disponíveis para adultos. Foram incluídos os pacientes internados com sepse durante 24 horas. O estudo mostrou que 30,2% dos leitos de UTI estavam ocupados com pacientes com sepse, mostrando o grande impacto médico e financeiro que essa doença representa para o Brasil. A incidência de sepse na UTI foi de 36,3 por 1000 dias-paciente (95% IC 29,8 - 44,0). A partir desses dados, estimou-se incidência de 290 por 100.000 habitantes (95% IC 237,9 - 351,2) de casos adultos de sepse tratados em UTI por ano, o que produz cerca de 420.000 casos por ano, dos quais 230.000 morrem no hospital. Já o estudo BASES, feito em 5 UTI do Brasil, mostrou taxa de incidência para sepse, sepse grave e choque séptico de 61,4 , 35,6 e 30,0 por 1000 pacientes-dia, respectivamente(11).

1.1.3 – Mortalidade

A sepse é uma doença grave, cuja mortalidade intra-hospitalar varia nos estudos entre 10 e 50% (4, 9, 12-14). Quanto mais sinais de disfunção orgânica o paciente apresenta maior a sua mortalidade. Pacientes que preenchem os critérios atuais de sepse apresentam pelo menos 10% de mortalidade, enquanto pacientes com choque séptico tem mortalidade em torno de 50%(4).

Apesar de ainda apresentar prognóstico ruim, a mortalidade da sepse tem diminuído nas últimas décadas(5, 15, 16). Estudo retrospectivo multicêntrico americano mostrou redução da mortalidade atribuída a esta doença de 27,8% em 1979 para 17,9% em 2000(5). Metanálise de estudos multicêntricos randomizados também mostrou redução de mortalidade da sepse de 46,9% entre os anos 1991 a 1995 para 29% entre os anos

2006 a 2009(16).Outro estudo retrospectivo envolvendo centros da Áustria e Nova Zelândia mostrou redução de mortalidade de pacientes com sepse de 35,0% para 18,4% entre 2000 e 2012(15).

Os pacientes sobreviventes de sepse permanecem com risco elevado de morte em longo prazo. Estudo de coorte com 14.529 pacientes mostrou que esses pacientes apresentam, de 31 dias a dois anos, aumento de 22.1% (17,5 – 26,7) no risco absoluto de morte (40,4% versus 18,3%) em relação a pacientes não-hospitalizados, aumento de 10,4% (5,4 – 15,4) no risco absoluto de morte (42,8% versus 32,4%) em relação a pacientes admitidos no hospital com infecção sem sepse e aumento de 16,2% (10,2 – 22,2) no risco absoluto de morte (43,5% versus 27,3%) em relação a pacientes admitidos no hospital com condições inflamatórias estéreis(17).

A mortalidade da sepse no Brasil parece mais elevada do que a encontrada em países desenvolvidos. Estudo realizado em 65 hospitais de todas as regiões do país mostrou mortalidade na sepse, sepse grave e choque séptico de 16,7%, 34,4% e 65,3%, respectivamente(18). Já estudo retrospectivo feito através de análise de atestado de óbito em todo o país mostrou que, no ano de 2010, sepse aparecia como uma das doenças responsáveis pelo óbito em 16,46% dos casos (19). No estudo SPREAD, a mortalidade observada foi de 55,7% (IC 95% 52,2 – 59,2), mostrando, mais uma vez, a elevada mortalidade desta doença em nosso país(10).

1.1.4 – Fisiopatologia

A resposta normal do organismo humano à infecção envolve complexa regulação entre fatores pró-inflamatórios e anti-inflamatórios. Inicia-se com o reconhecimento, especialmente através de macrófagos, de componentes de microorganismos(20). Esse reconhecimento gera uma cascata de reações que ativa genes responsáveis pela resposta pró-inflamatória, levando ao aumento de citocinas pró-inflamatórias como fator de necrose tumoral alfa (TNFa) e interleucina-1 (IL-1). Paralelamente, ocorre a ativação de neutrófilos e sua concentração no local acometido pela infecção, graças as moléculas de adesão expressas pelo endotélio. Essas células polimorfonucleares liberaram mediadores que são responsáveis pelo calor, eritema e edema que correm no local infectado(21). O correto equilíbrio entre mediadores pró-inflamatórios (especialmente TNFa, IL-1 e IL-6) e anti-inflamatórios (por exemplo IL-10) é o responsável pelo

sucesso no processo de morte do microorganismo infectante e remoção de tecidos lesados.

A sepse é consequência da exacerbação dos mediadores pró-inflamatórios, levando a danos não apenas ao local da infecção, mas a todo o organismo(22). Por causa disso, a sepse é entendida como um desajuste do processo de defesa do organismo, caracterizado por ser descontrolado, autossustentado e causado por resposta inflamatória exagerada através de mediadores intravasculares(23).

Vários fatores contribuem para uma infecção tornar-se sepse. O tipo de microorganismo e sua carga infectante, através de componentes celulares e produtos (como as toxinas) tem papel central levando ao aumento da produção de mediadores pró-inflamatório(24). Por outro lado, fatores do paciente, como presença de imunossupressão, idade, comorbidades e até variações dos genes que produzem proteínas pró-inflamatórias e anti-inflamatórias aumentam o risco de sepse(25).

Está envolvido na fisiopatologia da sepse o balanço entre dois processos de morte celular: apoptose e necrose(26). A apoptose, morte celular programada, ocorre mantendo-se a integridade da membrana plasmática da célula até quase o término do processo, evitando assim a liberação de substâncias tóxicas no ambiente circundante da célula. Pode ocorrer por estímulo externo (através de receptor celular) ou interno(27). A apoptose é um dos principais mecanismos de remoção de células desnecessárias, disfuncionais ou neoplásicas. A necrose, ao contrário, ocorre geralmente por lesão isquêmica da célula, levando a ruptura da membrana com liberação de enzimas proteolíticas. Essas enzimas tem papel da destruição de bactérias na fase inicial de infecção(25).

Embora ocorra necrose na sepse, a apoptose está presente em maior intensidade(28). Há apoptose acelerada de linfócitos, no baço, timo e porção linfoide de outros órgãos, além de apoptose acelerada de células epiteliais intestinais e dendríticas, o que compromete a capacidade de defesa do organismo(29). Estudos mostram que quanto maior a grau de apoptose maior a gravidade da sepse(30, 31). Por outro lado, a sepse causa apoptose atrasada nos neutrófilos, fazendo com que essas células permaneçam ativas por mais tempo que o ideal, aumentando o processo inflamatório e levando a dano celular adjacente(32).

A sepse provoca ainda disfunção circulatória, com liberação de mediadores vasoativos (especialmente óxido nítrico) e baixa liberação de vasopressiva levando a hipotensão(33). Contribui ainda para a hipotensão o aumento da permeabilidade endotelial com redistribuição de fluídos para o terceiro espaço. Esse edema leva à redução dos capilares funcionantes na microvasculatura, reduzindo a capacidade tecidual de extração de oxigênio(34).

A nefrotoxicidade da sepse é multifatorial, contribuindo tanto a hipoperfusão quanto a resposta inflamatória local provocada pelas endotoxinas e citocinas pró-inflamatórias.(35)

A sepse leva ainda à disfunção cardíaca sistólica e diastólica, causada, entre outros fatores, pela redução da extração de oxigênio da microvasculatura coronariana e cardiotoxicidade de radicais livres e citocinas pró-inflamatórias(36). Há também disfunção pulmonar caracterizada por edema alveolar, causado especialmente pelo aumento da permeabilidade alveolar, e disfunção hepática causada por endotoxinas e restos bacterianos(37).

O papel do sistema nervoso autônomo na sepse tem sido objeto cada vez maior de pesquisa. Estudo em modelo animal mostrou que estimulação do nervo vago leva ao aumento da secreção de hormônio liberador de corticotrofina, ACTH e cortisol(38). Da mesma forma, a vagotomia atenua a febre e liberação de corticóide provocada pela administração intravenosa de citocinas(39). Esses resultados sugerem que a inervação vagal está envolvida na comunicação entre citocinas e o cérebro. Acetilcolina, a principal neurotransmissor parassimpático, apresenta um efeito anti-inflamatório, atenuando a liberação de citocinas TNF, IL-1beta, IL-6 e IL-18 , além de prevenir o desenvolvimento de choque(40). Tratamento com nicotina, um agonista colinérgico seletivo(41), e com colina, um precursor na síntese de acetilcolina(42), aumentou a sobrevida em modelo experimental de sepse.

1.1.5 – Alterações Cardíacas na Sepse

A sepse classicamente pode levar a vasodilatação periférica com hipotensão. Entretanto, há pelo menos três décadas, são conhecidas alterações também na função cardíaca relacionadas a essa síndrome. O estudo clássico de Parker et al de 1984, utilizando medições através do cateter de Swan-Ganz e angiografia radionuclear em 20 pacientes

sépticos, mostrou que pacientes sobreviventes apresentavam queda da fração de ejeção do ventrículo esquerdo e aumento do volume sistólico e diastólico do ventricular esquerdo, enquanto os pacientes não-sobreviventes apresentavam fração de ejeção normal e volume ventricular normal(43). Além disso, mostrou que, nos pacientes sobreviventes, essas alterações eram normalizadas após 7 a 10 dias. Essa associação paradoxal entre morte e fração de ejeção normal foi atribuída pelos autores ao fato de os pacientes não-sobreviventes apresentarem resistência vascular sistémica significativamente mais baixa que os sobreviventes(43). Entretanto, como apontado por Zaky et al. os métodos utilizados neste estudo para as medições são sujeitos a falhas e os grupos eram heterogêneos (além de pequenos), com alguns pacientes no grupo sobreviventes apresentando doença cardíaca prévia(44). Ainda assim, dada a importância desse estudo pioneiro e baseado em seus achados, criou-se a teoria de que os pacientes sobreviventes a sepse apresentavam melhor complacência do VE, e que, por isso, conseguiam manter o volume sistólico, mesmo com a queda da fração de ejeção, através do aumento do volume ventricular(45). Estudo de maior qualidade metodológica feito com 90 pacientes com choque séptico na década de 1990 avaliou a função cardíaca na sepse através de ecocardiograma(46). Mostrou, como o estudo anterior, que os paciente sobreviventes apresentavam função ventricular pior que os não-sobreviventes no momento da inclusão (FEVE 43,9% ± 16,.4 vs FEVE 52,0% ± 14.0 com $P<0,05$). Entretanto, diferentemente do estudo feito por Parker, este estudo mostrou que os pacientes sobreviventes mantinham o volume sistólico principalmente porque apresentavam melhora progressiva da fração de ejeção ao longo dos dias de observação. Já a dilatação do VE, embora existisse, era discreta (14% de aumento contra 100% de aumento encontrado no estudo de Parker et al). De qualquer forma, o estudo confirmou que a gravidade da disfunção ventricular no paciente com choque séptico não acarreta risco maior de mortalidade a esses pacientes. Outro estudo, publicado em 2008, avaliou 67 pacientes com choque séptico e encontrou disfunção ventricular (definido como FEVE < 45%) em 60% dos pacientes (47). Mais uma vez a disfunção ventricular reversível não se mostrou associada com a mortalidade. Outro estudo, no entanto, encontrou associação em análise univariada entre a velocidade sistólica de pico no anel mitral (outro método para avaliação da função sistólica) e mortalidade na sepse(48). Entretanto, essa associação não se manteve na análise multivariada.

Resultados similares a esses, ou seja, disfunção ventricular e melhora progressiva no grupo sobrevivente, foi encontrado também para o ventrículo direito(49).

Com relação a função diastólica, estudo com 262 pacientes, mostrou que 50% dos pacientes sépticos e 58,5% dos pacientes com choque séptico apresentaram disfunção diastólica (caracterizada por velocidade da onda e′ < 8 cm/s)(48). A presença de disfunção diastólica esteve associada de maneira independente com a mortalidade.

Biomarcadores como Troponina e Peptídeo Natriurético Cerebral (BNP) também estão elevados em parte dos pacientes com sepse. Dois estudos diferentes mostram, em pacientes sépticos, associação entre elevação de troponina e mortalidade, assim como entre elevação de troponina e presença de disfunção ventricular esquerda(50, 51). De forma análoga, em coorte de pacientes com sepse, o BNP estava elevado (>100 pg/mL) em 42% dos pacientes no momento da inclusão(52). Além disso, BNP >230 pg/mL esteve associado com a presença de disfunção ventricular e BNP >210 pg/mL esteve associado com mortalidade.

O mecanismo fisiopatológico da cardiopatia na sepse é complexo e ainda não totalmente entendido. Sabe-se que as citocinas pró-inflamatórias contribuem para o quadro ao acarretaram o aumento da concentração de prostanóides (especialmente tromboxano e prostaciclinas) e óxido nítrico(53). Os prostanóides alteram a função endotelial coronariana(54) e o óxido nítrico em excesso contribui para a disfunção ventricular através de metabólicos citotóxicos(55).

Além disso, há, na sepse, disfunção mitocondrial que atinge também as mitocôndrias das células miocárdicas, gerando aumento de óxido nítrico e reduzindo a capacidade de produção de ATP(56). Associado a isso, haveria também aumento da apoptose dos miócitos induzida pela toxinas bacterianas, contribuindo para o dano miocárdico(57).

Contribuiria ainda para o processo de cardiopatia da sepse uma importante alteração no metabolismo e geração de energia. Na sepse, há aumento da concentração de triglicérides plasmático, como consequência da redução da lipólise intravascular e da redução da entrada de lipídios nos tecidos(58). Como 70% do ATP cardíaco é gerado através da oxidação lipídica, haveria hipóxia tecidual(59).

Como será detalhado mais adiante, outro mecanismo cada vez mais estudado na sepse é a desregulação autonômica, causando redução da variabilidade da frequência cardíaca(44).

1.2 – Variabilidade da Frequência Cardíaca

1.2.1 – Definição

A análise da Variabilidade da Frequência Cardíaca (VFC) é uma ferramenta indireta de avaliação do sistema nervoso autonômico(60). Seu interesse científico existe há mais de 50 anos, desde que Hon e Lee descreveram que o sofrimento fetal era precedido por alteração nos intervalos R-R, em uma fase em que a frequência cardíaca ainda não havia se alterado(61). Nas últimas décadas, o estudo da VFC justificou-se principalmente pelo entendimento do papel desempenhado pela atividade simpática na geração e manutenção de arritmias graves(62) e do papel do sistema nervoso autonômico na regulação da inflamação e da imunidade do organismo(63). Embora o sistema autonômico tenha papel preponderante, sabe-se que outros fatores neuro-humorais também interferem na VFC, fazendo com que sua interpretação seja complexa e não completamente compreendida(64).

1.2.2 – Métodos de Medida da VFC

1.2.2.1 – Domínio do Tempo

É a metodologia mais simples. Nela, cada intervalo RR (entre dois QRS) de um ritmo sinusal é chamado de intervalo NN (Normal-to-Normal) e várias medidas estatísticas desse intervalo ao longo do tempo são calculadas. Os índices mais utilizados estão na Tabela 3.

O índice mais utilizado é o desvio padrão do intervalo NN (SDNN), ou seja, a raiz quadrada da variância. O SDANN mede o desvio padrão da média de NN calculada a intervalos de 5 min, enquanto o SDNNi mede a média dos desvios-padrão de NN calculados a intervalos de 5 min, respectivamente. Portanto, esses dois índices devem ser usados em registros de longa duração. O pNN50 mede a percentagem de pares NN adjacentes que diferem em mais de 50 ms, enquanto o NN50 mede o número de pares NN adjacentes que diferem em mais de 50 ms. O rMSSD mede a raiz quadrada da média ao quadrado das diferenças entre NN sucessivos.

Além dos métodos estatísticos (SDNN, SDANN, SDNN, r-MSSD, pNN50, NN50), há também índices de VFC no domínio do tempo calculados através de métodos geométricos. Neles, a série de intervalos NN é convertida em um padrão geométrico. Os mais utilizados são o índice triangular de VFC e o TINN. Para o cálculo do índice triangular de VFC é construído um histograma de densidade dos intervalos RR normais, com o comprimento dos intervalos RR no eixo horizontal e a frequência com que cada um deles ocorreu no eixo vertical. A união dos pontos das colunas do histograma forma uma figura semelhante a um triângulo e a largura da base deste triângulo expressa a variabilidade dos intervalos RR. O índice triangular é calculado dividindo-se o total de intervalos RR (área) pelo número de intervalos RR com frequência modal (altura)(65). Já o TINN é a interpolação triangular do histograma dos intervalos RR, calculada a partir da largura da linha de base do triângulo, sendo a diferença dos mínimos quadrados utilizados na determinação do triângulo.

A principal vantagem dos métodos geométricos é sofrerem pouca influência da qualidade analítica da série de intervalos NN e da presença de extrassístoles (ficam fora do triângulo do índice triangular, por exemplo)(66). Por outro lado, esses índices necessitam de um número razoável de intervalos NN para construir o padrão geométrico, sendo inadequados para avaliar as mudanças de curto prazo na VFC(60).

1.2.2.2 – Domínio da Frequência

Essa técnica baseia-se no fato de todo sinal poder ser decomposto em vários componentes oscilatórios de frequências diferentes. Dessa forma, a Variabilidade da Frequência Cardíaca é representada através de um tacograma (gráfico dos RR normais em relação ao tempo), que é decomposto em componentes oscilatórios de frequências diferentes, por meio de algoritmos matemáticos. Essas técnicas matemáticas podem ser paramétricas (modelos autorregressivos - AR) ou não-paramétricas (Transformada Rápida de Fourier - Fast Fourier Transform - FFT)(65).

Tabela 3 - Principais índices da VFC no Domínio do Tempo e Frequência

Índice	Unidade	Significado
Domínio do Tempo		

SDNN	ms	Desvio-padrão dos intervalos RR normais
SDANN (SDANNi)	ms	Desvio-padrão das médias dos intervalos RR normais de todos os segmentos de cinco minutos do traçado de 24 horas
SDNN Index (SDNNi)	ms	Média dos desvios padrão dos intervalos RR normais calculados em intervalos de cinco minutos
r-MSSD	ms	Raiz quadrada da média da soma dos quadrados das diferenças sucessivas entre intervalos RR normais adjacentes
pNN50	%	Percentagem das diferenças entre intervalos RR normais adjacentes que excedem 50 milissegundos
NN50	-	Número de intervalos RR normais adjacentes cuja diferença excede 50 milissegundos
HRV triangular index	-	Calculado a partir do histograma de densidade dos intervalos RR normais, dividindo-se o total de intervalos RR pelo número de intervalos RR com frequência modal.
TINN	ms	Interpolação triangular de histograma dos intervalos RR
Domínio da Frequência		
Total power (TP)	ms^2	Mede a variância total da VFC (poder em frequência de até até 0,40 Hz).
Ultra low frequency (ULF) power	ms^2	Poder em frequência de até 0.0033 Hz
Very low frequency (VLF) power	ms^2	Poder em frequência entre 0.0033 e 0.04 Hz.
Low frequency (LF) powe	ms^2	Poder em frequência entre 0.04 e 0.15 Hz.
High frequency (HF) power	ms^2	Poder em frequência entre 0.15 e 0.40 Hz.
LF/HF ratio	-	Razão entre baixa e alta frequência

O método FFT é o mais utilizado, dada a sua facilidade de aplicação, boa representação gráfica e alta disponibilidade em softwares computacionais. É um método objetivo e sem perda de informação no seu processo: o tacograma original pode ser recuperado mesmo após a transformação pela FFT. Entretanto, apresenta como desvantagem resolução de frequência limitada(67).

No modelo AR, a estimativa dos parâmetros é feita através de resolução de equações lineares. Neste modelo, os componentes espectrais podem ser distinguidos independentemente de bandas de frequência pré-selecionadas(65). A limitação é a necessidade da escolha adequada do modelo paramétrico(67).

Potência Espectral da VFC é divida usualmente em quatro faixas de freqüência:

a) Freqüência Ultra Baixa - 0.0001 a 0.0033 Hz (Ultra Low Frequency - ULF)

b) Freqüência Muito Baixa - 0.0033 to 0.04 Hz (Very Low Frequency - VLF);

c) Freqüência Baixa - 0.04 to 0.15 Hz ((Low Frequency - LF);

d) Freqüência Alta - 0.15 to 0.40 Hz (High Frequency - HF).

Os componentes de energia VLF, LF e HF são geralmente representados em valores absolutos de poder (ms^2). Entretanto, pode ser realizada a normalização (n.u.) dos valores de LF e HF através da divisão da potência do componente (LF ou HF) pelo espectro de potência total, subtraída do componente de VLF e multiplicada por 100(65). A normalização tenderia a minimizar o efeito das mudanças na potência total sobre os valores de LF e HF(60).

1.2.2.3 – Métodos Não-Lineares

Os sistemas lineares são aqueles cujo comportamento é determinístico, ou seja, aqueles em que, se estudarmos seus componentes e o funcionamento de cada um, conseguimos prever o que ocorrerá com o todo. Já, nos sistemas não-lineares, as interações dos seus componentes são, aparentemente, aleatórias e não previsíveis. Nestes sistemas, estímulos de pequena magnitude podem gerar respostas inesperadas, mudança radical de comportamento do todo, e vice-versa. Mesmo estudando seus componentes torna-se muito difícil prever o comportamento do todo. Os métodos não-lineares partem do pressuposto de que os sistemas biológicos são complexos e dinâmicos (interação de fatores hemodinâmicos, eletrofisiológicos, humorais, autonômicos e do sistema nervoso central), não se enquadrando com perfeição em métodos lineares. Na verdade, portanto, existiria uma relação entre os componentes do sistema gerando o resultado do todo, mas a relação entre esses componentes é tão complexa e dinâmica (ora de adição, ora de subtração, ora de grande intensidade, ora de baixa intensidade), que é impossível explicar esse sistema através de uma equação estática. Eles utilizam a teoria do Caos, que descreve elementos manifestando comportamentos que são extremamente sensíveis às condições iniciais, dificilmente se repetem, mas, apesar de tudo, são determinísticos(68).

Várias metodologias não-lineares já foram desenvolvidas para a análise da VFC.

1.2.2.3.1 – Correção de longo alcance e escala fractal

Um modelo representativo dessa técnica é o modelo $1/f$. O termo fractal foi utilizado para objetivos geométricos muito irregulares para serem enquadrados nas formas geométricas tradicionais, mas que se assemelhavam a um padrão comum quando vistos em diferentes escalas (auto-similaridade). Analogamente, um processo temporal pode ser chamado de auto-semelhante, se suas flutuações em pequenas escalas de tempo são estatisticamente equivalentes àqueles em grandes escalas de tempo. Matematicamente isso pode ser representado da seguinte forma: duas séries temporais x e y são auto-semelhantes se x(t) e $y(t) = s^h x(t/s)$ apresentam propriedades estatísticas idênticas para um determinado intervalo de escalas (s). O número real h é chamado de expoente de escala e caracteriza a auto-similaridade da série x (t)(69).

Na análise fractal, a série NN seria gerada por um processo aleatório (como jogar uma moeda), em vez de por um sistema determinista (tal como uma equação matemática precisa). Os sinais auto-similares exibem uma densidade de potência espectral em frequências baixas(0,0001 e 0,01 Hz). O expoente α do modelo modelo $1/f$ mede a e escala de auto-similaridade. Os processos auto-similares (fractal) são muitas vezes referidos como "longmemory" (processos com dependência de longo alcance), já que a função de correlação generalizada de sinais NN fractais decai muito lentamente, o que implica que o valor contemporânea da série seja significativamente afectado pelo seu passado. O índice DFA (detrended fluctuation analysis) permite quantificar esse processo medindo como a variância é afetada pelo comprimento da série NN(69).

1.2.2.3.2 – Complexidade de Curto Prazo

Essas técnicas quantificam irregularidades em escalas curtas (cerca de10 intervalos NN). O DFA de curto prazo, por exemplo, estima a auto-similaridade da VFC em escalas curtas. Apesar de não descrever a auto-similaridade global da VFC, quantifica mudanças de curto prazo causadas por oscilações de intervalo NN. Essas mudanças de curto prazo podem ser afetadas por ativação autonômica e são muitas vezes, erroneamente, relacionadas apenas à respiração(70).

Outro método deste grupo é a análise do gráfico de dispersão de Poincaré (ou mapa de recorrência), que é gerado a partir dos valores de NNn+1 contra os valores de NNn . Do gráfico de dispersão de Poincaré, são extraídos índices como SD1, SD2 e SD12, que

estão diretamente relacionados com o SDNN, gerando informação semelhante(71). O benefício da análise visual do gráfico de dispersão de Poincaré seria a confirmação da correta edição dos intervalos NN, separando, por exemplo, os batimentos ectópicos (69).

Há ainda análises da capacidade de aceleração e desaceleração, que se baseiam na hipótese de as alterações da frequência cardíaca provocadas por um evento de disparo particular são repetitivas. Phase rectified signal averaging (sinal de alta resolução fase retificado - PRSA) seleciona casos de eventos particulares, alinha-os e executa o cálculo da média do sinal de tempo da série para extrair as informações de interesse. Isso permite a detecção e quantificação de oscilações mascaradas pela natureza não-estacionária do sinal analisado(69). Dessa forma, o PRSA permite quantificar a aceleração (acceleration capacity—AC) e desaceleração (deceleration capacity—DC) de séries NN(72).

1.2.2.3.3 – Entropia e regularidade

Entropia é um conceito da termodinâmica que mede a desordem (ou aleatoriedade) das partículas de um sistema físico. Expandido esse conceito para Teoria da Informação, a entropia passou a ser definida como o grau médio de incerteza a respeito de fontes de informação, o que consequentemente permite a quantificação da informação presente que flui no sistema. Como o matemático Claude Shannon foi seu criador, quando aplicada a uma sequência (por exemplo, um histograma), a entropia passou a ser frequentemente denominada entropia de Shannon e quantifica a sua complexidade por meio de um teor médio de informações. A taxa de entropia mede a variação na entropia de uma sequencia causada pela adição de uma amostra extra(69).

1.2.3 – Interpretação Fisiológica dos Índices de VFC

O SDNN reflete todos os componentes cíclicos responsáveis pela VFC no período de gravação e pode ser utilizada tanto em períodos longos (como 24 horas) quanto em curtos (5min, por exemplo)**(60)**. Em períodos longos, abriga tanto componentes de baixa frequência quanto de alta frequência e tente a ser maior quanto maior o período analisado. Dessa forma, não é correto comparar o SDNN de períodos com duração

diferentes, porque eles não representam os mesmos componentes**(73)**. Valores elevados de SDNN reflete uma VFC normal, o que representa bom prognóstico em várias condições clínicas, como infarto agudo do miocárdio**(74)** e insuficiência cardíaca**(75)**.

O SDANN e o ULF representam o ritmo circadiano e só devem ser feitas em gravações longas. Em gravação de 24 horas, o SDANN e o SDNN apresentam valores próximos, sendo o SDNN muito mais utilizado.

O HF Power reflete a ação vagal (parassimpática) sobre o nó sinusal através da respiração (arritmia respiratória). A administração de atropina, antagonista parassimpático, reduz em mais de 90% o valor do HF Power (76), enquanto a administração de propranolol não o altera significativamente(77). Dentro da faixa da normalidade, a variação da frequência respiratória não altera significativamente o HF Power, embora bradipnéia possa causar aumento do seu valor e taquipnéia extrema possa causar sua diminuição, refletindo perda da função vagal(78).

O significativo fisiológico do VLF Power e do LF Power ainda não são muito bem entendidos e parecem depender do contexto clínico. O VLF Power parece refletir a ação vagal, já que o bloqueio parassimpático reduz seu valor em até 92%(79), mas também sofre influência do sistema renina-angiotensina aldosterona (iECA reduzem seu valor) e da frequência respiratória(79). O LF Power parece sofrer influência tanto do sistema simpático quanto do parassimpático, de forma extremamente complexa, através de várias vias(80). Os receptores beta-adrenérgicos, por exemplo, podem exercer ações diferentes, como demostrado pelo aumento expressivo do LF Power com administração de propranolol (beta-bloqueador não-seletivo)(81) e não alteração significativa do seu valor com a administração de atenolol (B1-antagonista)(79).

A razão LF / HF é classicamente chamada de balanço simpático-vagal, já que o HF representaria a atuação vagal, e o LF a atuação simpática e parassimpática com predomínio da primeira(82). Entretanto, essa interpretação é por demais simplista e não pode ser sustentada para a maioria das situações clínicas(80). Diversos estudos já demonstraram que as intervenções fisiológicas podem provocar alterações recíprocas ou paralelas não-lineares complexas em qualquer divisão do sistema nervoso autônomo, tornando a interpretação da razão LF / HF um grande desafio ao conhecimento atual(83). Por exemplo, diversas situações em que se esperaria aumento dessa razão por aumento da atividade simpática, como infarto agudo do miocárdio e exercício físico, apesar de provocarem aumento da FC, não provocaram aumento da razão LF / HF(84).

Da mesma forma, a administração de atropina isoladamente falhou em aumentar essa razão(84), como seria esperado. Já o bloqueio concomitante simpático e parassimpático com a denervação parassimpática e a administração de beta-bloqueador aumentou essa razão em mais de 7 vezes(85).

Embora a interpretação fisiológica da VFC ainda não esteja completamente compreendida, os índices de VFC trazem informações sobre as influências autonômicas que podem ser úteis na avaliação prognóstica e diagnóstica de pacientes com cardiopatias, disautonomias, diabetes, sepse, entre outras condições(60).

1.2.4 – VFC e Doença Cardiovascular

A VFC já foi estudada no contexto de várias doenças cardíacas. Metanálise envolvendo 21.998 pessoas sem doença cardiovascular conhecida mostrou que apresentar VFC baixa está associado com aumento de 32 a 45% do risco de ter o primeiro evento cardiovascular(86). Em estudos com pacientes pós-infarto, a presenta de VFC baixa está associada a maior risco de morte. Estudo pioneiro publicado em 1987 por Kleiger et al com 808 pacientes pós-infarto mostrou que aqueles que, no dia 11 ± 3, tinham SDNN < 50ms apresentavam mortalidade 5,3 vezes maior em 31 meses(74). Estudos semelhantes feitos na era do tratamento trombolítico(87) e da angioplastia primária(88) confirmaram o SDNN<50ms como preditor de mortalidade pós-infarto, entretanto, com a melhora do tratamento do IAM, o grupo de pacientes com alteração tão pronunciada da VFC se tornou cada vez menor. Ainda com relação a doença coronariana, estudo com 470 pacientes que seriam submetidos a cineangiocoronarografia eletiva, mostrou, em análise multivariada, que ter o LF menor que 250ms, independente do escore de Framingham, estava associado a risco maior (OR 2,4, 95% CI 1,3-4,4, p=0,004) de apresentar lesão obstrutiva (definida como obstrução maior que 50%)(89).

Em pacientes com FA, após a cardioversão elétrica, a presença da razão LF/HF ≥ 2 apresentou, em coorte de 93 pacientes, sensibilidade de 76% e especificidade de 90% para recorrência da arritmia em 2 semanas(90).

A VFC também já foi relativamente bem estudada em pacientes com insuficiência cardíaca. Em diferentes estudos, ter parâmetros baixos de VFC esteve associado de forma independente ao risco de morte global, morte súbita, arritmia e necessidade de transplante(75, 91, 92).

1.2.4 – VFC e Sepse

Estudos demonstraram que pacientes sépticos apresentam diversos parâmetros da VFC reduzidos em relação a pacientes saudáveis(93, 94). Mais do que isso, Ahmad et al demonstrou, em um estudo com pacientes submetidos a transplante de medula óssea monitorados com Holter contínuo, que 86% dos pacientes apresentavam queda significativa (maior que 25%) do valor de diversos parâmetros da VFC (SDNN, r-MSSD, LF e HF) em média 35 horas antes do diagnóstico de sepse. Já os pacientes que não tiveram sepse, não apresentavam essa queda(95). Tais achados levantaram a hipótese de a VFC poder ser usada para predizer o risco de desenvolver sepse ou mesmo para o diagnóstico de sepse. Além disso, parâmetros da VFC estão correlacionados com alguns escores de gravidades já consagrados utilizados em pacientes sépticos. Por exemplo, em estudo de Barnaby et al, o LFnu esteve negativamente correlacionado com o APACHE II (r = -0,67) e SOFA (r = -0,80) (96). Outro estudo mostrou que o valor da Proteína C reativa (PCR) está negativamente correlacionada com LF (r = -0,78), LF/HF (r = -0,61) e SDNN (r = -0,79), e positivamente correlacionada com HF (r = 0,80) e SD1/SD2 (r = 0,66), enquanto a interleucina 10 está positivamente correlacionada com HF (r = 0,71) e negativamente correlacionada com LF (r = -0,89) e LF/HF (r = -0,66), em pacientes com choque séptico (97). Em pacientes já com o diagnóstico de sepse, alguns estudos demonstraram que a VFC pode ser usada para predizer o risco de desenvolver desfechos ruins, como choque séptico e falência orgânica múltiplas (MODS). Chen et al mostrou que pacientes sépticos admitidos em sala de emergência, inicialmente sem hipotensão e que evoluíam para choque séptico nas primeiras 6 horas de acompanhamento apresentavam valores significativamente menores de LH e LF/HF em relação àqueles pacientes que não evoluindo com choque(98). Pontet et al, em estudo de coorte com 46 pacientes sépticos inicialmente sem MODS, mostrou que os pacientes que desenvolveram MODS apresentavam, nas primeiras 24 horas, valor de LF e r-MSSD significativamente reduzidos em relação aos que não evoluíram com MODS(99). Outro estudo, mostrou que em pacientes sépticos o coeficiente de variação da frequência cardíaca nas primeiras 3 a 6 horas de inclusão esteve associado a independência de vasopressor em 24 horas(100). Dessa forma, a queda de diversos parâmetros da VFC parece ser um evento precoce da sepse e sua intensidade parece estar relacionada a gravidade do paciente.

Com todos esses achados, apesar de terem sido encontrados em estudos pequenos, parecia razoável que se tentasse estudar a capacidade da VFC de predizer mortalidade na sepse. Em 2008, Chen et al, em estudo de coorte com pacientes sépticos, demonstrou que o grupo de pacientes sépticos não-sobreviventes apresentava redução de diversos parâmetros da VFC (SDNN, TP, VLF, LF e LF/HF) em relação ao grupo de pacientes sobreviventes(101). Outros estudos também encontraram redução da VFC entre aqueles pacientes que não sobreviveram à sepse(102, 103). Entretanto, nenhum estudo ainda definiu qual o melhor parâmetro da VFC e qual o melhor ponto de coorte para predizer o risco de morte na sepse.

2 – JUSTIFICAVA E OBJETIVOS

Objetivo geral: avaliar, em pacientes sépticos, através de estudo prospectivo observacional e de revisão sistemática, o comportamento da variabilidade da frequência cardíaca (VFC) e sua relação com mortalidade.

Objetivos específicos:

- analisar a relação entre VFC e mortalidade em pacientes sépticos;

- realizar revisão sistemática sobre VFC e mortalidade na sepse

3 – DETALHAMENTO DA METODOLOGIA

Foram realizados dois estudos: um estudo de coorte prospectivo e uma revisão sistemática.

3.1 – Estudo de Coorte Prospectivo

Realizamos estudo de coorte prospectivo, como um subprojeto da pesquisa intitulada “Estudo da função endotelial e cardiovascular em pacientes portadores de sepse:

implicações diagnósticas, definição de risco e terapêutica", que foi aprovada pelo COEP da UFMG em 16/12/2011 (CAAE 0319.0.203.000-11).

Trata-se de estudo observacional constituído por série prospectiva de pacientes com diagnóstico de sepse internados no CTI do HC-UFMG. Foram incluídos no estudo todos os pacientes adultos (idade ≥ 18 anos) internados no CTI do HC-UFMG, de 10 de março de 2012 a 06 de fevereiro de 2014, com diagnóstico de sepse, motivo da internação ou adquirida durante a estadia no CTI e com pelo menos uma disfunção orgânica relacionada a sepse. Os critérios de exclusão foram: pacientes moribundos (previsão de óbito em menos de 24h), pacientes com proposta de cuidados paliativos, pacientes com sepse sob antibioticoterapia há mais de 48h, pacientes com ritmo não-sinusal e pacientes com marcapasso.

O diagnóstico de sepse foi baseado no 2º Consenso de Sepse(104), sendo, portanto, considerado presente nos pacientes com Síndrome da Resposta Inflamatória Sistêmica (SIRS) associada a uma infecção bacteriana ou fúngica confirmada ou fortemente suspeita.

Foram considerados portadores de SIRS pacientes com pelo menos dois dos quatro critérios abaixo:

. Temperatura > 38ºC ou < 36ºC

. Frequência cardíaca > 90 batimentos/minuto

. Frequência respiratória > 20 movimentos/minuto ou PaCO2< 32 mmHg (< 4,3 kPa)

. Leucócitos > 12.000 células/mm3, ou < 4.000 célula/mm3, ou > 10% de formas jovens

A presença de pelo menos uma disfunção orgânica baseou-se na definição de sepse grave do Surviving Sepsis Campaign(105).

3.1.1 – Protocolo do Estudo e Procedimentos Gerais

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (UFMG), e todos os pacientes incluídos ou seus familiares assinaram um Termo de consentimento livre e esclarecido. Os dados clínicos dos pacientes foram coletados na admissão e durante o acompanhamento, através de formulário específico

(Clinical Report Form). As principais variáveis coletadas foram: idade, gênero, comorbidades, diagnóstico principal, sítio primário da infecção, resultado de cultura micromiológica, uso de antibiótico, Sepsis related Organ Failure Assessment score (SOFA)(106) and Acute Physiology and Chronic Health Evaluation II (APACHE II)(107).

3.1.2 – Análise da Variabilidade da Frequência Cardíaca

Cada paciente incluído no estudo foi submetido a duas gravações de Holter de 3 canais (Cardios®, modelo CardioLight, São Paulo, Brasil) no primeiro dia de inclusão: uma gravação de 20 minutos, seguida de uma gravação de 24 horas. O primeiro registro (de 20 minutos) foi realizado com o paciente em posição supina e sem intervenções durante a gravação (como cuidados de enfermagem, fisioterapêuticos, etc.). A gravação de 24 horas não alterava a rotina de cuidados do CTI. A análise da VFC foi realizada utilizando um software específico para esse propósito, que calcula automaticamente vários parâmetros: no domínio do tempo: NN, SDNN, r-MSSD, pNN50; no domínio da frequência com o método FFT: Total Power, VLF power, LF Power, HF Power, LF / HF. Na gravação de 24 horas, a VFC foi analisada apenas no domínio do tempo. Foi realizada revisão manual da interpretação automática do Holter, incluindo análise do ritmo e dos complexos (por exemplo, QRS normal, extrassístole ventricular, extrassístole supraventricular, taquicardia, bradicardia, artefato, etc.). Artefatos e batimentos irregulares foram manualmente deletados antes da análise da VFC. No Holter de 24 horas, a VFC no domínio do tempo foi calculada sobre todo o período de 24 horas. No Holter de 20 minutos, a VFC foi calculada tanto no domínio do tempo quanto do domínio da frequência sobre os primeiros 10 minutos de gravação.

3.1.3 – Desfecho

O desfecho principal do estudo foi mortalidade em 28 dias por todas as causas. Os pacientes foram categorizados em sobreviventes e não-sobreviventes dependendo desse desfecho. Os parâmetros da VFC foram comparados entre esses dois grupos.

3.1.4 – Cálculo Amostral

O cálculo amostral considera testar a hipótese de que a distribuição de SDNN é a mesma entre os pacientes que sobrevivem e aqueles que morrem. O teste estatístico usado foi o teste não paramétrico de Mann-Whitney, que assume que os dados são medidos pelo menos em escala ordinal. As fórmulas adotadas para cálculo do tamanho da amostra estão descritas em Zhao, Rahardja, & Qu(108) e implementados no software R(109). Para estimativa dos parâmetros necessários para o cálculo amostral foi considerada uma amostra piloto constituída dos vinte primeiros pacientes no estudo (6 óbitos e 14 sobreviventes). Para esta amostra piloto foram calculados os tercis de SDNN e, em cada grupo, foi obtida a proporção de indivíduos em cada uma das três faixas definidas pelos tercis. Com base na distribuição observada dessas proporções e considerando um erro tipo I de 5%, poder de 80%, e mantendo a razão de alocação semelhante à observada na amostra, foi obtido um tamanho de amostra de 58 pacientes no total, sendo 44 sobreviventes e 14 óbitos.

3.1.5 – Análise Estatística

A normalidade de cada variável contínua foi verificada através do teste de Kolmogorov-Smirnov. Os dados obtidos de variáveis contínuas foram apresentados como média e desvio padrão, se eles apresentavam distribuição normal, ou mediana e percentis 25 e 75, se eles não apresentavam distribuição normal. Os dados obtidos de variáveis categóricas foram expressos como número absoluto e proporção. As características clínicas dos pacientes sobreviventes e não-sobreviventes foram comparadas usando os testes t de Student, Mann-Whitney e exato de Fisher, de acordo com o tipo e distribuição da variável. Para escolher o melhor ponto de corte do SDNN, foi construída uma curva ROC tendo a mortalidade em 28 dias como referência e SDNN como o parâmetro de teste(110). Então, o SDNN dicotomizado foi utilizado para a construção das curvas de sobrevivência de Kaplan-Meier, as quais foram comparadas com o teste de log-rank. A influência dos parâmetros da VFC na sobrevida dos pacientes foi estudada através de regressão de Cox. Considerando a amostra pequena deste estudo, foram utilizadas estratégias para evitar o risco de sobre-ajuste e "otimismo" excessivo do modelo(111). A calibração dos modelos com SOFA e com SDNN + SOFA dicotomizado foi avaliada com o teste Grønnesby e Borgan (teste GF). O desempenho desses modelos de predição foi avaliado usando a concordância, o coeficiente de

determinação (R^2) e o critério de informação de Akaike (AIC). Para avaliar a reclassificação do modelo SOFA+SDNN comparado ao modelo com SOFA apenas, fixamos o tempo de 28 dias para as predições dos riscos, e calculamos a melhoria integrada da discriminação (IDI) e a melhoria na reclassificação global ou Net Reclassification Improvement (NRI). O valor de $p < 0,05$ foi considerado estatisticamente significativo para todas as análises.

Todas as análises estatísticas foram realizadas através do SPSS versão 23 (SPSS Inc., Chicago, IL, EUA) e R versão 3.3.0 (The R Foundation for Statistical Computing)(109) usando os pacotes rms, survMisc e survIDINRI.

3.2 – Revisão Sistemática

Realizamos revisão sistemática sobre VFC como preditor de mortalidade na sepse. Seguindo a recomentados do PRISMA(112) e de guideline específico para revisão sistemática e metanálise de estudos não-randomizados(113), utilizamos três métodos bibliográficos para identificar potenciais resumos de artigos: busca remota em bases de dados eletrônica; avaliação de citações bibliográficas através de busca manual de textos; e contato por e-mail com autores. A busca foi realizada em três bases de dados: PubMed, LILACS e Cochrane. Três revisores participaram da busca e da seleção dos estudos. Na base PubMed, os revisores independentes foram Fábio Morato de Castilho (FMC), Marcos Roberto de Sousa (MRS) e Guilherme Barros (GB). Dois revisores independentes (FMC e GB) realizaram a busca e a seleção dos estudos nas bases de dados, enquanto MRS resolveu as divergências. Artigos adicionais foram pesquisados através de citações dos artigos selecionados, e através de busca e outros artigos dos mesmos autores dos artigos selecionados. Os artigos selecionados foram lidos na íntegra para confirmar a elegibilidade. Os critérios de inclusão foram definidos antes do início da busca. Esta revisão sistemática foi registrada no PROSPERO (Registro prospectivo internacional de revisões sistemáticas) sob o número de registro CRD42017062367. Nós incluímos estudos contendo mais de 10 pacientes que avaliaram a VFC como preditor de mortalidade na sepse, publicados, em inglês, português ou espanhol, antes de 20 de agosto de 2017. Os descritores utilizados na busca foram: "Systemic

Inflammatory Response Syndrome"(Mesh), "Systemic Inflammatory Response Syndrome"(All Fields), "Sepsis"(Mesh), "sepsis"(All Fields), "heart rate"(MeSH Terms), "heart rate"(Text Word), "variability"(Text Word), "turbulence" (All Fields), "Nonlinear Dynamics"(Mesh), "Entropy"(Mesh), “triangular index”, "incidence"(MeSH), "mortality"(MeSH), "follow-up studies"(MeSH), "prognos"(Text Word), "predict"(Text Word), "course"(Text Word). Além da seleção de termos textuais e MeSH, foi realizada pesquisa manual das referências de cada documento, e também foi utilizada a ferramenta do PubMed chamada "related citations", para aumentar a sensibilidade da pesquisa.

Dois pesquisadores (MRS e FMC) verificaram, independentemente, a extração de dados de cada estudo. As discrepâncias foram resolvidas por consenso após discussão. Foram extraídas as seguintes informações: desenho do estudo e dados metodológicos; características demográficas e clínicas dos pacientes; número de pacientes que morreram e valores de cada parâmetro da VFC nos grupos sobreviventes e não-sobreviventes.

A Escala Newcastle-Ottawa(114) foi utilizada para avaliar a qualidade dos estudos não randomizados. Usando esta escala, cada estudo incluído foi avaliado em três grandes aspectos: a seleção dos grupos do estudo; a comparabilidade dos grupos; e a verificação do resultado de interesse, conforme recomendado pelo Cochrane Non-Randomized Studies Methods Working Group Version 5.1.0 (113, 115).

Para analisar os resultados, dividimos os artigos entre aqueles que mediram VFC através de gravações de curto prazo (≤ 1 hora) e aqueles que fizeram gravações de longo prazo (≥ 24 horas), uma vez que sabemos que a gravação de longo prazo representa componentes oscilatórios diferentes em relação à gravação de curta duração(60).

Um dos estudos incluídos continha a mediana de SDNN para os grupos sobreviventes e não sobreviventes, mas não continha o valor p da comparação entre os grupos. Assim, estimamos a média e o desvio padrão de cada grupo com base na mediana e valor mínimo e máximo do SDNN da amostra, de acordo com o método elaborado por S.P. Hozo, B. Djulbegovic e I. Hozo(116). A média do SDNN de sobreviventes e não sobreviventes foi comparada através do teste t de Student, utilizando o SPSS versão 23 (SPSS Inc., Chicago, IL, EUA).

4 – ARTIGO 1 - Heart rate variability as predictor of mortality in sepsis: a prospective cohort study (publicado na revista PLOS ONE)

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Abstract

Background: Sepsis is a serious medical condition with increasing prevalence and high mortality. The role of the autonomic nervous system in pathophysiology of sepsis has been increasingly researched. The objective of this study is to evaluate the Heart rate variability (HRV) as a predictor of mortality in septic patients.

Methods: This was a prospective cohort of patients diagnosed with sepsis. Patient recruitment was carried out at ICU in tertiary university hospital between March 2012 and February 2014. Clinical data and laboratory exams were collected at admission. Each patient underwent a 20-minute Holter and a 24-hour Holter on the first day of enrollment. The primary outcome was the 28-day all-cause mortality.

Results: A total of 63 patients were included. Patients were categorized into nonsurvivor group (n=16) or survivor group (n=47) depending on this endpoint. Survivors were younger (48.6 years vs. 63.0 years), had better renal function and lower values in severity scores (APACHE II and SOFA) compared to nonsurvivors. In the 20-minute Holter, SDNN, Total Power, VLF Power, LF Power and LF/HF of nonsurvivors were significantly lower than those of survivors (p=<0.001, p=0.003, p=0.002, p=0.006, p=0.009 respectively). ROC curve of SDNN was built, showing area under the curve of 0.772 (0.638-0.906) for mortality. The value of 17ms was chosen as best SDNN cutoff to discriminate survivors and nonsurvivors. In the Cox proportional regression, adjusted for SOFA score and for APACHE II, a SDNN ≤ 17ms was associated with a greater risk of death, with hazard ratios of 6.3 (1.4 – 28.0; p = 0.015) and 5.5 (1,2 – 24,8; p= 0.027), respectively. The addition of the dichotomized SDNN to the SOFA model reduced AIC and increased the concordance statistic and the R^2, indicating that predictive power of the SDNN + SOFA model can be better than predictive power of SOFA only.

Conclusions: Several HRV parameters are reduced in nonsurviving septic patients. Although further studies are necessary to confirm this finding, SDNN ≤17 is suggested as an independent risk factor for death in septic patients.

Introduction

Sepsis is a serious medical condition which prevalence has increased significantly in recent decades(1), making 31.5 million new cases to be expected in hospitals around the world each year(2). Due to the high mortality associated with this condition, which can reach 48.6%(3), it is essential to search risk factors for death and predictive scoring systems to help clinical decision in septic patients. Predictive scoring systems such as APACHE II (Acute Physiology and Chronic Health disease Classification System II), SOFA (Sepsis-related Organ Failure Assessment), SAPS-3 (Simplified Acute Physiology Score III) and MODS (Multiple Organ Dysfunction **Score)** combine clinical and laboratory characteristics to assess the severity of illness. However, none of these scores considers in its composition changes in the autonomic nervous modulation caused by sepsis.

Heart rate variability (HRV) is a noninvasive indirect test to evaluate autonomic function(4, 5). In normal situations, heart rate varies, indicating the heart's capacity to adapt to different situations. HRV measures the oscillation of the intervals between consecutive heart beats, which are related to, the influences of the autonomic nervous system on the sinus node(6). Patients with sepsis have reduced HRV compared to healthy patients, as demonstrated in small studies(7-9). Furthermore, HRV parameters such as low frequency (LF) power are positively correlated with APACHE II and SOFA(10) and negatively correlated with interleukins(11). Small studies have suggested that sepsis survivors present HRV parameters (e.g., standard deviation of NN interval, SDNN) higher than nonsurvivors(12, 13). However, no study has defined a specific HRV parameter and a cut-off point that can be used in practice for the prediction of the risk of death in septic patients. Thus, the use of HRV as an independent predictor of death in sepsis deserves further investigation**.**

The objective of this study was to evaluate the role of HRV - recorded both with the 20 minute and the 24 hour-Holter - as a predictor of death in patients with severe sepsis, defined by the presence of infection, the Systemic Inflammatory Response Syndrome criteria and evidence of organ dysfunction.

Materials and methods

Study design

This was a prospective cohort of patients diagnosed with severe sepsis. This report follows "Strengthening the Reporting of Observational studies in Epidemiology", the STROBE Statement(14).

Patient population

Patient recruitment was carried out at one of the Intensive Care Units of Hospital das Clínicas of the Universidade Federal de Minas Gerais (ICU-UFMG), Brazil, a mixed ICU with eight beds. From March 10th, 2012 to February 06th, 2014, all adult (i.e., 18 year-old or older) patients, hospitalized in the ICU-UFMG that had suspicion of sepsis at admission or during the ICU stay, and at least one organ dysfunction supposedly related to the infectious condition were considered for potential eligibility. Sepsis was defined according to the Sepsis 2 Consensus(15) as being a Systemic Inflammatory Response Syndrome associated with a confirmed infection or strongly suspected infection. Systemic Inflammatory Response Syndrome was defined as the presence of at least two of the following: 1- Body temperature higher than 38°C or lower than 36°C; 2- Heart rate higher than 90/min, 3- Hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg; 4- White blood cell count higher than 12,000 cells/µl or lower than 4,000/µl or at least 10% of immature forms (16). The presence of at least one organ dysfunction was based on severe sepsis definition of Surviving Sepsis Campaign(17). Despite inclusion phase of this study was conducted prior to publication of the Sepsis 3 definitions(18), all included patients met the criteria for Sepsis proposed in this consensus.

Exclusion criteria were: moribund patients (death previewed for the next 24 hours), patients with proposal for exclusive palliative care, septic patients under antibiotic therapy for more than 48 hours prior to enrollment and patients with non-sinus rhythm or with pacemaker.

Study protocol and general procedures

This study was approved by the Ethics Research Committee of the Universidade Federal de Minas Gerais, Brazil, and all included patients or their family members signed a written informed consent. Clinical data was collected at admission and during the clinical follow-up of patients through a dedicated Clinical Report Form. The main

variables collected were: age, gender, comorbidities, main diagnosis at the time of inclusion, primary site of infection and microbiological findings, antibiotic used, Sepsis related Organ Failure Assessment score (SOFA)(19) and Acute Physiology And Chronic Health Evaluation II (APACHE II)(20), both evaluated at the time of inclusion.

Heart rate variability analysis

Each patient enrolled in the study underwent a 3-channel Holter (Cardios® CardioLight model, São Paulo, Brazil) on the first day of enrollment. Two recordings were made sequentially: 20 minutes record and 24 hours record. Both Holter monitors were placed and removed from the patients by one of the medical researchers. The first measure (20 minutes record) was made with the patient in supine position and no intervention (nursing, physiotherapy, etc.) was made during its recording. The 24-hour measure was made without interference in the normal ICU care routine. Data analysis to derive HRV was performed using system specifically developed for this purpose (Cardios®), which automatically calculates the following indices of HRV in the time domain: Normal-to-Normal (NN) average interval, standard deviation of the NN interval (SDNN), square root of the squared mean of the difference of successive NN-intervals (r-MSSD), percentage of NN intervals deviated by more than 50 ms from adjacent NN-intervals (pNN50); and frequency domain with fast Fourier Transform (FFT) method: Total Power, Very low frequency power (VLF Power), Low frequency Power (LF Power), High frequency power (HF Power) and Ratio between LF and HF (LF/HF) . In the 24-hour Holter, HRV analysis was performed only in the time domain. We have performed manual review of all Holter's automatic interpretation, including the rhythm and the complexes recorded (e.g., normal QRS, ventricular extrasystoles, supraventricular extrasystoles, tachycardia, bradycardia, artifacts etc.). Artifacts and irregular beats (extrasystoles, noise and missing beats) were manually deleted before HRV analyses. In the 24-hour Holter, HRV in the time domain was calculated over an entire 24-hour period. In the 20 minutes Holter, HRV was calculated both in the time domain and in the frequency domain over the entire first 10 minutes of recording.

Outcomes

The primary outcome of this study was the all-cause mortality at 28 days of follow-up. Patients were categorized into nonsurvivor group or survivor group depending on the primary endpoint. Several HRV parameters were compared between these two groups.

Sample size

The sample size calculation tested the hypothesis that SDNN distribution would be the same between surviving and nonsurviving patients. The statistical test used was the nonparametric Mann-Whitney that assumes that the data is measured at least in ordinal scale. The formulas adopted for sample size calculation are described in Zhao, Rahardja, & Qu(21) and implemented in software R(22). A pilot sample constituted by the first twenty patients included in the study (6 deaths and 14 survived) was considered to estimate the parameters required to calculate the final sample size. Tertiles of SDNN were calculated from this pilot sample, defining three ranges. The proportion of subjects in each of these three ranges was obtained. Keeping the allocation ratio (i.e., survivors and non survivors) similar to that observed in the pilot study, a requirement of 58 patients (44 survivors and 14 deaths) was defined for the final analysis. We considered a type I error of 5% and 80% power.

Statistical analysis

The normality of each continuous variable was assessed by means of the Kolmogorov-Smirnov test. Data obtained from continuous variables are expressed as either mean and standard deviation if they have normal distribution, or median and interquartile range (25th and 75th percentiles) if they have non-normal distribution. Data concerning categorical variables are expressed as absolute numbers and proportions. Clinical characteristics of survivors and nonsurvivors were compared using Student *t* test, Mann–Whitney test and exact Fisher test according to the type and the distribution of the variable. In order to choose the best cut-off point of SDNN, a ROC curve was used having death as the reference and SDNN as the parameter test(23). Then the dichotomized SDNN was used to build Kaplan–Meier survival curves, and they were compared by log-rank test. The influence of HRV variables on survival was studied with Cox regression. Considering the small sample, we used modeling strategies to avoid the risk of overfitting and the excessive “optimism” of the model (24). The calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the Grønnesby and Borgan test (GF Test). The performance of those prediction models was assessed using concordance measure, Explained variation (R^2) and Akaike Information Criterion (AIC). In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we set the time of 28 days for the predictions of the risks, and calculated continuous net reclassification

improvement (NRI) and integrated discrimination improvement (IDI). A $p < 0.05$ was considered statistically significant for all analyses.

All the statistical analyses were conducted in SPSS version 23 (SPSS Inc., Chicago, IL, USA) and R version 3.3.0 (The R Foundation for Statistical Computing)(22) using the packages *rms, survMisc and survIDINRI*.

Results

From a total of 99 patients with sepsis assessed during the study period, 79 patients were initially identified as eligible. Of these, two patients were excluded because of technical problems with the Holter equipment and 14 patients were excluded due to atrial fibrillation. Thus, 63 patients were included in the final analyses (Fig 1).

The baseline characteristics of the included patients are shown in Table 1, stratified according to the 28-day all-cause mortality. As presented, 16 (25.4%) out of the 63 patients died during the follow-up of 28 days. Survivors were younger (48.6 years vs. 63.0 years), had better renal function and lower values in severity scores (APACHE II and SOFA) compared to nonsurvivors. There were no significant differences in other baseline characteristics.

HRV measures of each group are listed in Table 2. In 20-minute Holter, SDNN, Total Power, VHF Power, LF Power and LF/HF of non-survivors were significantly lower than those of survivors. There was no statistically significant difference in HRV measured in the 24 hours Holter between the two subgroups.

An unadjusted Cox regression for HRV parameters that were different between the two groups was built. It can be seen in Table 3. Since SDNN reached the larger difference between survivors and nonsurvivors, ROC curve was built to evaluate the accuracy of this parameter to predict the 28-day all-cause-mortality; as depicted in Fig 2, an area under the curve of 0.772 (0.638-0.906) was found. Then, because it presents the best relationship between sensitivity and specificity, 17ms was chosen as the cutoff point for SDNN. In order to test the possible clinical application of this cut-off point as a predictor of mortality in sepsis, patients were divided into two groups (SDNN> 17ms and SDNN≤17ms). As can be seen in Table 4, there is no significant difference between the baseline features of these two groups, except for the value of C-reactive protein, which was higher in the SDNN≤17 group. Kaplan-Meier curve of these two groups (Fig

2) found log rank p = 0.003, showing higher mortality of the patient group with SDNN≤17ms. For the analysis of 28 days mortality, Cox regression for this dichotomous variable was made adjusted by the SOFA showing HR 6.3 (1.4 – 28.0; p = 0.015) for SDNN≤17ms and HR 1.3 (1.1 – 1.4; p = 0.001) for SOFA. Following a similar trend, Cox regression for dichotomous SDNN adjusted by the APACHE II showed HR 5.5 (1.2 – 24.8; p= 0.027) for SDNN≤17ms and HR 1.1 (1.02 – 1.12; p = 0.004) for APACHE II.

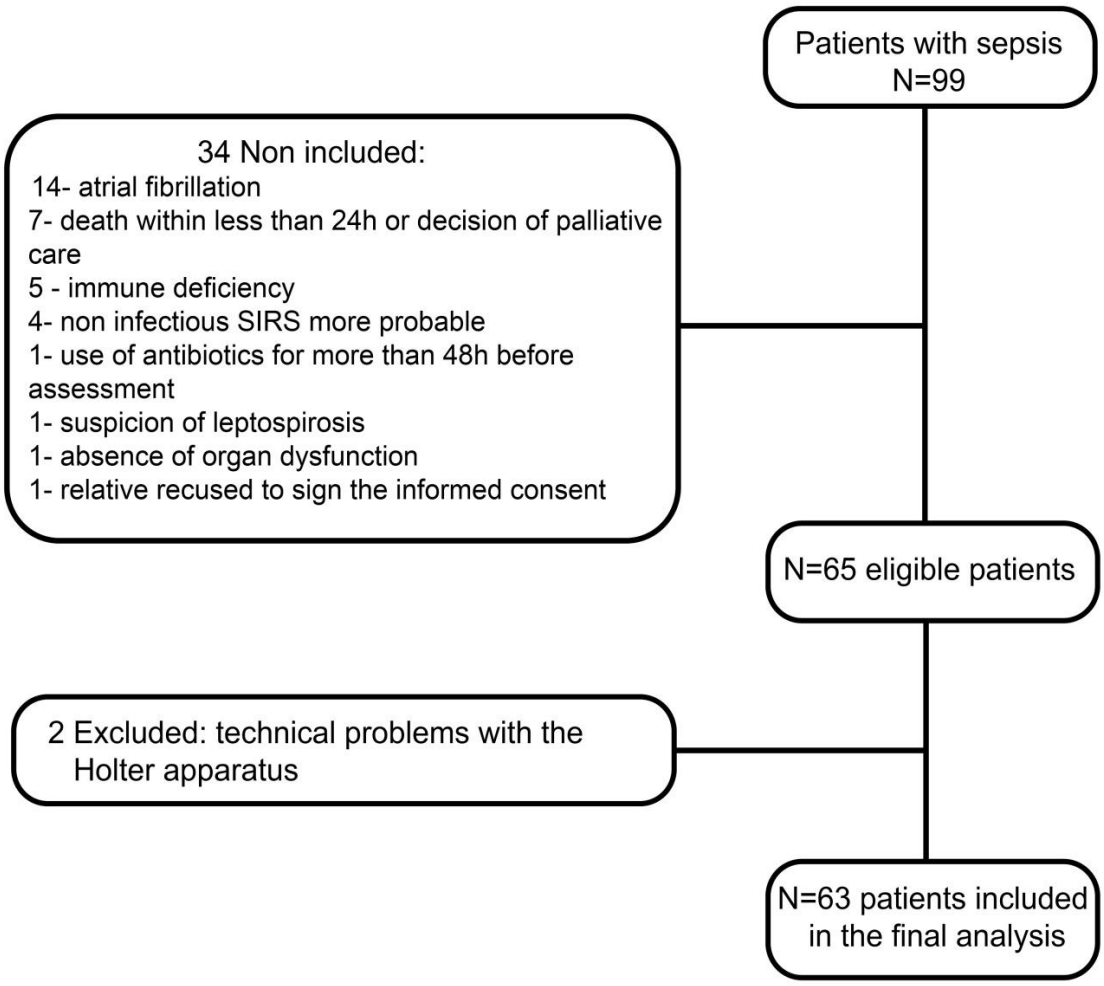

Fig 1. Flowchart of study procedures

Table 1: Baseline characteristics of the patients

Attribute	**Survivors (n=47)**	**Nonsurvivors (n=16)**	**p-Value**
Age (y), SD	49 (17.8)	63 (17.9)	0.007
Male gender, %	27 (57.4)	11 (68.8)	0.425
APACHE II, SD	14.15 (5.93)	21.94 (8.45)	<0.001
SOFA, SD	6.91 (2.84)	10.56 (4.21)	0.004
Mechanical Ventilation, %	24 (51.1)	12 (75.0)	0.095
Underlying disease, n (%)			
Cirrhosis	2 (4.3)	1 (6.2)	0.896
Dialytic patients	4 (8.5)	1 (6.2)	0.773
Hypertension	18 (38.3)	9 (56.2)	0.369
Diabetes	11 (23.4)	4 (25.0)	0.354
Stroke	7 (14.9)	2 (12.5)	0.793
Peripheral arterial disease	1 (2.1)	0 (0.0)	0.801
Heart Failure[b]	4 (8.5)	3 (18.8)	0.459
Coronary artery disease	4 (8.5)	3 (18.8)	0.288
Neoplasia	4 (8.5)	2 (12.5)	0.541
Chronic Obstructive Pulmonary Disease	3 (6.4)	0 (0.0)	0.453
Smoking	13 (27.7)	2 (12.5)	0.112
Laboratory data			
Hemoglobin (g/dL)	10.0 (1.86)	10.4 (3.15)	0.582
White blood cells (per mm3)	16171 (9653)	17929 (10005)	0.535
Platelet × 10^3	228 (121)	187 (116)	0.240
Lactate[a] (mmol/L)	1.80 (1.6 - 4.0)	2.55 (1.6 - 4.2)	0.059
C-reactive protein (mg/L)	229 (115)	287 (109)	0.082
Urea (mg/dL)	55.4 (34.4)	107.3 (47.4)	<0.001
Creatinine[a] (mg/dL)	0.72 (0.49 - 1.6)	2.28 (0.96 - 2.85)	0.004
Creatinine clearance[a] (mL/min)	108 (58 - 157)	29 (18 - 86)	0.005
Glucose (mg/dL)	144 (56)	171 (99)	0.176
International normalized ratio	1.2 (1.1 - 1.4)	1.4 (1.2 - 2.0)	0.026
Infection source, n (%)			
Respiratory tract	16 (34.0)	7 (43.8)	0.687
Intra-abdominal	8 (17.0)	4 (25.0)	0.737
Urinary tract	5 (10.6)	1 (6.3)	0.990
Catheter	7 (14.9)	2 (12.5)	0.860
Soft tissue	3 (6.4)	1 (6.3)	0.563
Central Nervous System	0 (0.0)	1 (6.3)	0.561
Undetermined	7 (14.9)	0 (0.0)	0.239
Miscellaneous	1 (2.1)	0 (0.0)	0.561

Footnote: Data presented as mean (SD), median (interquartile range) or absolute number (percentage). [a] = variables with non-normal distribution; [b] = Heart Failure was defined as previous echocardiogram with ejection fraction ≤ 50%.

Considering the small sample, we used modeling strategies to avoid the risk of overfitting and the excessive "optimism". For the model with SOFA and dichotomous SDNN, optimism was calculated at 0.1075 (and the shrinkage factor was 0.8925), calculated HR 5.2 (1.2 – 23.0) for SDNN ≤17, with p = 0.03. For the model with APACHEII and dichotomous SDNN, optimism was calculated at 0.1834 (and the shrinkage factor was 0.8166), calculated HR 4.0 (0.9 – 18.1) for SDNN ≤17, with p = 0.07.

Table 2: Heart rate variability measures

Parameter	**Survivors (n=47)**	**Nonsurvivors (n=16)**	**p-Value**
20 Minutes Holter			
Artifacts and irregular beats[a] (%)	2.0 (1.0 – 5.3)	2.5 (0.3 – 8.0)	0.112
Day recordings[a,b], %	37 (78.7)	13 (81.3)	0.829
NN (ms)	658.2 (166.9)	606.0 (130.4)	0.261
SDNN (ms)[a]	19.0 (10.0 - 36.0)	8.5 (5.0 - 14.5)	<0.001
rMSSD (ms)[a]	9.0 (6.0 - 28.0)	7.5 (6.0 - 12.8)	0.199
pNN50 (%)[a]	0.13 (0.00 - 4.73)	0.14 (0.00 - 0.63)	0.482
Total Power (ms^2)[a]	136.0 (46.0 - 590.0)	24.0 (5.0 - 173.5)	0.003
VLF Power (ms^2)[a]	90.0 (27.0 - 243.0)	9.5 (2.5 - 72.5)	0.002
LF Power (ms^2)[a]	18.0 (6.0 - 83.0)	2.0 (1.0 - 24.0)	0.006
HF Power (ms^2)[a]	9.0 (5.0 - 51.0)	6.5 (2.3 – 57.0)	0.343
LF/HF[a]	1.29 (0.47 - 3.63)	0.40 (0.21 - 1.84)	0.009
24-Hour Holter			
Artifacts and irregular beats[a] **(%)**	1.0 (1.0 – 1.0)	1.0 (1.0 – 2.3)	0.955
NN (ms)	661.0 (133.4)	622.9 (123.5)	0.345
SDNN (ms)	58.2 (39.4)	50.7 (24.5)	0.402
rMSSD (ms)[a]	14.0 (8.0 - 28.3)	15.5 (10.0 - 29.3)	0,944
pNN50 (%)[a]	0.55 (0.05 - 3.11)	0.66 (0.24 - 2.78)	0,688

Footnote: Data presented as mean (SD), median (interquartile range) [a] = variables with non-normal distribution; [b] = Day recordings was considered when the Holter monitor was placed between 8:00 a.m. and 6:00 p.m; NN = Normal-to-Normal; SDNN = standard deviation of the NN interval; rMSSD = Root Mean Square of the Successive Differences; pNN50 = proportion of adjacent NN intervals which differ by more than 50 ms; VLF Power = Very Low Frequency Power**;** LF Power = Low Frequency Power; HF Power = High Frequency Power; LF/HF = Low Frequency Power/ High Frequency Power

Finally, the calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the GF Test, showing, for the model with SOFA, p=0.550, and, for the model with SOFA + SDNN, p=0.600, indicating that there are no calibration problems. The GF test was valid under the usual assumption of proportional hazards of the Cox model. This assumption was not violated in the models considered,

since the global risk proportionality test found p=0.463 for the SOFA model only and p=0.633 for the model With SOFA + SDNN. The addition of the dichotomized SDNN to the SOFA model increased the concordance statistic from 0.725 to 0.805 and the R^2 of the model changed from 0.167 to 0.277. Furthermore, the AIC for the first model [SOFA] was 119.07 versus 112.17 for the second [SDNN + SOFA]. Greater values for concordance and R^2 indicate a better model while smaller values for AIC indicate a better model. In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we set the time of 28 days for the predictions of the risks, and calculated IDI (0.122; CI 0.043 – 0.235, p=0.00) and NRI (0.408; CI 0.168 – 0.643, p=0.01). These results suggest significant gains in the reclassification with the inclusion of SDNN in the model. All statistical analysis with the dichotomous SDNN can be seen in the Table 5.

In the survivor group, seven patients had undetermined infection source, while zero patients had undetermined infection source among the non-survivors. The results regarding the association of SDNN values and the outcome remained unchanged in the analysis excluding these seven patients. Thus, SDNN value was significantly higher among survivors as compared to non-survivors, when evaluated in the 20-minute Holter: 18.50 (10.00 – 34.50) and 8.50 (5.00 – 14.50), respectively, with p=0.003. Cox regression for dichotomous SDNN adjusted by the SOFA or APACHE II revealed similar results (HR 7.1 [1.6 – 32.8]; p = 0.012, for SDNN≤17ms and HR 1.3 [1.1 – 1.5]; p < 0.001, for SOFA. Following a similar trend, Cox regression for dichotomous SDNN, adjusted by the APACHE II, showed HR 5.1 (1.1 – 22.9; p= 0.033) for SDNN≤17ms, and HR 1.1 (1.03 – 1.12; p = 0.001) for APACHE II.

Discussion

In this prospective study with septic patients, we found that several HRV parameters obtained in the 20-minute Holter were correlated to 28-day all-cause mortality. In particular, SDNN ≤17 is associated with increased risk of death even after adjustment to SOFA or APACHE II. In contrast, HRV parameters in 24-hour Holter were not correlated to 28-day all-cause mortality.

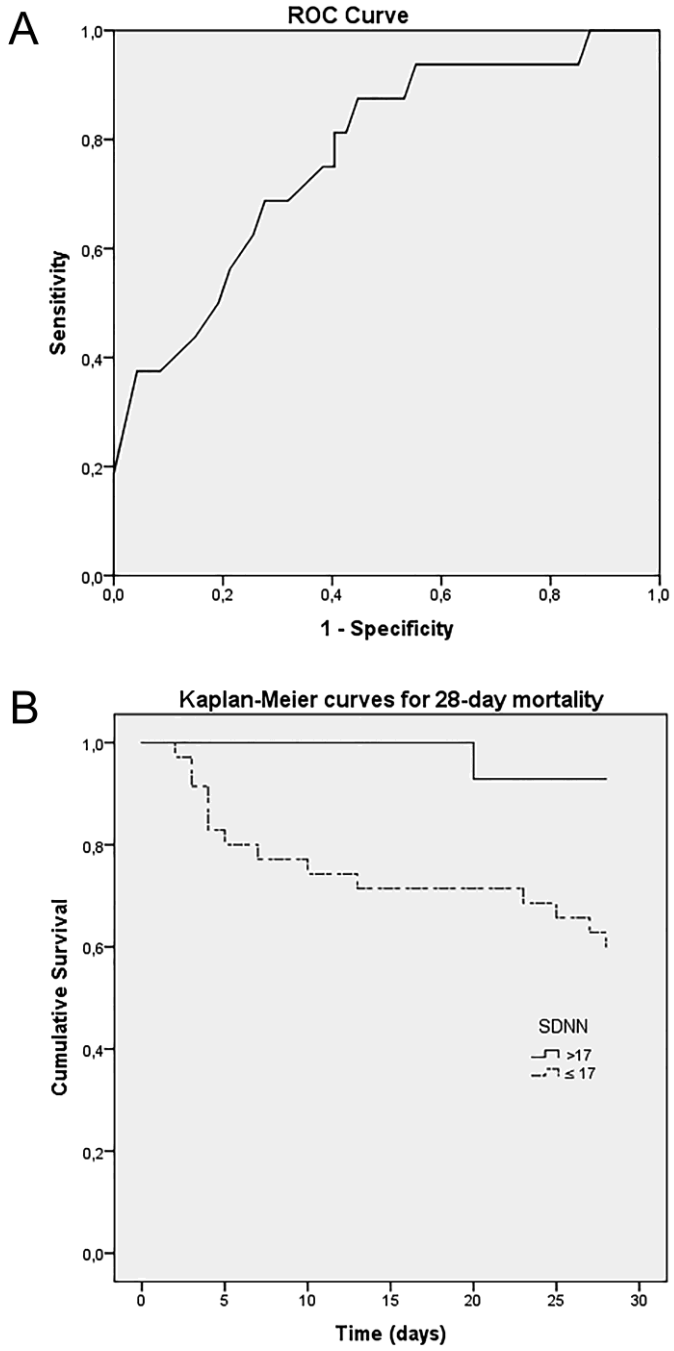

Fig 2. ROC Curve of SDNN and Kaplan-Meier curves for 28-day mortality. A: The ROC Curve of SDNN in 20-minute Holter in predicting 28-day mortality in patients with sepsis. The area under the curve was 0.772 (0.638-0.906). The value of 17ms was chosen as the cutoff point for SDNN (sensibility of 87.5%, specificity of 55.3%, positive likelihood ratio of 1.96 and negative likelihood ratio of 0.28). B: Kaplan-Meier curve showing 28-day mortality in septic patients with SDNN≤17ms (mean survival time of 21.3 days; 17.8 – 24.8) and SDNN>17ms **(**mean survival time of 27.4 days; 26.6 – 28.2). The survival curves were compared using log-rank test, p=0.003, showing higher mortality in the patient group with SDNN≤17ms.

Table 3: Cox regression for heart rate variability parameters in 20-minute Holter

Parameter	**HR**	**95% CI**	**p-Value**
SDNN (ms)	0.937	0.883 – 0.995	0.033
Total Power (ms^2)	0.999	0.997 – 1.001	0.273
VLF Power (ms^2)	0.998	0.996 – 1.001	0.269
LF Power (ms^2)	0.998	0.993 – 1.003	0.352
LF/HF	0.619	0.380 – 1.009	0.054

Footnote: HR = Hazard ratio; SDNN = standard deviation of the NN interval; VLF Power = Very Low Frequency **Power;** LF Power = Low Frequency Power; LF/HF = Low Frequency Power/ High Frequency Power

Table 4: Baseline characteristics of groups of SDNN≤17ms and SDNN>17ms

Attribute	**SDNN≤17 (n= 35)**	**SDNN>17 (n=28)**	**p-Value**
Age (y), SD	55 (20)	49 (17)	0.288
Male gender, %	22 (62.9)	16 (57.1)	0.796
APACHE II, SD	17.4 (8.16)	14.54 (6.16)	0.129
SOFA, SD	8.31 (3.68)	7.25 (3.43)	0.244
Mechanical Ventilation, %	22 (62.9)	14 (50.0)	0.306
Underlying disease, n (%)			
Cirrhosis	0 (0.0)	3 (10.7)	0.183
Dialytic patients	4 (11.4)	1 (3.6)	0.371
Hypertension	16 (45.7)	11 (39.3)	0.337
Diabetes	10 (28.6)	5 (17.9)	0.380
Stroke	4 (11.4)	5 (17.9)	0.192
Peripheral arterial disease	1 (2.86)	0 (0.0)	1.00
Heart Failure[b]	4 (11.4)	3 (10.7)	0.660
Coronary artery disease	6 (17.1)	1 (3.6)	0.234
Neoplasia	2 (5.7)	4 (14.3)	0.350
Chronic Obstructive Pulmonary Disease	2 (5.7)	1 (3.6)	0.899
Smoking	6 (17.1)	9 (32.1)	0.388
Laboratory data			
HB (g/dL)	10.3 (2.6)	9.9 (1.6)	0.528
White blood cells (per mm3)	14882 (7918)	18787 (11314)	0.113
Platelet × 103	205 (112)	233 (116)	0.365
Lactate[a] (mmol/L)	1.70 (1.2 - 2.8)	2.0 (1.15 - 2.83)	0.787
C-reactive protein (mg/L)	276 (100)	204 (124)	0.013
Urea (mg/dL)	73.1 (40.7)	63.0 (48.1)	0.382
Creatinine[a] (mg/dL)	1.27 (0.67 - 2.55)	0.71 (0.49 - 1.68)	0.223
Creatinine clearance[a] (mL/min)	77 (28 - 125)	106 (51 - 154)	0.307
Glucose (mg/dL)	158 (78)	141 (57)	0.322
International normalized ratio[a]	1.24 (1.10 - 1.43)	1.29 (1.13 - 1.59)	0.302

Footnote: Data presented as mean (SD), median (interquartile range) or absolute number (percentage). [a] = variables with non-normal distribution; [b] = Heart Failure was defined as previous echocardiogram with ejection fraction ≤ 50%.

Table 5: statistical analysis with the dichotomous SDNN

COX regression adjusted by SOFA			
Variable	**HR**	**CI**	**p-Value**
SDNN≤17ms	6.3	1.4 – 28.0	0.015
SOFA	1.3	1.1 – 1.4	0.001
COX regression adjusted by APACHE II			
Variable	**HR**	**CI**	**p-Value**
SDNN≤17ms	5.5	1.2 – 24.8	0.027
APACHE II	1.1	1.02 – 1.12	0.004
Model with dichotomous SDNN and SOFA optimism adjusted			
Variable	**HR**	**CI**	**p-Value**
SDNN≤17ms	5.2	1.2 – 23.0	0.03
Model with dichotomous SDNN and APACHE II optimism adjusted			
Variable	**HR**	**CI**	**p-Value**
SDNN≤17ms	4.0	0.9 – 18.1	0.07
Models of mortality prediction			
Model	**GF Test**	**Concordance**	**R^2**	**AIC**
SOFA	p=0.550	0.725	0.167	119.07
SOFA + SDNN≤17ms	p=0.600	0.805	0.277	112.17

Footnote: HR = Hazard Ratio; CI = Confidence Interval; R^2 = Explained variation; AIC = Akaike Information Criterion. For the analysis of 28 days mortality, Cox regression for dichotomous SDNN was made adjusted by the SOFA and adjusted by APACHE II. For the model with SOFA and dichotomous SDNN, optimism was calculated at 0.1075 (and the shrinkage factor was 0.8925). For the model with APACHEII and dichotomous SDNN, optimism was calculated at 0.1834 (and the shrinkage factor was 0.8166). The calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the GF Test, indicating that there are no calibration problems. The performance of those prediction models was assessed. Greater values for concordance and R^2 indicate a better model while smaller values for AIC indicate a better model. In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we calculated IDI (0.122; CI 0.043 – 0.235, $p<0.01$) and NRI (0.408; CI 0.168 – 0.643, $p=0.01$). These results suggest significant gains in the reclassification with the inclusion of SDNN in the model.

Normal immune and physiologic responses eradicate pathogens through complex process involving generation of proinflammatory and anti-inflammatory mediators. The pathophysiology of sepsis is due to the inappropriate regulation of these normal reactions that becomes generalized and deleterious(25). The role of the autonomic nervous system has been increasingly studied in the context of sepsis. Animal model studies suggest that vagus nerve stimulation increases the secretion of corticotropin-releasing hormone (CRH), ACTH, and cortisol(26). Likewise, vagotomy attenuated fever response and corticosterone response produced by cytokines(27). Acetylcholine,

the principle vagal neurotransmitter, has an anti-inflammatory effect, attenuating the release of cytokines TNF, IL-1beta, IL-6 and IL-18 and preventing the development of shock(28). Treatment with nicotine, a selective cholinergic agonist, and with choline, a precursor in the biosynthesis of acetylcholine, improved survival in experimental models of sepsis(29, 30). This results supports that vagal afferent pathway are involved in peripheral cytokine-to-brain communication.

Several methods have already been developed to evaluate the autonomic function. Some of the tests would not be adequate for this study because they require active participation of patients. This is the case of the Valsalva's manoeuvre, the deep breathing method, the isometric handgrip test, the mental arithmetic, and the active standing methods (31). Other methods require infusion of drugs (e.g. baroreflex sensitivity testing with intravenous administration of phenylephrine), which could interfere in the treatment of patients with sepsis, making its use unfeasible and potentially harmful(31). The serum catecholamines dosage can be used to evaluate the autonomic nervous system; however it has some limitations, providing information about the global autonomic function and not about organ-specific sympathetic function(32). Additionally, the plasma concentration of norepinephrine, for example, depends not only on sympathetic activity, but also on norepinephrine reuptake and noradrenaline clearance from circulation (33). Finally, patients with septic shock often receive external noradrenaline infusion as treatment. HRV is one of the most popular methods used to evaluate the autonomic function, presenting the advantages of being non-invasive and the fact that there are many commercial devices that provide the automated measurement of HRV(4).

The mechanism by which HRV is reduced in septic patients is not yet fully understood. In addition to the participation of the autonomic nervous system, recent studies in animals and cell cultures have shown that Lipopolysaccharides (amphiphilic components of the outer wall of Gram-negative bacteria) act in two ways on the hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN) of the atrial cells: directly inhibiting HCN-channels and indirectly sensitizing HCN-channels for sympathetic activation(34, 35).

Although there are no reference ranges of HRV parameters globally accepted, this study suggests that septic patients have reduced HRV compared to the general population. For example, in this study, the SDNN mean for surviving patients were 19.0ms and for

nunsurviving patients were 8.5ms, while Kim et al found SDNN mean of 39.6ms for normal Korean Population(36).

The physiological meaning of each HRV parameter is very complex and not fully known. SDNN reflects all the cyclic components responsible for HRV (including sympathetic and parasympathetic activity) and is the most commonly used parameter(4). HF Power reflects the vagal activity (parasympathetic) on the sinus node(37). LF Power reflects the sympathetic and parasympathetic activity, with alleged predominance of the first (38).The LF/HF ratio, in HRV, was classically described as an index of the sympathetic/parasympathetic balance(38). However, several studies have shown that this interpretation is imprecise and simplistic and that the physiological meaning of this ratio remains controversial(39). A reduced LF/HF ratio is associated with an increased risk of death in septic patients(40). In this study, nonsurviving patients had lower Total Power, VLF Power, LF Power and LF / HF ratio than survivors. This finding is similar to that found in previous research(10, 12, 41).

Unlike the study by Duke et al.(13), in ours, HRV parameters were significantly different between survivors and nonsurvivors only in the 20-minute Holter. Holter with shorter periods of record is potentially more useful for be used in critical care patients, including those with sepsis, because these patients present immediate risk of death and therefore need a fast tool for definition of severity. Moreover, in such a dynamic condition as sepsis, a long time recording may suffer interference from therapeutic measures instituted, which can partially explain the negative results found with the 24-hour Holter in this study.

Global HRV parameters such as SDNN and TP were lower in nonsurviving patients of this study, which is consistent with the findings of previous studies(12, 13). Chen et al(12) had demonstrated that SDNN would be a significant independent variable in the prediction of in-hospital mortality for emergency department patients with sepsis, although these authors did not present cut-off point for this HRV parameter. The cut-off of 17ms for SDNN obtained in a short time Holter record found in our study of might represent a useful tool due to identify patients with higher risk of death among septic patients in the daily practice. It worth mentioning that this result was maintained after adjustment for APACHE II or SOFA, indicating that this value could be an independent risk factor for mortality. Although the results found in the present study are statistically significant, the fact that the confidence intervals on the hazard ratio for SDNN are large

reflect the small number of patients in our study, which indicates the need of confirming these results in larger series of septic patients.

Furthermore, concordance measure, R^2, AIC, IDI and NRIindicate that predictive power of the SDNN + SOFA model is better than predictive power of SOFA only, which reinforces the possible clinical utility of this measure.

Study limitations

The small number of patients is the main limitation of this study. In order to minimize this problem, it was used advanced modeling techniques to avoid the risk of overfitting and also to adjust the coefficients for optimism. This analysis kept SDNN $\leq$17 as a risk factor for death for the model with SOFA but not for the model with APACHE II. Another limitation of this study is the possible influence of other clinical conditions known to affect HRV as congestive heart failure, coronary artery disease, diabetes or mechanical ventilation use(4). However, there was no difference between nonsurvivor and survivor groups about the frequency of these comorbidities (Table 1). Body temperature and medications (e.g., sedatives, beta-blockers, inotropic drugs) that can affect HRV were not evaluated in this study. Day-night variation in heart rate variability was not considered in the design of this study, although its existence has already been demonstrated in healthy volunteers with endotoxaemia(42). The majority of 20-minute Holter measures were made during the day, and there were no significant differences between the percentage of day recordings from surviving and non-surviving groups. Furthermore, we do not know whether this day-night difference occurs in ICU patients. Finally, all included patients were enrolled before the publication of the Sepsis 3 Consensus(18), reason for which we were not able to use the new definitions of sepsis in the present study. Despite this, all patients included in this study had a SOFA score $\geq$ 2 points and met criteria for Sepsis based on this new consensus in a post hoc analysis.

Considering the small number of patients in this single-center study, we believe that the results found here are preliminary, hinting at the potential predictive capability of a dichotomized SDNN, what should be confirmed in future studies through external validation of the results in a separate population.

Conclusions

Several HRV parameters are reduced in nonsurviving septic patients. Although further studies are necessary to confirm this finding, SDNN ≤17 is suggested as an independent risk factor for death in septic patients.

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5 – ARTIGO 2 - Heart rate variability as predictor of mortality in sepsis: a systematic review

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Abstract

Background: Autonomic dysregulation is one of the recognized pathophysiological mechanisms in sepsis, generating the hypothesis that heart rate variability (HRV) can be used to predict mortality in sepsis.

Methods: This was a systematic review of studies evaluating HRV as a predictor of death in patients with sepsis. The search was performed by independent researchers in PubMed, LILACS and Cochrane, including papers in English, Portuguese or Spanish, indexed until August 20th, 2017 with at least 10 patients. Study quality was assessed by Newcastle-Ottawa Scale. To analyze the results, we divided the articles between those who measured HRV for short-term recordings (≤ 1 hour), and those who did long-term recordings (≥ 24 hours).

Results: Nine studies were included with a total of 536 patients. All of them were observational studies. Studies quality varied from 4 to 7 stars in Newcastle-Ottawa Scale. The mortality rate in the studies ranged from 8 to 61%. Seven studies performed HRV analysis in short-term recordings. With the exception of one study that did not explain which group had the lowest results, all other studies showed reduction of several HRV parameters in the non-survivors in relation to the surviving septic patients. SDNN, TP, VLF, LF, LF/H, nLF, α1/α2 and r-MSSD of the non-survivor group were reduced in relation to the survivors in at least one study. Two studies found that SDNN is associated with mortality in sepsis, even after adjusting for possible confounding factors. Three studies performed HRV analysis using long-term recordings. Only one of these studies found difference between surviving and non-surviving groups, and even so, in only one HRV parameter: LogHF.

Conclusions: Several HRV parameters are reduced in nonsurviving septic patients in short-term recording. Two studies have found that SDNN is associated with mortality in sepsis, even after adjusting for possible confounding factors.

Introduction

Sepsis, a syndrome in which there is dysregulated host response to infection and presence of organ dysfunction(1), has a high mortality rate that can vary between 10 and 50%(1, 2). In addition to high lethality, its incidence has increased significantly in recent decades, making sepsis a serious global health problem (3). For all these reasons, it is useful to identify the most serious septic patients through predictive scoring systems. Although autonomic dysregulation is one of the recognized pathophysiological mechanisms in sepsis(4), existing predictive scoring systems such as APACHE II (Acute Physiology and Chronic Health disease Classification System II)(5), SOFA (Sepsis-related Organ Failure Assessment)(6), SAPS-3 (Simplified Acute Physiology Score III)(7, 8) and MODS (Multiple Organ Dysfunction Score)(9) do not considers in their composition changes in the autonomic nervous modulation.

Physiological variation of heart rate indicates heart's capacity to adapt to different situations, and is influenced, among other factors, by the autonomic nervous system (10). Heart rate variability (HRV) measures oscillations of the intervals between consecutive heart beats, being therefore a noninvasive indirect test to evaluate autonomic function (11). Studies have shown that patients with sepsis have reduced HRV compared to healthy patients(12, 13). Ahmad et al. demonstrated, in a small study, that patients with sepsis showed a significant drop in the value of several HRV parameters on average 35 hours before the diagnosis of sepsis(14). These findings raised the possibility that the HRV can be used to predict the risk of developing sepsis or even for the diagnosis of sepsis. In addition to the diagnosis of sepsis, HRV parameter reduction seems to be related to worse outcomes in septic patients, and has a correlation with APACHE II and SOFA(15). Finally, some studies have shown that HRV can be used to predict the risk of septic patients develop septic shock (16) and multiple organ dysfunction(17). Recently, our research group published the results of a cohort study with septic patients in which several parameters of HRV were reduced in those patients who died in comparison to their counterparts. (18).

The objective of this study was to perform a systematic review of studies evaluating HRV as a predictor of death in patients with sepsis.

Materials and methods

Following the PRISMA statement (19) for systematic reviews and specific guidelines for nonrandomized studies (20), three bibliographic methods were used to identify potential abstracts or investigations: remote search in electronic databases; evaluation of bibliographic citations from hand search of texts; and email contact with authors. The databases used were PubMed, LILACS and Cochrane. Independent reviewers participated in the search and selection of studies. Two independent reviewers (FMC and GB) made the search and selection of studies in the databases, while MRS resolved any divergences. Additional articles were searched by citation tracking of review articles and original articles, and by looking for additional articles authored by the same authors of the papers previously selected. After analyzing titles and abstracts**,** the selected articles were read in full to confirm eligibility, and doubts or disagreements were solved through discussions with senior researchers (ALPR, VN and MRS). Inclusion criteria were clearly defined before the beginning of search. This systematic review has been registered within PROSPERO (the NIHR International Prospective Register of Systematic Reviews), under the registration number CRD42017062367.

We included studies containing more than 10 patients which evaluated heart rate variability as predictor of mortality in sepsis, published before August 20th, 2017. Review studies and case series were excluded from this review. Publication languages included English, Portuguese and Spanish. The search-terms used were: "Systemic Inflammatory Response Syndrome"(Mesh), "Systemic Inflammatory Response Syndrome"(All Fields), "Sepsis"(Mesh), "sepsis"(All Fields), "heart rate"(MeSH Terms), "heart rate"(Text Word), "variability"(Text Word), "turbulence" (All Fields), "Nonlinear Dynamics"(Mesh), "Entropy"(Mesh), “triangular index”, "incidence"(MeSH), "mortality"(MeSH), "follow-up studies"(MeSH), "prognos"(Text Word), "predict"(Text Word), "course"(Text Word). Besides textual and MeSH terms selection, hand search within each paper’s references, and also "related citations", a search tool available in PubMed, were used to increase sensitivity of the search.

Two researchers (MRS and FMC) independently double checked the extraction of primary data from each study. Discrepancies were solved by consensus after discussion with the remaining researchers. The following information was extracted: study design and methodological data; demographic and clinical characteristics of patients; number

of patients who died and mean or median values of each HRV parameter in the surviving and non-surviving groups.

The Newcastle-Ottawa Scale(21) was used to assess the quality of the included studies. Using this 'star system' (ranges from 0 to 9) each included study was judged on three broad perspectives, as recommended by the Cochrane Non-Randomized Studies Methods Working Group Version 5.1.0 (20, 22): the selection of the study groups; the comparability of the groups; and the ascertainment of outcome of interest.

To analyze the results, we divided the articles between those who measured HRV for short-term recordings (≤ 1 hour), and those who did long-term recordings (≥ 24 hours), since we know that long-term recording have different oscillatory components as compared to those of short duration(11).

One of the included studies contained the median value of SDNN for surviving and non-surviving groups, but did not report the p-value of the comparison between groups. So, we estimated the mean and standard deviation of SDNN of each group on basis of the sample's reported median and range according to the method devised by S.P. Hozo, B. Djulbegovic, and I. Hozo(23). Subsequently, SDNN of survivors and nonsurvivors were compared using the Student´s *t* test, conducted in SPSS version 23 (SPSS Inc., Chicago, IL, USA).

Results

The selection process and the inclusion flow of studies are shown in Figure 1. Nine studies were included, with a total of 536 patients (18, 24-31). Table 1 shows the main methodological characteristics of the studies, while Table 2 describes the methodologies used in each study to measure HRV.

Study quality analysis by the Newcastle-Ottawa Scale showed that, in general, studies were representative of the sampled population, varying from 4 to 7 stars (mean 5.7). Except for the study of Chen and cols. 2012(27), in which patients with sepsis were included as controls of successfully resuscitated patients with out-of-hospital cardiac arrest (the main population of interest in the study), all other studies were prospective cohorts primary designed to include/primary focused on septic patients.

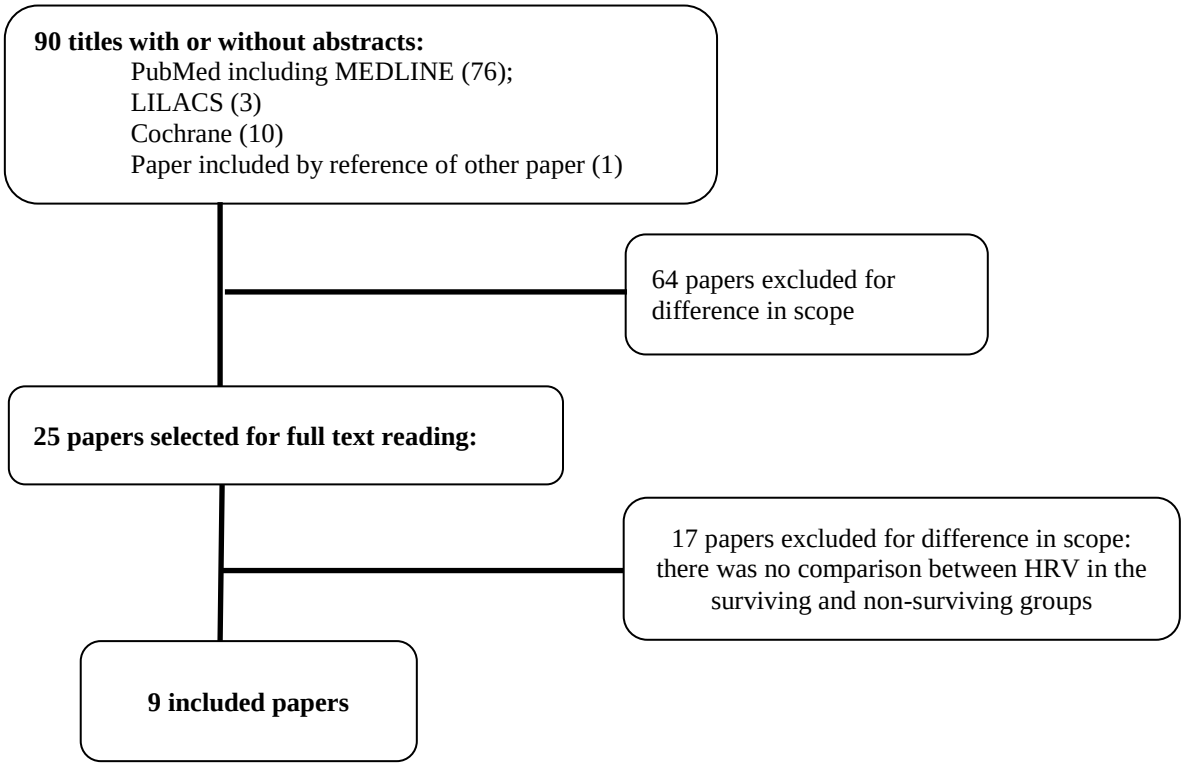

Figure 1 – Inclusion flow of studies:

Taken as whole, the included studies measured the following HRV parameters in the time domain: Normal-to-Normal (NN) average interval, Standard deviation of the NN interval (SDNN), Square root of the squared mean of the difference of successive NN-intervals (r-MSSD), Percentage of NN intervals deviated by more than 50 ms from adjacent NN-intervals (pNN50), Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording (NN50), Coefficient of variation (CV); frequency domain: Low Frequency Power (LF), High Frequency Power (HF), ratio of LF to HF (LF/HF), Total Power (TP), Very Low Frequency Power (VLF), Normalized Very Low Frequency (nVLF), Normalized Low Frequency Power (nLF) and Normalized High Frequency Power (nLF); nonlinear methods: Poincare standard deviation 1 (SD1), Poincare standard deviation 2 (SD2), Short-term (α1) and long-term (α2) fractal scaling coefficients from Detrended Fluctuation Analysis (DFA).

The small number of studies, their technical limitations and its great heterogeneity prevented a meta-analysis to be performed. Some studies only presented HRV results in graphs (29, 31) or summary descriptions in the body of the article(24, 25, 30), without informing the central value of each HRV parameter in the surviving and non-surviving groups. The outcome of one study was 24-hour mortality(27), not allowing comparison with the other studies, which assessed mortality at 28 days or the in-hospital mortality.

Table 1: Characteristics of included studies:

Study (1st author/year)	Country	Enrollment period	Sample Size	Age (Mean)	Male (%)	Mortality Endpoint	Mortality Rate	Population (Septic patients)	Exclusion criteria	NOS (stars)
Tateishi 2007	China	2002 to 2005	45	54	71	?	29	Adults in the ICU	DM or neurological disease	5
Nogueira 2008	Brasil	2003 to 2005	31	51	74	In-hospital mortality	61	Adults in the ICU receiving mechanical ventilation	MI, nonsinusal rhythm, use of a permanent pacemaker, CHF class III or IV, or DM	6
Chen 2008	Taiwan	2006	132	67	47	In-hospital mortality	8	Adults in the ED	Arrhythmia, cardiac pacing or respiratory failure under mechanical ventilator	7
Papaioannou 2009	Greece	2007 to 2008	20	58	76	?	20	Adults in the ICU receiving mechanical ventilation	Atrial flutter or fibrillation, ventricular ectopic beats, use of anti-arrhythmic medication, severe brain injuries or acquired immunodeficiencies	4
Duque 2012	Colombia	2009 to 2010	100	55	58	?	40	Adults in the ICU with the need for cardiovascular or ventilatory support	Clinical or electrocardioggraphic features complicating interpretation of the Holter recordings or coronary disease	6
Chen 2012	Taiwan	?	64	?	?	24-hour mortality	25	Age- and sex-matched patients with sepsis in the ED used as the negative controls	Persistent arrhythmia or cardiac pacing	5
Brown 2013	USA	2009 to 2011	48	57	46	28-day mortality	10	>15 years of age patients in the ICU with severe sepsis or septic shock	Pregnancy or non-sinus rhythm	7
Cedillo 2015	Spain	2012	33	62	39	In-hospital mortality	18	Non-smoking patients admitted to the ward	Infectious or inflammatory disorders, malignant diseases, CHF, nonsinusal rhythm, COPD, immunosuppression, use of beta or calcium-channel blockers, poorly-controlled DM, liver or renal failure or age > 80 years.	4
Castilho 2017	Brasil	2012 to 2014	63	53	60	28-day mortality	25	Adults in the ICU	Antibiotic therapy for more than 48 hours prior to enrollment, nonsinus rhythm or with pacemaker	7

Footnote: ICU = Intensive Care Unit; ED = Emergency Department; NOS = Newcastle-Ottawa Scale; DM= diabetes mellitus; MI = myocardial infarction; CHF = chronic heart failure; COPD = chronic obstructive pulmonary disease;

Table 2: HRV measurement in included studies:

Study (1st author/year)	Duration of measurement	Equipment	Recording day	Patient´s conditions during record	Were there patients receiving Mechanical Ventilation?	HRV parameters
Tateishi 2007	24-hour	Monitor	First and last	?	Yes	Frequency domain: LF, HF
Nogueira 2008	30-minute	Holter	1,3 and 6	Supine position, with ventilatory parameters completely controlled by the ventilator	All	Frequency domain: LF, HF, LF/HF
Chen 2008	10-minute	ECG	First	Supine position, room temperature around 25°C	No	Time domain: SDNN, r-MSSD; Frequency domain: TP, VLF, LF, HF, nVLF, nLF, nHF and LF/HF
Papaioannou 2009	10-minute	Holter	?	Supine position	All	Time domain: SDNN; Frequency domain: LF, HF, LF/HF; Nonlinear method: SD1/SD2
Duque 2012	48-hour	Holter	First	?	Yes	Time domain: SDNN, pNN50
Chen 2012	10-minute	ECG	First	?	Yes	Time domain: SDNN, CV; Frequency domain: TP, VLF, LF, HF, LF/HF
Brown 2013	30-minute	Monitor	First	?	Yes	Time domain: NN, SDNN, r-MSSD, pNN50, NN50; Frequency domain: TP, LF, HF, LF/HF; Nonlinear methods: SD1/SD2, Sample entropy, DFA short-term coefficient, DFA long-term coefficient, Ratio of DFA coefficients
Cedillo 2015	15-minute	ECG	First	Supine position after a 10-min resting period and normal breathing	No	Time domain: r-MSSD; Frequency domain: TP, LF, HF, LF/HF
Castilho 2017	20-minute and 24-hour	Holter	First	Supine position and no intervention was made during its recording	Yes	Time domain: NN, SDNN, r-MSSD, pNN50; Frequency domain: TP, VLF, LF, HF, LF/HF

Footnote: LF = Low Frequency Power; HF = High Frequency Power; LF/HF = Low Frequency Power / High Frequency Power; TP = Total Power; VLF = Very Low Frequency Power; nVLF = Normalized Very Low Frequency; nLF = Normalized Low Frequency Power; nHF = Normalized High Frequency Power; NN = Normal-to-Normal average interval; SDNN = Standard deviation of the NN interval; r-MSSD = Square root of the squared mean of the difference of successive NN-intervals; pNN50 = Percentage of NN intervals deviated by more than 50 ms from adjacent NN-intervals; NN50 = Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; SD1 = Poincare standard deviation 1; SD2 = Poincare standard deviation 2; DFA = Detrended Fluctuation Analysis; CV = Coefficient of variation

There was also a large difference in the population evaluated in the included studies: for instance, while two of the studies excluded mechanically ventilated patients(25, 26), two other studies restricted their analysis to patients who were on mechanical ventilation(29, 30). The mortality rate observed in the different studies varied considerably, ranging from 8 to 61%, probably indicating a difference in clinical spectrum among populations.

Seven studies performed HRV analysis in short-term recordings(18, 24-27, 29, 30), Three studies performed HRV analysis using long-term recordings (18, 28, 31). The main results of these studies are presented in Table 3 and Table 4, respectively. One of the studies made both a recording of short duration (20 minutes) and another one of long duration (24 hours)(18).

Regarding the studies that used short term recordings to measure HRV, one of them reported a statistically significant difference of SDNN, LF and HF values between survivors and non-survivors, but authors did not inform which patient group had the highest values, what does not allow a deeper analysis of these results and comparison with other studies(30). The remaining studies (n=6) showed reduction of several HRV parameters in the non-survivors in relation to the surviving septic patients: SDNN(18, 26), TP(18, 26), VLF(18, 26), LF(18, 26, 29), LF/HF ratio(18, 26, 27, 29), nLF(27), α1/α2(24) and r-MSSD(25). From these studies, four did not show the exact central value (mean or median) of the HRV parameters in survivor and non-survivor groups, presenting the results only in graphics or summarized in the body of the article. Chen et al, through the multiple logistic regression model, found that SDNN was a significant independent variable in the prediction of mortality in sepsis, with odds ratio of 0.719 (0.537-0.962), p=0.026 (26). Castilho et al defined a cut-off point for the SDNN of 17ms and found that Cox regression for dichotomous SDNN adjusted by the APACHE II showed Hazard ratio (HR) of 5.5 (1.2±24.8; p = 0.027) and Cox regression for this dichotomous variable adjusted by the SOFA showed HR of 6.3 (1.4±28.0; p = 0.015) (18).

There was a contradiction in the outcome prediction of HF and nHF, where some studies showed that their values were reduced in the non-survivor group (25, 29), while other studies showed higher values of these parameters were in the same group (26, 27).

From the studies using long-term recordings, only one article found statistically significant differences of any HRV parameter between survivors and non-survivors:

Table 3: Main results in short time record studies:

Study (1st author/year)	Duration of measurement	Does it show the values (mean or median) of HRV parameters in the surviving and non-survivors groups?	How are the results presented?	HRV parameters of nonsurvivors lower than those of survivors*	HRV parameters of nonsurvivors higher than those of survivors*	Additional results / Comments
Nogueira 2008	30-minute	No	Graph comparing LF, HF e LF/HF between surviving and non-surviving patients.	LF, HF and LF/HF		
Chen 2008	10-minute	Yes	Table with HRV parameters values for surviving and non-surviving groups	SDNN, TP, VLF, LF and LF/HF	nHF	Multiple logistic regression model identified SDNN and nHF as the significant independent variables in the prediction mortality.
Papaioannou 2009	10-minute	No	Only citation in the text of the article			The natural logarithms of SDNN, LF and HF were significantly different between survivors and non-survivors, but there is no information on which patient group had the highest values.
Chen 2012	10-minute	Yes	Table with HRV parameters values for surviving and non-surviving groups	nLF, and LF/HF	nHF and HF	
Brown 2013	30-minute	No	Only citation in the text of the article	α1/α2		
Cedillo 2015	15-minute	No	Only citation in the text of the article	r-MSSD and nHF		
Castilho 2017	20-minute	Yes	Table with HRV parameters values for surviving and non-surviving groups	SDNN, TP, VLF, LF and LF/HF		SDNN ≤17 is a risk factor for death in septic patients, even after adjusting for APACHE II or SOFA.

Footnote: *=Only results with statistical significance were shown; LF = Low Frequency Power; HF = High Frequency Power; LF/HF = Low Frequency Power / High Frequency Power; TP = Total Power; VLF = Very Low Frequency Power; nLF = Normalized Low Frequency Power; nHF = Normalized High Frequency Power; NN = Normal-to-Normal average interval; SDNN = Standard deviation of the NN interval; r-MSSD = Square root of the squared mean of the difference of successive NN-intervals; DFA = Detrended Fluctuation Analysis; CV = Coefficient of variation; α1/α2 = short-term and long-term fractal scaling coefficients from DFA;

Table 4: Main results in long time record studies:

Study (1st author/year)	Duration of measurement	Does it show the values of HRV parameters in the surviving and non-survivors groups?	How are the results presented?	HRV parameters of nonsurvivors lower than those of survivors*	HRV parameters of nonsurvivors higher than those of survivors*	Additional results / Comments
Tateishi 2007	24-hour	No	Graph comparing logLF and logHF between surviving and non-surviving patients		LogHF	
Duque 2012	48-hour	Yes	Table with SDNN and PNN50 values for surviving and non-surviving groups			Median SDNN non significantly higher in the surviving group than in the nonsurvivor group (72.5ms [IQR 42] vs 61ms [IQR 65],p value not reported in the article, but we calculated p=0.272)
Castilho 2017	24-hour	Yes	Table with HRV parameters values for surviving and non-surviving groups			There was no statistically significant difference in any HRV parameter measured in the 24 hours Holter between the two subgroups

Footnote: *=Only results with statistical significance were shown; Log = logarithm; LF = Low Frequency Power; HF = High Frequency Power; NN = Normal-to-Normal average interval; SDNN = Standard deviation of the NN interval;

LogHF was higher in the non-survivor than the survivor group(31). The other two articles found no statistically significant differences between survivors and non-survivors for any HRV parameter(18, 28).

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Discussion

In this systematic review, we found that HRV parameters measured in short-term recordings were reduced in septic patients who died in relation to those who survived. This finding raises the possibility that HRV measurement can be a useful tool to predict the risk death in sepsis. On the other hand, there was no clear evidence of association between HVR parameters in long-term recordings and sepsis outcome.

There have has been an increasing interest in the role played by the autonomic nervous system in the complexes mechanisms involved in sepsis physiopathology. It is known, for example, that vagus nerve stimulation increases the secretion of corticotropin-releasing hormone, ACTH and cortisol(32); it has been demonstrated that vagotomy attenuates fever response(33); and that acetylcholine, the main vagal neurotransmitter, has an anti-inflammatory effect, attenuating the release of cytokines such as TNF, IL-1beta, IL-6 and IL-18, and preventing the development of shock(34). Taken together, these findings suggest that the autonomic nervous system is involved in peripheral cytokine-to-brain communication, participating in the pathophysiology of sepsis.

Based on these results, some authors have investigated if the measurement of HRV, a noninvasive indirect test to evaluate autonomic function, in septic patients could be useful to predict outcome in these patients. HRV is measured using simple and non-invasive methods, requiring automated devices available on the market. Therefore, HRV is considered one of the most popular methods used to evaluate the autonomic function, being suitable for use in intensive care settings, where septic patients are usually taken(11).

The seven studies that analyzed HRV in short-term recordings(18, 24-27, 29, 30) showed significant difference between groups of surviving and non-surviving septic patients regarding different parameters. Except for one study that did not report which group had the highest value(30), the other six studies showed a reduction of at least one HRV parameter in the group of patients who died. These findings suggest that loss of heart rate oscillatory capacity, controlled, among other factors, by the autonomic

nervous system is related to the severity of sepsis and risk of death. The only conflicting results revealed by this systematic review referred to the HF Power, which was shown to be reduced in the non-survivor group in some studies, but increased in others. HF Power reflects the vagal activity (parasympathetic) on the sinus node(35)

Among all HRV parameters tested to predict risk of death in sepsis, SDNN proved to be the most promising one. In this context, two studies found this parameter to be associated with mortality in sepsis, even after adjusting for possible confounding factors(18, 26). SDNN seems to reflect all the cyclic components responsible for HRV (including sympathetic and parasympathetic activity)(11). Some studies used the ICU monitors themselves to perform the electrocardiographic recording(24, 31), assuming that, through the implementation of SDNN calculation software, the ICU monitors themselves could calculate the SDNN of septic patients as a measure of the risk of death.

Only one (31) from the three (18, 28, 31) studies that analyzed long-term recordings for HRV found differences between surviving and non-surviving groups, and even so, in only one HRV parameter. We believe that the difficulty of association between HRV parameters and mortality in sepsis in long-term recordings is due, among other factors, to the dynamic condition of sepsis, in which metabolic disturbances, hemodynamic and ventilatory evolution, as well as the therapeutic interventions instituted over short intervals of time (such as amine use, sedation, among others), may interfere with HRV parameters. Furthermore, in critical care patients, shorter periods of recording minimizes the interference with ICU routine activities, and have the advantage of being a fast tool for definition of severity, as these patients present immediate risk of death.

Study limitations

The main limitation of this systematic review is the low number and quality of the studies included. The great heterogeneity of the HRV recording and analysis methods used, as well as the great heterogeneity of the population of each study prevented us to perform a meta-analysis. Other limitations are the low number of patients in each study and the fact that all of them were unicentric. Thus, although it is possible to affirm that HRV fall seems to be related to sepsis mortality, it would be necessary to perform a larger, preferably multicenter study, to define the best HVR recordings and analysis

methodology, as well as what parameters and cutoff points should be adopted to predict the risk of death.

Conclusions

Several HRV parameters are reduced in nonsurviving septic patients in short-term recording. SDNN seems to be independently associated with mortality in sepsis, emerging as the most useful HRV parameter to predict sepsis outcome. These findings need to be confirmed in larger well-designed studies.

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6 – CONSIDERAÇÕES FINAIS

O principal achado desta tese é a associação entre parâmetros da VFC e mortalidade na sepse. O estudo de coorte mostrou que diversos parâmetros da VFC no Holter de 20 minutos estavam reduzidos no grupo de pacientes sépticos não-sobreviventes em relação aos sobreviventes. A revisão sistemática reforçou esse achado ao mostrar que todos os artigos que fizeram gravação de curta duração (excetuando um que não mostrou em qual grupo os parâmetros estavam reduzidos) encontraram redução de pelo menos um parâmetro da VFC do grupo não-sobrevivente em relação ao grupo de paciente sépticos que sobreviveram.

O papel do sistema nervoso autônomo na fisiopatologia da sepse é cada vez mais conhecido. Ele parece atuar na comunicação entre citocinas periféricas e o cérebro(38, 39). Neste cenário, o uso da VFC em paciente sépticos mostra-se promissor, já que a VFC é um método indireto de avaliação do sistema nervoso autônomo com muitas vantagens: não é invasivo, não dependente de colaboração ativa do paciente e é amplamente difundido, havendo diversos aparelhos que fazem seu cálculo automaticamente(60).

Embora não haja valores de referência universalmente validados para VFC, os valores encontrados no estudo de coorte que fizemos são reduzidos em relação a outros estudos populacionais. Como exemplo, podemos citar que estudo de Kim et al(117) encontrou média de SDNN de 39,6ms para a população coreana normal, enquanto, em nossa coorte, a média de SDNN dos pacientes sobreviventes foi de 19,0ms e dos não-sobreviventes foi de 8,5ms. Esse achado vai ao encontro de outros estudos que já mostravam haver redução de VFC em pacientes com sepse em relação a pessoas saudáveis(93, 94).

Diversos parâmetros da VFC estavam reduzidos no grupo de pacientes sépticos não-sobreviventes em relação aos sobreviventes na gravação de 20 minutos de nosso estudo de coorte: SDNN, Total Power, VHF, LF e LF/HF. Escolhendo o ponto de coorte de 17ms para o SDNN e dividindo os pacientes a partir desse valor (pacientes com SDNN≤17ms e com SDNN > 17ms), criou-se dois grupos sem diferenças estatisticamente significativas em suas características de base. A curva de Kaplan-Meier desses dois grupos mostrou log rank p = 0,003, mostrando maior mortalidade do grupo

de pacientes com SDNN≤17ms. A regressão de Cox para essa variável dicotômica ajustada pelo SOFA ou pelo APACHE II apresentou para o SDNN≤17ms HR de 6,3 (1,4±28,0; p=0,015) e de 5,5 (1,2±24,8; p=0,027), respectivamente. Esses achados apontam o SDNN como uma ferramenta útil para identificar entre pacientes sépticos aqueles com maior risco de morte. Além disso, o fato de esse resultado ter se mantido após ajuste para APACHEII ou SOFA sugere que o SDNN ≤17ms possa ser um fator de risco independente para a mortalidade na sepse. As medidas de concordância, R^2, AIC, IDI e NRI indicam que o poder preditivo do modelo SOFA+SDNN é melhor do que o poder preditivo do SOFA isoladamente, o que reforça a possível utilidade clínica dessa variável.

O principal resultado da revisão sistemática foi o achado de que todos os estudos que permitiam essa análise encontraram redução de pelo menos um parâmetro da VFC no grupo de pacientes sépticos não-sobreviventes em relação ao grupo de sobreviventes, em gravações de curto período. O fato de esse achado estar presente em diferentes estudos sugere que a perda de capacidade de oscilação da frequência cardíaca, que é controlada, entre outros fatores, pelo sistema nervoso autônomo, está relacionada à gravidade da sepse e ao risco de morte. Um dos estudos encontrou, através do modelo de regressão logística múltipla, que o SDNN era uma variável independente na predição da mortalidade na sepse, com odds ratio de 0,719 (0,537-0,962), p = 0,026(101). Esse resultado vai ao encontro do que encontramos em nosso estudo de coorte, apontando o SDNN como uma variável promissora para predizer risco de morte na sepse.

Os resultados em gravações de longa duração foram menos significativos. Em nosso estudo de coorte não houve diferença estatisticamente significativa em nenhum parâmetro de VFC entre os grupos de pacientes sépticos sobreviventes e não-sobreviventes. Na revisão sistemática, dos três estudos que fizeram gravação de longa duração, apenas um estudo encontrou diferença e, mesmo assim, em apenas uma variável: o logHF foi maior no grupo não-sobrevivente em relação ao grupo sobrevivente no estudo de Tateishi et al(118).

A dificuldade de associação entre os parâmetros de VFC e a mortalidade em sepse em gravações de longa duração pode-se dever, entre outros fatores, à condição dinâmica da sepse, em que distúrbios metabólicos, hemodinâmicos e ventilatórios, bem como as intervenções terapêuticas instituídas (como uso de amina, sedação, entre outros) podem interferir nos parâmetros de VFC. Além disso, em pacientes críticos, períodos mais

curtos de gravação apresentam a vantagem de minimizar a interferência que poderiam causar na rotina da UTI e serem uma ferramenta rápida para a definição de gravidade em pacientes com risco imediato de morte.

Ambos os estudos conduzidos nesta tese têm limitações. O estudo de coorte apresenta pequeno número de pacientes, porém a amostra atingida confere poder razoável conforme cálculo amostral. As técnicas avançadas de modelagem foram utilizadas para minimizar o risco de sobre-ajuste (overfitting) e "otimismo" excessivo do modelo. Esta análise manteve SDNN≤17 como fator de risco para a morte no modelo com o escore SOFA, mas não para o modelo com APACHE II. Outra limitação deste estudo é a possível influência de outras condições clínicas que afetam a VFC como insuficiência cardíaca congestiva, doença arterial coronariana, diabetes ou ventilação mecânica. No entanto, não houve diferença na frequência dessas condições entre os grupos de pacientes não-sobreviventes e sobreviventes. A temperatura corporal e os medicamentos (por exemplo, sedativos, beta-bloqueadores e drogas inotrópicas) que podem interferir na VFC não foram avaliados neste estudo. A influência do fator dia-noite na VFC não foi avaliada neste estudo, embora sua existência já tenha sido demonstrada em voluntários saudáveis com endotoxemia(119). A maioria das medidas de Holter de 20 minutos foi feita durante o dia e não houve diferenças significativas entre a porcentagem de gravações realizadas durante o dia nos grupos sobreviventes e não sobreviventes. Além disso, não sabemos se essa diferença dia-noite ocorre em pacientes em UTI. Todos os pacientes incluídos nesta coorte o foram antes da publicação do Terceiro Consenso de Sepse(1), razão pela qual não foram utilizadas as novas definições de sepse no presente estudo. Apesar disso, todos os pacientes incluídos neste estudo tinham um escore SOFA≥ 2 pontos, sendo possível que preenchessem os critérios para o diagnóstico de sepse segundo o novo consenso.

Já a revisão sistemática apresenta como principal limitação o baixo número e a qualidade dos estudos incluídos. A grande heterogeneidade dos métodos de gravação e análise de HRV utilizados, bem como a grande heterogeneidade da população de cada estudo, impediu-nos de realizar metanálise. Outras limitações são o baixo número de pacientes em cada estudo e o fato de que todos os estudos serem unicêntricos.

Em conclusão, podemos afirmar que vários parâmetros de VFC são reduzidos em pacientes séptico não-sobreviventes em relação aos sobreviventes. O SDNN parece estar associado de forma independente à mortalidade na sepse, emergindo como o mais

útil parâmetro da VFC para prever o resultado da sepse. Essas descobertas precisam ser confirmadas em estudos maiores.

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8 – APÊNDICES

8.1 – CRF Geral do Estudo

Estudo da função endotelial e cardiovascular em pacientes portadores de sepse: implicações diagnósticas, definição de risco e terapêutica

1-VARIÁVEIS SÓCIO-DEMOGRÁFICAS **(*) INDICA AS VARIÁVEIS A SEREM REPETIDAS NOS CRFs DOS SUBPROJETOS**	
1.1*-Nome:	
1.2*-Iniciais: __ __ __ __	**I__I__I__I__I**
1.2*- Número inclusão estudo:__ __ __	**I__I__I__I**
1.3- Data inclusão estudo: __ __/__ __/__ __	I__I__I__I__I__I__I
1.4- SAME: __ __ __ __ __ __	I__I__I__I__I__I__I__I
1.5- Data de nascimento: __ __/__ __/__ __	I__I__I__I__I__I__I
1.6- Data internação HC: __ __/__ __/__ __	I__I__I__I__I__I__I
1.7- Data internação UCO: __ __/__ __/__ __	I__I__I__I__I__I__I
1.8- Data alta HC: __ __/__ __/__ __	I__I__I__I__I__I__I
1.9- Data alta UCO: __ __/__ __/__ __	I__I__I__I__I__I__I
1.10- Sexo: __ *1- M 2- F*	I__I
Contatos telefônicos: Fixo: (__ __) __ __ __ __ __ __ __ __ Celular: (__ __) __ __ __ __ __ __ __ __ **Endereço:** Rua/Av: _____ No.:__ __ __ __ Complemento: __ __ __ __ __ __ Bairro:_____Cidade: _____ CEP: __ __. __ __ __ - __ __ __	**Obseervações:**
2-VARIÁVEIS CLÍNICAS À INCLUSÃO	
2.1- Tipo de internação: __ ***1**. Clínica **2**. Cirúrgica*	I__I
2.2 Principal causa internação clínica em CTI ***1**.Insuf. respiratória **2**.Insuf. cardíaca **3**.Choque **4**.Coma **5**.Distúrbio hidroeletrolítico* ***6**.Insuf. renal **9**.NSA Outras:_____*	I__I
2.3 Principal causa internação cirúrgica: __ ***1**.Relacionada à cirurgia entre 1º. e 7º. DPO **2**. PO cirurgia urgência **3**.PO eletivo alto-risco **9**.NSA*	I__I
2.4- APACHE II: __ __	I__I__I
2.5- SOFA total: __ __	I__I__I
2.6- SOFA respiratório: __	I__I
2.7- SOFA coagulação: __	I__I
2.8- SOFA hepático: __	I__I
2.9- SOFA cardiovascular: __	I__I
2.10- SOFA neurológico (Glasgow) : __	I__I

2.11- SOFA renal: __	I__I
3-HISTÓRIA PREGRESSA/COMORBIDADES	
3.1- Insuficiência cardíaca congestiva: __ *0- não 2-sim 8- IGN*	I__I
3.2- DPCO: __ *0- não 2-sim 8- IGN*	I__I
3.3- Cirrose hepática: __ *0- não 2-sim 8- IGN*	I__I
3.4- Insuficiência renal crônica: __ *0- não 2-sim 8- IGN*	I__I
3.5- HAS: __ *0- não 2-sim 8- IGN*	I__I
3.6- Diabetes mellitus: __ *0- não 2-sim 8- IGN*	I__I
3.7- Hipercolesterolemia: __ *0- não 2-sim 8- IGN*	I__I
3.8- AVE prévio: __ *0- não 2-sim 8- IGN*	I__I
3.9- Doença arterial periférica: __ *0- não 2-sim 8- IGN*	I__I
3.10- Evento tromboembólico prévio: __ *0- não 2-sim 8- IGN*	I__I
3.11- Evento coronariano prévio: __ *0- não 2-sim 8- IGN*	I__I
3.12- Tipo evento coronariano prévio: __ *1. angina 2. IAM sem SST 3.IAM com SST 8.IGN 9.NSA*	I__I
5.13- Uso de estatina: *0- não 2-sim 8- IGN*	I__I
5.14-Tabagismo ativo ou interrompido há menos de 6 meses: *0- não 2-sim 8- IGN*	I__I
5.15- Carga tabágica (anos/maço): __ __	I__I__I
5.16- Ex-tabagista: __ *0- não 2-sim 8- IGN*	
5.17- História familiar de DAC: *0- não 2-sim 8- IGN*	I__I
3.18- Uso de corticoides (referência ≥15mg/dia prednisona): __ *0- não 2-sim 8- IGN*	I__I
3.19- Uso de imunossupressores: __ *0- não 2-sim 8- IGN*	I__I
4- DADOS MICROBIOLÓGICOS À INCLUSÃO	
4.1- Tipo de infecção: __ *1. Comunitária 2. Nosocomial*	I__I
4.2- Sítio de infecção: __ ***1**.Pulmonar **2**.Intra-abdominal **3**.Renal **4**.Cateter **5**.Partes moles **6**.Neurológico **7**.Indeterminado **11**.Misto*	I__I__I
4.3- Tipo de pneumonia: __ ***1**.Comunitária **2**.Nosocomial não VAP **3**.Nosocomial VAP **9**.NSA*	I__I
4.4- Confirmação microbiológica da sepse: __ *0.Não 1.Sim*	I__I
4.5- Hemocultura positiva: __ *0.Não 1.Sim*	I__I
4.6- Germe isolado como causa da sepse_1: __ __ __	I__I__I__I
4.7- Germe isolado como causa da sepse_2: __ __ __	I__I__I__I

5- VARIÁVEIS LABORATORIAIS À INCLUSÃO	
5.1- Hb: __ __,__g/L	I__I__I,I__I
5.2- Leucócitos totais: __ __ __ __ __ __ __	I__I__I__I__I__I__I__I
5.3- Bastonestes: __ __ __ __ __ cls/mm^3	I__I__I__I__I__I__I
5.4- Eosinófilos: __ __ __ __ __ cls/mm^3	I__I__I__I__I__I__I
5.5- Plaquetas: __ __ __ __ __ __ cls/mm^3	I__I__I__I__I__I__I
5.6- Lactato: __ __,__ mmol/L	I__I__I,I__I
5.7- PCR: __ __ __ mg/dL	I__I__I__I
5.10 Ureia: __ __ __	I__I__I__I
5.11- Creatinina: __ __,__	I__I__I.I__I
5.12-ClCreatinina estimado: __ __ __ ml/min	I__I__I__I
5.13- CK total: __ __ __ __ __ __	I__I__I__I__I__I
5.14- CK MB: __ __ __	I__I__I__I
5.15- Troponina: __,__	I__ I,I __I
5.16- Colesterol total: __ __ __	I__I__I__I
5.17-LDL:__ __ __	I__I__I__I
5.18- HDL: __ __ __	I__I__I__I
5.19- Triglicérides: __ __ __ __	I__I__I__I
5.20- Glicemia de jejum: __ __ __	I__I__I__I
5.21- Procalcitonina: __ __ __ ,__	I__I__I__I,I__I
5.22- BNP: __ __ __ __, __	I__I__I__I__I,I__I
6- INTERVENÇÕES TERAPÊUTICAS PRIMEIRAS 72h INCLUSÃO	
6.1-Uso de corticoide durante as primeiras 72h de inclusão: __ *0. não 1.sim*	I__I
6.2- Corticoide usado para choque séptico: __ *0. não 1.sim 9.NSA*	I__I
6.3 Uso de ventilação mecânica durante as primeiras 72h: __ *0. não 1.sim*	I__I
6.4- Tipo de ventilação mecânica: __ 0. invasiva 1. não invasiva 9. NSA	I__I
6.5- Uso de aminas vasopressoras nas primeiras 72h: __ *0. não 1.sim*	I__I
6.5- Uso de inotrópicos positivos nas primeiras 72h: __ *0. não 1.sim*	I__I
6.6- Submetido a hemodiálise nas primeiras 72h: __ *0. não 1.sim*	I__I
7- SEGUIMENTO 3º. DIA INCLUSÃO	**DATA:** I__ __I__ __I__ __I

7.1- Procalcitonina: __ __ __.__	I__I__I__I,I__I
7.2- BNP: __ __ __ __.__	I__I__I__I__I,I__I
7.3- PCR: __ __ __	I__I__I__I
7.4- SOFA total: __ __	I__I__I
7.5- Está em uso de corticoide no 3º. dia: __ *0. não 1.sim Se sim, dose (equivalente prednisona):__ __ __*	I__I
8- SEGUIMENTO 7º. DIA INCLUSÃO **DATA:** I__ __I__ __I__ __I	
8.1- Procalcitonina: __ __ __.__	I__I__I__I,I__I
8.2- BNP: __ __ __ __.__	I__I__I__I__I,I__I
8.3- PCR: __ __ __	I__I__I__I
8.4- SOFA total: __ __	I__I__I
8.5- Está em uso de corticoide no 7º. dia: __ *0. não 1.sim Se sim, dose (equivalente prednisona):__ __ __*	I__I
9- DADOS FINAL SEGUIMENTO (28º. INCLUSÃO) **DATA:** I__ __I__ __I__ __I	
9.1- Condição no 28º. dia incluso 0. óbito 1. Sobrevivente	I__I
9.2- Submetido à VM invasiva durante o seguimento *0. não 1.sim*	I__I
9.2- Submetido à hemodiálise durante o seguimento *0. não 1.sim*	I__I
10- DADOS 90º. DIA INCLUSÃO **DATA:** I__ __I__ __I__ __I	
10.1- Condição alta UCO: __ 0. óbito 1. sobrevivente	I__I
10.2- Condição alta HC: __ 0. óbito 1. sobrevivente	I__I
10.3- Condição no 90º. dia inclusão 0. óbito 1. sobrevivente	I__I
11- DADOS 1 ANO INCLUSÃO **DATA:** I__ __I__ __I__ __I	
11.1- Condição 1 ANO: __ 0. óbito 1. sobrevivente	I__I

8.2 – CRF complementar do estudo de VFC na Sepse

Estudo da Variabilidade da Frequência Cardíaca na Sepse	
1.1*-Nome:	
Registro HC:	
1.2*-Iniciais: __ __ __ __	I__I__I__I__I
1.2*- Número inclusão estudo:__ __ __	I__I__I__I
2.1- Ritmo sinusal? S/N	I__I
2.2- Qual __ __ __ __ __ __	I__I__I__I__I__I__I__I
3.1- Uso de MP? S / N	I__I
4.1- Comorbidades:S / N	I__I
4.2- Qual __ __ __ __ __ ____ __	I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I
4.2- Qual __ __ __ __ __ ____ __	I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I
4.2- Qual __ __ __ __ __ ____ __	I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I I__I__I__I__I__I__I__I
5.1- IAM no último mês? S / N	I__I
6.1- SOFA 1: __ __	I__I__I
6.2- SOFA 2: __ __	I__I__I
6.3- SOFA 3: __ __	I__I__I
6.4- SOFA 4: __ __	I__I__I
6.5- SOFA 5: __ __	I__I__I
6.6- SOFA 6: __ __	I__I__I
6.7- SOFA 7: __ __	I__I__I
7.1- Data do Holter 20min: __ __/__ __/__ __	I__I__I__I__I__I__I
7.1- Hora do Holter 20min: __ __:__ __	I__I__I__I__I
7.1- Data do Holter 24h: __ __/__ __/__ __	I__I__I__I__I__I__I
7.1- Hora do Holter 24h: __ __:__ __	I__I__I__I__I

8.3 – Termos de Consentimento

8.3.1 – Termo de Consentimento Livre e Esclarecido (Para o paciente)

Projeto de pesquisa: Estudo da função endotelial e cardiovascular em pacientes portadores de sepse: implicações diagnósticas, definição de risco e terapêutica

Pesquisador responsável: Vandack Alencar Nobre Jr

Instituição: Faculdade de Medicina da Universidade Federal de Minas Gerais

A Universidade Federal de Minas Gerais convida o Sr. / a Sra. a participar de uma pesquisa sobre infecções graves (septicemia) causadas por bactérias. Muitas questões sobre esse tipo de doença permanecem desconhecidas pelos médicos. As infecções graves são motivo de diversas pesquisas no mundo todo. Os pesquisadores do Hospital das Clínicas da UFMG estão propondo uma pesquisa sobre essa doença. Trata-se de um projeto para conhecer melhor o problema, e tentar descobrir formas de diagnosticá-lo mais rapidamente, e tratar melhor os pacientes.

Sabe-se que nos pacientes com septicemia ocorre uma significativa inflamação. Vários exames realizados no sangue podem ajudar a descobrir se esta inflamação está muito intensa, e se terá maiores conseqüências. Além disso, o funcionamento de vários órgãos fica prejudicado quando o paciente tem uma infecção grave. Os pesquisadores da UFMG querem estudar o funcionamento do coração e dos vasos sanguíneos nesses pacientes, utilizando exames que são feitos por cima da pele, e não doem. Não haverá nenhuma mudança no seu tratamento se você participar da pesquisa. Além disso, não se prevê benefícios diretos para você, caso você participe.

Serão coletadas amostras de sangue diariamente, juntamente com os exames de rotina, dos pacientes que aceitarem participar da pesquisa. O sangue será enviado para o Laboratório do Hospital das Clínicas, onde serão realizadas dosagens de várias substâncias. Essas substâncias poderão ajudar a entender melhor o funcionamento do corpo nas infecções graves. O sangue dos pacientes não será utilizado para outros fins, e será descartado após os exames da pesquisa.

Os procedimentos propostos nesta pesquisa e os seus respectivos riscos são os seguintes:

1. Coleta de sangue: procedimento feito rotineiramente nos pacientes internados no CTI. A coleta de sangue apresenta riscos mínimos e pode causar:

- **hematomas no local de coleta**
- **infecção no local de coleta**

2. Eletrocardiografia / Holter: procedimento realizado frequentemente em pacientes internados, e que não trazem risco algum para o paciente.

3. Ultrassonografia para avaliação do diâmetro de veia cava inferior: exame não-invasivo, e que não traz qualquer risco ao paciente. Pode haver pequeno desconforto devido ao posicionamento do paciente, que às vezes precisa ficar deitado de lado.

4. Ecocardiograma bidimensional: também frequentemente realizado em pacientes internados. Trata-se de exame não-invasivo, e que não traz qualquer risco ao paciente. Pode haver pequeno desconforto devido ao posicionamento do paciente, que às vezes deve ficar deitado de lado.

5. Teste de hiperemia reativa: assemelha-se a uma medida de pressão arterial. No entanto, o manguito fica insuflado por 5 (cinco) minutos, o que pode gerar algum desconforto, mas não implica em riscos para o paciente.

Os pesquisadores assumem o dever de dar assistência aos participantes da pesquisa para problemas relacionados aos procedimentos citados acima ou mesmo para quaisquer outros problemas da saúde apresentados durante a pesquisa.
Os registros (informações dos pacientes) serão mantidos de forma confidencial com códigos de identificação de acesso limitado e o nome do paciente não será mencionado em nenhum momento.
Eu tenho o direito de recusar ou cancelar o meu consentimento de participação a qualquer instante, sem penalização alguma e sem prejuízo ao seu cuidado, bastando comunicar a minha decisão aos organizadores da pesquisa.

EU_____,
ENTENDI O QUE ME FOI PROPOSTO E TIVE OPORTUNIDADE DE ESCLARECER MINHAS DÚVIDAS. ESTOU CIENTE E CONSINTO QUE SE REALIZE COLETA DE SANGUE (50 ML DE SANGUE AO TODO). SEI QUE OS RESULTADOS DO ESTUDO SERÃO MANTIDOS EM SIGILO, PODENDO SER INFORMADOS SOMENTE A MINHA PESSOA. ESTOU CIENTE DE QUE OS RESULTADOS SERÃO PUBLICADOS SOMENTE EM CONJUNTO, NÃO PERMITINDO A IDENTIFICAÇÃO INDIVIDUAL.

Belo Horizonte, __/__/__ _____
Assinatura

_____
Impressão digital (se analfabeto – nesse caso ler o consentimento em voz alta após explicá-lo)

_____

Pesquisador responsável

Os telefones abaixo podem lhe ser úteis para esclarecimentos:
Pesquisadores
1-Dr. Vandack Alencar Nobre Jr (Hospital das Clínicas): (31) 3409-9436 / 9831-0004
2-Dra. Luisa Caldeira Brant (Hospital das Clínicas): (31) 34099436

Av. Alfredo Balena, 190 – Departamento de Clínica Médica da Faculdade de Medicina da UFMG – Santa Efigênia – Belo Horizonte, MG – Brasil – CEP 30130-100

Comitês de Ética em Pesquisa
Comitê de Ética em Pesquisa da UFMG - COEP: Telefax (31) 3409-4592
Av. Antônio Carlos, 6627 – Unidade Administrativa II – 2º andar, sl 2005 – Campus Pampulha, Belo Horizonte, MG – Brasil – CEP 31270-901

8.3.2 – Termo de Consentimento Livre e Esclarecido (Familiar ou responsável)
<u>Projeto de pesquisa</u>: Estudo da função endotelial e cardiovascular em pacientes portadores de sepse: implicações diagnósticas, definição de risco e terapêutica

<u>Pesquisador responsável</u>: Vandack Alencar Nobre Jr

<u>Instituição</u>: Faculdade de Medicina da Universidade Federal de Minas Gerais

A Universidade Federal de Minas Gerais convida o seu familiar / amigo a participar de uma pesquisa sobre infecções graves (septicemia) causadas por bactérias. Muitas questões sobre esse tipo de doença permanecem desconhecidas pelos médicos. As infecções graves são motivo de diversas pesquisas no mundo todo. Os pesquisadores do Hospital das Clínicas da UFMG estão propondo uma pesquisa sobre essa doença. Trata-se de um projeto para conhecer melhor o problema, e tentar descobrir formas de diagnosticá-lo mais rapidamente, e tratar melhor os pacientes.

Sabe-se que nos pacientes com septicemia ocorre uma significativa inflamação. Vários exames realizados no sangue podem ajudar a descobrir se esta inflamação está muito intensa, e se terá maiores conseqüências. Além disso, o funcionamento de vários órgãos fica prejudicado quando o paciente tem uma infecção grave. Os pesquisadores da UFMG querem estudar o funcionamento do coração e dos vasos sanguíneos nesses pacientes, utilizando exames que são feitos por cima da pele, e não doem. Não haverá nenhuma mudança no tratamento do seu familiar / amigo se ele(ela) participar da pesquisa. Além disso, não se prevê benefícios diretos para ele(ela), caso ele(ela) participe.

Serão coletadas amostras de sangue diariamente, juntamente com os exames de rotina, dos pacientes que aceitarem participar da pesquisa. O sangue será enviado para o Laboratório do Hospital das Clínicas, onde serão realizadas dosagens de várias substâncias. Essas substâncias poderão ajudar a entender melhor o funcionamento do corpo nas infecções graves. O sangue dos pacientes não será utilizado para outros fins, e será descartado após os exames da pesquisa.

Os procedimentos propostos nesta pesquisa e os seus respectivos riscos são os seguintes:

1. Coleta de sangue: procedimento feito rotineiramente nos pacientes internados no CTI. A coleta de sangue apresenta riscos mínimos e pode causar:

-hematomas no local de coleta
-infecção no local de coleta

2. Eletrocardiografia / Holter: procedimento realizado frequentemente em pacientes internados, e que não trazem risco algum para o paciente.

3. Ultrassonografia para avaliação do diâmetro de veia cava inferior: exame não-invasivo, e que não traz qualquer risco ao paciente. Pode haver pequeno desconforto devido ao posicionamento do paciente, que às vezes precisa ficar deitado de lado.

4. Ecocardiograma bidimensional: também frequentemente realizado em pacientes internados. Trata-se de exame não-invasivo, e que não traz qualquer risco ao paciente. Pode haver pequeno desconforto devido ao posicionamento do paciente, que às vezes deve ficar deitado de lado.

5. Teste de hiperemia reativa: assemelha-se a uma medida de pressão arterial. No entanto, o manguito fica insuflado por 5 (cinco) minutos, o que pode gerar algum desconforto, mas não implica em riscos para o paciente.

Os pesquisadores assumem o dever de dar assistência aos participantes da pesquisa para problemas relacionados aos procedimentos citados acima ou mesmo para quaisquer outros problemas da saúde apresentados durante a pesquisa.
Os registros (informações dos pacientes) serão mantidos de forma confidencial com códigos de identificação de acesso limitado e o nome do paciente não será mencionado em nenhum momento. eito de recusar ou cancelar o seu consentimento de participação a qualquer instante, sem penalização alguma e sem prejuízo ao seu cuidado, bastando comunicar a sua decisão aos organizadores da pesquisa.

EU_____(.....
do paciente .....) ENTENDI O QUE ME FOI PROPOSTO E TIVE OPORTUNIDADE DE ESCLARECER MINHAS DÚVIDAS. ESTOU CIENTE E CONSINTO QUE SE REALIZE COLETA DE SANGUE (50 ML DE SANGUE AO TODO). DO MEU FAMILIAR / AMIGO. SEI QUE OS RESULTADOS DO ESTUDO SERÃO MANTIDOS EM SIGILO, PODENDO SER INFORMADOS SOMENTE A MINHA PESSOA. ESTOU CIENTE DE QUE OS RESULTADOS SERÃO PUBLICADOS SOMENTE EM CONJUNTO, NÃO PERMITINDO A IDENTIFICAÇÃO INDIVIDUAL.

Belo Horizonte, __/__/__ _____
Assinatura

_____
Impressão digital (se analfabeto – nesse caso ler o consentimento em voz alta após explicá-lo)

_____

Pesquisador responsável

Os telefones abaixo podem lhe ser úteis para esclarecimentos:

<u>Pesquisadores</u>
1-Dr. Vandack Alencar Nobre Jr (Hospital das Clínicas): (31) 3409-9436 / 9831-0004
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8.4 – Artigo 1 da tese (publicação da revista PLOS ONE)

PLOS ONE

RESEARCH ARTICLE

Heart rate variability as predictor of mortality in sepsis: A prospective cohort study

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Abstract

Background

Sepsis is a serious medical condition with increasing prevalence and high mortality. The role of the autonomic nervous system in pathophysiology of sepsis has been increasingly researched. The objective of this study is to evaluate the Heart rate variability (HRV) as a predictor of mortality in septic patients.

Methods

This was a prospective cohort of patients diagnosed with sepsis. Patient recruitment was carried out at ICU in tertiary university hospital between March 2012 and February 2014. Clinical data and laboratory exams were collected at admission. Each patient underwent a 20-minute Holter and a 24-hour Holter on the first day of enrollment. The primary outcome was the 28-day all-cause mortality.

Results

A total of 63 patients were included. Patients were categorized into nonsurvivor group (n = 16) or survivor group (n = 47) depending on this endpoint. Survivors were younger (48.6 years vs. 63.0 years), had better renal function and lower values in severity scores (APACHE II and SOFA) compared to nonsurvivors. In the 20-minute Holter, SDNN, Total Power, VLF Power, LF Power and LF/HF of nonsurvivors were significantly lower than those of survivors (p = <0.001, p = 0.003, p = 0.002, p = 0.006, p = 0.009 respectively). ROC curve of SDNN was built, showing area under the curve of 0.772 (0.638–0.906) for mortality. The value of 17ms was chosen as best SDNN cutoff to discriminate survivors and nonsurvivors. In the Cox proportional regression, adjusted for SOFA score and for APACHE II, a SDNN $\leq$ 17ms was associated with a greater risk of death, with hazard ratios of 6.3 (1.4–28.0; p = 0.015) and 5.5 (1,2–24,8; p = 0.027), respectively. The addition of the dichotomized SDNN to the SOFA model reduced AIC and increased the concordance statistic and the R^2, indicating that predictive power of the SDNN + SOFA model is better than predictive power of SOFA only.

Competing interests: The authors have declared that no competing interests exist.

Conclusions

Several HRV parameters are reduced in nonsurviving septic patients. SDNN ≤17 is a risk factor for death in septic patients, even after adjusting for severity scores.

Introduction

Sepsis is a serious medical condition which prevalence has increased significantly in recent decades[1], making 31.5 million new cases to be expected in hospitals around the world each year[2]. Due to the high mortality associated with this condition, which can reach 48.6%[3], it is essential to search risk factors for death and predictive scoring systems to help clinical decision in septic patients. Predictive scoring systems such as APACHE II (Acute Physiology and Chronic Health disease Classification System II), SOFA (Sepsis-related Organ Failure Assessment), SAPS-3 (Simplified Acute Physiology Score III) and MODS (Multiple Organ Dysfunction *Score)* combine clinical and laboratory characteristics to assess the severity of illness. However, none of these scores considers in its composition changes in the autonomic nervous modulation caused by sepsis.

Heart rate variability (HRV) is a noninvasive indirect test to evaluate autonomic function [4, 5]. In normal situations, heart rate varies, indicating the heart's capacity to adapt to different situations. HRV measures the oscillation of the intervals between consecutive heart beats, which are related to, the influences of the autonomic nervous system on the sinus node[6]. Patients with sepsis have reduced HRV compared to healthy patients, as demonstrated in small studies[7–9]. Furthermore, HRV parameters such as low frequency (LF) power are positively correlated with APACHE II and SOFA[10] and negatively correlated with interleukins [11]. Small studies have suggested that sepsis survivors present HRV parameters (e.g., standard deviation of NN interval, SDNN) higher than nonsurvivors[12, 13]. However, no study has defined a specific HRV parameter and a cut-off point that can be used in practice for the prediction of the risk of death in septic patients. Thus, the use of HRV as an independent predictor of death in sepsis deserves further investigation.

The objective of this study was to evaluate the role of HRV—recorded both with the 20 minute and the 24 hour-Holter—as a predictor of death in patients with severe sepsis, defined by the presence of infection, the Systemic Inflammatory Response Syndrome criteria and evidence of organ dysfunction.

Materials and methods

Study design

This was a prospective cohort of patients diagnosed with severe sepsis. This report follows "Strengthening the Reporting of Observational studies in Epidemiology", the STROBE Statement[14].

Patient population

Patient recruitment was carried out at one of the Intensive Care Units of Hospital das Clínicas of the Universidade Federal de Minas Gerais (ICU-UFMG), Brazil, a mixed ICU with eight beds. From March 10th, 2012 to February 06th, 2014, all adult (i.e., 18 year-old or older) patients, hospitalized in the ICU-UFMG that had suspicion of sepsis at admission or during the ICU stay, and at least one organ dysfunction supposedly related to the infectious condition

were considered for potential eligibility. Sepsis was defined according to the Sepsis 2 Consensus[15] as being a Systemic Inflammatory Response Syndrome associated with a confirmed infection or strongly suspected infection. Systemic Inflammatory Response Syndrome was defined as the presence of at least two of the following: 1- Body temperature higher than 38˚C or lower than 36˚C; 2- Heart rate higher than 90/min, 3- Hyperventilation evidenced by respiratory rate higher than 20/min or PaCO2 lower than 32 mmHg; 4- White blood cell count higher than 12,000 cells/μl or lower than 4,000/μl or at least 10% of immature forms [16]. The presence of at least one organ dysfunction was based on severe sepsis definition of Surviving Sepsis Campaign[17]. Despite inclusion phase of this study was conducted prior to publication of the Sepsis 3 definitions[18], all included patients met the *criteria* for *Sepsis* proposed in this consensus.

Exclusion criteria were: moribund patients (death previewed for the next 24 hours), patients with proposal for exclusive palliative care, septic patients under antibiotic therapy for more than 48 hours prior to enrollment and patients with non-sinus rhythm or with pacemaker.

Study protocol and general procedures

This study was approved by the Ethics Research Committee of the Universidade Federal de Minas Gerais, Brazil, and all included patients or their family members signed a written informed consent. Clinical data was collected at admission and during the clinical follow-up of patients through a dedicated Clinical Report Form. The main variables collected were: age, gender, comorbidities, main diagnosis at the time of inclusion, primary site of infection and microbiological findings, antibiotic used, Sepsis related Organ Failure Assessment score (SOFA)[19] and Acute Physiology And Chronic Health Evaluation II (APACHE II)[20], both evaluated at the time of inclusion.

Heart rate variability analysis

Each patient enrolled in the study underwent a 3-channel Holter (Cardios® CardioLight model, São Paulo, Brazil) on the first day of enrollment. Two recordings were made sequentially: 20 minutes record and 24 hours record. Both Holter monitors were placed and removed from the patients by one of the medical researchers. The first measure (20 minutes record) was made with the patient in supine position and no intervention (nursing, physiotherapy, etc.) was made during its recording. The 24-hour measure was made without interference in the normal ICU care routine. Data analysis to derive HRV was performed using system specifically developed for this purpose (Cardios®), which automatically calculates the following indices of HRV in the time domain: Normal-to-Normal (NN) average interval, standard deviation of the NN interval (SDNN), square root of the squared mean of the difference of successive NN-intervals (r-MSSD), percentage of NN intervals deviated by more than 50 ms from adjacent NN-intervals (pNN50); and frequency domain with fast Fourier Transform (FFT) method: Total Power, Very low frequency power (VLF Power), Low frequency Power (LF Power), High frequency power (HF Power) and Ratio between LF and HF (LF/HF). In the 24-hour Holter, HRV analysis was performed only in the time domain. We have performed manual review of all Holter's automatic interpretation, including the rhythm and the complexes recorded (e.g., normal QRS, ventricular extrasystoles, supraventricular extrasystoles, tachycardia, bradycardia, artifacts etc.). Artifacts and irregular beats (extrasystoles, noise and missing beats) were manually deleted before HRV analyses. In the 24-hour Holter, HRV in the time domain was calculated over an entire 24-hour period. In the 20 minutes Holter, HRV was calculated both in the time domain and in the frequency domain over the entire first 10 minutes of recording.

Outcomes

The primary outcome of this study was the all-cause mortality at 28 days of follow-up. Patients were categorized into nonsurvivor group or survivor group depending on the primary endpoint. Several HRV parameters were compared between these two groups.

Sample size

The sample size calculation tested the hypothesis that SDNN distribution would be the same between surviving and nonsurviving patients. The statistical test used was the nonparametric Mann-Whitney that assumes that the data is measured at least in ordinal scale. The formulas adopted for sample size calculation are described in Zhao, Rahardja, & Qu[21] and implemented in software R[22]. A pilot sample constituted by the first twenty patients included in the study (6 deaths and 14 survived) was considered to estimate the parameters required to calculate the final sample size. Tertiles of SDNN were calculated from this pilot sample, defining three ranges. The proportion of subjects in each of these three ranges was obtained. Keeping the allocation ratio (i.e., survivors and non survivors) similar to that observed in the pilot study, a requirement of 58 patients (44 survivors and 14 deaths) was defined for the final analysis. We considered a type I error of 5% and 80% power.

Statistical analysis

The normality of each continuous variable was assessed by means of the Kolmogorov-Smirnov test. Data obtained from continuous variables are expressed as either mean and standard deviation if they have normal distribution, or median and interquartile range (25th and 75th percentiles) if they have non-normal distribution. Data concerning categorical variables are expressed as absolute numbers and proportions. Clinical characteristics of survivors and nonsurvivors were compared using Student *t* test, Mann–Whitney test and exact Fisher test according to the type and the distribution of the variable. In order to choose the best cut-off point of SDNN, a ROC curve was used having death as the reference and SDNN as the parameter test[23]. Then the dichotomized SDNN was used to build Kaplan–Meier survival curves, and they were compared by log-rank test. The influence of HRV variables on survival was studied with Cox regression. Considering the small sample, we used modeling strategies to avoid the risk of overfitting and the excessive "optimism" of the model [24]. The calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the Grønnesby and Borgan test (GF Test). The performance of those prediction models was assessed using concordance measure, Explained variation (R^2) and Akaike Information Criterion (AIC). In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we set the time of 28 days for the predictions of the risks, and calculated continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI). A $p < 0.05$ was considered statistically significant for all analyses.

All the statistical analyses were conducted in SPSS version 23 (SPSS Inc., Chicago, IL, USA) and R version 3.3.0 (The R Foundation for Statistical Computing)[22] using the packages *rms, survMisc and survIDINRI*.

Results

From a total of 99 patients with sepsis assessed during the study period, 79 patients were initially identified as eligible. Of these, two patients were excluded because of technical problems with the Holter equipment and 14 patients were excluded due to atrial fibrillation. Thus, 63 patients were included in the final analyses (Fig 1).

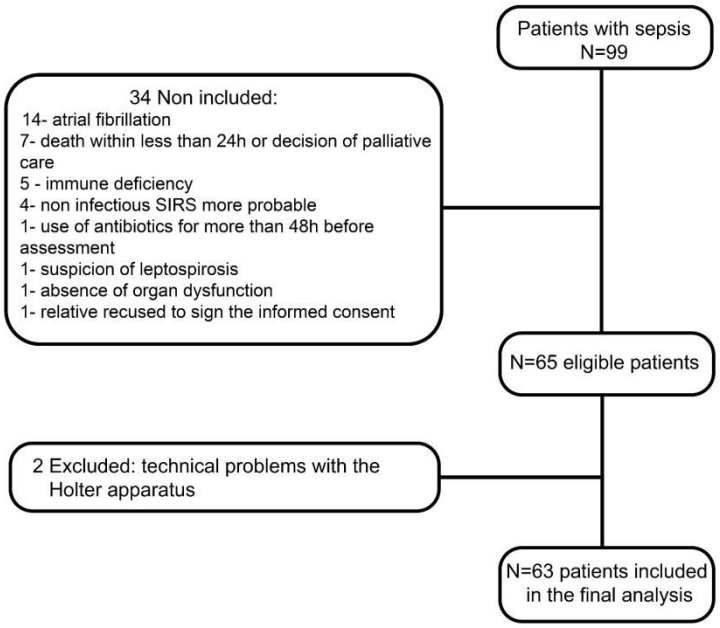

Fig 1. Flowchart of study procedures.

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The baseline characteristics of the included patients are shown in Table 1, stratified according to the 28-day all-cause mortality. As presented, 16 (25.4) out of the 63 patients died during the follow-up of 28 days. Survivors were younger (48.6 years vs. 63.0 years), had better renal function and lower values in severity scores (APACHE II and SOFA) compared to nonsurvivors. There were no significant differences in other baseline characteristics.

HRV measures of each group are listed in Table 2. In 20-minute Holter, SDNN, Total Power, VHF Power, LF Power and LF/HF of non-survivors were significantly lower than those of survivors. There was no statistically significant difference in HRV measured in the 24 hours Holter between the two subgroups.

An unadjusted Cox regression for HRV parameters that were different between the two groups was built. It can be seen in Table 3. Since SDNN reached the larger difference between survivors and nonsurvivors, ROC curve was built to evaluate the accuracy of this parameter to predict the 28-day all-cause-mortality; as depicted in Fig 2, an area under the curve of 0.772 (0.638–0.906) was found. Then, because it presents the best relationship between sensitivity and specificity, 17ms was chosen as the cutoff point for SDNN. In order to test the possible clinical application of this cut-off point as a predictor of mortality in sepsis, patients were divided into two groups (SDNN$>$ 17ms and SDNN$\leq$17ms). As can be seen in Table 4, there is no significant difference between the baseline features of these two groups. Kaplan-Meier curve of these two groups (Fig 2) found log rank p = 0.003, showing higher mortality of the patient group with SDNN$\leq$17ms. For the analysis of 28 days mortality, Cox regression for this dichotomous variable was made adjusted by the SOFA showing HR 6.3 (1.4–28.0; p = 0.015)

Table 1. Baseline characteristics of the patients.

Attribute	Survivors (n = 47)	Nonsurvivors (n = 16)	p-Value
Age (y), SD	49 (17.8)	63 (17.9)	0.007
Male gender, %	27 (57.4)	11 (68.8)	0.425
APACHE II, SD	14.15 (5.93)	21.94 (8.45)	<0.001
SOFA, SD	6.91 (2.84)	10.56 (4.21)	0.004
Mechanical Ventilation, %	24 (51.1)	12 (75.0)	0.095
Underlying disease, n (%)			
Cirrhosis	2 (4.3)	1 (6.2)	0.896
Dialytic patients	4 (8.5)	1 (6.2)	0.773
Hypertension	18 (38.3)	9 (56.2)	0.369
Diabetes	11 (23.4)	4 (25.0)	0.354
Stroke	7 (14.9)	2 (12.5)	0.793
Peripheral arterial disease	1 (2.1)	0 (0.0)	0.801
Heart Failure[b]	4 (8.5)	3 (18.8)	0.459
Coronary artery disease	4 (8.5)	3 (18.8)	0.288
Neoplasia	4 (8.5)	2 (12.5)	0.541
Chronic Obstructive Pulmonary Disease	3 (6.4)	0 (0.0)	0.453
Smoking	13 (27.7)	2 (12.5)	0.112
Laboratory data			
Hemoglobin (g/dL)	10.0 (1.86)	10.4 (3.15)	0.582
White blood cells (per mm3)	16171 (9653)	17929 (10005)	0.535
Platelet × 10^3	228 (121)	187 (116)	0.240
Lactate[a] (mmol/L)	1.80 (1.6–4.0)	2.55 (1.6–4.2)	0.059
C-reactive protein (mg/L)	229 (115)	287 (109)	0.082
Urea (mg/dL)	55.4 (34.4)	107.3 (47.4)	<0.001
Creatinine[a] (mg/dL)	0.72 (0.49–1.6)	2.28 (0.96–2.85)	0.004
Creatinine clearance[a] (mL/min)	108 (58–157)	29 (18–86)	0.005
Glucose (mg/dL)	144 (56)	171 (99)	0.176
International normalized ratio	1.2 (1.1–1.4)	1.4 (1.2–2.0)	0.026
Infection source, n (%)			
Respiratory tract	16 (34.0)	7 (43.8)	0.687
Intra-abdominal	8 (17.0)	4 (25.0)	0.737
Urinary tract	5 (10.6)	1 (6.3)	0.990
Catheter	7 (14.9)	2 (12.5)	0.860
Soft tissue	3 (6.4)	1 (6.3)	0.563
Central Nervous System	0 (0.0)	1 (6.3)	0.561
Undetermined	7 (14.9)	0 (0.0)	0.239
Miscellaneous	1 (2.1)	0 (0.0)	0.561

Data presented as mean (SD), median (interquartile range) or absolute number (percentage).

[a] = variables with non-normal distribution;

[b] = Heart Failure was defined as previous echocardiogram with ejection fraction ≤ 50%.

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for SDNN≤17ms and HR 1.3 (1.1–1.4; p = 0.001) for SOFA. Following a similar trend, Cox regression for dichotomous SDNN adjusted by the APACHE II showed HR 5.5 (1.2–24.8; p = 0.027) for SDNN≤17ms and HR 1.1 (1.02–1.12; p = 0.004) for APACHE II.

Considering the small sample, we used modeling strategies to avoid the risk of overfitting and the excessive "optimism". For the model with SOFA and dichotomous SDNN, optimism

Table 2. Heart rate variability measures.

Parameter	Survivors (n = 47)	Nonsurvivors (n = 16)	p-Value
20 Minutes Holter			
Artifacts and irregular beats[a] (%)	2.0 (1.0–5.3)	2.5 (0.3–8.0)	0.112
Day recordings[a,b], %	37 (78.7)	13 (81.3)	0.829
NN (ms)	658.2 (166.9)	606.0 (130.4)	0.261
SDNN (ms)[a]	19.0 (10.0–36.0)	8.5 (5.0–14.5)	<0.001
rMSSD (ms)[a]	9.0 (6.0–28.0)	7.5 (6.0–12.8)	0.199
pNN50 (%)[a]	0.13 (0.00–4.73)	0.14 (0.00–0.63)	0.482
Total Power (ms^2)[a]	136.0 (46.0–590.0)	24.0 (5.0–173.5)	0.003
VLF Power (ms^2)[a]	90.0 (27.0–243.0)	9.5 (2.5–72.5)	0.002
LF Power (ms^2)[a]	18.0 (6.0–83.0)	2.0 (1.0–24.0)	0.006
HF Power (ms^2)[a]	9.0 (5.0–51.0)	6.5 (2.3–57.0)	0.343
LF/HF[a]	1.29 (0.47–3.63)	0.40 (0.21–1.84)	0.009
24-Hour Holter			
Artifacts and irregular beats[a] (%)	1.0 (1.0–1.0)	1.0 (1.0–2.3)	0.955
NN (ms)	661.0 (133.4)	622.9 (123.5)	0.345
SDNN (ms)	58.2 (39.4)	50.7 (24.5)	0.402
rMSSD (ms)[a]	14.0 (8.0–28.3)	15.5 (10.0–29.3)	0,944
pNN50 (%)[a]	0.55 (0.05–3.11)	0.66 (0.24–2.78)	0,688

NN = Normal-to-Normal; SDNN = standard deviation of the NN interval; rMSSD = Root Mean Square of the Successive Differences; pNN50 = proportion of adjacent NN intervals which differ by more than 50 ms; VLF Power = Very Low Frequency *Power*; LF Power = Low Frequency Power; HF Power = High Frequency Power; LF/HF = Low Frequency Power/ High Frequency Power. Data presented as mean (SD), median (interquartile range)

[a] = variables with non-normal distribution;

[b] = Day recordings was considered when the Holter monitor was placed between 8:00 a.m. and 6:00 p.m;

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was calculated at 0.1075 (and the shrinkage factor was 0.8925), calculated HR 5.2 (1.2–23.0) for SDNN $\leq$17, with p = 0.03. For the model with APACHEII and dichotomous SDNN, optimism was calculated at 0.1834 (and the shrinkage factor was 0.8166), calculated HR 4.0 (0.9–18.1) for SDNN $\leq$17, with p = 0.07.

Finally, the calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the GF Test, showing, for the model with SOFA, p = 0.550, and, for the model with SOFA + SDNN, p = 0.600, indicating that there are no calibration problems. The GF test was valid under the usual assumption of proportional hazards of the Cox model. This assumption was not violated in the models considered, since the global risk proportionality test found p = 0.463 for the SOFA model only and p = 0.633 for the model With

Table 3. Cox regression for heart rate variability parameters in 20-minute Holter.

Parameter	HR	95% CI	p-Value
SDNN (ms)	0.937	0.883–0.995	0.033
Total Power (ms^2)	0.999	0.997–1.001	0.273
VLF Power (ms^2)	0.998	0.996–1.001	0.269
LF Power (ms^2)	0.998	0.993–1.003	0.352
LF/HF	0.619	0.380–1.009	0.054

HR = Hazard ratio; SDNN = standard deviation of the NN interval; VLF Power = Very Low Frequency *Power*; LF Power = Low Frequency Power; LF/HF = Low Frequency Power/ High Frequency Power

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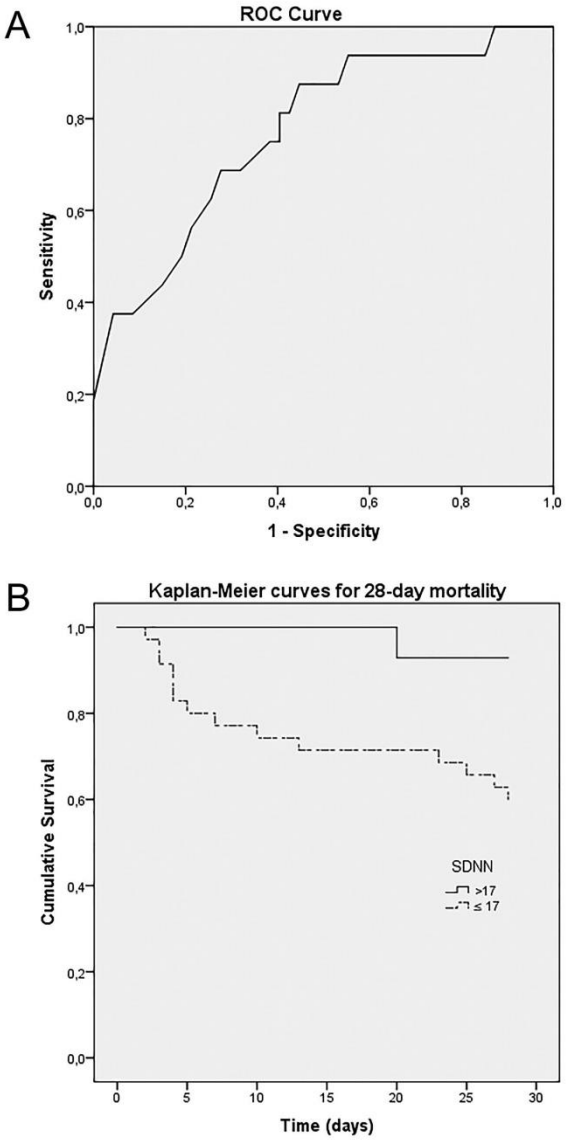

Fig 2. ROC Curve of SDNN and Kaplan-Meier curves for 28-day mortality. A: The ROC Curve of SDNN in 20-minute Holter in predicting 28-day mortality in patients with sepsis. The area under the curve was 0.772 (0.638–0.906). The value of 17ms was chosen as the cutoff point for SDNN (sensibility of 87.5%, specificity of 55.3%, positive likelihood ratio of 1.96 and negative likelihood ratio of 0.28). B: Kaplan-Meier curve showing 28-day mortality in septic patients with SDNN≤17ms (mean survival time of 21.3 days; 17.8–24.8) and

SDNN>17ms (mean survival time of 27.4 days; 26.6–28.2). The survival curves were compared using log-rank test, p = 0.003, showing higher mortality in the patient group with SDNN≤17ms.

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SOFA + SDNN. The addition of the dichotomized SDNN to the SOFA model increased the concordance statistic from 0.725 to 0.805 and the R^2 of the model changed from 0.167 to 0.277. Furthermore, the AIC for the first model [SOFA] was 119.07 versus 112.17 for the second [SDNN + SOFA]. Greater values for concordance and R^2 indicate a better model while smaller values for AIC indicate a better model. In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we set the time of 28 days for the predictions of the risks, and calculated IDI (0.122; CI 0.043–0.235, p = 0.00) and NRI (0.408; CI 0.168–0.643, p = 0.01). These results suggest significant gains in the reclassification with the inclusion of SDNN in the model. All statistical analysis with the dichotomous SDNN can be seen in the Table 5.

Table 4. Baseline characteristics of groups of SDNN≤17ms and SDNN>17ms.

Attribute	SDNN≤17 (n = 35)	SDNN>17 (n = 28)	p-Value
Age (y), SD	55 (20)	49 (17)	0.288
Male gender, %	22 (62.9)	16 (57.1)	0.796
APACHE II, SD	17.4 (8.16)	14.54 (6.16)	0.129
SOFA, SD	8.31 (3.68)	7.25 (3.43)	0.244
Mechanical Ventilation, %	22 (62.9)	14 (50.0)	0.306
Underlying disease, n (%)			
Cirrhosis	0 (0.0)	3 (10.7)	0.183
Dialytic patients	4 (11.4)	1 (3.6)	0.371
Hypertension	16 (45.7)	11 (39.3)	0.337
Diabetes	10 (28.6)	5 (17.9)	0.380
Stroke	4 (11.4)	5 (17.9)	0.192
Peripheral arterial disease	1 (2.86)	0 (0.0)	1.00
Heart Failure[b]	4 (11.4)	3 (10.7)	0.660
Coronary artery disease	6 (17.1)	1 (3.6)	0.234
Neoplasia	2 (5.7)	4 (14.3)	0.350
Chronic Obstructive Pulmonary Disease	2 (5.7)	1 (3.6)	0.899
Smoking	6 (17.1)	9 (32.1)	0.388
Laboratory data			
HB (g/dL)	10.3 (2.6)	9.9 (1.6)	0.528
White blood cells (per mm3)	14882 (7918)	18787 (11314)	0.113
Platelet × 103	205 (112)	233 (116)	0.365
Lactate[a] (mmol/L)	1.70 (1.2–2.8)	2.0 (1.15–2.83)	0.787
C-reactive protein (mg/L)	276 (100)	204 (124)	0.013
Urea (mg/dL)	73.1 (40.7)	63.0 (48.1)	0.382
Creatinine[a] (mg/dL)	1.27 (0.67–2.55)	0.71 (0.49–1.68)	0.223
Creatinine clearance[a] (mL/min)	77 (28–125)	106 (51–154)	0.307
Glucose (mg/dL)	158 (78)	141 (57)	0.322
International normalized ratio[a]	1.24 (1.10–1.43)	1.29 (1.13–1.59)	0.302

Data presented as mean (SD), median (interquartile range) or absolute number (percentage).

[a] = variables with non-normal distribution;

[b] = Heart Failure was defined as previous echocardiogram with ejection fraction ≤ 50%.

https://doi.org/10.1371/journal.pone.0180060.t004

Table 5. statistical analysis with the dichotomous SDNN.

COX regression adjusted by SOFA				
Variable		**HR**	**CI**	**p-Value**
SDNN≤17ms		6.3	1.4–28.0	0.015
SOFA		1.3	1.1–1.4	0.001
COX regression adjusted by APACHE II				
Variable		**HR**	**CI**	**p-Value**
SDNN≤17ms		5.5	1.2–24.8	0.027
APACHE II		1.1	1.02–1.12	0.004
Model with dichotomous SDNN and SOFA optimism adjusted				
Variable		**HR**	**CI**	**p-Value**
SDNN≤17ms		5.2	1.2–23.0	0.03
Model with dichotomous SDNN and APACHE II optimism adjusted				
Variable		**HR**	**CI**	**p-Value**
SDNN≤17ms		4.0	0.9–18.1	0.07
Models of mortality prediction				
Model	**GF Test**	**Concordance**	**R^2**	**AIC**
SOFA	p = 0.550	0.725	0.167	119.07
SOFA + SDNN≤17ms	p = 0.600	0.805	0.277	112.17

HR = Hazard Ratio; CI = Confidence Interval; R^2 = Explained variation; AIC = Akaike Information Criterion. For the analysis of 28 days mortality, Cox regression for dichotomous SDNN was made adjusted by the SOFA and adjusted by APACHE II. For the model with SOFA and dichotomous SDNN, optimism was calculated at 0.1075 (and the shrinkage factor was 0.8925). For the model with APACHEII and dichotomous SDNN, optimism was calculated at 0.1834 (and the shrinkage factor was 0.8166). The calibration of the models with only SOFA and with dichotomized SDNN + SOFA was assessed with the the GF Test, indicating that there are no calibration problems. The performance of those prediction models was assessed. Greater values for concordance and R^2 indicate a better model while smaller values for AIC indicate a better model. In order to evaluate the reclassification of the SOFA + SDNN model compared to the SOFA model only, we calculated IDI (0.122; CI 0.043–0.235, p<0.01) and NRI (0.408; CI 0.168–0.643, p = 0.01). These results suggest significant gains in the reclassification with the inclusion of SDNN in the model.

https://doi.org/10.1371/journal.pone.0180060.t005

In the survivor group, seven patients had undetermined infection source, while zero patients had undetermined infection source among the non-survivors. The results regarding the association of SDNN values and the outcome remained unchanged in the analysis excluding these seven patients. Thus, SDNN value was significantly higher among survivors as compared to non-survivors, when evaluated in the 20-minute Holter: 18.50 (10.00–34.50) and 8.50 (5.00–14.50), respectively, with p = 0.003. Cox regression for dichotomous SDNN adjusted by the SOFA or APACHE II revealed similar results (HR 7.1 [1.6–32.8]; p = 0.012, for SDNN≤17ms and HR 1.3 [1.1–1.5]; p < 0.001, for SOFA. Following a similar trend, Cox regression for dichotomous SDNN, adjusted by the APACHE II, showed HR 5.1 (1.1–22.9; p = 0.033) for SDNN≤17ms, and HR 1.1 (1.03–1.12; p = 0.001) for APACHE II.

Discussion

In this prospective study with septic patients, we found that several HRV parameters obtained in the 20-minute Holter were correlated to 28-day all-cause mortality. In particular, SDNN ≤17 is associated with increased risk of death even after adjustment to SOFA or APACHE II. In contrast, HRV parameters in 24-hour Holter were not correlated to 28-day all-cause mortality.

Normal immune and physiologic responses eradicate pathogens through complex process involving generation of proinflammatory and anti-inflammatory mediators. The

pathophysiology of sepsis is due to the inappropriate regulation of these normal reactions that becomes generalized and deleterious[25]. The role of the autonomic nervous system has been increasingly studied in the context of sepsis. Animal model studies suggest that vagus nerve stimulation increases the secretion of corticotropin-releasing hormone (CRH), ACTH, and cortisol[26]. Likewise, vagotomy attenuated fever response and corticosterone response produced by cytokines[27]. Acetylcholine, the principle vagal neurotransmitter, has an anti-inflammatory effect, attenuating the release of cytokines TNF, IL-1beta, IL-6 and IL-18 and preventing the development of shock[28]. Treatment with nicotine, a selective cholinergic agonist, and with choline, a precursor in the biosynthesis of acetylcholine, improved survival in experimental models of sepsis[29, 30]. This results supports that vagal afferent pathway are involved in peripheral cytokine-to-brain communication.

Several methods have already been developed to evaluate the autonomic function. Some of the tests would not be adequate for this study because they require active participation of patients. This is the case of the Valsalva's manoeuvre, the deep breathing method, the isometric handgrip test, the mental arithmetic, and the active standing methods [31]. Other methods require infusion of drugs (e.g. baroreflex sensitivity testing with intravenous administration of phenylephrine), which could interfere in the treatment of patients with sepsis, making its use unfeasible and potentially harmful[31]. The serum catecholamines dosage can be used to evaluate the autonomic nervous system; however it has some limitations, providing information about the global autonomic function and not about organ-specific sympathetic function[32]. Additionally, the plasma concentration of norepinephrine, for example, depends not only on sympathetic activity, but also on norepinephrine reuptake and noradrenaline clearance from circulation [33]. Finally, patients with septic shock often receive external noradrenaline infusion as treatment. HRV is one of the most popular methods used to evaluate the autonomic function, presenting the advantages of being non-invasive and the fact that there are many commercial devices that provide the automated measurement of HRV[4].

The mechanism by which HRV is reduced in septic patients is not yet fully understood. In addition to the participation of the autonomic nervous system, recent studies in animals and cell cultures have shown that Lipopolysaccharides (amphiphilic components of the outer wall of Gram-negative bacteria) act in two ways on the hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN) of the atrial cells: directly inhibiting HCN-channels and indirectly sensitizing HCN-channels for sympathetic activation[34, 35].

Although there are no reference ranges of HRV parameters globally accepted, this study suggests that septic patients have reduced HRV compared to the general population. For example, in this study, the SDNN mean for surviving patients were 19.0ms and for nunsurviving patients were 8.5ms, while Kim et al found SDNN mean of 39.6ms for normal Korean Population[36].

The physiological meaning of each HRV parameter is very complex and not fully known. SDNN reflects all the cyclic components responsible for HRV (including sympathetic and parasympathetic activity) and is the most commonly used parameter[4]. HF Power reflects the vagal activity (parasympathetic) on the sinus node[37]. LF Power reflects the sympathetic and parasympathetic activity, with alleged predominance of the first [38]. The LF/HF ratio, in HRV, was classically described as an index of the sympathetic/parasympathetic balance[38]. However, several studies have shown that this interpretation is imprecise and simplistic and that the physiological meaning of this ratio remains controversial[39]. A reduced LF/HF ratio is associated with an increased risk of death in septic patients[40]. In this study, nonsurviving patients had lower Total Power, VLF Power, LF Power and LF / HF ratio than survivors. This finding is similar to that found in previous research[10, 12, 41].

Unlike the study by Duke et al.[13], in ours, HRV parameters were significantly different between survivors and nonsurvivors only in the 20-minute Holter. Holter with shorter periods of record is potentially more useful for be used in critical care patients, including those with sepsis, because these patients present immediate risk of death and therefore need a fast tool for definition of severity. Moreover, in such a dynamic condition as sepsis, a long time recording may suffer interference from therapeutic measures instituted, which can partially explain the negative results found with the 24-hour Holter in this study.

Global HRV parameters such as SDNN and TP were lower in nonsurviving patients of this study, which is consistent with the findings of previous studies[12, 13]. Chen et al[12] had demonstrated that SDNN would be a significant independent variable in the prediction of in-hospital mortality for emergency department patients with sepsis, although these authors did not present cut-off point for this HRV parameter. The cut-off of 17ms for SDNN obtained in a short time Holter record found in our study of might represent a useful tool due to identify patients with higher risk of death among septic patients in the daily practice. It worth mentioning that this result was maintained after adjustment for APACHE II or SOFA, indicating that this value could be an independent risk factor for mortality. Although the results found in the present study are statistically significant, the fact that the confidence intervals on the hazard ratio for SDNN are large reflect the small number of patients in our study, which indicates the need of confirming these results in larger series of septic patients.

Furthermore, concordance measure, R^2, AIC, IDI and NRIindicate that predictive power of the SDNN + SOFA model is better than predictive power of SOFA only, which reinforces the possible clinical utility of this measure.

Study limitations

The small number of patients is the main limitation of this study. In order to minimize this problem, it was used advanced modeling techniques to avoid the risk of overfitting and also to adjust the coefficients for optimism. This analysis kept SDNN $\leq$17 as a risk factor for death for the model with SOFA but not for the model with APACHE II. Another limitation of this study is the possible influence of other clinical conditions known to affect HRV as congestive heart failure, coronary artery disease, diabetes or mechanical ventilation use[4]. However, there was no difference between nonsurvivor and survivor groups about the frequency of these comorbidities (Table 1). Body temperature and medications (e.g., sedatives, beta-blockers, inotropic drugs) that can affect HRV were not evaluated in this study. Day-night variation in heart rate variability was not considered in the design of this study, although its existence has already been demonstrated in healthy volunteers with endotoxaemia[42]. The majority of 20-minute Holter measures were made during the day, and there were no significant differences between the percentage of day recordings from surviving and non-surviving groups. Furthermore, we do not know whether this day-night difference occurs in ICU patients. Finally, all included patients were enrolled before the publication of the Sepsis 3 Consensus[18], reason for which we were not able to use the new definitions of sepsis in the present study. Despite this, all patients included in this study had a SOFA score $\geq$ 2 points and met criteria for Sepsis based on this new consensus in a post hoc analysis.

Considering the small number of patients in this single-center study, we believe that the results found here are preliminary, hinting at the potential predictive capability of a dichotomized SDNN, what should be confirmed in future studies through external validation of the results in a separate population.

Conclusions

Several HRV parameters are reduced in nonsurviving septic patients. Although further studies are necessary to confirm this finding, SDNN $\leq$17 is suggested as an independent risk factor for death in septic patients.

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Author Contributions

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Project administration: Fábio M. de Castilho, Antonio Luiz P. Ribeiro, José Luiz P. da Silva, Vandack Nobre, Marcos R. de Sousa.

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9 – ANEXOS

9.1 – Aprovação do Comitê de Ética em Pesquisa (COEP) da UFMG

UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 0319.0.203.000-11

Interessado(a): Prof. Vandack Alencar Nobre Jr.
Departamento de Clínica Médica
Faculdade de Medicina - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 16 de dezembro de 2011, após atendidas as solicitações de diligência, o projeto de pesquisa intitulado **"Estudo da função endotelial e cardiovascular em pacientes portadores de sepse: implicações diagnósticas, definição de risco e terapêutica"** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
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UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 0319.0.203.000-11

Interessado(a): Prof. Vandack Alencar Nobre Jr.
Departamento de Clínica Médica
Faculdade de Medicina - UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP analisou e aprovou, no dia 03 de setembro de 2012, as alterações, abaixo relacionadas, no projeto de pesquisa intitulado **"Estudo da função endotelial e cardiovascular em pacientes portadores de sepse: implicações diagnósticas, definição de risco e terapêutica"**:

- Inclusão de avaliações clínicas e laboratoriais que visam investigar a ocorrência de alterações neuropsiquiátricas dos pacientes;
- Termo de Consentimento Livre e Esclarecido relacionado.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto.

Profa. Maria Teresa Marques Amaral
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2° andar – Sala 2005 – Cep:31270-901 – BH-MG
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9.2 – Declaração de Defesa

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS APLICADAS À SAÚDE DO ADULTO

UFMG

FOLHA DE APROVAÇÃO

VARIABILIDADE DA FREQUÊNCIA CARDÍACA COMO PREDITOR DE MORTALIDADE NA SEPSE

FÁBIO MORATO DE CASTILHO

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIAS APLICADAS À SAÚDE DO ADULTO, como requisito para obtenção do grau de Doutor em CIÊNCIAS APLICADAS À SAÚDE DO ADULTO, área de concentração CIÊNCIAS APLICADAS À SAÚDE DO ADULTO.

Aprovada em 24 de novembro de 2017, pela banca constituída pelos membros:

Prof. Marcos Roberto de Sousa - Orientador
HC-UFMG

Prof. Antonio Luiz Pinho Ribeiro - Coorientador
UFMG

Prof. Vandack Alencar Nobre Júnior - Coorientador
UFMG

Prof. Fernando Antônio Botoni
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Prof. Marco Paulo Tomaz Barbosa
HC-UFMG

Prof. Valdo Jose Dias da Silva
UFTM

Profa. Flávia Ribeiro Machado
UNIFESP

Belo Horizonte, 24 de novembro de 2017.