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Reprogramação metabólica mitocondrial em linfócitos como um biomarcador de desfechos clínicos em pacientes com choque séptico

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Reprogramação metabólica mitocondrial em linfócitos como um biomarcador de desfechos clínicos em pacientes com choque séptico

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de doutor em Ciências Biológicas: Bioquímica.

Orientador: Prof. Dr. Luis Valmor Cruz Portela

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“Em Deus nós confiamos, todos os demais tragam dados”

William Edwards Deming (1900-1993)

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A todos, o meu muito obrigado.

RESUMO

A sepse é definida como uma disfunção orgânica causada por uma resposta desregulada do hospedeiro a uma infecção. A sua forma mais grave, o choque séptico, é caracterizado como sendo o subgrupo da sepse no qual as anormalidades circulatórias e metabólicas associadas a infecção são profundas o suficiente para aumentar substancialmente a mortalidade destes pacientes. Isso acontece devido ao desenvolvimento de falências multiorgânicas, as quais, atualmente, são reconhecidas como sendo em decorrência, principalmente, de uma disfunção metabólica; cenário no qual se destaca a ineficiência na produção de ATP via fosforilação oxidativa. Este estudo, realizado com pacientes com choque séptico, propõe mudanças no metabolismo mitocondrial de linfócitos como biomarcador clínico. Considerando este contexto, investigamos: i. uma coorte prospectiva avaliando o papel da disfunção mitocondrial em linfócitos isolados de pacientes com choque séptico, sua evolução temporal e a sua associação com o prognóstico destes pacientes; ii. um estudo avaliando a associação entre a bioenergética mitocondrial, as variáveis perfusionais e os níveis de lactato numa população de choque séptico, na fase pós-ressuscitação volêmica; iii. um estudo analisando as diferentes assinaturas metabólicas da mitocôndria e sua possível modulação por antimicrobianos usados na rotina da UTI; iv. a associação entre o metabolismo mitocondrial dos linfócitos e a atividade de interleucinas pró- e anti-inflamatórias no choque séptico; e finalmente, v. realizamos uma revisão sobre o estado da arte da disfunção mitocondrial na sepse e sua importância na fisiopatologia, as possíveis técnicas

para avaliação do metabolismo mitocondrial no ambiente clinico, e o seu valor prognóstico.

ABSTRACT

Sepsis is defined as an organ dysfunction caused by a dysregulated host response to an infection. Its most severe form, septic shock, is characterized as the sepsis subgroup in which the circulatory and metabolic abnormalities associated with infection are profound enough to substantially increase the mortality of these patients. This evolves through the development of multiorgan failures, which are currently recognized as being mainly due to metabolic dysfunction; scenario in which the inefficiency in the production of ATP via oxidative phosphorylation stands out. This work carried out with patients with septic shock proposes that changes in the mitochondrial metabolism of lymphocytes may serve as a clinical biomarker. Taking into account this context, we performed: i. a prospective cohort evaluating the role of mitochondrial dysfunction in lymphocytes isolated from patients with septic shock, its temporal evolution, and its association with the prognosis of these patients; ii. a study evaluating the association between mitochondrial activity, perfusion variables, and lactate levels in a population with septic shock, in the post-volemic resuscitation phase; iii. a study analyzing the different metabolic signatures of mitochondria and their possible modulation by antimicrobials routinely used in the ICU; iv. a study on the association between the mitochondrial metabolism of lymphocytes and the activity of pro and anti-inflammatory interleukins in septic shock; and finally, v. we performed a review of the state of the art of mitochondrial dysfunction in sepsis and its importance in pathophysiology, possible methods to assess mitochondrial metabolism in the clinical settings, and its prognostic value.

APRESENTAÇÃO

Esta tese está organizada em três Partes, cada uma sendo constituída dos seguintes itens:

Parte I: Resumo, Resumo em inglês (abstract), Lista de figuras, Lista de abreviações, Introdução e Objetivos;

Parte II: Resultados, apresentados na forma capítulos constituídos de artigos científicos;

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oxigênio acoplado a produção de ATP (Estágio III). Azido de sódio mais antimicina A foram adicionados para inibir o Complexo IV mitocondrial, permitindo a estimativa do fluxo de oxigênio não-OXPHOS (ROX), e estes fluxos foram descontados para obter uma taxa de fluxo autêntica derivada de todos os estágios acima mencionados, e fluxos de oxigênio específicos para a massa tissular foram comparados em diferentes substratos, estágios de acoplamento e inibidores (protocolo SUIT). Linfócitos foram permeabilizados com digitonina a 0.005% w/v. Extraído de Mitochondrial Physiology Network 07.08(09):1-7 (2017) Version 09: 2017-11-16.

LISTA DE ABREVIATURAS

ADP – difosfato de adenosina

ATP – trifosfato de adenosina

BCE – *biochemical coupling efficiency*

DAMP - *danger-associated molecular patterns*

DNA – ácido desoxirribonucleico

IFN - interferon

IL - interleucina

NLR – nucleotide-like receptor

NK – *natural killer*

NOD - *nucleotide-binding oligomerization domain*

OXPHOS – fosforilação oxidativa

PAMP - *pathogen-associated molecular patterns*

PCR – proteína C reativa

PIICS - inflamação-imunosupressão e catabolismo persistente

ROS – espécies reativas de oxigênio

SAPS – *Simplified Acute Physiology Score*

SOFA - *sequential organ failure assessment*

TLR – *toll like receptor*

TNF – *tumoral necrosis factor*

UTI – Unidade de Terapia Intensiva

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Capítulo 1 - Introdução

A sepse é uma das síndromes cujo relato é um dos mais antigos da medicina, já sendo descrita desde Hipócrates (Angus DC, van der Poll T, 2013). Atualmente, a sepse é definida como “uma disfunção orgânica causada por uma resposta desregulada do hospedeiro a uma infecção” (Singer M, Deutschman CS, Seymour CW, 2016). A sua forma mais grave, o choque séptico, é caracterizado como sendo o subgrupo da sepse no qual as anormalidades circulatórias e metabólicas associadas a infecção são profundas o suficiente para aumentar substancialmente a mortalidade (Singer M, Deutschman CS, Seymour CW, 2016). Há um quadro de hipotensão irresponsiva a administração de fluidos, necessitando-se o uso de drogas vasoativas (vasopressores) para se manter um alvo pressórico minimamente aceitável, almejando-se uma oferta adequada de oxigênio para os tecidos periféricos (Cecconi M, Evans L, Levy M, 2018). O reconhecimento destas condições traz o benefício de poder gerar uma pronta ação em resposta a esta condição, na intenção de diminuir sua morbimortalidade.

Trata-se de um problema de saúde global, com uma estimativa de 19 milhões de casos anuais e uma mortalidade da ordem de 5 milhões, com maior prevalência em países de baixa e de média renda (Rudd KE, Kissoon N, Limmathurotsakul D, 2018). É considerada, pela Organização Mundial da Saúde, como uma prioridade global de saúde desde 2017 (Cecconi M, Evans L, Levy M, 2018). No Brasil, a sepse tem uma elevada incidência, especialmente em UTIs, acarretando uma elevada taxa de mortalidade (Machado FR, Cavalcanti AB, Bozza FA, 2017), sem melhora significativa desde o início deste século (Silva E,

Pedro MA, Sogayar ACB, 2004). A taxa de incidência projetada no nosso país é de 420000 casos de adultos internados em UTI por ano, com uma mortalidade estimada de 230000 pessoas (Machado FR, Cavalcanti AB, Bozza FA, 2017). Além disso, é associada a elevados custos relacionados aos cuidados de saúde, especialmente nos não-sobreviventes, e a uma maior mortalidade em pacientes internados no sistema público de saúde, em comparação aos pacientes tratados no sistema privado (Sogayar AMC, Machado FR, Rea-Neto A, 2008).

A resposta desregulada do hospedeiro é o evento que vai diferenciar uma infecção com uma resposta auto-limitada de um processo que evolua com disfunções orgânicas e a uma maior letalidade, como é o caso da sepse. Uma exposição a PAMPs, tais como lipopolissacarídeo, peptideoglicanas e flagelinas gera dano tissular, estimulando a geração de DAMPs, tais como *heat shock proteins*, catelicidinas, ácido úrico, fibrinogênio, DNA mitocondrial e celular livre (Conway-Morris A, Wilson J, Shankar-Hari M, 2018). Estas DAMPs e PAMPs acabam por ativar receptores de conhecimento de padrão, tais como TLR e NLR, induzindo a transcrição de uma ampla gama de citocinas pró e antinflamatórias (Singer M, 2017).

O desenvolvimento da disfunção orgânica é o evento clínico mais relevante durante a sepse, uma vez que ele se relaciona diretamente com a morbimortalidade do paciente (Pool R, Gomez H, Kellum JA, 2018). A despeito de uma terapêutica adequada, uma série de eventos podem levar ao quadro de falência multiorgânica (Kozlov AV, Bahrami S, Calzia E, 2011). Mesmo terapias que forneçam uma oferta adequada de oxigênio não implicam necessariamente em um desfecho mais favorável, uma vez que uma disfunção mitocondrial coexistente pode prejudicar o consumo de oxigênio, contribuindo com a

progressão de falências orgânicas e até mesmo para o óbito (Jang DH, Greenwood JC, Spyres MB, 2017) (Messina A, Greco M, Cecconi M, 2019).

Dentro deste cenário, um dos biomarcadores mais avaliados na prática clínica no manejo da sepse é a mensuração do lactato arterial. Atualmente ele é considerado como critério diagnóstico para o choque séptico (Singer M, Deutschman C, Seymour CW, 2016), assim como é estudado como alvo terapêutico no manejo da síndrome (Hernández G, Ospina-Tascón G, Damiani LP, 2019). Uma diminuição nos níveis de lactato ao longo do tempo é sistematicamente associada a uma menor mortalidade nesta população, tornando-se, portanto, uma ferramenta de grande relevância (Vincent JL, Quintairos e Silva A, Couto Jr, L, 2016). A redução dos níveis de lactato ao longo do tempo durante as intervenções terapêuticas é proposta como um desfecho na ressuscitação da sepse, sendo comumente interpretada como um derivado da adequação da entrega de oxigênio aos tecidos periféricos, indicando, assim, a resolução da hipóxia tissular (Garcia-Alvarez M, Marik P, Bellomo R, 2014) (Jones AE, Shapiro NI, Trzeciak S, 2010) (Sterling SA, Puskarich MA, Shapiro NI, 2013). No entanto, durante a resposta inflamatória intrínseca a sepse, a produção de ATP também pode ser derivada através da glicólise, com a produção subsequente de piruvato, acarretando acúmulo de lactato ao invés da sua transformação através do Ciclo de Krebs (Park DW, Zmijewski JW, 2017). Logo, a disfunção mitocondrial pode resultar num excesso de lactato, caso a capacidade metabólica mitocondrial esteja comprometida (Iepsen UW, Plovsing RR, Tjelle K, et al, 2021) (Masyuk M, Wernly B, Lichtenauer M, 2019). Uma das fontes mais significativas de lactato na sepse é oriunda das células imunes, mobilizadas durante a resposta inflamatória (Certo, M., Tsai, C. H., Pucino, V.,

2021). Portanto, justifica-se a interpretação do lactato em conjunto com variáveis de metabolismo mitocondrial de células imunes como também com outras variáveis perfusionais, que possam indicar um déficit de oferta de oxigênio às células (Bakker J, Postelnicu R, Mukherjee V, 2020) (Kushimoto S, Akaishi S, Sato T, et al, 2016). Um modelo conceitual de hiperlactatemia dependente do consumo de oxigênio é descrito na Figura 1.

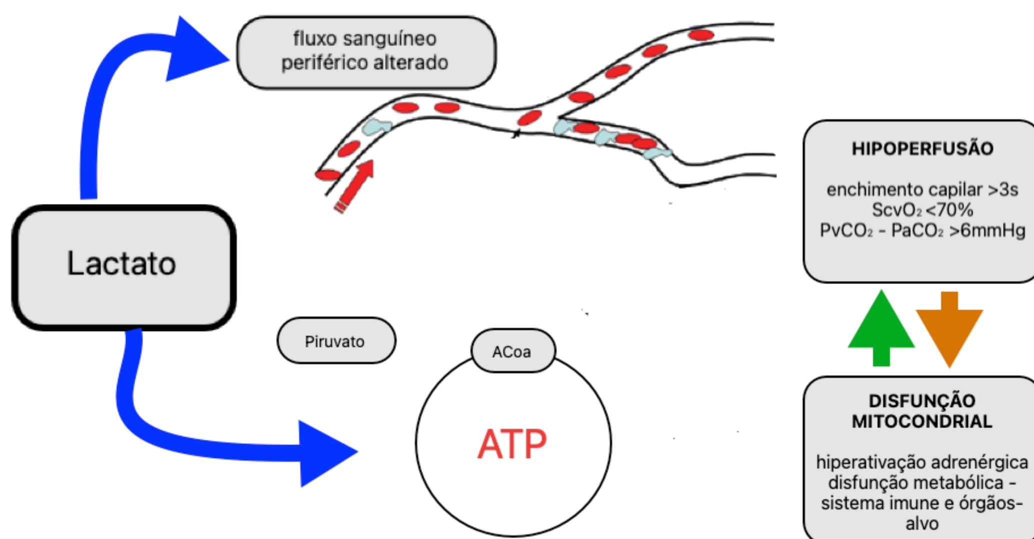

Figura 1. Modelo conceitual da produção de lactato dependente do consumo arterial de oxigênio no choque séptico, com as suas variáveis contribuintes – hipoperfusão tissular e disfunção mitocondrial celular. Legenda: ACoA, acetil-coenzima A; ATP, trifosfato de adenosina; $PvCO_2$-$PaCO_2$, gradiente veno-arterial de CO_2; $ScvO_2$, saturação venosa central de O2. Figura do autor.

A forma mais objetiva e mais tradicional de se mensurar a magnitude das falências orgânicas se dá através do escore SOFA (Vincent JL, Moreno R, Takala J, 1996) (Karakike E, Kyriazopoulou E, Tsangaris I, 2019). Trata-se de um escore que varia entre 0 e 24 pontos, graduando quantitativamente a intensidade das falências em distintos sistemas (sistema nervoso central, sistema circulatório, respiratório, renal, digestivo e hematológico). Em adição ao escore máximo de um determinado período, a mudança no escore (ou a

diferença entre as medidas em dois intervalos de tempo – delta SOFA) também se relaciona de maneira significativa com a mortalidade de um paciente portador de sepse (Lambden S, Laterre PF, Levy MM, 2019). A trajetória do delta SOFA é considerado um preditor acurado e consistente de mortalidade em pacientes criticamente enfermos (de Grooth H, Geenen IL, Girbes AR, 2017).

Um dos mecanismos relacionados a falência orgânica se dá através de uma estratégia de reprogramação metabólica celular, readequando o seu consumo de oxigênio com a intenção de se limitar danos adicionais por espécies reativas de oxigênio (ROS), prevenindo dano ao DNA e preservando os componentes celulares (Pool R, Gomez H, Kellum JA, 2018). O déficit na oferta de oxigênio aos tecidos periféricos, assim como a atividade inflamatória intrínseca a sepse impõe um estresse celular, afetando a fisiologia da mitocôndria. A integridade celular é diretamente relacionada a manutenção da homeostase mitocondrial, e a produção de ROS é vista como sendo a grande força motriz da disfunção mitocondrial (Kozlov AV, Bahrami S, Calzia E, 2011).

Este fenômeno é observado, por exemplo, em cardiomiócitos, onde há uma diminuição da atividade do citocromo C oxidase mitocondrial durante o curso da sepse (Piel DA, Gruber PJ, Weinheimer CJ, et al, 2007). A melhora da atividade do citocromo C oxidase se associa a melhora da contratilidade miocárdica, sugerindo uma relação direta entre as alterações mitocondriais e a perda de função orgânica (Piel DA, Gruber PJ, Weinheimer CJ, et al, 2007).

Avanços atuais na compreensão da fisiopatologia da sepse têm incorporado o conceito da existência de uma interação entre o metabolismo das células imunes e a própria resposta imune, atualmente chamada de imunometabolismo. Células mononucleares são repletas de mitocôndria, e tanto

estudos clínicos quanto estudos experimentais suportam a tese de que uma resposta imune efetiva contra a sepse seja dependente de uma OXPHOS que suporte a demanda energética da síndrome (Mills EL, Kelly B, O'Neill LAJ, 2017) (Brealey D, Brand M, Hargreaves I, 2002) (Cheng SC, Scicluna BP, Arts RJW, 2016), (Pearce EJ, Pearce EL, 2018) (Kramer PA, Ravi S, Chacko B, 2014).

A OXPHOS depende da energia dos elétrons transferidos através dos complexos mitocondriais I, II, III e IV para o oxigênio, que gera uma força próton-motora para a síntese de ATP no complexo V – F_1F_O-ATP sintase. As taxas de consumo de oxigênio associadas com os complexos mitocondriais em particular, e o parâmetro bioquímico derivado, a BCE, podem ser reveladas através de protocolos de respirometria. A BCE é uma variável refinada que pontua o quão eficientemente a maquinaria mitocondrial consegue empreender a síntese de ATP (Makrecka-Kuka M, Krumschnabel G, Gnaiger E, 2015) (Gnaiger, 2014).

Marcadamente, o curso altamente catabólico e inflamatório do choque séptico desafiam os leucócitos a melhorar a produção de anticorpos e de moléculas de sinalização ao longo do tempo, que particularmente gera um microambiente metabólico estressado para mitocôndria, causando defeitos bioenergéticos junto com a diminuição da função imune (Gotts JE, Matthay MA, 2016) (Cheng SC, Scicluna BP, Arts RJW, 2016). Além disso, tem sido proposto que o metabolismo das células imunes circulantes possa funcionar como um sensor biológico (Pearce EJ, Pearce EL, 2018). Baseada na sua pronta interação com o ambiente, as células imunes requerem uma reprogramação metabólica constante para fornecer imunidade, refletido por maior consumo de substratos energéticos, e maiores taxas respiratórias (Kramer PA, Ravi S, Chacko B, 2014). Estas características únicas sugerem que as células imunes possam apresentar

assinaturas metabólicas em pacientes com choque séptico que possam se relacionar intimamente com as disfunções orgânicas apresentadas por eles, assim como com os desfechos clínicos que eles venham a desenvolver (Puskarich MA, Kline JA, Watts JA, 2016), (Sjovall F, Morota S, Hansson MJ, 2010) (Sjövall F, Morota S, Persson J, 2013) (Carré JE, Orban J-C, Re L, 2010) (Brealey D, Brand M, Hargreaves I, 2002). Uma resposta imune desregulada na sepse pode acarretar num quadro de inflamação e imunossupressão persistentes, no qual a linfopenia é um dos seus principais sinalizadores laboratoriais. A Figura 2 esquematiza um modelo teórico no qual podemos sugerir um papel da disfunção do metabolismo linfocitário nesta evolução clínica insatisfatória.

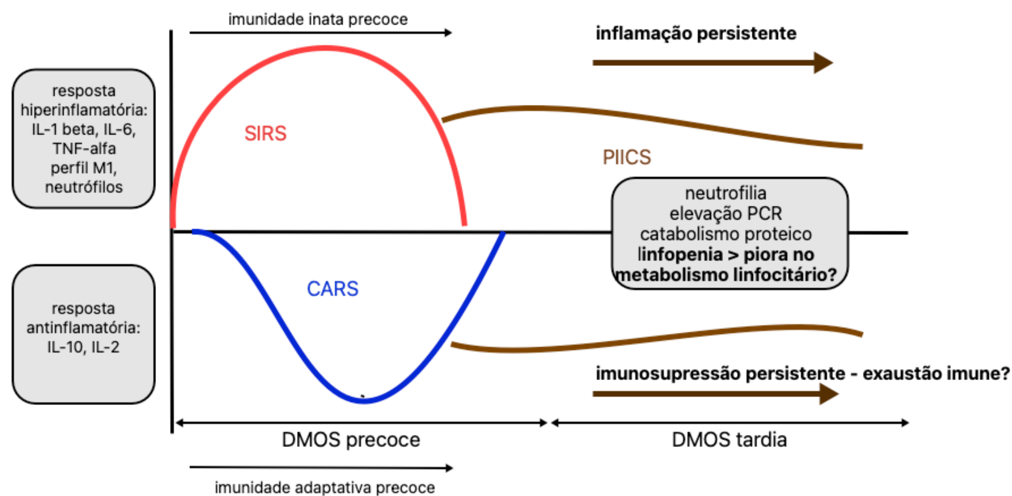

Figura 2. Esquematização da resposta imune na sepse, com suas fases pró- e antinflamatória. A linfopenia é um sinalizador de progressão para um quadro de PIICS. Neste contexto, propõe-se um papel associado da disfunção mitocondrial linfocitária. Legenda: CARS, *compensatory anti-inflammatory response syndrome*; DMOS, disfunção múltipla de órgãos e sistemas; IL, interleucina; PCR, proteína C reativa; PIICS, *persistent inflammation,*

immunosupression and catabolism syndrome; SIRS, *systemic inflammatory response syndrome*. Figura do autor.

Alterações na capacidade de reprogramação metabólica em resposta a sepse pode levar a mudanças na produção de ATP (da OXPHOS para a glicólise) e inibição da respiração mitocondrial (Pool R, Gomez H, Kellum JA, 2018). A falência em reestabelecer a OXPHOS como fonte primária de ATP em estágios mais tardios da sepse se associa com a perpetuação do estado pro-inflamatório, que limita o reestabelecimento da função orgânica (Yang L, Xie M, Yang M, et al, 2014). Em infecções agudas, os linfócitos alteram dinamicamente seu programa metabólico após a ativação. A ativação dos linfócitos, principalmente das células T, é altamente anabólica e apresenta aumento proeminente da glicólise e captação de glicose (Pearce EL, Poffenberger MC, Chang CH, 2013). Os linfócitos T mudam seu metabolismo de oxidativo (OXPHOS) para glicolítico (glicólise), independentemente da presença de hipóxia, como um fenótipo mais favorável para promover crescimento e proliferação celular (efeito Warburg) (Toro J, Manrique-Caballero CL, Gómez H, 2021). A glicólise é importante para a ativação e proliferação das células T induzidas pela ativação, não apenas porque pode produzir ATP, mas também porque seus intermediários metabólicos podem se desviar para diferentes vias metabólicas para atender às necessidades biossintéticas das células T (Liu H, Yang H, Chen X, 2014). Consequentemente, a inibição do metabolismo anabólico afeta a ativação e proliferação de linfócitos (Chapman NM, Chi H, 2022).

Um modelo conceitual da reprogramação metabólica é explicitado na Figura 3.

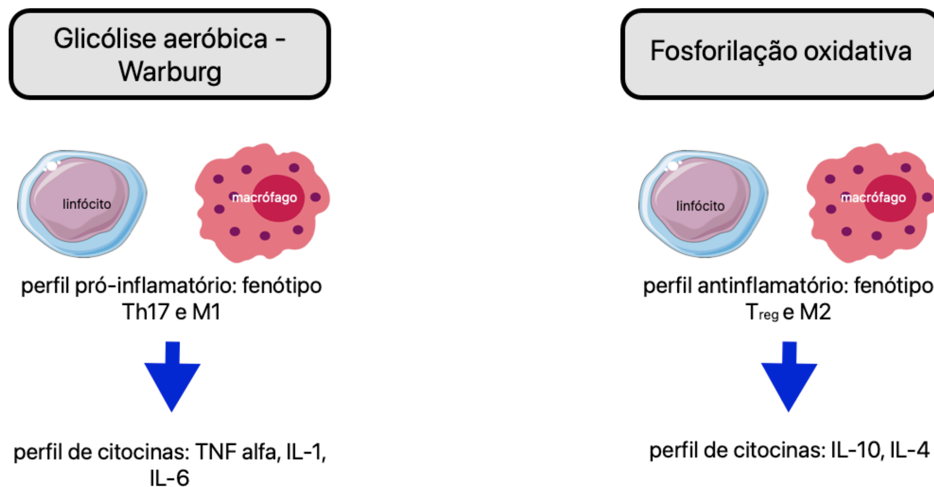

Figura 3. Esquematização da reprogramação metabólica em linfócitos e em monócitos. Estas células, ativadas em perfil pró-inflamatório, tornam-se ativas através da expressão de citocinas pró-inflamatórias. O perfil antinflamatório, com a expressão das suas respectivas citocinas, se dá no momento de transição para a fosforilação oxidativa. Legenda: IL, interleucina; T_h, célula T *helper*; TNF, *tumoral necrosis factor*; T_{reg}, célula T regulatória. Figura do autor.

O efeito da sepse na respiração mitocondrial e em pontos específicos da cadeia transportadora de elétrons foi documentado previamente em estudos clínicos (Belikova I, Lukaszewicz AC, Faivre V; 2007) (Brealey D, Brand M, Hargreaves I; 2002). A atividade de complexo I e de complexo IV na cadeia transportadora de elétrons são significativamente reduzidas na musculatura esquelética de doentes sépticos, com os sobreviventes apresentando maiores concentrações de ATP tissular e maior atividade mitocondrial (Brealey D, Brand M, Hargreaves I, et al, 2002).

1.1 Isolamento de linfócitos e respirometria mitocondrial

Mensurar o metabolismo mitocondrial, num cenário clínico, nem sempre é viável. Isso se deve às questões éticas e práticas relacionadas a obtenção de amostras mitocondriais adequadas de órgãos vitais em pacientes (Sjovall F, Morota S, Hansson MJ, 2010). Portanto, é lógico avaliar a disfunção mitocondrial em células fáceis de coletar, especialmente aquelas que podem refletir um efeito "sistêmico" da sepse no organismo. As células sanguíneas periféricas têm sido usadas como fonte para acessar a função bioenergética. As células mononucleares do sangue periférico, em especial os linfócitos, são ricas em mitocôndrias com altas taxas de respiração (Kraft BD, Chen L, Suliman HB, 2019) e, portanto, são as principais candidatas no sangue circulante para fornecer informações confiáveis de controle de qualidade mitocondrial, especialmente quando permeabilizados (Pecina P, Houšťková H, Mráček T, 2014). Estas células desempenham um papel importante na fisiopatologia da sepse porque sua ativação pode induzir inflamação remota de órgãos não infectados (Belikova I, Lukaszewicz AC, Faivre V, 2007). Portanto, este perfil fornece uma base teórica para medir sua atividade mitocondrial como um 'proxy' para o metabolismo sistêmico.

Por exemplo, propomos avaliar a respiração mitocondrial relacionada ao Complexo I, II e F_1F_O-ATP sintase em linfócitos permeabilizados isolados no D1 e no D3, dos quais derivamos a BCE. Assim, com pequeno volume de sangue coletados em frascos de EDTA no dia 1 (D1) e no dia 3 (D3) do diagnóstico do choque séptico podemos revelar o perfil temporal de resposta metabólica durante o manejo clínico. O isolamento dos linfócitos e posterior permeabilização para estudos de bioenergética já foi descrito previamente (Pecina P, Houšťková H, Mráček T, 2014).

O BCE (também conhecido como o fator ≈P) permite estimar o fluxo de oxigênio mitocondrial acoplado a produção de ATP. O BCE é calculado pelo fluxo P – L / P; (J≈P = (P-L)/P), como descrito previamente (Makrecka-Kuka M, Krumschnabel G, Gnaiger E, 2015) (Gnaiger, 2014), sendo P a taxa de fluxo máximo de oxigênio consumido acoplado a produção de ATP, e L a respiração de leak (Carteri RB, Kopczynski A, Rodolphi MS, 2019). A Figura 4 ilustra o protocolo empregado para avaliação do metabolismo mitocondrial por respirometria de alta resolução.

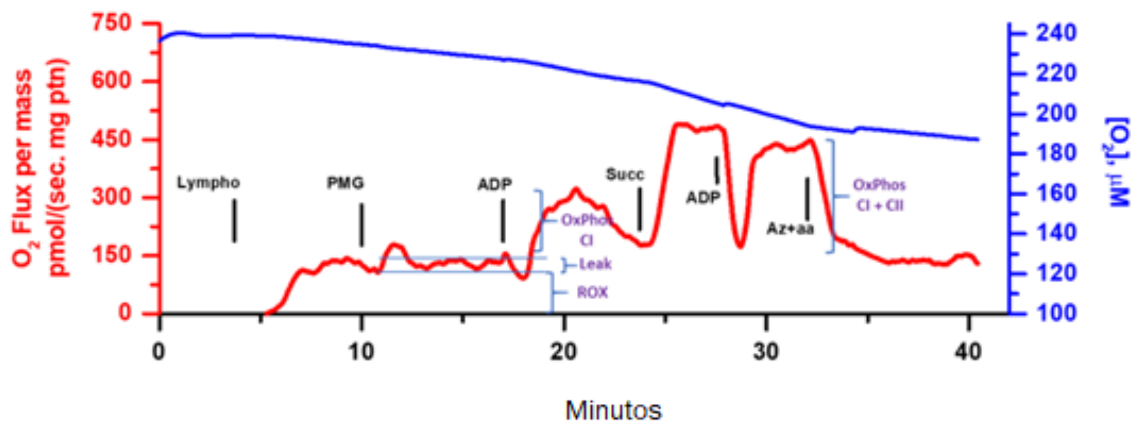

Figura 4. Imagem representativa de um ensaio de respirometria em linfócitos de um controle. Após atingir a respiração basal, foram adicionados piruvato, malato e glutamato (10, 10 and 20 mM, respectivamente), levando a respiração de leak (L), seguido por adições sequenciais de 2.5 mM de ADP, 10 mM de succinato, e uma segunda alíquota de 2.5 mM de ADP, permitindo a taxa de estado estacionário de consumo de oxigênio de cada etapa (fluxo de oxigênio por volume ou por massa). Esse protocolo de titulação de substrato permite a derivação do Complexo I (CI), Complexo II (CII) e consumo máximo de fluxo de oxigênio acoplado a produção de ATP (Estágio III). Azido de sódio mais antimicina A foram adicionados para inibir o Complexo IV mitocondrial, permitindo a estimativa do fluxo de oxigênio não-OXPHOS (ROX), e estes fluxos foram descontados para obter uma taxa de fluxo autêntica derivada de todos os estágios acima mencionados, e fluxos de oxigênio específicos para a massa tissular foram comparados em diferentes substratos, estágios de

acoplamento e inibidores (protocolo SUIT). Linfócitos foram permeabilizados com digitonina a 0.005% w/v. Extraído de Mitochondrial Physiology Network 07.08(09):1-7 (2017) Version 09: 2017-11-16.

1.2 Principais estudos abordando a atividade mitocondrial na sepse

Diversos estudos, em diferentes cenários clínicos e em diferentes amostras, avaliaram a função mitocondrial através da respirometria em pacientes sépticos. As amostras constituem-se, basicamente, de tecidos musculares ou de células mononucleares periféricas.

No que se refere a avaliação em células imunes, pequenos estudos de coorte abordaram este tópico. Weiss et al (Weiss SL, Selak MA, Tuluc F, 2015), ao comparar pacientes sépticos pediátricos (n = 13) com controles, encontrou uma menor capacidade respiratória de reserva (um índice de reserva bioenergética) em sépticos em comparação aos controles, que normalizou entre os dias 5 e 7. Pacientes sépticos também apresentaram uma maior relação de leak/respiração máxima do que os controles, também com normalização na fase tardia da sepse, sugerindo um desacoplamento mitocondrial precoce. No entanto, as amostras de pacientes sépticos não apresentaram diferenças no consumo de oxigênio basal, no consumo associado a ATP ou no potencial transmembrana mitocondrial. O consumo de oxigênio mitocondrial não se associou, neste estudo, a uma maior incidência ou magnitude de falências orgânicas. Os principais estudos que compararam as diferentes formas de se avaliar a atividade mitocondrial e a sobrevivência de pacientes sépticos, em diferentes amostras e tecidos, em pacientes adultos, estão descritos abaixo, na Tabela 1.

Tabela 1. Estudos que abordaram a disfunção mitocondrial na sepse em pacientes adultos

Estudo	Número de pacientes incluídos	Mensuração da atividade mitocondrial	Principais desfechos (sobreviventes vs não-sobreviventes)
Belikova, et al. 2007	18	Respirometria de mononucleares em sangue periférico	Mononucleares de pacientes sépticos com maior respiração basal comparada aos controles não-sépticos, porém com menor aumento na respiração quando estimuladas por ADP
Brealey, et al. 2002	28	Concentração de ATP, atividade de Complexo I, II e IV em amostra de células musculares	Níveis aumentados de ATP e aumento na atividade de complex I e IV em sobreviventes, comparados aos não-sobreviventes
Carré, et al. 2010	16	Morfologia mitocondrial (densidade de superfície e volume), RT-PCR de fatores de biogênese mitocondrial e concentração de proteínas de Complexo I e de Complexo IV, transcriptômica de OXPHOS em amostras de biópsia musculares	Não-sobreviventes apresentaram um maior declínio na densidade da superfície mitocondrial, e um volume mitocondrial similar entre os grupos Transcriptômica da OXPHOS mais abundante em sobreviventes Aumento na concentração de ATP em sobreviventes Sem diferença entre os grupos na atividade de Complexo I e de Complexo IV
Kraft, et al. 2009	37	qRT-PCR de genes que regulam a biogênese mitochondrial em mononucleares de sangue periférico	Aumento da ativação gênica da biogênese mitochondrial no dia 3, quando comparado ao dia 1 Diminuição na concentração de mtDNA em sépticos, quando comparados aos controles, com recuperação no dia 5 Ativação precoce da biogênese mitochondrial no dia 1 foi associada com maior probabilidade de alta da UTI Aumento nos níveis de mRNA em sobreviventes
Japiassú, et al. 2011	20	Respirometria de mononucleares em sangue periférico, avaliando-se estado 3, 4 e razão de controle respiratório	Sem diferença na respiração estimulada por ADP em não-sobreviventes, quando comparada aos sobreviventes
Puskarich, et al. 2016	28	Respirometria de plaquetas	Respiração basal e de estado 3 foram significativamente maiores em não-sobreviventes em comparação aos sobreviventes. Respiração de estado 4 apresentou um aumento não-significativo em não-sobreviventes
Pyle, et al. 2010	Não reportado	Concentração de mtDNA de células mononucleares	Sem relação entre a concentração de mtDNA e sobrevida em 180 dias

	(147 pacientes, incluindo sépticos)		
Sjovall, et al. 2010	18	Respirometria de plaquetas isoladas avaliando atividade de Complexo I, Complexo II, estado 3, estado 4 e razão de controle respiratório	Não-sobreviventes apresentaram um aumento na atividade de Complexo I, complex II, respiração em estado 3 e um aumento na razão de controle respiratório entre os dias 6 e 7, quando comparados aos sobreviventes.
Sjovall, et al. 2013	20	Respirometria de células imunes periféricas permeabilizadas	Sobreviventes e não-sobreviventes em 90 dias pós sepse não apresentaram diferença na respiração de Complexo I e de Complexo II normalizada para a concentração de citrato sintase, mtDNA, e citocromo c

Legenda: ADP, difosfato de adenosina; ATP, trifosfato de adenosina; qRT-PCR, reação de cadeia de polimerase-transcriptase reversa quantitativa; mtDNA, ácido desoxirribonucleico mitocondrial; OXPHOS, fosforilação oxidativa; UTI, unidade de terapia intensiva.

Tanto as alterações na função mitocondrial em tecidos musculares (Brealey D, Brand M, Hargreaves I, 2002) (Carré JE, Orban J-C, Re L, 2010), quantos em células sanguíneas (Puskarich MA, Kline JA, Watts JA, 2016) (Sjovall F, Morota S, Hansson MJ, 2010) (Sjövall F, Morota S, Persson J, 2013) se associaram a pior prognóstico em diferentes estudos. No entanto, estes trabalhos que analisaram o metabolismo da OXPHOS na sepse apresentam resultados controversos principalmente acerca dos desfechos metabólicos, e a sua capacidade de predizer desfechos clínicos desfavoráveis nos pacientes - particularmente falências orgânicas e mortalidade (Puskarich MA, Kline JA, Watts JA, 2016) (Sjovall F, Morota S, Hansson MJ, 2010) (Sjövall F, Morota S, Persson J, 2013) (Carré JE, Orban J-C, Re L, 2010) (Japiassú AM, Santiago APSA, D'Avila JDCP, 2011) (Brealey D, Brand M, Hargreaves I, 2002). Por

exemplo, pacientes com choque séptico, quando comparados com controles, apresentam uma diminuição significativa na síntese de ATP em monócitos periféricos, no entanto, sem associação estatisticamente significativa em ao menos um estudo (Japiassú AM, Santiago APSA, D'Avila JDCP, 2011). O uso de células do sangue para uma mensuração da atividade mitocondrial tem a possibilidade de ser feito de modo repetitivo, acompanhando-se a evolução clínica do paciente (Hsiao CP, Hoppel C, 2018). Além disso, apresentam uma correlação com a atividade mitocondrial em tecidos periféricos (Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, 2016). Neste contexto, se destacam os estudos avaliando a atividade mitocondrial plaquetária (Puskarich MA, Kline JA, Watts JA, 2016) (Sjovall F, Morota S, Hansson MJ, 2010) e os mensurando atividade em mono e polimorfonucleares, com resultados distintos. Em polimorfonucleares a falência energética se associa a uma modulação da resposta imune, também dependente do plasma (Belikova I, Lukaszewicz AC, Faivre V, 2007) e em monócitos a falência energética secundária a disfunção mitocondrial se associa a desfechos negativos (Martínez-García JJ, Martínez-Banaclocha H, Angosto-Bazarra D, 2019) (Merz TM, Pereira AJ, Schürch R, 2017) (Weiss SL, Zhang D, Bush J, 2019) (Japiassú AM, Santiago APSA, D'Avila JDCP, 2011), porém com respostas distintas conforme a linhagem celular avaliada e o momento específico da respiração celular (Merz TM, Pereira AJ, Schürch R, 2017). Sugere-se que a mensuração da atividade mitocondrial de linfócitos seja uma das mais promissoras, por ser adequadamente validada em cenários distintos de distúrbios mitocondriais (Pecina P, Houšťková H, Mráček T, 2014).

A mensuração da respiração mitocondrial em um paciente com uma condição aguda como na sepse tem o potencial de identificar aqueles sob um

risco de uma maior deterioração do quadro clínico, monitorando o curso clínico e a resposta às intervenções terapêuticas em tempo real, transformando-se num biomarcador ideal para o contexto agudo de doença (Jang DH, Greenwood JC, Spyres MB, 2017).

Foi estabelecido que uma das prioridades em pesquisa na área da sepse é proporcionar um melhor entendimento da função celular, especialmente no que se refere a produção de ATP em diferentes grupos celulares, com destaque às células imunes, em especial monócitos e linfócitos (Coopersmith CM, De Backer D, Deutschman C, 2018). A pesquisa experimental é a linha-mestra do entendimento dos processos fisiopatológicos na medicina. No entanto, o seu modelamento às patologias clínicas constitui-se num desafio, especialmente na área de cuidados de pacientes críticos. Nesse sentido, há necessidade de se conciliar com mecanismos fisiopatológicos de grande complexidade com as elevadas mortalidades, e, para tanto, o diálogo entre os estudos clínicos observacionais e a pesquisa experimental se torna um caminho inevitável para proporcionar um melhor entendimento da patologia e a respostas ao manejo clinico (Pène F, Ait-Oufella H, Taccone FS, et al, 2015), como é o objetivo dos estudos aqui apresentados.

Os leucócitos circulantes são programados para papéis fisiológicos distintos, que incluem a mediação do processo inflamatório sistêmico. Os linfócitos são células unicelulares importantes na modulação da imunidade adaptativa (Conway-Morris A, Wilson J, Shankar-Hari M, 2018). Esta população está, normalmente, em um estado quiescente, utilizando, primariamente, as mitocôndrias para as suas demandas energéticas. A ativação dos linfócitos é associada com uma transição para um fenótipo metabólico com um aumento no

consumo de oxigênio mitocondrial (Macintyre AN, Rathmell JC, 2013). Este fenômeno é essencial para as funções imunes, que incluem a expansão clonal, a produção de citocinas e de anticorpos. A abundância de células, a sua heterogeneidade e a sua reatividade ao estímulo infeccioso as candidatam como células ideias para o estudo da bioenergética celular na sepse (Kramer, Ravi, Chacko, 2014).

No entanto, a avaliação da função bioenergética mitocondrial através de um preparado inespecífico de células mononucleares periféricas não se justifica, levando-se em consideração o diferente comportamento entre monócitos e linfócitos. Por exemplo, a concentração de Complexo IV mitocondrial é maior em monócitos e a de Complexo III maior em linfócitos (Kramer, Ravi, Chacko, 2014).

1.3 Interações potenciais do metabolismo mitocondrial na doença crítica

Notavelmente, as células imunes, como monócitos/macrófagos, células dendríticas e neutrófilos, liberam citocinas pró-inflamatórias que podem servir como estímulos adicionais para desafiar os componentes da maquinaria imune dos linfócitos (de Pablo R, Monserrat J, Prieto A, 2014). Citocinas pró-inflamatórias, incluindo IL-1 β e IL-6, medeiam principalmente respostas imunes precoces na sepse (Koutroulis I, Batabyal R, McNamara B, 2019), regulando positivamente a síntese de mediadores secundários, atraindo células imunes e estimulando a produção de proteínas de fase aguda. Os níveis aumentados de IL-6 durante a fase inicial da sepse são biomarcadores valiosos para se avaliar a resposta clinica do paciente (Yang Y, Xie J, Guo F, 2016) (Vivas MC, Villamarin Guerrero HF, Tascon AJ, 2021) (Song J, Park DW, Moon S, , 2019) (Carta A, de

Lucca MG, Pires MD, 2016). Além disso, como efeito “downstream” direto relacionado ao aumento de IL-6 (Song J, Park DW, Moon S, 2019) e, em menor grau, de IL-1ß (Ho KM, Lipman J), o fígado aumenta a biossíntese da PCR (Ryoo SM, Han KS, Ahn S, 2019). Até o momento, a PCR exerce uma dupla função: pró-inflamatória, ativando a parte inicial da cascata do complemento, aumentando a fagocitose e aumentando os níveis de IL-1ß e IL-6; e antinflamatória, refletindo na expressão do antagonista do receptor de IL-1 e aumento da liberação de IL-10 (Ho KM, Lipman J, 2009), o que amortece a resposta imune (Williams NC, O'Neill LAJ, 2018) (Gleeson LE, Sheedy FJ, 2016) (Toro J, Manrique-Caballero CL, Gómez H, 2021). Elevações crônicas nos níveis de IL-10 e a diminuição dos linfócitos T CD4+, CD8+ e células NK (de Pablo R, Monserrat J, Prieto A, 2014) podem representar um estado de imunossupressão (Nolt B, Tu F, Wang X, 2018).

Nesse contexto fisiopatológico, uma estratégia de biomarcadores baseada na quantificação de um perfil imunológico composto por IL-1ß, IL-6, PCR, IL-10 e contagem de linfócitos poderia fornecer uma compreensão clínica do estado inflamatório em pacientes com sepse e suas implicações sobre o estado bioenergético dos linfócitos. Diante disso, levantamos a hipótese de que os mediadores inflamatórios podem afetar os complexos mitocondriais, levando a uma resposta bioenergética deficiente nos linfócitos.

O uso de catecolaminas acelera a captação de glicose, estimulando a glicólise aeróbica e, de modo associado, impactando no metabolismo mitocondrial (Hartmann C, Radermacher P, Wepler M, 2017). As catecolaminas promovem o desacoplamento mitocondrial nas células immunes, de modo dose-dependente, o que pode inibir o consumo de oxigênio, e consequentemente

impactando na resposta imune do hospedeiro (Lunemann JD, Buttgereit F, Tripmacher R, 2001).

Objetivo geral

Avaliar a evolução temporal do metabolismo mitocondrial de linfócitos em pacientes portadores de choque séptico, e sua associação com a mortalidade em curto e em longo prazo nesta população.

Objetivos específicos

- Avaliar a associação entre os marcadores de metabolismo mitocondrial e os marcadores perfusionais, assim como a sua associação com os níveis de lactato sérico;
- Avaliar o impacto da administração de diferentes antimicrobianos com o metabolismo mitocondrial em pacientes portadores de choque séptico;
- Avaliar a associação entre a expressão de interleucinas pró- e antinflamatórias na fase inicial da sepse e sua associação com o metabolismo celular de linfócitos;
- Investigar a associação entre reprogramação metabólica mitocondrial e mortalidade
- Revisar os estudos que abordam o metabolismo mitocondrial na sepse, apontando perspectivas futuras para esta área de pesquisa.

Capítulo 2 – “*Association between hyperlactatemia, perfusional parameters, and lymphocyte mitochondrial dysfunction in septic shock patients.”*

No capítulo 2, apresentamos o artigo publicado na revista *Shock*. Este artigo apresenta a análise das variáveis de metabolismo mitocondrial (BCE, respiração basal, respiração de Complexo I e respiração de Complexo II) e a sua associação com as variáveis de fluxo e perfusionais, em especial o enchimento capilar e o delta PvCO2-PaCO2. Também avaliamos a associação entre as variáveis de metabolismo mitocondrial com os níveis de lactato arterial e o diagnóstico de hipoperfusão.

Foram incluídos 90 pacientes com a avaliação concomitante das variáveis em estudo. Esta análise foi realizada no D1, logo após a ressuscitação hemodinâmica ter sido considerada completa pela equipe assistente, durante a administração de vasopressores.

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ASSOCIATION BETWEEN HYPERLACTATEMIA, PERFUSIONAL PARAMETERS, AND LYMPHOCYTE MITOCHONDRIAL DYSFUNCTION IN SEPTIC SHOCK PATIENTS

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ABSTRACT—**Introduction:** In septic shock, mitochondrial dysfunction, and hypoperfusion are the main triggers of multi-organ failure. Little is known about the crosstalk between mitochondrial dysfunction and hemodynamic alterations, especially in the post-resuscitation phase. Here, we assess whether hypoperfusion and lactate levels are associated with oxygen consumption linked to mitochondrial bioenergetic activity in lymphocytes of patients admitted with septic shock. **Patients and Methods:** Prospective cohort study in patients with septic shock defined as the requirement of vasopressors to maintain a mean arterial pressure 65 mm Hg after initial fluid administration. Basal mitochondrial and Complex I respiration was measured to evaluate mitochondrial activity. Both variables and capillary refill time were compared with arterial lactate post-fluid resuscitation. We also compared mitochondrial activity measurements between patients with and without hypoperfusion status. **Results:** A total of 90 patients were included in analysis. The median arterial lactate at the time of septic shock diagnosis was 2.0 mmol/Dl (IQR 1.3–3.0). Baseline respiration at the time of septic shock diagnosis was correlated with lactate (Spearman −0.388, 95% CI −0.4893 to −0.1021; $P = 0.003$), as well as Complex I respiration (Spearman −0.403, 95% CI −0.567 to −0.208; $P < 0.001$). Patients with hypoperfusion status had no difference in basal respiration when compared with patients who did not have hypoperfusion status ($P = 0.22$) nor in Complex I respiration ($P = 0.09$). **Conclusion:** Changes in lymphocytic mitochondrial metabolism are associated with post-resuscitation arterial lactate in septic shock; however, they are not associated with the presence of a hypoperfusional status. In this scenario, it is therefore suggested that systemic perfusion and mitochondrial metabolism have different courses.

KEYWORDS—Basal respiration, complex I respiration, lactate, microcirculation, mitochondrial dysfunction, sepsis, shock

INTRODUCTION

Sepsis is a systemic and deleterious host response to infection that could evolve to septic shock, a major healthcare problem associated with a high mortality rate worldwide (1). Septic shock is clinically characterized by elevated serum lactate levels and signs of tissue hypoperfusion (2), which are abnormalities that require therapeutic intervention to avoid unfavorable clinical outcomes (3, 4).

Early identification of tissue hypoperfusion is a cornerstone of septic shock management, as prompt resuscitation represents a key factor in limiting progression to multiple organ dysfunction and death (5). The Surviving Sepsis Campaign recommends guiding hemodynamic resuscitation therapy by performing repeated measurement of blood lactate levels until normalization (6), suggesting that effective fluid resuscitation is crucial for stabilization of sepsis-induced hypoperfusion and metabolic derangements. The main objective of monitoring lactate levels is to improve tissue oxygen delivery. This is based on the assumption that decreased lactate levels mirror decreased tissue metabolic stress and increased oxidative metabolism. However, attempts to aggressively increase oxygen delivery to supraphysiologic levels in the setting of established sepsis have failed to demonstrate metabolic benefit (7). After adequate resuscitation, the levels of tissue oxygen tension in various organ beds are within normal levels or even elevated (8), indicating the availability of oxygen that meets or even exceeds cellular metabolic demands (9).

These reported findings are not compatible with a strict pathophysiological mechanism, in which tissue hypoxia sustains elevated lactate levels and alters oxidative metabolism, leading to cell damage and likely organ dysfunction (10, 11). Given this paradox, it seems reasonable to postulate that beyond availability, the utilization of oxygen at the cellular and molecular level by mitochondria is potentially affected by challenging mechanisms linked to septic shock. Remarkably, the transference of electrons, starting in mitochondrial complexes I or II ultimately results in the formation of an electrochemical gradient, reduction of oxygen, and synthesis of adenosine triphosphate (ATP) in the complex V- FoF1- ATP synthase (12). Although such mitochondrial properties are widely investigated in experimental studies using isolated organs to understand the oxidative metabolic behavior of specific cell types, few studies have addressed these relevant issues in the intensive care setting, once impaired metabolism

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associated with organ failure is a hallmark of septic shock (13). Although a direct approach to mitochondrial metabolism seems clinically relevant, it has been limited by the need for organ biopsy. Thus, to reveal peripheral blood readouts, which present metabolic associations with septic shock severity, metabolic responsiveness with organ similarities, and indicate the response to therapies may help surpass the difficulties of accessing mitochondria from the tissue of patients.

In this context, lymphocytes cells have well-established roles in the immune response to septic shock. They participate in the deterioration of patients' health through an exacerbated release of cytokines, but on the opposite, in the resolution of septic shock by building up an appropriate immunity. Lymphocytes are highly oxidative cells and consume glucose as the main substrate for ATP synthesis. Also, lymphocytes produce and extrude lactate via monocarboxylate transporters (MCT1 and 4) as a strategy to induce lymphocytes proliferation and build up an appropriate immunity (14, 15). Interestingly, specific metabolic signatures in lymphocyte mitochondria can reflect the level of oxygen consumption by specific complexes, which influences lactate production, but also have the potential to reflect clinical outcomes and response to therapies (14). In this context, it is uncertain whether perfusion status and hemodynamic therapeutic interventions impact mitochondrial metabolism of lymphocytes from septic shock patients.

Therefore, the primary objective of this study is to assess whether hypoperfusion and lactate levels are associated with oxygen consumption linked to mitochondrial bioenergetic activity in lymphocytes of patients admitted with septic shock.

PATIENTS AND METHODS

We prospectively included consecutive patients in the study who had been admitted to four different intensive care units (ICU) in one tertiary academic hospital (Grupo Hospitalar Conceição, Porto Alegre, Brazil), between 2017 and 2018. This study was approved by the local ethics committee (Plataforma Brasil number 66240017.0.0000.5530). Written informed consent was signed by the patient or next of kin.

Patients

Clinical admissions to the ICU refer to admissions for septic shock whose primary infectious focus is managed through the treatment of clinical diseases (e.g., pneumonia, meningitis, primary bloodstream infection, cellulitis). All other admissions are of surgical origin, that is, ICU admissions for septic shock whose control of the primary infectious focus required surgical intervention (e.g., cholecystectomy to treat acute cholecystitis, colectomy to treat perforated colonic diverticulitis, drainage of pleural empyema). Adult patients (>18 years old) admitted for septic shock (16) were enrolled in this study. Septic shock was defined as the requirement of vasopressors to sustain a mean arterial pressure ≥65 mm Hg after initial fluid administration (17). Patients were excluded when presented with known mitochondrial disease, pregnancy, refusal of the patient or next of kin to sign informed consent, patients with imminent death, patients with withholding or withdrawing treatment.

Patients' epidemiologic characteristics and treatments were prospectively recorded, including the Simplified Acute Physiology Score (SAPS III) and the Sequential Organ Failure Assessment (SOFA) at ICU admission. We collected the following data: patient's gender, age, height, primary site of infection, community-acquired or hospital-acquired infection, comorbidities, catecholamine dose, lactate level, capillary refill time (CRT), arterial lactate, central venous saturation ($SvcO_2$), delta $PvCO_2$-$PaCO_2$, SOFA score, and SAPS III score at ICU admission.

Blood samples and isolation of lymphocytes

We performed a respirometric assay in permeabilized lymphocytes collected at the time of diagnosis of septic shock. Cells were isolated from a blood sample obtained of each patient after the following steps; septic shock diagnosis, after fluid resuscitation phase, and after the start of a vasopressor drug (norepinephrine). We confirmed the absence of other cell types in the preparation using a cell counter. The time delay from blood sampling to respirometry assay was no more than 3 h.

Mitochondrial high-resolution respirometry

Measurements were performed in high-resolution oxygraph (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) at 37°C. Oxygen concentration (micromolar) and oxygen flux (expressed in pmol $O_2 \cdot s^{-1} \cdot 10^{-6}$ cells) were recorded with DatLab software 6.0 (Oroboros Instruments, Innsbruck, Austria). Oxygen consumption background was measured in each plasma sample immediately after calibration and automatically corrected for the ensuing experiments. After stabilization of O_2 consumption, the basal ("routine") respiration was reached. Basal respiration was the first measure common to all O_2 consumption measurements experiments.

When basal respiration was reached, 2.5 μL of Pyruvate, Malate, and Glutamate (10 mM, 10 mM, and 20 mM, respectively) were added, followed by stepwise additions of 6 μL of ADP 2.5 mM, 2 μL of Succinate 10 mM, and a second 6 μL of ADP 2.5 mM, allowing for stabilization of oxygen consumption in each step. This substrate titration protocol enables the obtainment of Complex I oxygen consumption (18).

Residual oxygen consumption was extracted from all the above-mentioned states, and tissue-mass-specific oxygen fluxes were compared in different substrate and coupling states. We calculated the flux control factors for Complex I, which express the change of flux in a single step of the SUIT protocol, normalized to the high flux as a specific reference state (19, 20). Authors involved in sample analysis were blinded to clinical outcomes, and authors involved in clinical data collection were blinded to mitochondrial outcomes. All chemicals used for high-resolution respirometry analysis were analytical grade and purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, Mo).

Hemodynamic variables

All patients underwent arterial catheterization, into the radial or femoral artery. A venous catheter was inserted through the jugular, femoral, or subclavian vein. Arterial and central venous blood samples were withdrawn simultaneously for the determination of the respirometric assay and measurement of the following variables: arterial oxygen tension, arterial carbon dioxide tension ($PaCO_2$), $ScvO_2$, arterial oxygen saturation (SaO_2), and venous carbon dioxide tension ($PvCO_2$). $ScvO_2$ was collected in patients with venous catheter tip properly positioned for its measurement in superior vena cava. Hemoglobin concentration was also measured. The venous-arterial carbon dioxide tension (delta $PvCO_2$-$PaCO_2$) was calculated using the formula: delta $PvCO_2$-$PaCO_2 = PvCO_2 - PaCO_2$, because it could be used as a surrogate for the difference in the mixed venous-arterial CO_2 content. The arterial blood lactate concentration was measured using an enzymatic method. CRT was measured by applying a firm pressure to the ventral surface of the right index finger distal phalanx, immediately after blood collection. The pressure was maintained for 10 s, the time for the return of the normal skin color was registered with a chronometer (6). The hypoperfusion context was defined by the presence of ScvO2 <70%, or delta $PvCO_2$-$PaCO_2$ <6 mm Hg, or a CRT <4 s together with hyperlactatemia (>2 mmol/L) after initial fluid resuscitation in the ICU (21).

Statistical analysis

Descriptive statistics included frequencies and percentages for categorical variables and means, standard deviation, confidence intervals, medians, and interquartile ranges (IQR) for continuous variables. To compare continuous variables, we used Student *t* test, Mann–Whitney *U* test, and Pearson or Spearman correlation test, as appropriate. We performed an exploratory linear logistic regression model on the variables associated with lactate: basal or Complex I respiration, CRT, and SvcO2. Statistical tests were two-tailed with significance defined as a *P* value less than 0.05. All *P* values were two-tailed. We use R version 0.4.2 (R project) for all analyses.

RESULTS

A total of 90 patients were included in the analysis. Overall ICU, hospital, and 6-month mortality were 45%, 57%, and 66%, respectively. The mean age was 68 (±15.9) years, and the patients were predominantly men (55%). Sixty-one percent had a clinical ICU admission and 39% a surgical ICU admission.

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Table 1. **Main patient characteristics**

Characteristics	N (%)	Mean/median (SD/IQR)
Surgical patients	35 (38%)	
Male	50 (55%)	
Sepsis foci		
Abdome	36 (40%)	
Cutaneous	2 (2%)	
Blood	7 (8%)	
Urinary	4 (4%)	
Lung	41 (46%)	
Solid cancer	7 (8%)	
Blood cancer	7 (8%)	
HIV	2 (2%)	
Cirrhosis	7 (8%)	
Chronic kidney disease	10 (11%)	
Diabetes	25 (27%)	
Hypertension	32 (35%)	
Age		64.8 (15.9)
SAPS 3		75.8 (12.8)
SOFA at ICU admission		8 (3.1)
SvO_2		70 (10.2)
$\Delta PaCO_2-PvCO_2$		7.4 (4.3–11.5)
Gap CO_2:$CavO_2$		2.3 (1.4–3.3)
Cumulative fluid balance (first day)		2,874 (1,635–5,493)
Cappilary refil time		4 (2–6)

$CavO_2$ indicates arteriovenous O_2 content difference; $PaCO_2$, carbon dioxide arterial pressure; $PvCO_2$, carbon dioxide venous pressure; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment.

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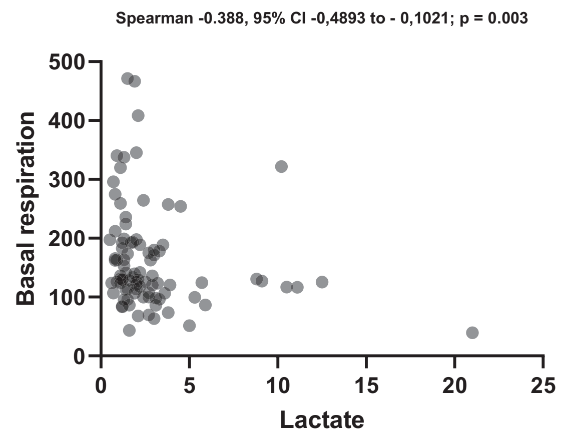
The most frequent sources of sepsis were lung (n = 41, 46%) and abdominal (n = 36, 40%). The mean SAPS 3 score was 75.8 (±12.9) points and the mean SOFA score at sepsis diagnosis was 8.5 (±3.2) points. During the study period, 32% of the patients underwent hemodialysis and 85% under mechanical ventilation. Median arterial lactate at septic shock diagnosis was 2.0 mmol/Dl (IQR 1.3–3.0), and the median volume of fluid resuscitation in septic shock was 44 mL/kg (IQR 30.7–65.6). The median basal mitochondrial respiration was 130 pmol/(sec.mg ptn) (IQR 108–191), mean mitochondrial Complex I respiration was 251 pmol/(sec.mg ptn) (IQR 173–406), and the mean CRT was 4 s (2–6 s). Fluid balance on the first day of admission to the ICU was 2874 mL (IQR 1,635–5,493). The main clinical characteristics are described in Table 1.

We further investigated possible associations between routine clinical parameters in the ICU and mitochondrial oxygen consumption in lymphocytes, trying to provide proxy indicators of tissue metabolism, and the impact of perfusion status. We found that lactate levels at septic shock diagnosis were significantly correlated with mitochondrial basal respiration (Spearman −0.388, 95% CI −0.4893 to −0.1021; $P = 0.003$) and Complex I respiration (Spearman −0.403, 95% CI −0.567 to −0.208; $P < 0.001$) (Fig. 1 A and B).

CRT showed an association with lactate levels (Pearson 0.246, 95% CI 0.017–0.45; $P = 0.03$). Additionally, lactate was correlated with $SvcO_2$ (Pearson −0.347; 95% CI −0.557 to −0.095 $P = 0.008$), but not with delta $PvCO_2$-$PaCO_2$ (Pearson 0.189 95% CI −0.088 to 0.439; $P = 0.18$). We performed two exploratory linear logistic regression models with variables associated

A Basal respiration x lactate



B Complex I respiration x lactate

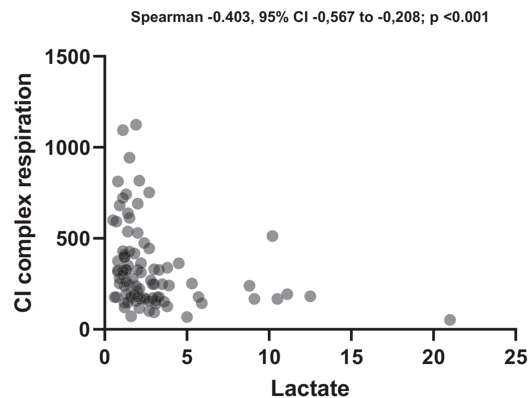


Fig. 1. **Correlation between variables and arterial lactate.**

with lactate (Basal or Complex I, CRT, $SvcO_2$) and found no significant associations. Complex I respiration was associated with CRT (Spearman −0.217; 95% CI −0.4111 to −0.004289; $P = 0.039$), but not basal respiration (Spearman −0.072; 95% CI −0.2807 to 0.1433; $P = 0.5$). Basal respiration was also not related to delta $PvCO_2$-$PaCO_2$ (Spearman −0.129; 95% CI −0.3756 to 0.1342; $P = 0.321$) or Complex I (Spearman 0.018; 95% CI −0.2417 to 0.2760, $P = 0.888$). The correlation between mitochondrial variables (basal respiration and Complex I respiration), clinical variables (maximum noradrenaline—in μg/kg/min, cumulative fluid balance and fluid replacement in sepsis resuscitation—in mL/kg), and perfusion variables (CRT, delta PvCO2-PaCO2, and $SvcO_2$), both between them and among them with lactate is shown in Figure 2. There is a correlation of mitochondrial variables only with lactate and a weak correlation between Complex I respiration and CRT. The influence of fluid replacement on mitochondrial variables was not demonstrated ($P > 0.05$).

Five patients received thiamine and did not display a difference in lactate value as compared with patients who did not receive thiamine: 1 mmol/Dl (IQR 0.65–3.05) versus 1.75 mmol/Dl (IQR 1–2.47) respectively, $P = 0.22$. Sixteen patients received beta-2-agonist in this period and did not show

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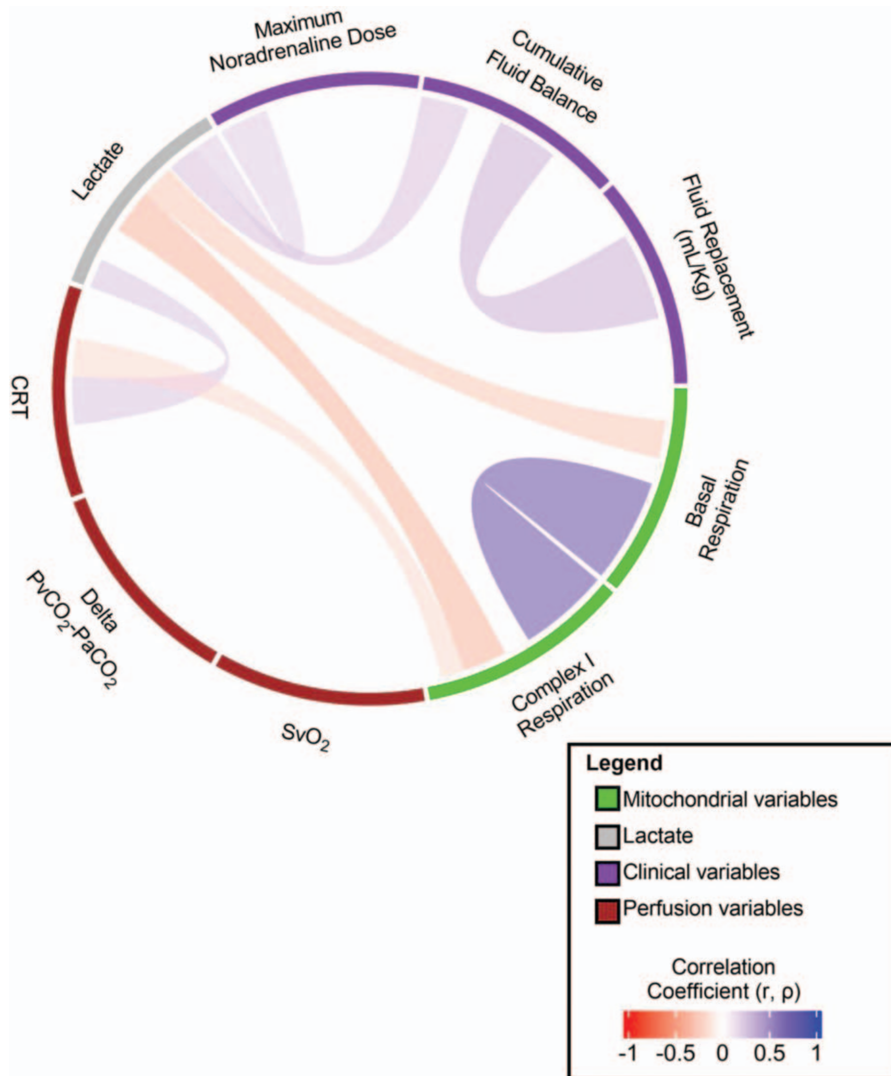

Fig. 2. **Correlation between clinical, perfusional, mitochondrial variables, and lactate. Correlations not shown have $P > 0.05$. CRT indicates capillary refill time.**

a significant association with increased lactate levels: 1.7 mmol/Dl (IQR 0.72–2.1) in beta-2-agonist users versus 1.7 mmol/Dl in those who did not receive beta-2-agonists (IQR 1–2.6), $P = 0.35$.

Evaluating basal respiration in patients with a diagnostic criterion for septic shock using the Sepsis 3 criteria (2), we found that the median basal mitochondrial respiration was lower in those with diagnostic septic shock (n = 42) 144 pmol/(sec.mg ptn) (±75) compared with those who did not meet the septic shock criteria (n = 31) 180 pmol/(sec.mg ptn) (±78) ($P = 0.04$). Patients who performed the diagnostic criteria for Sepsis 3 also had lower levels of Complex I activity compared with those who did not: 192 pmol/(sec.mg.ptn) ± 161 versus 325 pmol/(sec.mg.ptn) ± 324, respectively ($P = 0.007$). Also, septic shock patients had a CRT of 4 (2.5–6.5), while those who did not meet diagnostic criteria had a CRT of 6 (2–6) ($P = 0.04$).

Patients with hypoperfusion status had no difference in mitochondrial basal respiration when compared with patients who did not have hypoperfusion status: 148 pmol/(sec.mg ptn) (±78) versus 173 pmol/(sec.mg ptn) (±76) ($P = 0.22$). Similarly, mitochondrial complex I respiration in lymphocytes of patients with hypoperfusion was not different from those without hypoperfusion: 239 pmol/(sec.mg ptn) (±162) versus 318 pmol/(sec.mg ptn) (±273), respectively ($P = 0.09$).

DISCUSSION

In this work, the decreased mitochondrial basal and complex I stimulated respiration profile in lymphocytes was inversely associated with arterial lactate levels, providing a mechanistic perspective that oxidation of pyruvate in mitochondria is not favored, thus pyruvate is being predominantly diverted to cytosolic conversion to lactate. Today, several mechanisms are known to be involved in hyperlactatemia in patients with septic shock (22, 23). An increase in resting metabolic rate increased glucose metabolism with a glycolytic flux exceeding the capacity of pyruvate dehydrogenase to catalyze the conversion of pyruvate into acetyl coenzyme A and, therefore, increased conversion of pyruvate to lactate should be an

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important etiologic agent in this context (22). The production of energy as ATP occurs through a flow of electrons that passes through the five molecular complexes of the electron transport chain (24), and the process of oxidative phosphorylation is closely associated with the functioning of the electron transport chain. The flow of electrons from nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) is processed in Complex I, and NADH is closely related to the lactate concentration. Thus, Complex I respiration is intrinsically related to lactate concentration. Basal respiration, known as ''routine'' respiration, is a measure of oxygen consumption under steady state conditions (25), reflecting the ''usual'' leukocyte metabolism in the course of sepsis.

Mitochondrial dysfunction in sepsis has received more attention when decreased oxygen availability at the cellular level, namely, cytopathic hypoxia, was first mentioned as a plausible mechanism contributing to tissue hypoxia and therefore multiple organ failure (MOF) in sepsis (26). Tissue hypoxia in sepsis, first thought to result from microcirculatory disruption, does not, however, explain the increased tissue oxygen tensions, and minimal cell death observed in postmortem samples from various organs affected by sepsis, and more importantly the near-complete recovery of organ function in MOF survivors (8). In the post-resuscitation phase of sepsis, as in our cohort, increases in oxygen delivery did not cause an increase in oxygen consumption by lymphocytes and there is no consistent relationship between oxygen delivery and lactic acidosis (27). In this work, we did not find an association between hypoperfusional state and mitochondrial dysfunction in this phase of resuscitation and just a weak association between CRT and Complex I respiration. Therefore, it is possible to believe that variables associated with perfusion and variables associated with mitochondrial metabolism have an independent course of disease, at least in the post-resuscitation phase.

In experimental sepsis, a decrease in blood lactate levels during the first hours of resuscitation is associated with changes in mitochondrial respiration that are specific to each organ studied (28). Meaningful real-time analyses of the altered cells and tissues of failing organs are not feasible in living humans, as they would require a biopsy for tissue collection, which is unfeasible in clinical practice. Therefore, the assessment of the mitochondrial activity of lymphocytes is justified since they are cells circulating in the organism, easy to collect and analyze, and coupled with their metabolism with the immune response necessary to establish a favorable inflammatory profile (29). Previous studies have also evaluated the association of mitochondrial mononuclear and platelet metabolism with serum lactate levels (13, 30). Understanding the contribution of various organs to circulating lactate, however, is complicated by the observation that organs appear to simultaneously release and take up lactate in different profiles (31). It is known that muscle tissue is an important source of lactate during septic shock, and that there is an important correlation between lactate levels (both arterial and muscle) with mitochondrial activity in myocytes (32). Mitochondrial metabolism, both in lymphocytes and muscle cells, is potentially subject to the influence of septic plasma, thus assuming the presence of common triggers for metabolic failure of both myocytes and lymphocytes (33). Therefore, it is inferred that the same triggers for mitochondrial dysfunction in lymphocytes are present in muscle cells, for example, justifying, pathophysiologically, the use of mitochondrial activity in lymphocytes as a proxy metabolic indicator of general oxidative metabolism. A similar approach is performed with the measurement of a systemic lactate in the clinical setting, a metabolite extruded from several organs but with a well-defined synthetic pathway. In this context, lymphocytes oxidatively metabolize glucose producing ATP, CO_2, and H_2O, but pyruvate is also diverted to lactate biosynthesis in these cells as a fundamental mechanism of immune response. MCTs one and four highly expressed in lymphocytes make the eflux of lactate take up by other lymphoxytes and proliferate as an immune strategy. These MCTs are also expressed in brain, heart, lungs, skeletal muscles, small intestine, testis, ovaries, placenta. It was shown that under stressful imunological activation, a hallmak of septic shock, lymphocyte metabolism favors lactate production (14). Here, we added a piece to this puzzle suggesting that the mitocondrial respiratory Complex I is a component of this lactate response.

Activation of lymphocytes in an immunological phenomenon such as sepsis is associated with a switch to a metabolic phenotype with an increase in mitochondrial oxygen consumption (34). To improve the validity of our translational approach, we also use ''systemic'' hypoperfusion assessments, which further support our analysis. In this study, we cannot establish whether evaluation of regional perfusion by near-infrared spectroscopy or regional microdialysis, for example, would be associated with organ-specific mitochondrial dysfunction (muscle tissue, for example). We also did not evaluate the glycolytic pathway, which is another important limitation for a better understanding of lactate metabolism in this scenario (35). The sample size studied may also have been insufficient to establish refute associations between variables.

CONCLUSION

Lymphocytic mitochondrial metabolism had an inverse correlation with arterial lactate in the post-resuscitation phase of septic shock. Alterations in mitochondrial activity were not associated with hypoperfusional signs, suggesting an independent course of metabolic and hemodynamic alterations in this phase of the disease.

REFERENCES

1. Machado FR, Cavalcanti AB, Bozza FA, Ferreira EM, Angotti Carrara FS, Sousa JL, Caixeta N, Salomao R, Angus DC, Pontes Azevedo LC, et al.:, SPREAD Investigators, Latin American Sepsis Institute Network. The epidemiology of sepsis in Brazilian intensive care units (the Sepsis PREvalence Assessment Database, SPREAD): an observational study. *Lancet Infect Dis* 17(11):1180–1189, 2017.
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al.: Assessment of clinical criteria for sepsis for the third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 315(8):762–774, 2016.
3. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, Deane AM, Shehabi Y, Hajjar LA, Oliveira G, et al.: Effect of Vitamin C, hydrocortisone, and thiamine vs hydrocortisone alone on time alive and free of vasopressor support among patients with septic shock: the VITAMINS Randomized Clinical Trial. *JAMA* 323(5): 423–431, 2020.
4. Seymour CW, Rosengart MR: Septic shock: advances in diagnosis and treatment. *JAMA* 314(7):708–717, 2015.

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5. Cecconi M, de Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, et al.: Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. *Intensive Care Med* 40(12):1795–1815, 2014.
6. Hernández G, Ospina-Tascón GA, Damiani LP, Estenssoro E, Dubin A, Hurtado J, Friedman G, Castro R, Alegría L, Teboul JL, et al.: Effect of a resuscitation strategy targeting peripheral perfusion status vs serum lactate levels on 28-day mortality among patients with septic shock: The ANDROMEDA-SHOCK Randomized Clinical Trial. *JAMA* 321(7):654–664, 2019.
7. Pool R, Gomez H, Kellum JA: Mechanisms of organ dysfunction in sepsis. *Crit Care Clin* 34(1):63–80, 2018.
8. Singer M: Critical illness and flat batteries. *Crit Care* 21(S3):309, 2017.
9. Crouser ED: Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion* 4(5–6):729–741, 2004.
10. Garofalo AM, Lorente-Ros M, Goncalvez G, Carriedo D, Ballén-Barragán A, Villar-Fernández A, Peñuelas Ó, Herrero R, Granados-Carreño R, Lorente JA: Histopathological changes of organ dysfunction in sepsis. *Intensive Care Med Exp* 7(Suppl 1):45, 2019.
11. Takasu O, Gaut JP, Watanabe E, To K, Fagley RE, Sato B, Jarman S, Efimov IR, Janks DL, Srivastava A, et al.: Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. *Am J Respir Crit Care Med* 187(5):509–517, 2013.
12. Galley HF: Oxidative stress and mitochondrial dysfunction in sepsis. *Br J Anaesth* 107(1):57–64, 2011.
13. Puskarich MA, Kline JA, Watts JA, Shirey K, Hosler J, Jones AE: Early alterations in platelet mitochondrial function are associated with survival and organ failure in patients with septic shock. *J Crit Care* 31(1):63–67, 2016.
14. Murray CM, Hutchinson R, Bantick JR, Belfield GP, Benjamin AD, Brazma D, Bundick RV, Cook ID, Craggs RI, Edwards S, et al.: Monocarboxylate transporter Mctl is a target for immunosuppression. *N Chem Biol* 1(7):371–376, 2005.
15. Broer S: Lactate transportation is required for lymphocyte activation. *Nat Chem Biol* 1(7):356–357, 2005.
16. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, et al.: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 39(2):165–228, 2013.
17. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G: 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* 31(4):1250–1256, 2003.
18. Carteri RB, Kopczynski A, Rodolphi MS, Strogulski NR, Sartor M, Feldmann M, De Bastiani MA, Duval Wannmacher CM, De Franceschi ID, Hansel G, et al.: Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. *J Neurotrauma* 36:2246–2259, 2019.
19. Gnaiger E: Mitochondrial Pathways and Respiratory Control An Introduction to OXPHOS Analysis The Blue Book Mitochondrial Pathways and Respiratory Control An Introduction to OXPHOS Analysis. 4th Ed, OROBOROS MiPNet Publications; 2014.
20. Makrecka-Kuka M, Krumschnabel G, Gnaiger E: High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate and isolated mitochondria. *Biomolecules* 5(3):1319–1338, 2015.
21. Alegría L, Vera M, Dreyse J, Castro R, Carpio D, Henriquez C, Gajardo D, Bravo S, Araneda F, Kattan E, et al.: A hypoperfusion context may aid to interpret hyperlactatemia in sepsis-3 septic shock patients: a proof-of-concept study. *Ann Intensive Care* 7(1):29, 2017.
22. Suetrong B, Walley KR: Lactic acidosis in sepsis: it's not all anaerobic: implications for diagnosis and management. *Chest* 149(1):252–261, 2016.
23. Hernandez G, Bellomo R, Bakker J: The ten pitfalls of lactate clearance in sepsis. *Intensive Care Med* 45(1):82–85, 2019.
24. Galley HF: Oxidative stress and mitochondrial dysfunction in sepsis. *Br J Anaesth* 107:57–64, 2011.
25. Jang DH, Greenwood JC, Spyres MB, Eckmann DM: Measurement of mitochondrial respiration and motility in acute care. *J Intensive Care Med* 32(1):86–94, 2017.
26. Gotts JE, Matthay MA: Sepsis: pathophysiology and clinical management. *BMJ* 353:i1585, 2016.
27. Astiz ME, Rackow EC, Kaufman B, Falk JL, Weil MH: Relationship of oxygen delivery and mixed venous oxygenation to lactic acidosis in patients with sepsis and acute myocardial infarction. *Crit Care Med* 16(7):655–658, 1988.
28. Corrêa TD, Pereira AJ, Brandt S, Vuda M, Djafarzadeh S, Takala J, Jakob SM: Time course of blood lactate levels, inflammation, and mitochondrial function in experimental sepsis. *Crit Care* 21(1):1–12, 2017.
29. Wallace DC, Fan W, Procaccio V: Mitochondrial energetics and therapeutics. *Annu Rev Pathol* 5:297–348, 2010.
30. Wittayachamnankul B, Apaijai N, Sutham K, Chenthanakij B, Liwsrisakun C, Jaiwongkam T, Chattipakorn SC, Chattipakorn N: High central venous oxygen saturation is associated with mitochondrial dysfunction in septic shock: a prospective observational study. *J Cell Mol Med* 24(11):6485–6494, 2020.
31. Levitt DG, Levitt JE, Levitt MD: Quantitative assessment of blood lactate in shock: measure of hypoxia or beneficial energy source. *Biomed Res Int* 2020:2608318, 2020.
32. Levy B, Gibot S, Franck P, Cravoisy A, Bollaert PE: Relation between muscle Na+K+ ATPase activity and raised lactate concentrations in septic shock: a prospective study. *Lancet* 365(9462):871–875, 2005.
33. Maestraggi Q, Lebas B, Clere-Jehl R, Ludes PO, Chamaraux-Tran TN, Schneider F, Diemunsch P, Geny B, Pottecher J: Skeletal muscle and lymphocyte mitochondrial dysfunctions in septic shock trigger ICU-acquired weakness and sepsis-induced immunoparalysis. *Biomed Res Int* 2017:7897325, 2017.
34. Kramer PA, Ravi S, Chacko B, Johnson MS, Darley-Usmar VM: A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: Implications for their use as bioenergetic biomarkers. *Redox Biology* 2(1):206–210, 2014.
35. Suistomaa M, Ruokonen E, Kari A, Takala J: Time-pattern of lactate and lactate to pyruvate ratio in the first 24 hours of intensive care emergency admissions. *Shock* 14(1):8–12, 2000.

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Capítulo 3 – *“Short-term inflammatory biomarker profiles are associated with deficient mitochondrial bioenergetics in lymphocytes of septic shock patients - a prospective cohort study.”*

Neste capítulo, apresentamos outro estudo publicado na revista *Shock*. Analisamos 64 pacientes portadores de choque séptico, avaliando a associação entre as variáveis de metabolismo mitocondrial (BCE, respiração basal, respiração de Complexo I e respiração de Complexo II) e as dosagens séricas de interleucinas pró e antinflamatórias: IL-1β, IL-6, IL-10, além da proteína C reativa. Neste estudo, avaliamos a dosagem no primeiro e no terceiro dia dos biomarcadores, assim como o gradiente (delta) entre as medidas do dia 3 e do dia 1 da detecção do choque séptico.

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SHORT-TERM INFLAMMATORY BIOMARKER PROFILES ARE ASSOCIATED WITH DEFICIENT MITOCHONDRIAL BIOENERGETICS IN LYMPHOCYTES OF SEPTIC SHOCK PATIENTS—A PROSPECTIVE COHORT STUDY

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ABSTRACT—**Introduction:** A biomarker strategy based on the quantification of an immune profile could provide a clinical understanding of the inflammatory state in patients with sepsis and its potential implications for the bioenergetic state of lymphocytes, whose metabolism is associated with altered outcomes in sepsis. The objective of this study is to investigate the association between mitochondrial respiratory states and inflammatory biomarkers in patients with septic shock. **Methods:** This prospective cohort study included patients with septic shock. Routine, complex I, complex II respiration, and biochemical coupling efficiency were measured to evaluate mitochondrial activity. We measured IL-1ß, IL-6, IL-10, total lymphocyte count, and C-reactive protein levels on days 1 and 3 of septic shock management as well as mitochondrial variables. The variability of these measurements was evaluated using delta counts (days 3–1 counts). **Results:** Sixty-four patients were included in this analysis. There was a negative correlation between complex II respiration and IL-1ß (Spearman ρ, −0.275; $P = 0.028$). Biochemical coupling efficiency at day 1 was negative correlated with IL-6: Spearman ρ, −0.247; $P = 0.05$. Delta complex II respiration was negatively correlated with delta IL-6 (Spearman ρ, −0.261; $P = 0.042$). Delta complex I respiration was negatively correlated with delta IL-6 (Spearman ρ, −0.346; $P = 0.006$), and delta routine respiration was also negatively correlated with both delta IL-10 (Spearman ρ, −0.257; $P = 0.046$) and delta IL-6 (Spearman ρ, −0.32; $P = 0.012$). **Conclusions:** The metabolic change observed in mitochondrial complex I and complex II of lymphocytes is associated with a decrease in IL-6 levels, which can signal a decrease in global inflammatory activity.

KEYWORDS—Lymphocytes, sepsis, septic shock, interleukin 6, interleukin 1, interleukin 10, immunometabolism

INTRODUCTION

Sepsis is a syndrome caused by dysregulated host response to an infection, characterized by vascular and metabolic disturbances associated with acute phase inflammatory reaction that may evolve to immune suppression (1,2).

The early-onset activation of innate immune cells after infection releases proinflammatory cytokines that further mediate responses in the target tissues and in adaptive immune cells like lymphocytes (3). In this context, proinflammatory cytokines, like IL-1 β and IL-6, seem to exert a primary role in mediating (4) autocrine and paracrine signaling, with reflections on biosynthetic pathways (5–9). For instance, an increase in IL-6 stimulates the liver synthesis of reactant C-reactive protein (CRP), a pro- and anti-inflammatory effector (10,11). The anti-inflammatory effect is mediated by an increased IL-10 level (11), which dampens the immune response (12–14). However, chronic elevations in IL-10 levels coupled with decreased lymphocytes count (3) are linked with immunosuppression (15). Moreover, the onset of inflammatory response, termination, and transition to the homeostasis after sepsis are ATP demanding processes and require biosynthetic precursors provided by metabolic pathways. While studies have shown that an altered inflammatory response in the acute and prolonged phases of sepsis is tightly connected with impaired immune cells metabolism and unfavorable outcomes, the mechanistic mediators of this misconnection are not completely elucidated yet (5–9).

Recent clinical evidence from our laboratory demonstrated an association between decreased mitochondrial complex II (CII) respiration and biochemical coupling efficiency (BCE) with mortality of septic patients up to 3 days intensive care unit (ICU) admission (16). On the contrary, septic patients whose lymphocytes improved mitochondrial bioenergetics in this short period presented an increased survival rate up to 6 months after admission. Whether this mitochondrial metabolic reprogramming in lymphocytes might be concurrently affected by the inflammatory signaling fluctuations still remains a relevant question. It is known that lymphocytes shift their metabolism from mitochondrial

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The authors report no conflicts of interest.

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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oxidative phosphorylation toward aerobic glycolysis and lactate production, even when oxygen is available, as an apparent more favorable metabolic phenotype to supply ATP and substrates for cytokines production, cell growth, and proliferation (1,17–21). Nonetheless, the increase of aerobic glycolisis in lieu of oxidative phosphorylation does not reduce the importance of mitochondrial respiration to regulate and energetically support proinflammatory immune response mechanisms. In macrophages for instance, it has been shown that mitochondrial electron transport system is required for NLRP3 inflammasome activation and that this is fueled by glutamine oxidation (22,23). In addition, the role of mitochondrial metabolism in immune response surpasses energy production, because the accumulation of intermediaries of the tricarboxylic acid cycle (TCA) cycle, such as succinate and itaconate, may also serve as signaling triggers that drive pro-inflammatory IL-1β and anti-inflammatory IL-10 production, respectively (24–26). On the other hand, immune signals, such as LPS or IL-4, also can elicit the remodeling of immune cell mitochondrial metabolism. Beyond well-described proglycolytic effects, LPS signaling in macrophages for instance elicits an inhibition of pyruvate dehydrogenase and citrate synthase, effectively restricting entry of glycolysis metabolites to the TCA cycle and increasing reliance on glycolysis-derived ATP and glutaminolysis (27). In the contrary, anti-inflammatory signaling provided by IL-4 leads to a robust increase in macrophage mitochondrial respiration, driven by lactate oxidation through the TCA cycle (28), suggesting that lymphocyte mitochondrial metabolism may also mediate immune response to pathogens. While preclinical studies have shown that functional interactions between metabolism and immune cells involve a myriad of mechanistic components with pathophysiological significance for sepsis, few studies have explored such association in the clinical settings (16,29,30). Thus, beyond potential complementary clinical biomarkers of severity, response to therapies, and prognosis, immune effectors might inform whether preclinical findings can be functionally connected with patients settings. Given this, we propose here a multimodal biomarker strategy based on classic inflammatory biomarkers linked to sepsis (IL-1ß, IL-6, CRP, IL-10, and lymphocyte counts), components of mitochondrial bioenergetics machinery, and clinical scores of organic failure.

Hence, the main objective of this study was to investigate in patients admitted with sepsis associations between mitochondrial respiratory states linked to CI and CII with inflammatory biomarkers and clinical outcomes.

MATERIALS AND METHODS

We prospectively included consecutive patients in the study who had been admitted to 4 different ICUs in a tertiary, academic hospital (Grupo Hospitalar Conceição, Porto Alegre, Brazil), between 2017 and 2018. This study was approved by the local ethics committee (Plataforma Brazil number 66240017.0.0000.5530). Written informed consent was obtained from the patient or the next kin.

Patients

Adult patients (>18 years of age) admitted for septic shock (31) were enrolled in this study. Septic shock was defined as the requirement of vasopressors to maintain MAP <65 mm Hg after the initial fluid administration. Patients were excluded if they presented with known mitochondrial disease, pregnancy, refusal of the patient or next of kin to sign informed consent, imminent death, or withholding or withdrawing treatments. Corticosteroids were administered according to guidelines (32): hydrocortisone 200 mg/d in four divided doses, between 5 and 14 days. In this study, we defined the patient as having a chronic critical illness as those who underwent tracheostomy as a result of prolonged mechanical ventilation after sepsis (33).

Epidemiological characteristics and treatments of patients were prospectively recorded, including the Simplified Acute Physiology Score (SAPS III) and Sequential Organ Failure Assessment (SOFA) score at admission to the ICU and at first (D1) and third (D3) day, respectively. We collected the following data: sex, age, height, primary infection site, community- or hospital-acquired infection, comorbidities, IL-1ß, IL-6, IL-10, CRP, absolute lymphocyte count, SOFA score, SAPS III score at admission to the ICU (D1), and SOFA score at 3 days (D3) of septic shock treatment. Arterial lactate was measured at the first day of sepsis.

Blood samples and lymphocyte isolation

Blood was collected after the diagnosis of septic shock: after the fluid resuscitation phase, and after the start of the vasopressor drug (norepinephrine). Six milliliters of blood of each patient were sampled in EDTA tubes at D1 and D3 and lymphocytes obtained through gradient centrifugation as previously described (34). Briefly, lymphocytes were isolated by layering 4 mL of blood over 4 mL of Ficoll 1.077 g/dL (GE Healthcare, Little Chalfont, United Kingdom) in a 15-mL sterile centrifuge tube. Samples were centrifuged at 800*g* for 20 min at 4°C. An amount of 2 mL from the Buffy coat present on the interface between centrifuged blood and Ficoll solution was placed in a second sterile centrifuge tube and then diluted in 38 mL of RBC lysis solution (NH_4Cl 155 mM, K_2HCO_3 10 mM, EDTA 0.1 mM) and left for 30 min on ice. This was then centrifuged for additional 20 min at 800*g* for 20 min at 4°C. The obtained pellet was resuspended in 750-μL respiration buffer (mannitol 320 mM, TRIS 100 mM, KCl 50 mM, $MgCl_2$ 4 mM, NaH_2PO_4 4 mM, EDTA 0.73 mM, pH 7.4). We confirmed the predominant presence of lymphocytes (95%) in the isolated fraction using a hemocytometer. The time delay from blood sampling to respirometry assay was no more than 3 h.

Mitochondrial high-resolution respirometry

For real-time respirometry assay, respiration buffer was supplemented with 0.005% wt/vol digitonin to allow permeabilization of mitochondrial membranes to polar substrates. Adequate permeabilization in this isolation protocol was investigated before the analysis, assessing oxygen consumption after substrate addition to permeabilized and nonpermeabilized isolates from the same sample. Measurements were made using a high-resolution oxygraph (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria) at 37°C. The oxygen concentration (micromolar) and flux (expressed in pmol $O_2 \cdot s^{-1} \cdot 10^{-6}$ cells) were recorded using DatLab software (version 6.0; Oroboros Instruments, Innsbruck, Austria). The background oxygen consumption was measured for each plasma sample immediately after calibration and automatically corrected for subsequent experiments. After stabilization of O_2 consumption, baseline respiration was reached (“routine” respiration). Baseline respiration was the first measurement common to all O_2 consumption measurements.

When basal respiration was reached, 2.5 μL of pyruvate, malate, and glutamate (10, 10, and 20 mM, respectively) were added, followed by stepwise additions of 6 μL of ADP (2.5 mM), 2 μL of succinate (10 mM), and a second 6 μL of ADP (2.5 mM), allowing stabilization of oxygen consumption in each step (35).

We performed a protocol as previously described (16). This protocol assesses the steady-state, “basal” (state 1) respiration, the respiration linked to CI, CII (state 2) and maximal oxygen flow rate consumption coupled to ATP production (state 3), and nonmitochondrial oxygen consumption. In addition, BCE (also known as P control factor) was measured as an indicator of the effectiveness of mitochondrial oxygen flow coupled with ATP production (36). After respirometric analyses, total protein concentration was assessed using BCA kits (Thermo Fisher Scientific Inc., Waltham, Massachussets, USA), using 25 μL of lymphocyte isolate.

Residual oxygen consumption was extracted from all the previously mentioned states, and tissue mass–specific oxygen fluxes were compared for different substrate and coupling states. We calculated the flux control factors for CI, which express the change in flux in a single step of the SUIT protocol normalized for the high flux as a specific reference state (37). The authors involved in the analysis of the sample were blinded to the clinical results, and those involved in the collection of clinical data were blinded to the mitochondrial results. All chemicals used for high-resolution respirometry analysis were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO).

Inflammatory variables

All blood samples were collected through an arterial line, into the radial or femoral artery, or through venous puncture at the same time as the blood samples for the lymphocyte respirometric assay. The samples were centrifuged, supernatant was aliquoted in 1.5-mL Eppendorf tubes identified, and stored at −80°C for subsequent laboratory tests. The interleukins IL-1ß (Ref #BMS224-2), IL-6 (Ref #BMS213-2), and IL-10 (Ref #BMS215-2) were measured using a commercially available microplate enzyme-linked immunosorbent assay (Invitrogen by Thermo Fisher Scientific, Waltham, MA), and concentrations were expressed in picograms per milligram. The technical procedures were carried out according to the manufacturer instructions without adaptations. For carry out dosages two tubes with samples were slowly thawed in ice and spinned for homogenization. Components of each reagent kit were

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prepared few hours before running the technical protocol. Briefly, target-specific antibody has been precoated in the wells of the supplied microplate. Samples, standards, or internal controls of the kit (high and low) were added in duplicates to the microwells precoated with a target-specific antibody, which capture the interleukin of interest. After incubation, the microwells were washed, and a biotinylated detection antibody (detector antibody) was added. The addition of a colorimetric substrate solution reacts with this labeled antibody and produce color, whose intensity is proportional to the concentration of target interleukin present in the microwell. All interleukins were assayed in the same day by blinded experimenters. The variation between duplicates of standards, internal controls, and samples was less than 5%.

The following analyses were performed in the hospital clinical laboratory: CRP was examined according to the manufacturer using the quantitative analyzer of the CRP by turbidimetric immunoassay (Cobas c311 quantitative C-Reactive Protein, reference number 07876033190; Roche Diagnostics, Mannheim, Germany), and the concentration was in milligrams per liter. In this particle-enhanced immunoturbidimetric assay, human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The aggregates are determined turbidimetrically. The laboratory protocol is subject to the quality control established by the supplier and also to internal quality controls, which is based on running blinded samples with predefined concentration ranges. Routine hemogram monitoring was performed using an automated hemocytometer analyzer, which provides global lymphocytes count expressed as cells per liter. Arterial blood lactate concentration was measured using an enzymatic method running in an automatized biochemistry analyzer. The laboratory hospital is submitted to the Program of Quality Control for Clinical Laboratories of the Brazilian Society of Clinical Pathology, and all analysis complies international standard patterns.

Statistical analysis

Descriptive statistics included frequencies and percentages for categorical variables, and means, standard deviation (SD), confidence intervals (CIs), medians, and interquartile ranges for continuous variables. To compare continuous variables, we used the Student *t* test, Mann-Whitney *U* test, Kruskal-Wallis test, and Pearson or Spearman correlation test, as appropriate, according to normal distribution. Categorical variables were analyzed using the chi-square test. We performed a simple linear regression analysis exploring the SOFA score on day 1 of septic shock and each inflammatory variable (IL-1ß day 1, IL-6 day 1, IL-10 day 1, CRP, and lymphocyte count). We performed an exploratory multiple linear regression model to explore the association between SOFA score on day 1 as the independent variable in the model and routine respiration on day 1, CI respiration on day 1, CII respiration on day 1, BCE day 1, IL-1ß day 1, IL-6 d 1, IL-10 d 1, and CRP day 1 as independent variables. We defined the delta of inflammatory and mitochondrial variables as the measurements of these variables on day 3 minus the measurements on day 1. We also performed a binomial logistic regression exploring the delta of mitochondrial variables and the delta of the inflammatory variables with SOFA score improvement at day 3. All *P* values were two-tailed, and a 2-sided unadjusted *P* value of 0.05 or less was considered statistically significant. We used R version 0.4.2 (R project) and Jamovi (The jamovi project) version 1.6.23 for all analyses (38,39).

RESULTS

Clinical characteristics

A total of 64 patients were included in the analysis. The main clinical characteristics of patients are presented in Table 1. Intensive care unit mortality, hospital mortality, and 28-day mortality were 32%, 48%, and 35%, respectively. Median ICU length of stay was 7 days (interquartile range, 4–13 days). The patients showed an improvement in the SOFA score (mean difference [MD], −2; 95% CI, −3.3 to −0.8; *P* = 0.002), an increase in routine respiration (MD, 47.5; 95% CI, 21.2 to 73.8; *P* < 0.001), in CI respiration (MD, 116; 95% CI, 53 to 175; *P* = 0.01), in CII respiration (MD, 217; 95% CI, 103 to 341; *P* < 0.01), and in BCE (MD, 0.04; 95% CI, 0.006 to 0.08; *P* = 0.02) on day 3 compared with day 1. There was an increase in IL-10 levels (MD, 34.2 pg/mL; 95% CI, 0.1 to 105; *P* = 0.05) and a decrease in CRP (MD, −72 mg/L; 95% CI, −97 to −47; *P* < 0.01) during this period. These populations did not decrease IL-1ß levels (MD, −2.3 pg/ml; 95% CI, −13.4 to 10.2; *P* = 0.669), IL-6 (MD, −18.6 pg/ml; 95% CI, −63 to 2; *P* = 0.094), and lymphocyte count (MD, −139 × 103/μL; 95% CI, −96 to 393; *P* = 0.246).

Table 1. **Descriptive characteristics of the cohort**

Variable	n (%)	Mean/median (SD/IQR)
Age		70 (56–76)
Male:female	38 (59%):26 (41%)	
Primary focus of infection		
Abdominal	26 (41%)	
Cutaneous	2 (3%)	
Lung	31 (48%)	
Primary bloodstream infection	3 (5%)	
Urinary	1 (2.6%)	
Neoplasy	16 (25%)	
Chronic hypertension	22 (34%)	
Diabetes	19 (27%)	
Chronic kidney disease	6 (9%)	
COPD	10 (15%)	
Cirrhosis	5 (8%)	
Culture-positive sepsis: culture-negative sepsis	30 (47%):34 (53%)	
SAPS III score at ICU admission		76 (65–85)
Maximum noradrenaline dose		0.21 (0.1–0.36)
SOFA day 1		7 (6–10)
SOFA day 3		4 (2–8)
BCE day 1		0.345 (0.243–0.395)
BCE day 3		0.414 (0.201–0.52)
CI respiration day 1		260 (176–488)
CI respiration day 3		431 (301–630)
CII respiration day 1		529 (379–788)
CII respiration day 3		818 (596–1,069)
IL-6 day 1, pg/mL		66 (30–212)
IL-6 day 3, pg/mL		67 (34–144)
IL-1 day 1, pg/mL		34 (15–506)
IL-1 day 3, pg/mL		40 (11–487)
IL-10 day 1, pg/mL		191 (149–240)
IL-10 day 3, pg/mL		182 (150–481)
CRP day 1, mg/L		156 (84–223)
CRP day 3, mg/L		84 (38–166)
Total lymphocyte count day 1, cells/μL		1,001 (632–1,593)
Total lymphocyte count day 3, cells/μL		1,191 (780–1,458)
Lactate day 1		2.83 (1.4–3.05)

BCE, biochemical coupling efficiency; CI, complex I; CII, complex II; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; SAPS, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment.

Correlation between mitochondrial and inflammatory variables

The correlation matrix, exploring the correlation between mitochondrial and inflammatory variables on day 1, showed a negative and weak correlation between CII respiration and IL-6 (Spearman ρ, −0.275; *P* = 0.028). Moreover, BCE at day 1 was negative correlated with IL-6: Spearman ρ, −0.247; *P* = 0.05. Complex II respiration was also negatively correlated with IL-1ß (Spearman ρ, −0.253; *P* = 0.044). All analyses are described in ESM 1, http://links.lww.com/SHK/B593. In ESM 2, http://links.lww.com/SHK/B594, a correlation matrix exploring the correlation between the delta of mitochondrial and inflammatory variables is described. Delta CII respiration was negatively correlated with delta IL-6 (Spearman ρ, −0.261; *P* = 0.042). Delta CI respiration was negatively correlated with delta IL-6 (Spearman ρ, −0.346; *P* = 0.006), and delta routine respiration was also negatively correlated with both delta IL-10 (Spearman ρ, −0.257; *P* = 0.046) and delta IL-6 (Spearman ρ, −0.32; *P* = 0.012).

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Patients who had an improvement in routine respiration did not have a greater improvement in the inflammatory variables when compared with those who did not have an improvement in routine respiration: IL-10 (MD, −20.56; 95% CI, −207.4 to 166.3; $P = 0.826$), IL-6 (MD, 197.84; 95% CI, −300.3 to 696; $P = 0.43$), IL-1ß (MD, 27.9; 95% CI, −99.7 to 155.5; $P = 0.663$), lymphocytes (MD, −427; 95% CI, −1057 to 203; $P = 0.181$), or CRP (MD, 8.53; 95% CI, −72 to 89; $P = 0.832$). Patients who had an improvement in CI respiration did not have a greater improvement in IL-1ß when compared with those who did not have an improvement in CI respiration (MD, − 6.59; 95% CI, −45.6 to 14.3; $P = 0.563$). These patients have greater improvement in IL-6 (MD, −42.57; 95% CI, −79.7 to −12; $P = 0.01$), but not in IL-10 (MD, −3.84; 95% CI, −76.8 to 173.9; $P = 0.943$) nor in CRP (MD, −18; 95% CI, −87 to 44; $P = 0.615$). Patients who improved CI respiration also did not show greater improvement in lymphocyte counts when compared with those who did not (MD, 220; 95% CI, −272 to 803; $P = 0.368$). Patients who had improved CII respiration did not have a greater improvement in IL-1ß when compared with those who did not improve CII respiration (MD, −20.3; 95% CI, −61.9 to 3.4; $P = 0.1$). These patients have greater improvement in IL-6 (MD, −36.6; 95% CI, −92.1 to −3.4; $P = 0.03$), but not in IL-10 (MD, −13.2; 95% CI, −72.7 to 171.1; $P = 0.748$) nor in CRP (MD, 22.5; 95% CI, −42 to 83; $P = 0.416$). Patients who improved CII respiration also did not show greater improvement in lymphocyte counts when compared with those who did not (MD, 42; 95% CI, −503 to 651; $P = 0.887$). Patients who had improved BCE have a greater decrease in IL-10 levels when compared with those who did not improve BCE (MD, −67.7; 95% CI, −201.7 to −0.2; $P = 0.049$). These patients did not have great improvement in IL-6 (MD, 15.1; 95% CI, −26 to 50.8; $P = 0.431$), IL-1ß (MD, −3.22; 95% CI, −23.2 to 22.5; $P = 0.793$), CRP (MD, 27.6; 95% CI, −33 to 87; $P = 0.421$), and lymphocyte count (MD, −294; 95% CI, −861 to 176; $P = 0.218$).

Lactate concentration at day 1 was not associated with mitochondrial variables: routine respiration (Spearman ρ, −0.025; $P = 0.844$), CI respiration (Spearman ρ, −0.053; $P = 0.679$), CII respiration (Spearman ρ, −0.068; $P = 0.595$), and BCE at day 1 (Spearman ρ, −0.025; $P = 0.848$). Lactate was also not associated with inflammatory biomarkers: IL-1ß (Spearman ρ, 0.091; $P = 0.479$), IL-6 (Spearman ρ, 0.167; $P = 0.192$), IL-10 (Spearman ρ, 0.149; $P = 0.245$), CRP (Spearman ρ, 0.029; $P = 0.832$), and lymphocyte count (Spearman ρ, 0.114; $P = 0.383$).

Association between mitochondrial and inflammatory variables with SOFA score

In the bivariate analysis, CI respiration and BCE on day 1 of septic shock were associated with the SOFA score at this time, and in the multivariate analysis, no variable remained associated with the outcome. We also evaluated the association between the delta of mitochondrial and inflammatory variables and the improvement in SOFA score on day 3, compared with SOFA score on the first day. Patients who did not improve SOFA scores did not show a lesser improvement compared with those who improved SOFA scores, with respect to delta routine respiration, delta CI respiration, delta CII respiration, and delta BCE. Patients who did not improve SOFA also did not have a different variation in inflammatory variables compared with those who improved SOFA on day 3: delta IL-1ß, delta IL-6, delta IL-10, delta CRP, and delta lymphocyte count. In multivariate analysis, only delta BCE was included in the model and did not reach a statistical significant association with the outcome. All these results are presented in Table 2.

In subsequent analysis, four patients underwent tracheostomy, in a state of chronic critical illness. These patients did not have differences in mitochondrial and inflammatory variables when compared with those who did not undergo tracheostomy. Twenty-seven patients received corticosteroids during the study. Patients who received corticosteroids had greater improvement in BCE when compared with those who did not receive corticosteroids (MD, 0.09; 95% CI, 0.01 to 0.16; $P = 0.016$). These results are summarized in ESM 3, http://links.lww.com/SHK/B595.

DISCUSSION

In this study, we observed an improvement in lymphocytic oxidative phosphorylation, expressed by both CI, CII activity, and BCE, associated with decreased levels of IL-6 on the third day of sepsis. Lymphocytes attenuate the potentially harmful effects of the proinflammatory response through metabolic reprogramming, and this modulation of the immune response has a major impact on the prognosis of these patients (3). In addition, IL-6 promotes the differentiation of T and B lymphocytes, leading to increased immune responses targeted at infected cells (40) and upregulates the synthesis of CRP (41,42). Despite this known interaction, we did not find any association between CRP levels and lymphocytic metabolic activity, implying that this biomarker cannot serve as a "proxy" for lymphocytic metabolic response in these patients.

The IL-10 downregulates the immune system through the inhibition of monocyte-macrophage activation and suppression of production of TNF-α, IL-1, and IFN-γ from lymphocytes (43). In our study, a BCE improvement was associated with decrease in IL-10 levels on day 3 after sepsis diagnosis. Biochemical coupling efficiency should be interpreted as a biomarker of an integral function of mitochondrial bioenergetic components, which may lead to an effective coupling between oxygen consumption with ATP synthesis (44). In addition, decreased IL-10 may serve as an immunomodulator after the initial phase of sepsis (45). Considering the changes in the dynamics of the BCE, on the first day and its delta (D3-D1), with the dynamics of IL-6 and IL-10 within these same intervals, we assumed that a "proinflammatory" activity expressed by an increase in IL-6 mediates decreased lymphocyte bioenergetic efficiency. On the other hand, an anti-inflammatory immune modulation, expressed by an increase in IL-10, seems to be connected with more effective mitochondrial metabolic function, in terms of ATP biosynthesis. We found only a weak negative correlation between serum levels of IL-1ß and CII respiration. Interleukin 1ß is fundamental in the host's innate immune response to infection, and glycolytic reprogramming is crucial in macrophage IL-1ß production (13), with an infection-driven reorganization of electron transport chain specifically, with an increase in CII activity relative to CI, suggesting that sepsis causes a bypass from CI to CII regarding electron transfer. Thus, our findings suggest a different pattern of macrophages and lymphocytes in the early stages of sepsis. To our knowledge, this

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Table 2. **Inflammatory and mitochondrial variables and its association with SOFA score at ICU admission**

Outcome: SOFA score at day 1				
	Bivariate analysis		Multivariate analysis	
Predictor	Estimative	*P*	Estimative	*P*
Routine respiration	−0.002	0.476		
CI respiration	−0.003	0.034	−0.008	0.073
CII respiration	−0.001	0.156	0.004	0.172
BCE	−7.35	0.039	−4.103	0.282
IL-1ß	<0.0001	0.638		
IL-6	<0.0001	0.577		
IL-10	<0.0001	0.743		
CRP	−0.003	0.45		
Lymphocyte count	<0.0001	0.818		

Outcome: SOFA improvement at day 3				
	Bivariate analysis		Multivariate analysis	
Predictor	MD (95% CI)	*P*	OR (95% CI)	*P*
Delta routine respiration	−6.83 (−64 to 71.78)	0.859		
Delta CI respiration	−23.36 (−158.78 to 205.27)	0.844		
Delta CII respiration	−205.16 (−494 to 139.24)	0.205		
Delta BCE	−0.08 (−0.19 to 0.01)	0.117	77.2 (0.53 to 11,216.7)	0.086
Delta IL-1ß	6.2 (−13.3 to 72.4)	0.543		
Delta IL-6	18.27 (−29 to 106.9)	0.495		
Delta IL-10	−15.24 (−115.2 to 36.5)	0.586		
Delta CRP	35 (−27 to 115)	0.25		
Delta lymphocyte count	250 (−287 to 965)	0.363		

BCE, biochemical coupling efficiency; CI, confidence interval; CI, complex I; CII, complex II; CRP, C-reactive protein; IL, interleukin; MD, mean difference; OR, odds ratio; SOFA, Sequential Organ Failure Assessment.

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is the first clinical study to explore lymphocytic immunometabolism in patients with sepsis/septic shock, which has the merit of identifying associations between lymphocyte metabolism and levels of inflammatory biomarkers in this population. Previous studies suggest that changes in the mitochondrial activity of leukocytes is associated with the prognostic of septic patients (16,29,30), albeit this association needs to be conciliated with pathophysiological mechanisms, as we trend to propose in our work. According to our results, this interaction, at least in the case of lymphocytes, does not occur an altered expression in the classic inflammatory interleukins clinically linked with sepsis, in the production of CRP or in the total concentration of lymphocytes. The metabolic reprogramming expected in sepsis could lead to increased lactate production by immune cells due to an increased glycolysis, with a potentially impact on immune response (15,46). This phenomenon, however, does not contributed to an increase in arterial blood lactate levels in our study.

Our study has some limitations that should be stated. First, screening-specific inflammatory phenotype of lymphocytes subpopulations linked with particular mitochondrial bioenergetic signatures may reinforce particular effects on target cells and its relevance for the favorable and unfavorable clinical outcomes. However, we were not able to completely pave the way in this direction. Even with this limitation, our global estimate of lymphocyte mitochondrial metabolism revealed some level of biological plausibility regarding the modulation of CI and CII by IL-6. Traditionally, there is a clinical response assessment within 72 h after starting treatment in patients with sepsis (47,48), and a reassessment of mitochondrial activity is warranted in this context. Thus, we cannot rule out different responses over longer periods after starting treatment. Therefore, the association between early lymphocyte metabolism in septic shock and the phenotype of persistent inflammation, immunosuppression, and catabolism syndrome would be better characterized with further evaluation. In our analysis, a major sign of persistent inflammation, immunosuppression, and catabolism syndrome was the need for tracheostomy due to prolonged mechanical ventilation (49); the low number of patients who used it, however, does not allow us to draw conclusions about this hypothesis. A combined effect of all interleukins analyzed over mitochondrial endpoints would reflect more realistically their behavior in a clinical scenario. Considering the absence of this analysis as an intrinsic limitation in a clinical study, we estimated interactions between specific interleukins with mitochondrial metabolic endpoints. Finally, the low number of patients included in this cohort may lead to a type II error in this context, and the multiple analyses are not submitted to a correction of the *P* value, because of the hypothesis-generating characteristic of this study.

CONCLUSIONS

There was an increase in routine CI and CII respiration as well as in the BCE of lymphocytes during the early phase of septic shock. This metabolic reprograming is associated with a decrease in IL-6 levels, which can signal a decrease in the global inflammatory activity.

REFERENCES

1. Preau S, Vodovar D, Jung B, Lancel S, Zafrani L, Flatres A, Oualha M, Voiriot G, Jouan Y, Joffre J, et al.: Energetic dysfunction in sepsis: a narrative review. *Ann Intensive Care* 11:104, 2021.
2. McBride MA, Owen AM, Stothers CL, Hernandez A, Luan L, Burelbach KR, Patil TK, Bohannon JK, Sherwood ER, et al.: The metabolic basis of immune dysfunction following sepsis and trauma. *Front Immunol* 11:1043, 2020.

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3. de Pablo R, Monserrat J, Prieto A, Alvarez-Mon M: Role of circulating lymphocytes in patients with sepsis. *Biomed Res Int* 2014:671087, 2014.
4. Koutroulis I, Batabyal R, McNamara B, Ledda M, Hoptay C, Freishtat RJ: Sepsis immunometabolism: from defining sepsis to understanding how energy production affects immune response. *Crit Care Explor* 1:e0061, 2019.
5. Yang B, Chang X, Huang J, Pan W, Si Z, Zhang C, Li H: The role of IL-6/lymphocyte ratio in the peripheral blood of severe patients with COVID-19. *Int Immunopharmacol* 97:107569, 2021.
6. Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, Fine J, Krichevsky A, Delude RL, Angus DC, et al.: Understanding the inflammatory cytokine response in pneumonia and sepsis results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med* 167:1655–1663, 2007.
7. Vivas MC, Villamarin Guerrero HF, Tascon AJ, Valderrama-Aguirre A: Plasma interleukin-6 levels correlate with survival in patients with bacterial sepsis and septic shock. *Interv Med Appl Sci* 11:224–230, 2021.
8. Song J, Park DW, Moon S, Cho HJ, Park JH, Seok H, Choi WS: Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the sepsis-3 definitions. *BMC Infect Dis* 19:968, 2019.
9. Carta A, de Lucca MG, Pires MD, Lobo SM: Sepsis-associated organ dysfunction and increased supportive care are associated with high serum interleukin-6 levels. *J Bras Patol Med Lab* 52:367–373, 2016.
10. Ryoo SM, Han KS, Ahn S, Shin TG, Hwang SY, Chung SP, Hwang YJ, Park YS, Jo YH, Chang HL, et al.: The usefulness of C-reactive protein and procalcitonin to predict prognosis in septic shock patients: a multicenter prospective registry-based observational study. *Sci Rep* 9:6579, 2019.
11. Ho KM, Lipman J: An update on C-reactive protein for intensivists. *Anaesth Intensive Care* 37:234–241, 2009.
12. Williams NC, O'Neill LAJ: A role for the Krebs cycle intermediate citrate in metabolic reprogramming in innate immunity and inflammation. *Front Immunol* 9:141, 2018.
13. Gleeson LE, Sheedy FJ: Metabolic reprogramming & inflammation: fuelling the host response to pathogens. *Semin Immunol* 28:450–468, 2016.
14. Toro J, Manrique-Caballero CL, Gómez H: Metabolic reprogramming and host tolerance: a novel concept to understand sepsis-associated AKI. *J Clin Med* 10:4184, 2021.
15. Nolt B, Tu F, Wang X, Ha T, Winter R, Williams DL, Li C: Lactate and immunosuppression in sepsis. *Shock* 49:120–125, 2018.
16. Nedel WL, Kopczynski A, Rodolphi MS, Strogulski NR, de Bastiani M, Montes THM, Abruzzi J Jr., Galina A, Horvath TL, Portela LV: Mortality of septic shock patients is associated with impaired mitochondrial oxidative coupling efficiency in lymphocytes: a prospective cohort study. *Intensive Care Med Exp* 9:1–14, 2021.
17. Pearce EL, Poffenberger MC, Chang CH, Jones RG: Fueling immunity: insights into metabolism and lymphocyte function. *Science* 342:6155, 2013.
18. Donnelly RP, Finlay DK: Glucose, glycolysis and lymphocyte responses. *Mol Immunol* 68:513–519, 2015.
19. Assmann N, O'Brien KL, Donnelly RP, Dyck L, Zaiatz-Bittencourt V, Loftus RM, Heinrich P, Oefner PJ, Lynch L, Gardiner CM, et al.: Srebp-controlled glucose metabolism is essential for NK cell functional responses. *Nat Immunol* 18:1197–1206, 2017.
20. Liu H, Yang H, Chen X, Lu Y, Zhang Z, Wang J, Zhang M, Xue L, Xue F, Liu G: Cellular metabolism modulation in T lymphocyte immunity. *Immunology* 2014.
21. Chapman NM, Chi H: Metabolic adaptation of lymphocytes in immunity and disease. *Immunity* 55:14–30, 2022.
22. McManus R, Komes MP, Griep A, Santarelli F, Schwartz S, Perea JR, et al.: NLRP3-mediated glutaminolysis regulates microglia in Alzheimer's disease. *SSRN Electronic J* 2022. Available at: https://papers.ssrn.com/abstract=4178538. Accessed November 8, 2022.
23. Billingham LK, Stoolman JS, Vasan K, Rodriguez AE, Poor TA, Szibor M, Jacobs HT, Reczek CR, Rashidi A, Zhang P: Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. *Nat Immunol* 23:692–704, 2022.
24. Tannahill GM, Curtis AM, Adamik J, Palsson-Mcdermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, et al.: Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 496:238–242, 2013.
25. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, Chmielewski K, Stewart KM, Ashall J, Everts B, et al.: Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* 42:419–430, 2015.
26. Mills EL, Ryan DG, Prag HA, Dikovskaya D, Menon D, Zaslona Z, Jedrychowski MP, Costa ASH, Higgins M, Hams E: Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature* 556:113–117, 2018.
27. Seim GL, Britt EC, John SV, Yeo FJ, Johnson AR, Eisenstein RS, Pagliarini DJ, Fan J: Two-stage metabolic remodelling in macrophages in response to lipopolysaccharide and interferon-γ stimulation. *Nat Metab* 1:731–742, 2019.
28. Noe JT, Rendon BE, Geller AE, Conroy LR, Morrissey SM, Young LEA, Bruntz RC, Kim EJ, Wise-Mitchell A, Barbosa de Souza Rizzo M, et al.: Lactate supports a metabolic-epigenetic link in macrophage polarization. *Sci Adv* 7:eabi8602, 2021.
29. Japiassú AM, Santiago AP, d'Avila JC, Garcia-Souza LF, Galina A, Castro Faria-Neto HC, Bozza FA, Oliveira MF: Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5′-triphosphate synthase activity. *Crit Care Med* 39:1056–1063, 2011.
30. Weiss SL, Selak MA, Tuluc F, Perales Villarroel J, Nadkarni VM, Deutschman CS, Becker LB: Mitochondrial dysfunction in peripheral blood mononuclear cells in pediatric septic shock. *Ped Crit Care Med* 16:e4–e12, 2015.
31. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, et al.: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 39:165–228, 2013.
32. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC, et al.: Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021. *Crit Care Med* 49:E1063–E1143, 2021.
33. Carson SS: Definitions and epidemiology of the chronically critically ill. *Respir Care* 57:848–858, 2012.
34. Pecina P, Houšťková H, Mráček T, Pecinová A, Nůsková H, Tesařová M, Hansíková H, Janota J, Zeman J, Houštěk J: Noninvasive diagnostics of mitochondrial disorders in isolated lymphocytes with high resolution respirometry. *BBA Clin* 2:62–71, 2014.
35. Carteri RB, Kopczynski A, Rodolphi MS, Strogulski NR, Sartor M, Feldmann M, De Bastiani MA, Duval Wannmacher CM, de Franceschi ID, Hansel G, et al.: Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. *J Neurotrauma* 36:2246–2259, 2019.
36. Chance B, Williams GR: Respiratory enzymes in oxidative phosphorylation. *J Biol Chem* 217:383–393, 1955.
37. Makrecka-Kuka M, Krumschnabel G, Gnaiger E: High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate and isolated mitochondria. *Biomolecules* 5:1319–1338, 2015.
38. The jamovi project. jamovi (Version 2.3) [Computer Software]. Sydney, Australia. 2022. Available at: https://www.jamovi.org. Accessed December 29, 2022.
39. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013. Available at: http://www.R-project.org/. Accessed December 29, 2022.
40. del Giudice M, Gangestad SW: Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. *Brain Behav Immun* 70:61–75, 2018.
41. Póvoa P: C-reactive protein: a valuable marker of sepsis. *Intensive Care Med* 28: 235–243, 2002.
42. Li X, Xu Z, Pang X, Huang Y, Yang B, Yang Y, Chen K, Liu X, Mao P, Li Y: Interleukin-10/lymphocyte ratio predicts mortality in severe septic patients. *PLoS One* 12:e0179050, 2017.
43. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G: Interleukin 10 (IL-10) inhibits human lymphocyte interferon 3,-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 178:1041–1048, 1993.
44. Jang DH, Greenwood JC, Spyres MB, Eckmann DM: Measurement of mitochondrial respiration and motility in acute care. *J Intensive Care Med* 32:86–94, 2017.
45. Greenfield KG, Badovinac VP, Griffith TS, Knoop KA: Sepsis, cytokine storms, and immunopathology: the divide between neonates and adults. *Immunohorizons* 5:512–522, 2021.
46. Girardot T, Rimmelé T, Monneret G, Textoris J, Venet F: Intra-cellular lactate concentration in T lymphocytes from septic shock patients—a pilot study. *Intensive Care Med Exp* 6:1–14, 2018.
47. Jones AE, Trzeciak S, Kline JA: The Sequential Organ Failure Assessment score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. *Crit Care Med* 37: 1649, 2009.
48. de Grooth HJ, Geenen IL, Girbes AR, Vincent JL, Parienti JJ, Oudemans-van Straaten HM: SOFA and mortality endpoints in randomized controlled trials: a systematic review and meta-regression analysis. *Crit Care* 21:1–9, 2017.
49. Mira JC, Brakenridge SC, Moldawer LL, Moore FA: Persistent Inflammation, Immunosuppression and Catabolism Syndrome (PICS). *Crit Care Clin* 33: 245, 2017.

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ESM 1. Correlation matrix: correlation between mitochondrial and inflammatory variables on day 1

Correlation Matrix

		Routine respiration day 1	**Complex I respiration day 1**	**Complex II respiration day 1**	**BCE day 1**	**IL-6 day 1**	**IL-10 day 1**	**IL-1 day 1**	**Lymphocytes day 1**
Routine respiration day 1	Spearman's rho	—	0.811	0.770	0.189	-0.225	-0.017	-0.279	0.065
	p-valor	—	< .001	< .001	0.134	0.074	0.892	0.026	0.615
Complex I respiration day 1	Spearman's rho		—						
	p-value		—						
Complex II respiration day 1	Spearman's rho		0.882	—					
	p-value		< .001	—					
BCE day 1	Spearman's rho		0.372	0.253	—				
	p-value		0.003	0.044	—				
IL-6 day 1	Spearman's rho		-0.227	-0.275	-0.247	—			
	p-value		0.071	0.028	0.050	—			
IL-10 day 1	Spearman's rho		-0.091	-0.088	-0.046	0.052	—		
	p-value		0.477	0.487	0.719	0.686	—		
IL-1 day 1	Spearman's rho		-0.228	-0.253	-0.119	0.515	0.338	—	

Correlation Matrix

		Routine respiration day 1	Complex I respiration day 1	Complex II respiration day 1	BCE day 1	IL-6 day 1	IL-10 day 1	IL-1 day 1	Lymphocytes day 1
	p-value		0.070	0.044	0.349	< .001	0.006	—	
Lymphocytes day 1	Spearman's rho		0.033	0.004	0.002	0.019	-0.132	-0.163	—
	p-value		0.798	0.973	0.990	0.882	0.308	0.206	—

ESM 2. Correlation matrix: correlation between delta (day 3 – day 1) mitochondrial and inflammatory variables

Correlation Matrix

		Delta Routine respiration	Delta Complex I respiration	Delta Complex II respiration	Delta BCE	Delta IL-10	Delta IL-6	Delta IL-1	Delta lymphocytes	Delta CRP
Delta Routine respiration	Spearman's rho	—								
	p-value	—								
Delta Complex I respiration	Spearman's rho	0.723 ***	—							
	p-value	< .001	—							
Delta Complex II respiration	Spearman's rho	0.734 ***	0.930 ***	—						
	p-value	< .001	< .001	—						
Delta BCE	Spearman's rho	0.129	-0.019	0.081	—	-0.226	0.098	-0.116	-0.171	0.168
	p-value	0.322	0.884	0.533	—	0.081	0.454	0.373	0.187	0.281
Delta IL-10	Spearman's rho	-0.257 *	-0.214	-0.220	-0.226	—				
	p-value	0.046	0.097	0.089	0.081	—				
Delta IL-6	Spearman's rho	-0.320 *	-0.346 **	-0.261 *	0.098	0.393 **	—			
	p-value	0.012	0.006	0.042	0.454	0.001	—			
Delta IL-1	Spearman's rho	-0.204	-0.096	-0.167	-0.116	0.236	-0.024	—		

Correlation Matrix

		Delta Routine respiration	Delta Complex I respiration	Delta Complex II respiration	Delta BCE	Delta IL-10	Delta IL-6	Delta IL-1	Delta lymphocytes	Delta CRP
	p-value	0.116	0.464	0.197	0.373	0.061	0.848	—		
Delta lymphocytes	Spearman's rho	0.015	-0.016	-0.050	-0.171	0.030	0.131	0.032	—	
	p-value	0.908	0.905	0.702	0.187	0.817	0.301	0.804	—	
Delta CRP	Spearman's rho	0.032	-0.002	0.069	0.168	0.086	0.151	0.098	0.157	—
	p-value	0.841	0.991	0.659	0.281	0.577	0.322	0.524	0.304	—

Nota. * p < .05, ** p < .01, *** p < .001

ESM 3. Association between tracheostomy and corticosteroid use with mitochondrial and inflammatory variables.

Outcome: tracheostomy

Variable	MD [tracheostomized vs non-tracheostomized (95% CI)]	p
Delta routine respiration	-34.7 (-178 to 84)	0.53
Delta complex I respiration	-58.8 (-432 to 160)	0.55
Delta complex II respiration	14.7 (-706 to 354)	0.35
Delta BCE	0.07 (-0.08 to 0.22)	0.45
Delta IL-1ß	-15 (-345 to 27)	0.51
Delta IL-6	0.5 (-127 to 124)	0.98
Delta IL-10	7.8 (-424 to 176)	0.81
Delta CRP	-23.1 (-130 to 73)	0.59
Delta lymphocytes	130 (-780 to 830)	0.79

Outcome: corticosteroid use

Variable	MD [corticosteroid use vs non-corticosteroid use (95% CI)]	p
Delta routine respiration	7 (-44.3 to 54.5)	0.8
Delta complex I respiration	-1.9 (-148.5 to 105.3)	0.97
Delta complex II respiration	14.7 (-217 to 282)	0.9
Delta BCE	0.09 (0.01 – 0.16)	0.01
Delta IL-1ß	8.5 (-12.2 to 32)	0.4
Delta IL-6	-20.8 (-74.5 to 17.3)	0.26
Delta IL-10	-59 (-246 to 4)	0.07
Delta CRP	13.6 (-37 to 82)	0.59
Delta lymphocytes	-262 (-705 to 212)	0.25

Capítulo 4 – "*Antibiotic therapy does not alter mitochondrial bioenergetics in lymphocytes of patients with septic shock – A prospective cohort study*."

Estudo publicado na revista *Mitochondrion*. Avaliamos a variabilidade dos marcadores mitocondriais (delta BCE, delta respiração basal, delta respiração de Complexo I e delta respiração de Complexo II) em pacientes recebendo diferentes antimicrobianos, de diferentes classes. Também realizamos análises em controles saudáveis, em pacientes que utilizaram terapia antimicrobiana combinada (em comparação àqueles que utilizaram em monoterapia), e em casos de sepse nosocomial, comparados aos casos de sepse adquirida na comunidade.

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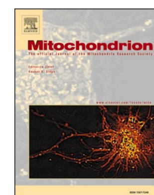

Antibiotic therapy does not alter mitochondrial bioenergetics in lymphocytes of patients with septic shock – A prospective cohort study

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ABSTRACT

Antibiotics may trigger alterations in mitochondrial function, which has been explored in cells culture, and in animal model of sepsis. This study sought to evaluate whether antibiotic therapy affects mitochondrial bioenergetics in a 68-patients clinical study. We studied mitochondrial respiratory rates at two time points: the first day of antibiotic administration and three days after. The Δbasal, ΔCI, ΔCII respiration, and ΔBCE respiratory rates were not different between patients administered with polymyxin, vancomycin, amoxicillin-clavulanate, and azithromycin compared to those who were not administered. Specific beta-lactams are associated with specific modifications in mitochondrial respiratory endpoints – patients who used meropenem had higher delta C2 values compared to those who did not ($p = 0.03$). Patients who used piperacillin-tazobactam had lower delta C1 ($p = 0.03$) values than those who did not, but higher delta C2 values ($p = 0.02$). These mitochondrial metabolic signatures in isolated lymphocytes challenges the proposed effects of antibiotics in mitochondrial bioenergetics of cell cultures, but at current status have an uncertain clinical significance.

1. Introduction

Critically-ill patients are the highest per-capita consumers of antimicrobials (Vincent et al., 2009), and are at greatest risk of harm from untreated or undertreated infection (Zasowski et al., 2020). However, this fact is associated with a dark side. Particularly, there is growing evidence of the harm caused to individual patients by unnecessary or unduly prolonged antimicrobial use (Arulkumaran et al., 2020; Zheng et al., 2019). Although not yet demonstrated in critically septic patients, the harmful effects associated with prolonged use of antimicrobials could affect already compromised cell function, resulting in consequences that could amplify pathophysiological mechanisms.

For instance, altered metabolism is a pathophysiological component of organ dysfunction in sepsis and, it was demonstrated that at clinically relevant doses, exposure of various human cells line and mice to bactericidal antimicrobials resulted in mitochondrial abnormalities including overproduction of reactive oxygen species (ROS) and oxidative damage to protein, DNA and lipid membranes (Kalghatgi et al., 2013). Such undesirable effects were observed at different dose and time regimens of quinolone (ciprofloxacin), beta-lactams (ampicillin) and aminoglycoside (kanamycin) (Kalghatgi et al., 2013). Acute kidney injury is a common complication in these patients, and antibiotic use is a major risk factor (Perazella, 2019). Antibiotic-induced kidney injury occurs mainly with the use of aminoglycosides and vancomycin, and mitochondrial dysfunction of tubular cells is one of the key mechanisms for its emergence (Blair et al., 2021; Perazella, 2019; Stefano et al., 2017).

Although beta-lactams (like meropenem, amoxicilline-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam) and cephalosporins have been implicated as reversible inhibitors of carriers for mitochondrial substrates, such as succinate carriers, prolonged exposure to beta-lactams caused irreversible acylation of mitochondrial Complex II

Abbreviations: ATP, adenosine triphosphate; BCE, biochemical coupling efficiency; CI, complex I; CII, complex II; ETC, electron transport chain; ICU, intensive care unit; PTC, peptidyl transferase center; ROS, reactive oxygen species; SAPS, Simplified Acute Physiology score; SOFA, Sequential Organ Failure Assessment.

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(Tune, 1990), accompanied by decreased activity. Also, at high doses, several antimicrobials are able to inhibit mitochondrial oxidative phosphorylation (Duewelhenke et al., 2007). An combined impairment in substrate availability, ETC complex activity, and decreased adenosine triphosphate (ATP) production triggered by antimicrobials has the potential to act synergistically to influence organ dysfunction in septic patients (Brealey et al., 2002). Piperacillin-tazobactam can penetrate blood–brain barrier and cause mitochondrial dysfunction in neuron cells as shown by the reduction of mitochondrial respiration, membrane potential, and ATP production (Jiang et al., 2018). This damage has clinical consequences, such as encephalopathy, mental confusion and psychosis induced not only by piperacillin-tazobactam but also by other beta-lactams (Roger and Louart, 2021).

Although preclinical studies emphasizes that antimicrobials impose risks to mitochondrial function and cells viability, this association has not been addressed in human studies, particularly in patients with septic shock, a more severe spectrum of sepsis. While the antimicrobials act as key supporters of septic shock resolution in humans, there are still gaps regarding its influence on mitochondrial bioenergetics and whether it conciliates results from preclinical studies (Arulkumaran et al., 2020). Furthermore, an appropriate bioenergetic function of lymphocytes is fundamental to produce an adequate immune response and signaling molecules (Brady et al., 2020). Mitochondrial metabolism of lymphocytes are closely related with citokynes production and clonal expansion, fundamental in the immune response of sepsis (Kramer et al., 2014). Thus, is relevant to know whether antimicrobials interact with specific targets in lymphocyte mitochondria to influence its metabolism (Barnhill et al., 2012; Duewelhenke et al., 2007).

In this study, our objective is to evaluate the association between the use of different classes of antibiotics commonly used in septic shock and their potential impact on the mitochondrial metabolism of lymphocytes, after the start of their administration.

2. Material & methods

2.1. Participants and study design

This is a prospective cohort study designed to evaluate the mitochondrial metabolism of circulating lymphocytes in patients with septic shock admitted to four different intensive care units (ICUs) of a university hospital in Brazil. This study was approved by the local ethics committee (Plataforma Brasil number 66240017.0.0000.5530). We prospectively enrolled 68 adult patients (greater than18 years old) admitted to the ICU due to septic shock between November 2017 and July 2018. Patients were excluded if they had known mitochondrial disease, pregnancy, refusal of the patient or the next of kin to sign the informed consent, patients with imminent death, and patients with withholding or withdrawing treatments. No patients used metformin during the study. The patients included in this study had not previously used other antibiotics during the management of septic shock, thus allowing the assessment of the isolated impact of starting the study antibiotics, and maintained the use of antibiotics during the study period, without adding or removing any of them. Septic shock was defined as the presence of persistent hypotension with the need for vasopressor therapy to maintain a mean arterial pressure of 65 mmHg or greater (Levy et al., 2003). Patients were treated for septic shock according to best clinical practice (Evans et al., 2021). The following demographic and clinical characteristics were prospectively recorded: gender, age, primary site of infection, community-acquired or hospital-acquired infection, antibiotic use, ICU and 28-day mortality. Also, Simplified Acute Physiology score (SAPS 3) and the Sequential Organ Failure Assessment (SOFA) score were assessed.

Clinical and laboratory endpoints, including mitochondrial respirometry in permeabilized lymphocytes (digitonin 0.005% w/v), were evaluated at two time points: the first day of admission to the ICU (D1) and three days after admission (D3). Pairwise variations between D1 and D3 (Δ) were used to estimate the improvement or worsening of the clinical, laboratory, and mitochondrial endpoints. Additionally, we performed an analysis evaluating the mithocondrial metabolism measurements (Routine, Complex I respiration, Complex II respiration and BCE) of six healthy individuals with the study population, comparing the variables at the two moments (D1 and D3) in which the septic shock patients were analyzed. The primary outcome was the Δ between those who used and those who did not use each type of antibiotic.

2.2. Isolation of lymphocytes and mitochondrial respirometry

Six milliliters of blood were sampled in EDTA tubes at D1 and D3 and Lymphocytes obtained through gradient centrifugation as described by (Pecina et al., 2014). Briefly, lymphocytes were isolated by layering 4 mL of blood over 4 mL of Ficoll 1.077 g/dL (GE® healthcare, Little Chalfont, UK) in a 15 mL sterile centrifuge tube. Samples were centrifuged at 800 × g for 20 min at 4 °C. An amount of 2 mL from the Buffy coat present on the interface between centrifuged blood and Ficoll solution was placed in a second sterile centrifuge tube and then diluted in 38 mL of RBC lysis solution (NH_4Cl 155 mM, K_2HCO_3 10 mM, EDTA 0.1 mM) and left for 30 min on ice. This was then centrifuged for additional 20 min at 800 X g for 20 min at 4 °C. The obtained pellet was resuspended in 750 μL respiration buffer (Mannitol 320 mM, TRIS 100 mM, KCl 50 mM, $MgCl_2$ 4 mM, NaH_2PO_4 4 mM, EDTA 0.73 mM, pH 7.4), and added to an Oroboros O2k High-resolution oxygraphy (Oroboros Instruments, Innsbruck, AT). Respiration buffer was supplemented with 0.005% w/v digitonin to allow permeabilization of mitochondrial membranes to polar substrates. Adequate permeabilization in this isolation protocol was investigated prior to the analysis, assessing oxygen consumption following substrate addition to permeabilized and non-permeabilized isolates from the same sample (ESM 8). We performed a protocol as previously described (Nedel et al., 2021). This protocol assess the steady-state, "basal" (State 1) respiration, the respiration linked to Complex I (CI), Complex II (CII) (State 2) and maximal oxygen flow rate consumption coupled to ATP production (State 3), and nonmitochondrial oxygen consumption. Additionally, biochemical coupling efficiency (BCE) (also known as P control factor) was measured as an indicator of the effectiveness of mitochondrial oxygen flow coupled with ATP production (Chance and Williams, 1955). Following respirometric analyses, total protein concentration was assessed using BCA kits (Thermo Scientific, USA), using 25 μL of lymphocyte isolate.

The researchers involved in the mitochondrial analysis were blinded to the clinical outcomes, and the researchers involved in the clinical data collection were blinded to the mitochondrial outcomes. All chemicals used for high-resolution respirometry analysis were analytical grade and purchased from Sigma-Aldrich (Sigma Aldrich, St. Louis, MO, USA).

2.3. Statistical analysis

Descriptive statistics included frequencies and percentages for categorical variables and means, and standard deviation, confidence intervals, medians, and interquartile ranges for continuous variables. Mann-Whitney *U* test and One-Way ANOVA test were used to compare continuous variables. To access the impact of each antibiotic on the outcomes, a multiple linear regression was performed with Δ Basal, Δ CI, Δ CII and Δ BCE as the dependent variable, and monotherapy (versus combined antibiotic therapy), SOFA score at septic shock diagnosis, pulmonary sepsis (versus non-pulmonary sepsis), nosocomial sepsis (versus community-acquired sepsis) as independent variables in the model. The antibiotics analyzed were selected for the model because they presented a p value of$<$0.20 in the univariate analysis when compared with the variation of mitochondrial variables (Δ Basal, Δ CI, Δ CII and Δ BCE). Since the BCE assessment is a global marker of the adequacy of mitochondrial metabolism, and given the fact that all patients received antibiotic therapy as a result of the management of sepsis, we performed an exploratory analysis evaluating the association between

improvement in BCE in the second mensuration and the use of combination antimicrobial therapy (compared to the use of antibiotic monotherapy). In this multivariate analysis, we constructed a binomial logistic regression with the presence of potentially confounding clinical variables of the outcome (BCE improvement): SOFA score at sepsis diagnosis, SAPS 3 at admission, clinical admission (versus surgical admission), nosocomial sepsis (versus community-acquired sepsis), pulmonary sepsis (versus non-pulmonary sepsis foci). We also performed analysis of collinearity between independent variables in the different models. Statistical tests were two-tailed with significance defined as a p-value<0.05. All p-values were two-tailed. We used R 4.1.0 (R foundation) and Jamovi (The jamovi project) version 1.6.23 for all analyses.

3. Results

A total of 68 patients were included in the study, 43 are men and 25 are women. The mean age was 64.5 (±16.1) years, 38 had a clinical admission and 30 had a surgical admission to the ICU, and 43 patients had nosocomial sepsis. The most prevalent septic foci were pulmonary (31 patients) and abdominal (29 patients). ICU mortality was 38% and hospitalar mortality was 50%. Fifty-eight (85%) patients received mechanical ventilation and 41% received hemodialysis, and the mean SOFA score at admission to the ICU was 8 (±3.2) points. The clinical characteristics of the population studied are described in Table 1.

Eighteen patients received polymyxin, 21 received meropenem, 17 received vancomycin, 9 received amoxicillin-clavulanate, 10 received azithromycin, 6 received ampicillin-sulbactam, and 32 received piperacillin-tazobactam, in mono or combined therapy.

Results of mitochondrial variables (Basal D1 and D3, Complex I respiration D1 and D3, Complex II respiration D1 and D3, BCE D1 and D3 and its respectively deltas (D3 – D1) are shown in ESM 1 – 7. ESM 8 shows representative images of high-resolution respirometry assay in lymphocytes. There were no differences in the ΔBasal, ΔCI, ΔCII, and ΔBCE values between patients treated with polymyxin, vancomycin, amoxicillin-clavulanate, and azithromycin compared to those not treated with these antimicrobials. Patients who used meropenem had higher ΔCII values compared to those who did not, but there were no statistical differences in ΔCI ($p = 0.09$), ΔBCE ($p = 0.07$) and ΔBasal ($p = 0.17$) – ESM 2. Patients who used ampicillin-sulbactam had decreased ΔBasal respiration compared to those who did not ($p = 0.039$), and no significant differences was found relative to ΔCI values ($p = 0.07$), and ΔCII values ($p = 0.16$) – ESM 6. Patients who used piperacillin-tazobactam displayed decreased ΔCI respiratory values and increased ΔCII values than those who did not use. Additionally, no statistical differences in ΔBasal and ΔBCE values were observed – ESM 7. We did not find statistically significant differences in D1 and D3 measurements in the different analysis performed (ESM 1 – 7).

Since the antibiotics studied have specific indications depending on the focus of sepsis and its origin (nosocomial or community-acquired), we performed an exploratory analysis regarding the influence of the primary site of infection on mitochondrial respiratory endpoints. Patients who had nosocomial septic shock did not show altered mitochondrial bioenergetic function compared to those who acquired community septic shock: ΔBasal ($p = 0.39$), ΔCI ($p = 0.08$), ΔCII ($p = 0.42$), and ΔBCE ($p = 0.84$). Furthermore, the foci of septic shock were not associated with ΔBasal ($p = 0.26$), ΔCI ($p = 0.30$), ΔCII ($p = 0.20$), and ΔBCE ($p = 0.75$). There was no statistically significant differences in pairwise comparisons (abdominal, catheter, cutaneous, pulmonary, primary bloodstream and urinary foci). We also performed an analysis evaluating the hypothesis that the use of antibiotics in monotherapy would present a smaller change in the values of Δ Basal, Δ CI, Δ CII and Δ BCE compared to those patients who used combined antibiotic therapy. We did not find difference between the groups regarding Δ Basal (56.9 [IQR 4 to 113] in monotherapy versus 37 [IQR 3 to 93] in combined therapy, $p = 0.37$), Δ CI (103 [IQR −7 to 188] in monotherapy versus 108 [IQR 12 to 240] in combined therapy, $p = 0.54$), Δ CII (171 [IQR −49 to 366] in monotherapy versus 155 [IQR −115 to 462] in combined therapy, $p = 0.73$) and Δ BCE (0 [IQR −0.11 to 0.13] in monotherapy versus 0.03 [IQR −0.08 to 0.14], $p = 0.57$). In the several multivariate models in which we analyzed the impact of each antibiotic in mitochondrial variables (Table 2), piperacillin-tazobactam was associated with a lesser Δ Complex II improvement in 72 h ($p = 0.001$) when compared with non-users, and ampicillin-sulbactam was associated with a greater improvement in Δ Basal when compared with non-users ($p = 0.034$). In the another models, only SOFA score at septic shock diagnosis were independently associated with changes in mitochondrial variables. The complete data of these statistical analyzes are published in a data repository (zenodo.org, https://doi.org/10.5281/zenodo.6575297).

Healthy controls had higher BCE measurements compared to patients with septic shock in D1 (0.5 [IQR 0.4 – 0.5] versus 0.3 [0.3 – 0.4], $p = 0.02$, respectively) but not in D3 (0.4 [IQR 0.2 – 0.5] in patients with septic shock, $p = 0.28$ in comparison between groups). These populations did not differ in Routine measurements, both in D1 (127.9 [IQR 85.2 – 448.9] in healthy controls and 134.7 [IQR 113.6 – 197.2] in septic patients, $p = 0.81$) and in D3 (211.4 [IQR 153.4 – 272, $p = 0.41$). Healthy controls had lower CI respiration measurements when compared with septic shock patients, both in D1 (81.6 [IQR 20.9 – 232.9] versus 274.2 [IQR 176.4 – 446.7, respectively, $p = 0.01$) and in D3 (408.5 [IQR 287.4 – 620.3], $p = 0.01$). Healthy controls also had lower CII respiration measurements when compared with septic shock patients, both in D1 (46.5 [IQR 36.4 – 118.9] versus 548.5 [IQR 389.7 – 787.2], respectively, $p < 0.01$) and in D3 (787.8 [IQR 552.9 – 1053.6], $p < 0.01$).

We performed an analysis that evaluated the impact of combination antimicrobial therapy (compared to monotherapy) on improving BCE on day 3, corrected for potentially confounding variables. Monotherapy was not associated with the improvement of BCE (OR 0.80, 95% CI 0.28 – 2.29; $p = 0.69$), SAPS 3 score (OR 0.99, 95% CI 0.95 – 1.04; $p = 0.97$),

Table 1
Main clinical characteristics of the study population.

Variable	N (%)
Clinical ICU admission	38 (55%)
Male:female sex	43:28
Nosocomial sepsis	43 (68%)
Sepsis foci	
Abdominal	29
Catheter	1
Cutaneous	2
Lung	31
Primary bloodstream	4
Urinary	1
ICU mortality	25 (36%)
Hospitalar mortality	34 (50%)
Hemodialysis	28 (41%)
Mechanical ventilation	58 (85%)
Polymixin	18
Meropenem	21
Vancomycin	17
Amoxillin-clavulanate	9
Azitromycin	10
Ampicillin-sulbactam	6
Piperacillin-tazobactam	32
SAPS 3 score – mean (dp)	75 (13)
SOFA score – mean (dp)	8 (3.2)
Age – mean (dp)	64.5 (16.1)
CRP – median (iqr)	220 (153 to 270)
Δ Basal – median (iqr)	42.7 (4.2 to 106.3)
Δ Complex I respiration – median (iqr)	105.3 (1.1 to 224.9)
Δ Complex II respiration – median (iqr)	163.1 (−111.3 to 409.8)
ΔBCE – median (iqr)-	0.03 (−0.09 to 0.14)

Legend: BCE, biochemical coupling efficiency; CRP, c-reactive protein; ICU, intensive care unit; SOFA, sequential organ failure assessment; SAPS, simplified acute physiology score, Δ: Day 3 - Day 1.

Table 2
Multivariate analysis exploring the impact of different antimicrobials in mitochondrial variables.

Δ BCE: meropenem use	Coefficient (95% CI)	p
Intercept	0.52 (0.19 – 0.85)	0.002
Meropenem use (vs non-use)	−0.23 (−0.52 to 0.04)	0.10
Monotherapy (vs combined therapy)	−0.08 (−0.2 to 0.04)	0.19
SOFA at sepsis diagnosis	−0.02 (−0.03 to −0.01)	<0.001
Pulmonary sepsis (vs non-pulmonary sepsis)	0.001 (−0.06 to 0.06)	0.959
Nosocomial sepsis (vs community-acquired sepsis)	0.02 (−0.04 to 0.09)	0.486
Δ Basal: meropenem use	**Coefficient (95% CI)**	**p**
Intercept	116.92 (−20.05 to 446.85)	0.072
Meropenem use (vs non-use)	−94.89 (−300.79 to 111)	0.360
Monotherapy (vs combined therapy)	−9.82 (−100.68 to 81.03)	0.827
SOFA at sepsis diagnosis	−2.15 (−9.34 to 5.03)	0.551
Pulmonary sepsis (vs non-pulmonary sepsis)	21.3 (−25.08 to 67.69)	0.362
Nosocomial sepsis (vs community-acquired sepsis)	−20.26 (−68.36 to 27.85)	0.403
Δ Complex I: meropenem use	**Coefficient (95% CI)**	**P**
Intercept	340.2 (−368. 05 to 1048.46)	0.34
Meropenem use (vs non-use)	181.42 (−443.24 to 806.08)	0.564
Monotherapy (vs combined therapy)	−117.03 (−392.67 to 158.6)	0.399
SOFA at sepsis diagnosis	1.68 (−20.11 to 23.48)	0.877
Pulmonary sepsis (vs non-pulmonary sepsis)	73.78 (−66.95 to 214.51)	0.299
Nosocomial sepsis (vs community-acquired sepsis)	−120.76 (−266.72 to 25.18)	0.103
Δ Complex II: meropenem use	**Coefficient (95% CI)**	**P**
Intercept	849.80 (−368.05 to 1048.46)	0.154
Meropenem use (vs non-use)	174.92 (−443.24 to 806.08)	0.737
Monotherapy (vs combined therapy)	−312.51 (−392.67 to 158.61)	0.177
SOFA at sepsis diagnosis	−8.42 (−20.11 to 23.49)	0.644
Pulmonary sepsis (vs non-pulmonary sepsis)	157.5 (−66.95 to 214.51)	0.183
Nosocomial sepsis (vs community-acquired sepsis)	−113.81 (−266.72 to 25.19)	0.352
Δ BCE: amoxicillin-clavulanate use	**Coefficient (95% CI)**	**P**
Intercept	0.21 (0.055 to 0.371)	0.009
Amoxicillin-clavulanate use (vs non-use)	−0.01 (−0.12 to 0.005)	0.063
Monotherapy (vs combined therapy)	−0.056 (−0.143 to 0.116)	0.386
SOFA at sepsis diagnosis	−0.017 (−0.027 to −0.007)	0.001
Pulmonary sepsis (vs non-pulmonary sepsis)	−0.002 (−0.069 to 0.063)	0.937
Δ Complex I: piperacillin-tazobactam use	**Coefficient (95% CI)**	**P**
Intercept	0.21 (−71.03 to 617.25)	0.009
Piperacillin-tazobactam use (vs non-use)	−0.01 (−342.85 to −25.60)	0.063
Monotherapy (vs combined therapy)	−0.056 (−278.07 to 303.41)	0.386
SOFA at sepsis diagnosis	−0.02 (−23.55 to 21.41)	0.001
Pulmonary sepsis (vs non-pulmonary sepsis)	−0.002 (−44.2 to 240.47)	0.937
Nosocomial sepsis (vs community-acquired sepsis)	0.023 (−228.42 to 70.49)	0.506
Δ Complex II: piperacillin-tazobactam use	**Coefficient (95% CI)**	**P**
Intercept	574.85 (3.73 to 1145.96)	0.049
Piperacillin-tazobactam use (vs non-use)	− 361.40 (−624.63 to −98.16)	0.007
Monotherapy (vs combined therapy)	−57.99 (−540.46 to 424.49)	0.811
SOFA at sepsis diagnosis	−13.78 (−51.1 to 23.54)	0.463
Pulmonary sepsis (vs non-pulmonary sepsis)	199.64 (−36.57 to 435.84)	0.096
Nosocomial sepsis (vs community-acquired sepsis)	−30.62 (−278.65 to 217.4)	0.806
Δ Basal: ampicillin-sulbactam use	**Coefficient (95% CI)**	**P**
Intercept	83 (−26.69 to 192.69)	0.135
Ampicillin-sulbactam use (vs non-use)	95.2 (7.02 to 183.38)	0.034
Monotherapy (vs combined therapy)	−23.07 (−114.4 to 68.25)	0.615
SOFA at sepsis diagnosis	−3.14 (−10.34 to 4.05)	0.386
Pulmonary sepsis (vs non-pulmonary sepsis)	28.05 (−17.75 to 73.87)	0.226
Nosocomial sepsis (vs community-acquired sepsis)	−4.54 (−52.7 to 43.61)	0.851
Δ Complex I: ampicillin-sulbactam use	**Coefficient (95% CI)**	**P**
Intercept	249.13 (−105.92 to 604.19)	0.166
Ampicillin-sulbactam use (vs non-use)	135.52 (−149.9 to 420.95)	0.347
Monotherapy (vs combined therapy)	−114.62 (−410.24 to 181	0.442
SOFA at sepsis diagnosis	1.5 (−21.78 to 24.8)	0.898
Pulmonary sepsis (vs non-pulmonary sepsis)	99.29 (−49 to 247.59)	0.186
Nosocomial sepsis (vs community-acquired sepsis)	−90.52 (−246.34 to 65.35)	0.251
Δ Complex II: ampicillin-sulbactam use	**Coefficient (95% CI)**	**P**
Intercept	526.3 (−70.64 to 1123.25)	0.083
Ampicillin-sulbactam use (vs non-use)	252.44 (−227.44 to 732.33)	0.297
Monotherapy (vs combined therapy)	−304.53 (−801.55 to 192.49)	0.226
SOFA at sepsis diagnosis	−8.51 (−47.68 to 30.66)	0.666
Pulmonary sepsis (vs non-pulmonary sepsis)	200.95 (−48.42 to 450.23)	0.112
Nosocomial sepsis (vs community-acquired sepsis)	−54.82 (−316.89 to 207.24)	0.678

1.
BCE, biochemical coupling efficiency.

clinical admission (OR 1.85, 95% CI 0.5 – 6.82, p = 0.35), nosocomial sepsis (OR 1.09, 95% CI 0.34 – 3.45; p = 0.87) or pulmonary sepsis (OR 0.94, 95% CI 0.28 – 3.17; p = 0.92). There was, however, a statistically significant association between BCE improvement at day 3 and SOFA score at septic shock diagnosis: OR 0.75, 95% CI 0.6 – 0.93; p = 0.01.

4. Discussion

Lymphocytes mitochondrial bioenergetics is an essential component of immune function (Kramer et al., 2014). Energy metabolism regulates the response of both innate and adaptive immune cells and, hence, potential interactions between antimicrobials with mitochondria in patients with septic shock may enhance immune cell responsiveness or alternatively conduct immunoparalysis (Cheng et al., 2016).

It has been reported that more than 40% of antibiotics interfere with bacterial and also host protein biosynthetic machinery, more specifically with ribosomal sites which may lead to damage in mitochondrial proteins involved in bioenergetic activity like the electron transfer complexes and ATP synthase (McCoy et al., 2011). Antibiotics may also interact with other functional effectors present in the cells of the host, resulting in adverse effects organ specific (Blair et al., 2021; Roger and Louart, 2021). Remarkably, several classes of antimicrobials are involved with different levels of mitochondrial damage, implying they impair energy metabolism and ATP-dependent mechanisms. Actually, in preclinical studies some antibiotics increases the reactive oxygen species production and oxidative damage to proteins and tissues reflecting they may cause metabolic uncoupling (Barnhill et al., 2012). (Cheng et al., 2016). Our data demonstrate a lower BCE in septic shock patients compared with healthy individuals, with increased Complex I and Complex II respiration in these population, which may corroborate these data. Differentiating the intrinsic effect of sepsis and the additional effect of antibiotic administration, however, cannot be estimated.

In our study, different classes of antibiotics did not alter mitochondrial metabolism in lymphocytes of septic shock patients; therefore, we were not able of reconciliating the detrimental effects of antimicrobials on mitochondria metabolism reported in cell culture and in animal models. However, antimicrobials traditionally associated with

mitochondrial damage have a reduction in their use (Timsit et al., 2019) due to limitations in their antimicrobial spectrum, such as aminoglycosides, the magnitude of side effects, such as chloramphenicol, or in their induction of bacterial resistance, such as quinolones.

Beta-lactams are the guideline in the treatment of several relevant clinical conditions, such as nosocomial and community-acquired pneumonia as well as intra-abdominal infection, being the class of antimicrobials mostly consumed nowadays (Tamma et al., 2021). Here, we found an impairment in complex II respiration in lymphocytes of patients who received meropenem, compared with those who not received; and there was a tendency of improvement in ΔBCE and ΔComplex I respiration in those who received meropenem. In others beta-lactams studied, we found distinct patterns of response. In patients receiving ampicillin-sulbactam, there was an impairment in ΔBasal respiration but a trend of improvement in ΔComplex I respiration. In patients who received piperacillin-tazobatam, there was an impairment in ΔComplex I respiration and an improvement in ΔComplex II respiration being this oscillation in CI and CII respiration a suggestion of compensatory mechanism.

When variations in mitochondrial metabolism (delta) are evaluated for the presence of potential confounders, both as markers of underlying disease severity (such as the SOFA score), and the primary focus of sepsis, the associated use of multiple antibiotics and the origin of infection, the use of different types of antibiotics is not independently associated with alterations in mitochondrial metabolism, except when we evaluate the use of piperacillin-tazobactam and Δ CII and the use of ampicillin-sulbactam and the ΔBasal. However, due to the small sample size when patients are stratified by each antimicrobial employed, and the multiple comparisons performed, the analyzes are subject to type I and type II statistical errors. Based on this limitation, we suggest "statistical trends" regarding improvement or worsening of mitochondrial metabolic endpoints, depending on the antibiotic studied. However, it is important to note that we found alterations only in antibiotics of the beta-lactam class, with intrinsic effects of each drug on specific mitochondrial respiratory endpoints.

In this work, our aim was to minimize the confusion bias inherent in the use of drugs for nosocomial infections, and not for community infections, as well as their use for infections from different sites (e.g., pulmonary and abdominal). We did not find significant differences in mitochondrial respiration between nosocomial patients compared to those with community-acquired infections, nor in those with infections at the pulmonary or abdominal site, the most prevalent in our sample. The small sample size, especially the very small numbers with any one antibiotic, is a great limitation of our work, because a previously related risk of a type II error in this context. Critically-ill patients are commonly exposed to an insufficient antibiotic exposure (Hagel et al., 2022), and this is a relevant limitation in our study, since there was no monitoring of serum levels of antibiotics. Thus, is impossible to know if levels of plasma antibiotics approached those used in in-vitro assays demonstrating impairment of bioenergetics.

Another potential limitation of this study is the use of different antibiotics, in different combinations, which could theoretically limit a better understanding of the role of each of them in mitochondrial function. However, this heterogeneity is in line with current parameters of antibiotic use in critically ill patients (Curcio and Group†, 2013; Lonsdale and Lipman, 2021). In addition, this heterogeneity reflects a real-life scenario, in which, although the use of a particular antibiotic may affect the mitochondrial function of an immune cell, this impact is possibly masked by other conditions that also alter the patient's mitochondrial function, in particular the severity of their disease (measured by SOFA score in our study). The fact that we found no difference in mitochondrial metabolism between those patients who used only one antibiotic, when compared with those who used a combination of two or more antimicrobials, also corroborates this finding. Possibly, therefore, the use of a particular antibiotic has a lesser effect on mitochondrial metabolism than the improvement of the infection per se. In this study, the usual regimen of antibiotics for the treatment of septic shock does not produce a large enough change in lymphocyte bioenergetics over 72 h of administration to be detected amidst the effect of initial administration of antibiotics and the critical illness itself.

In conclusion, mitochondrial metabolic signatures in isolated lymphocytes challenges the proposed effects of antibiotics in mitochondria bioenergetics of cell cultures, but at current status have an uncertain clinical significance.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement.

The data that supports the findings of this study are available upon reasonable request to the corresponding author.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mito.2022.07.001.

References

Arulkumaran, N., Routledge, M., Schlebusch, S., Lipman, J., Conway Morris, A., 2020. Antimicrobial-associated harm in critical care: a narrative review. Intensive Care Med. 46, 225–235. https://doi.org/10.1007/s00134-020-05929-3.

Barnhill, A.E., Brewer, M.T., Carlson, S.A., 2012. Adverse effects of antimicrobials via predictable or idiosyncratic inhibition of host mitochondrial components. Antimicrob. Agents Chemother. 56, 4046–4051. https://doi.org/10.1128/AAC.00678-12.

Blair, M., Côté, J.M., Cotter, A., Lynch, B., Redahan, L., Murray, P.T., 2021. Nephrotoxicity from vancomycin combined with piperacillin-tazobactam: A comprehensive review. Am. J. Nephrol. https://doi.org/10.1159/000513742.

Brady, J., Horie, S., Laffey, J.G., 2020. Role of the adaptive immune response in sepsis. Intensive Care Med. Experiment. 8 https://doi.org/10.1186/s40635-020-00309-z.

Brealey, D., Brand, M., Hargreaves, I., Heales, S., Land, J., Smolenski, R., Davies, N.A., Cooper, C.E., Singer, M., 2002. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 360, 219–223. https://doi.org/10.1016/S0140-6736(02)09459-X.

Chance, B., Williams, G.R., 1955. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J. Biol. Chem. 217, 383–393.

Cheng, S.C., Scicluna, B.P., Arts, R.J.W., Gresnigt, M.S., Lachmandas, E., Giamarellos-Bourboulis, E.J., Kox, M., Manjeri, G.R., Wagenaars, J.A.L., Cremer, O.L., Leentjens, J., van der Meer, A.J., van de Veerdonk, F.L., Bonten, M.J., Schultz, M.J., Willems, P.H.G.M., Pickkers, P., Joosten, L.A.B., van der Poll, T., Netea, M.G., 2016. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. Nat. Immunol. 17, 406–413. https://doi.org/10.1038/ni.3398.

Curcio, D., Group†, O. behalf of the L.A.A.U. in I.C.U., 2013. Antibiotic prescriptions in critically-Ill patients: A Latin American experience. Ann. Med. Health Sci. Res. 3, 220. https://doi.org/10.4103/2141-9248.113666.

Duewelhenke, N., Krut, O., Eysel, P., 2007. Influence on mitochondria and cytotoxicity of different antibiotics administered in high concentrations on primary human osteoblasts and cell lines. Antimicrob. Agents Chemother. 51, 54–63. https://doi.org/10.1128/AAC.00729-05.

Evans, L., Rhodes, A., Alhazzani, W., Antonelli, M., Coopersmith, C.M., French, C., Machado, F.R., Mcintyre, L., Ostermann, M., Prescott, H.C., Schorr, C., Simpson, S., Wiersinga, W.J., Alshamsi, F., Angus, D.C., Arabi, Y., Azevedo, L., Beale, R.,

Beilman, G., Belley-Cote, E., Burry, L., Cecconi, M., Centofanti, J., Coz Yataco, A., de Waele, J., Dellinger, R.P., Doi, K., Du, B., Estenssoro, E., Ferrer, R., Gomersall, C., Hodgson, C., Møller, M.H., Iwashyna, T., Jacob, S., Kleinpell, R., Klompas, M., Koh, Y., Kumar, A., Kwizera, A., Lobo, S., Masur, H., McGloughlin, S., Mehta, S., Mehta, Y., Mer, M., Nunnally, M., Oczkowski, S., Osborn, T., Papathanassoglou, E., Perner, A., Puskarich, M., Roberts, J., Schweickert, W., Seckel, M., Sevransky, J., Sprung, C.L., Welte, T., Zimmerman, J., Levy, M., 2021. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 47, 1181–1247. https://doi.org/10.1007/S00134-021-06506-Y.

Hagel, S., Bach, F., Brenner, T., Bracht, H., Brinkmann, A., Annecke, T., Hohn, A., Weigand, M., Michels, G., Kluge, S., Nierhaus, A., Jarczak, D., König, C., Weismann, D., Frey, O., Witzke, D., Müller, C., Bauer, M., Kiehntopf, M., Neugebauer, S., Lehmann, T., Roberts, J.A., Pletz, M.W., 2022. Effect of therapeutic drug monitoring-based dose optimization of piperacillin/tazobactam on sepsis-related organ dysfunction in patients with sepsis: a randomized controlled trial on behalf of the TARGET Trial Investigators. Intensive Care Med. 48, 311–321. https://doi.org/10.1007/s00134-021-06609-6.

Kalghatgi, S., Spina, C.S., Costello, J.C., Liesa, M., Morones-Ramirez, J.R., Slomovic, S., Molina, A., Shirihai, O.S., Collins, J.J., 2013. Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in mammalian cells. Sci. Transl. Med. 5 https://doi.org/10.1126/scitranslmed.3006055.

Kramer, P.A., Ravi, S., Chacko, B., Johnson, M.S., Darley-Usmar, V.M., 2014. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: Implications for their use as bioenergetic biomarkers. Redox Biol. 2, 206. https://doi.org/10.1016/J.REDOX.2013.12.026.

Jiang, S., Li, T., Zhou, X., Qin, W., Wang, Z., Liao, Y., 2018. Antibiotic drug piperacillin induces neuron cell death through mitochondrial dysfunction and oxidative damage. Can J Physiol Pharmacol. 2018 Jun;96(6):562-568. doi: 10.1139/cjpp-2016-0679.

Levy, M.M., Fink, M.P., Marshall, J.C., Abraham, E., Angus, D., Cook, D., Cohen, J., Opal, S.M., Vincent, J.L., Ramsay, G., 2003. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference, in: Intensive Care Medicine. https://doi.org/10.1007/s00134-003-1662-x.

Lonsdale, D.O., Lipman, J., 2021. Global personalization of antibiotic therapy in critically ill patients. https://doi.org/10.1080/23808993.2021.1874823 6, 87–93. https://doi.org/10.1080/23808993.2021.1874823.

McCoy, L.S., Xie, Y., Tor, Y., 2011. Antibiotics that target protein synthesis. Wiley Interdiscip. Rev.: RNA 2, 209–232. https://doi.org/10.1002/WRNA.60.

Nedel, W.L., Kopczynski, A., Rodolphi, M.S., Strogulski, N.R., de Bastiani, M., Montes, T. H.M., Abruzzi Jr, J., Galina, A., Horvath, T.L., Portela, L.V., 2021. Mortality of septic shock patients is associated with impaired mitochondrial oxidative coupling efficiency in lymphocytes: a prospective cohort study. Intensive Care Med Exp 9. https://doi.org/10.1186/S40635-021-00404-9.

Pecina, P., Houšťková, H., Mráček, T., Pecinová, A., Nůsková, H., Tesařová, M., Hansíková, H., Janota, J., Zeman, J., Houštěk, J., 2014. Noninvasive diagnostics of mitochondrial disorders in isolated lymphocytes with high resolution respirometry. BBA Clin. 2, 62–71. https://doi.org/10.1016/j.bbacli.2014.09.003.

Perazella, M.A., 2019. Drug-induced acute kidney injury: Diverse mechanisms of tubular injury. Curr. Opin. Crit. Care. https://doi.org/10.1097/MCC.0000000000000653.

Roger, C., Louart, B., 2021. Beta-lactams toxicity in the intensive care unit: An underestimated collateral damage? Microorganisms. https://doi.org/10.3390/microorganisms9071505.

Stefano, G.B., Samuel, J., Kream, R.M., 2017. Antibiotics may trigger mitochondrial dysfunction inducing psychiatric disorders. Med. Sci. Monit. 23, 101–106. https://doi.org/10.12659/MSM.899478.

Tamma, P.D., Aitken, S.L., Bonomo, R.A., Mathers, A.J., van Duin, D., Clancy, C.J., 2021. Infectious Diseases Society of America Antimicrobial Resistant Treatment Guidance: Gram-Negative Bacterial Infections A Focus on Extended-Spectrum β-lactamase Producing Enterobacterales (ESBL-E), Carbapenem-Resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with Difficult-to-Treat Resistance (DTR-P. aeruginosa). Clin Infect Dis 72, e169–e183. https://doi.org/10.1093/cid/ciaa1478.

Timsit, J.F., Bassetti, M., Cremer, O., Daikos, G., de Waele, J., Kallil, A., Kipnis, E., Kollef, M., Laupland, K., Paiva, J.A., Rodríguez-Baño, J., Ruppé, É., Salluh, J., Taccone, F.S., Weiss, E., Barbier, F., 2019. Rationalizing antimicrobial therapy in the ICU: a narrative review. Intensive Care Med. https://doi.org/10.1007/s00134-019-05520-5.

Tune, M., 1990. The renal mitochondrial toxicity of beta-lactam antibiotics: in vitro effects of cephaloglycin and imipenem. J Am Soc Nephrol 1, 815–821. https://doi.org/10.1681/ASN.V15815.

Vincent, J.L., Rello, J., Marshall, J., Silva, E., Anzueto, A., Martin, C.D., Moreno, R., Lipman, J., Gomersall, C., Sakr, Y., Reinhart, K., 2009. International study of the prevalence and outcomes of infection in intensive care units. JAMA – J. Am. Med. Assoc. 302, 2323–2329. https://doi.org/10.1001/jama.2009.1754.

Zasowski, E.J., Bassetti, M., Blasi, F., Goossens, H., Rello, J., Sotgiu, G., Tavoschi, L., Arber, M.R., McCool, R., Patterson, J.V., Longshaw, C.M., Lopes, S., Manissero, D., Nguyen, S.T., Tone, K., Aliberti, S., 2020. A systematic review of the effect of delayed appropriate antibiotic treatment on the outcomes of patients with severe bacterial infections. Chest 158, 929–938. https://doi.org/10.1016/j.chest.2020.03.087.

Zheng, R., Wang, H., Liu, Z., Wang, X., Li, J., Lei, X., Fan, Y., Liu, S., Feng, Z., Shang, H., 2019. A real-world study on adverse drug reactions to Xuebijing injection: hospital intensive monitoring based on 93 hospitals (31,913 cases). Ann. Transl. Med. 7, 117. https://doi.org/10.21037/atm.2018.09.26.

ESM 1. Polymixin use and its association with mitochondrial parameters in septic patients

Table 1. Summary statistics of patients that had use compared with those who did not use polymyxin.

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	632	0.572	8.71332	
Complex I D1	Mann-Whitney U	620	0.495	24.82015	
Complex II D1	Mann-Whitney U	572	0.248	75.47212	
BCE D1	Mann-Whitney U	604	0.401	-0.02154	
Basal D3	Mann-Whitney U	645	0.661	-8.82382	
Complex I D3	Mann-Whitney U	621	0.500	-43.63294	
Complex II D3	Mann-Whitney U	603	0.394	-99.86379	
BCE D3	Mann-Whitney U	480	0.767	-0.01243	
Delta BCE	Mann-Whitney U	494	0.905	-0.00997	
Delta Basal	Mann-Whitney U	454	0.533	13.69644	
Delta Complex I	Mann-Whitney U	471	0.682	-20.24700	
Delta Complex II	Mann-Whitney U	441	0.431	-93.82934	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	69	160.8606	130.3825	80.535	9.6953
	1	20	164.5468	123.3731	110.072	24.6128
Complex I D1	0	69	336.6349	251.5307	227.698	27.4116
	1	20	311.9815	243.9455	233.398	52.1894
Complex II D1	0	69	625.7839	557.6072	315.943	38.0350
	1	20	535.7112	485.5082	271.063	60.6114
BCE D1	0	69	0.3003	0.3155	0.114	0.0137
	1	20	0.3196	0.3520	0.113	0.0252
Basal D3	0	69	178.3576	184.4232	121.168	14.5869
	1	20	189.6433	188.3835	96.759	21.6360
Complex I D3	0	69	403.7014	355.9639	337.436	40.6225
	1	20	446.0006	377.1989	300.822	67.2659
Complex II D3	0	69	702.2946	645.9623	550.769	66.3048
	1	20	791.8178	734.2167	465.225	104.0275
BCE D3	0	56	0.3643	0.3696	0.196	0.0262
	1	18	0.3872	0.3675	0.226	0.0534

Group Descriptives

	Group	N	Mean	Median	SD	SE
Delta BCE	0	56	0.0354	0.0300	0.143	0.0190
	1	18	0.0461	0.0350	0.168	0.0396
Delta Basal	0	56	52.6100	53.4650	100.704	13.4572
	1	18	32.9028	33.8050	84.653	19.9530
Delta Complex I	0	56	132.8079	100.3550	324.078	43.3067
	1	18	155.5044	131.6350	271.176	63.9167
Delta Complex II	0	56	216.1411	171.9750	549.893	73.4826
	1	18	309.3722	236.8050	422.409	99.5628

Legend: 0, no polymyxin use; 1, polymyxin use; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error

Figure 1. Polymixin mitochondrial parameteres

Legend: 0, no polymyxin use; 1, polymyxin use; black square, mean of measurement; D1, first day of polymyxin use; D3, third day of polymyxin use.

Figure 1.1 Polymixin Routine D1. Routine in graphic refers to Basal respiration.

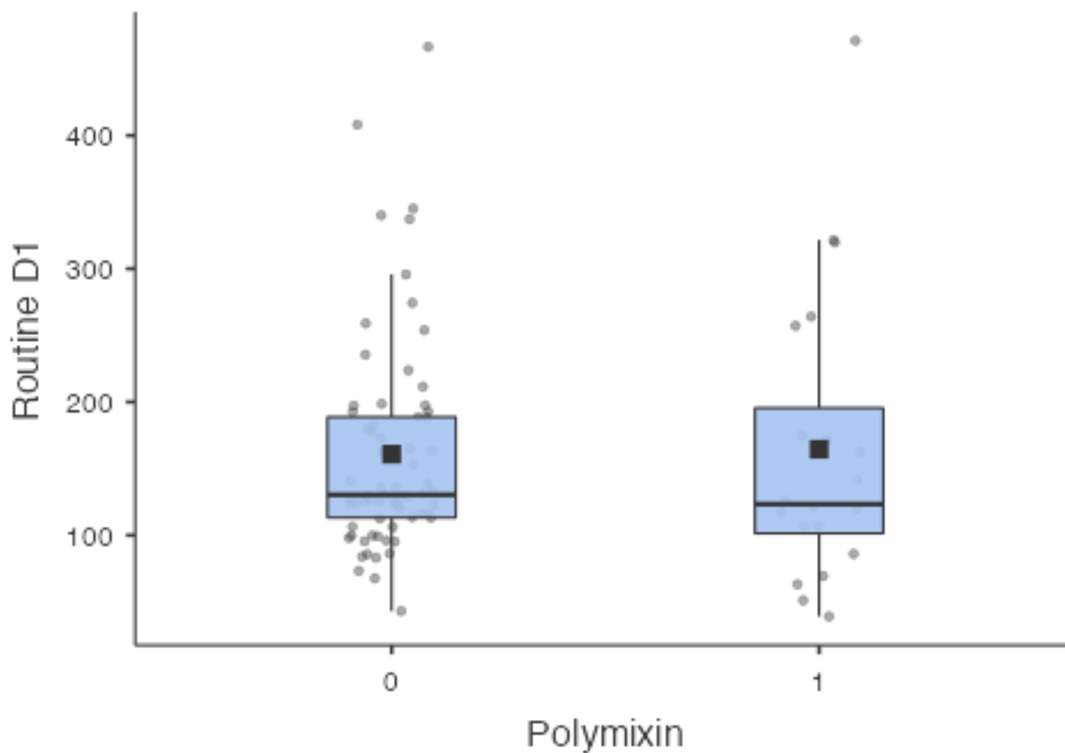


Figure 1.2 Polymixin Complex I D1

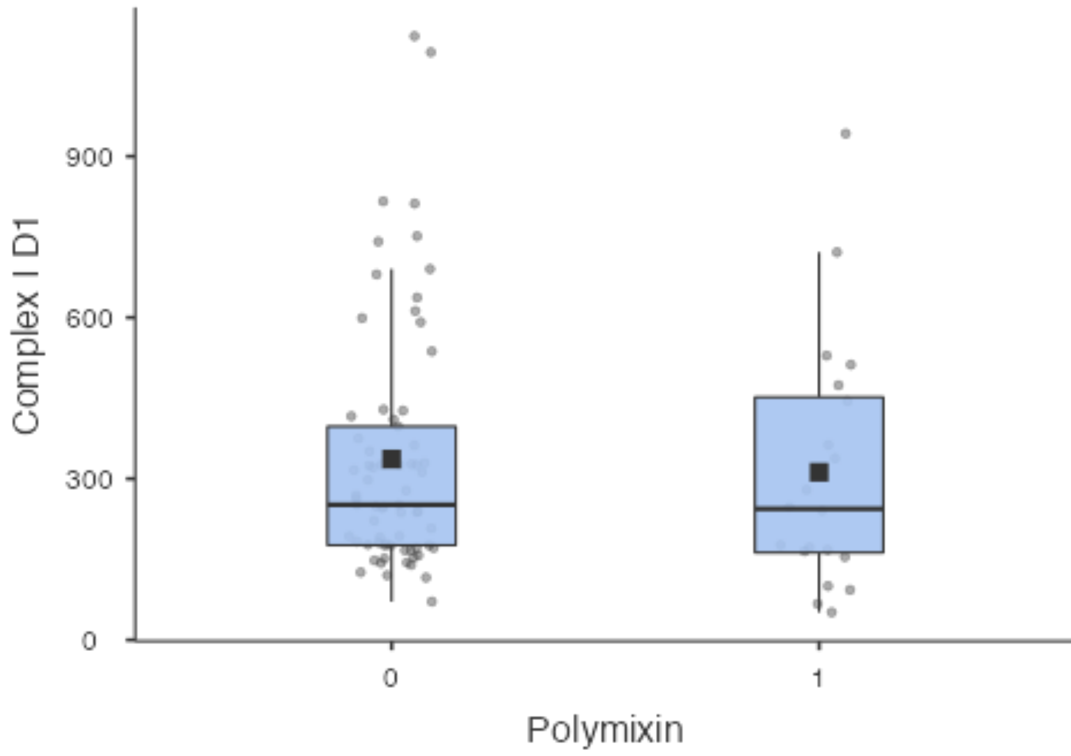


Figure 1.3 Polymyxin Complex II D1

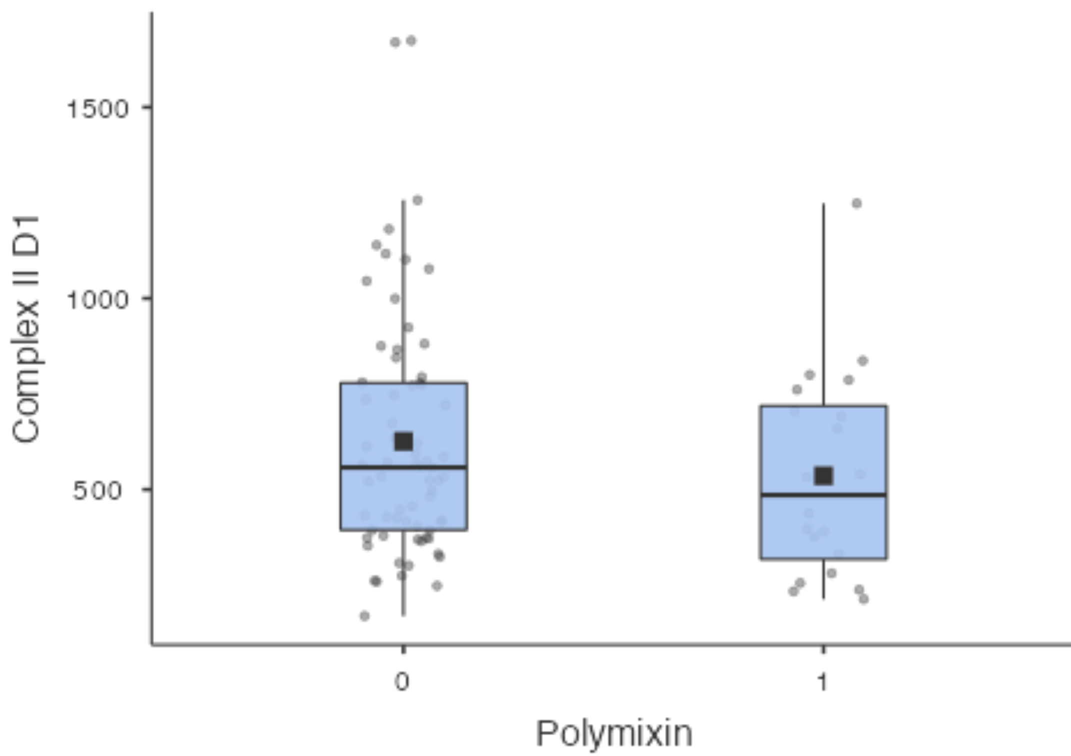


Figure 1.4 Polymyxin BCE D1

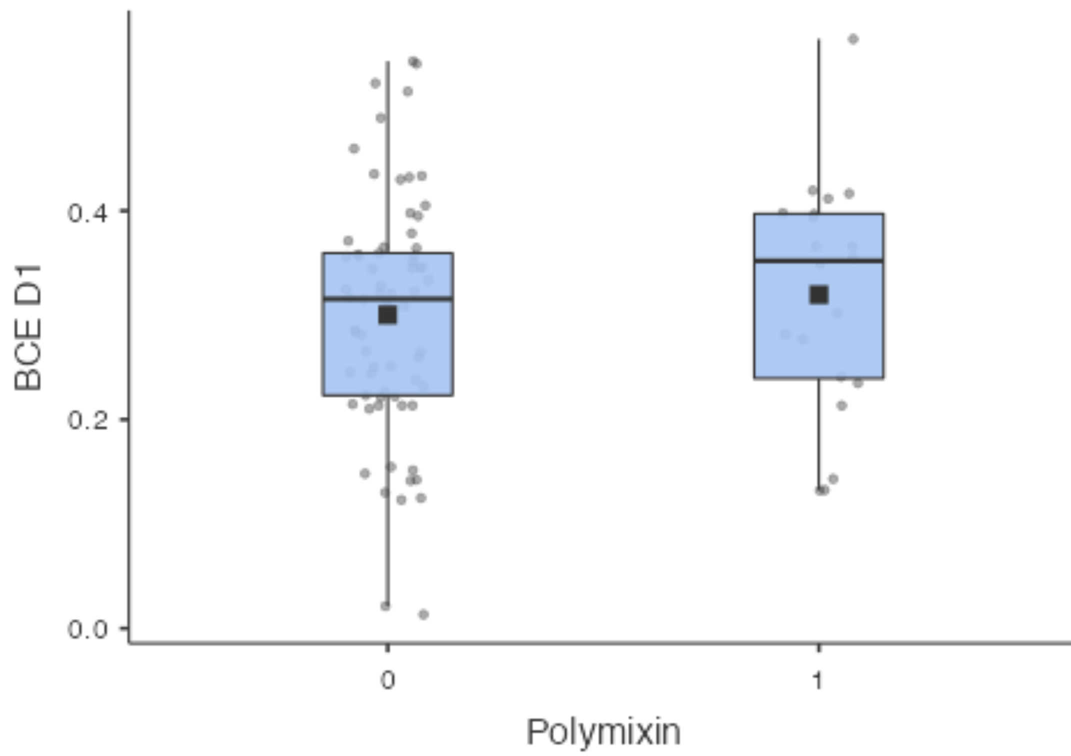


Figure 1.5 Polymyxin Routine D3. Routine in graphic refers to Basal respiration.

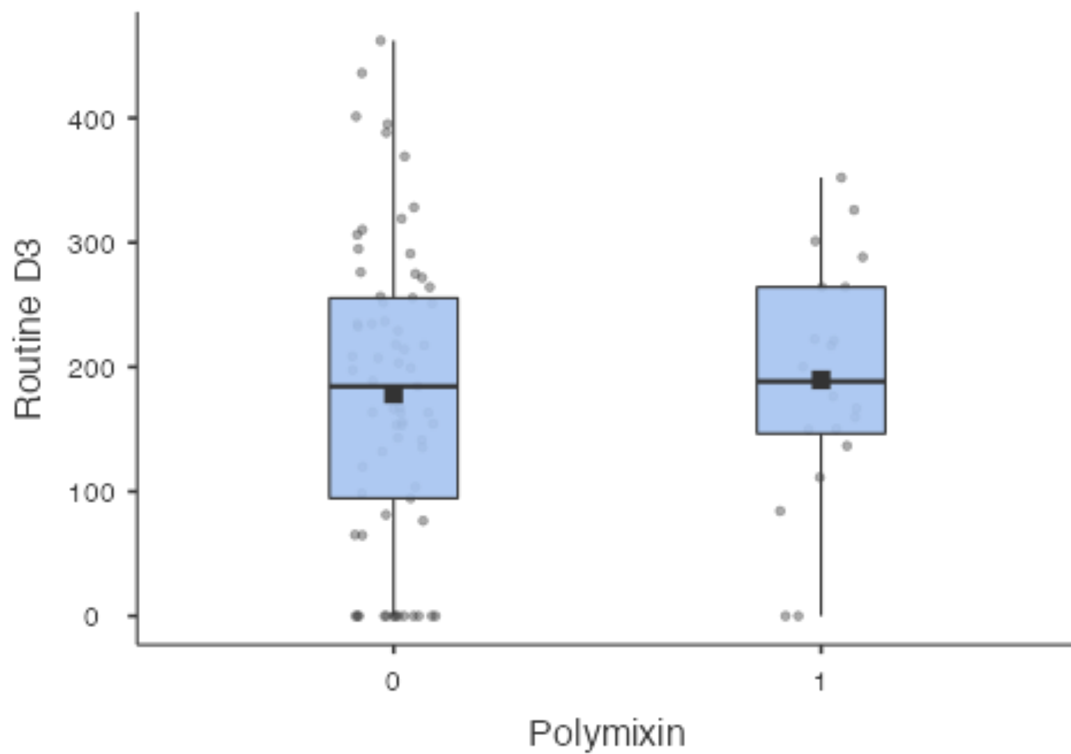


Figure 1.6 Polymyxin Complex I D3

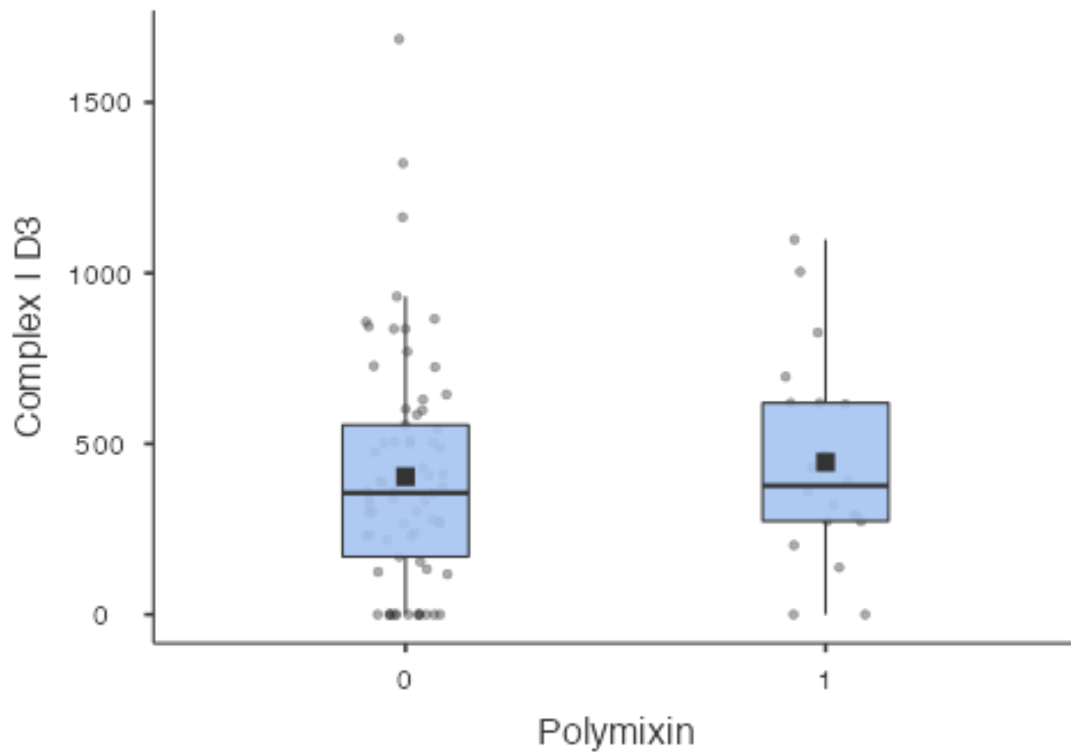


Figure 1.7 Polymyxin Complex II D3

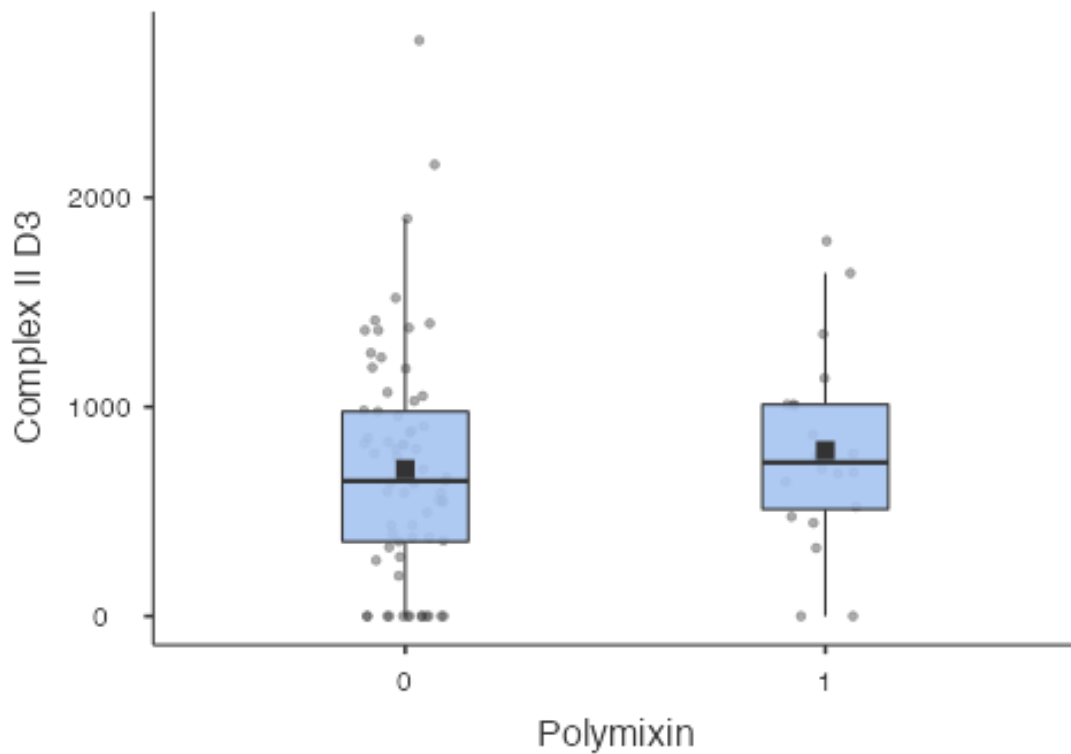


Figure 1.8 Polymyxin BCE D3

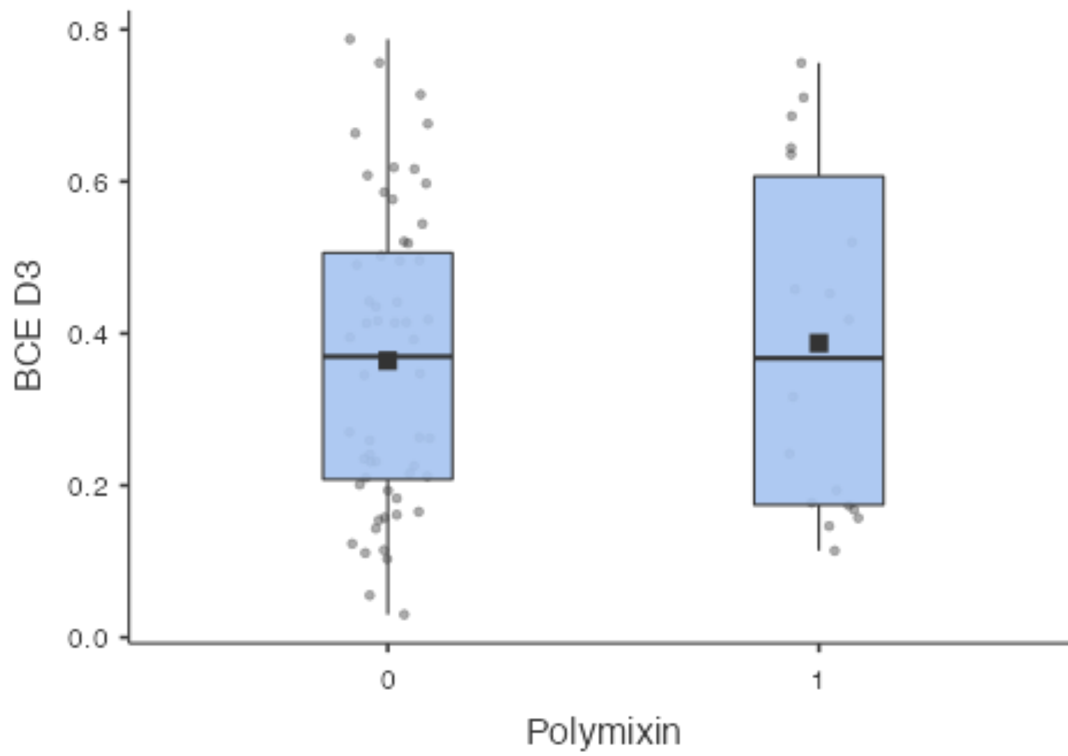


Figure 1.9 Polymyxin Delta BCE

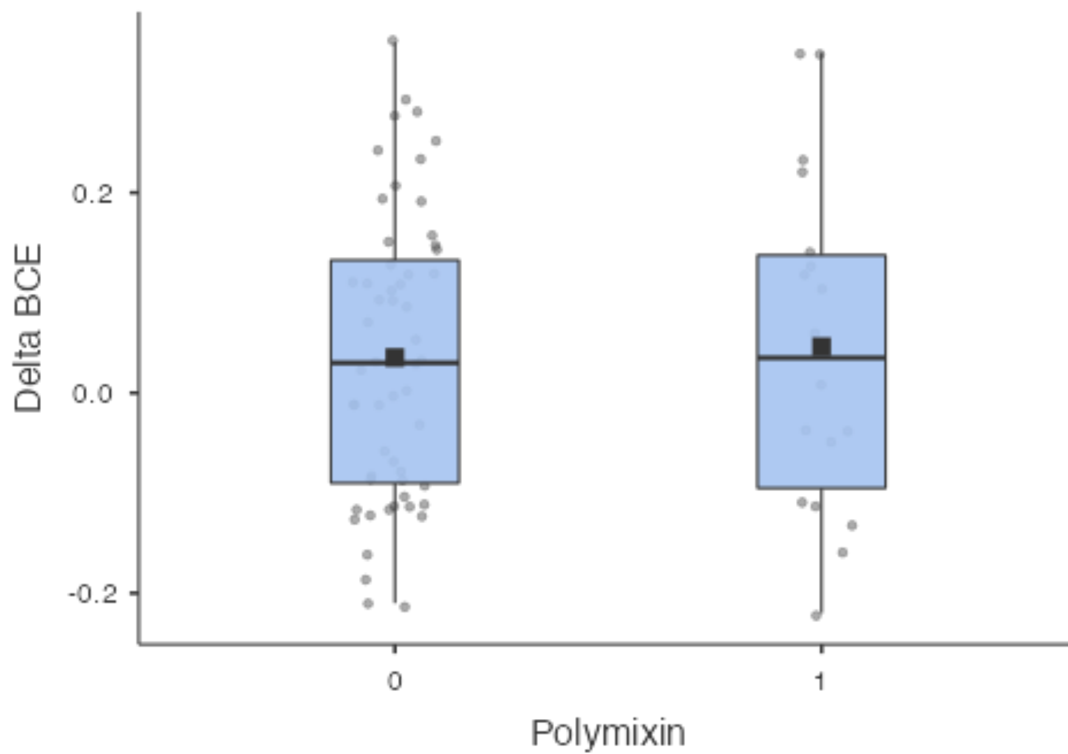


Figure 1.10 Polymyxin Delta Routine. Routine in graphic refers to Basal respiration.

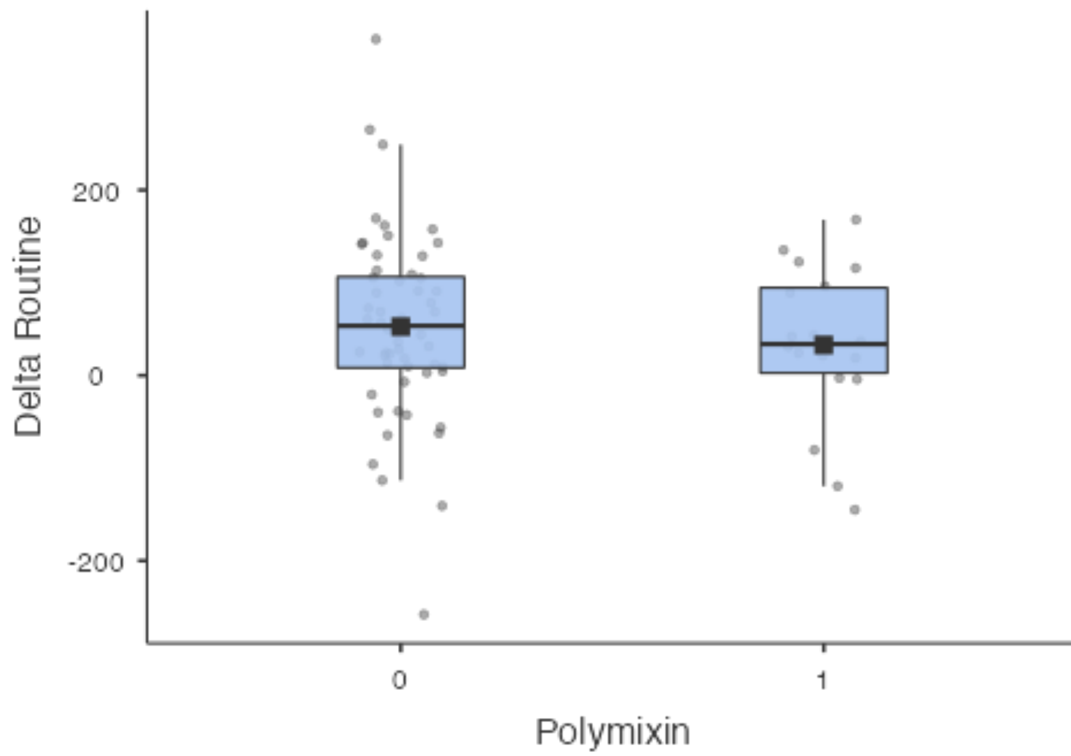


Figure 1.11 Polymyxin Delta Complex I

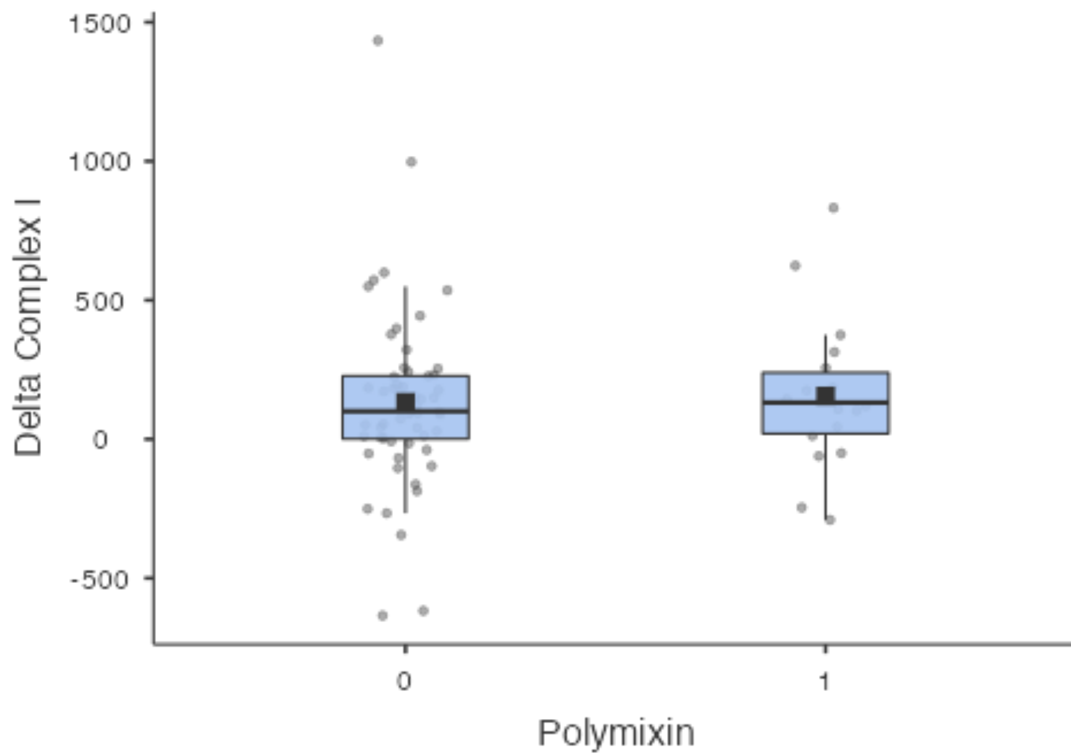
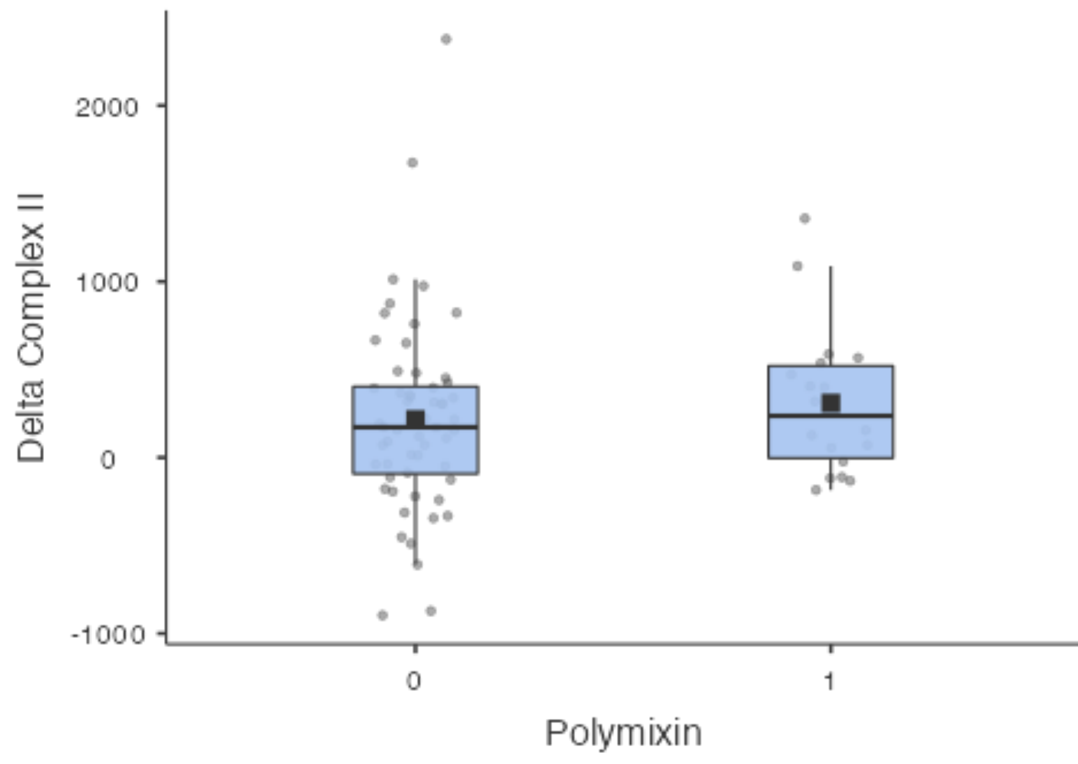


Figure 1.12 Polymyxin Delta Complex II



Delta Complex II
2000
1000
0
-1000
0
1
Polymixin

ESM 2. Meropenem use and its association with mitochondrial parameters in septc patients

Difference in mitochondrial variables between use or non-use of meropenem

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	781	0.866	2.48393	
Complex I D1	Mann-Whitney U	782	0.873	-4.04463	
Complex II D1	Mann-Whitney U	706	0.393	50.53094	
BCE D1	Mann-Whitney U	758	0.705	-0.00949	
Basal D3	Mann-Whitney U	659	0.197	-33.71290	
Complex I D3	Mann-Whitney U	636	0.135	-118.21938	
Complex II D3	Mann-Whitney U	655	0.186	-159.57375	
BCE D3	Mann-Whitney U	447	0.191	-0.06236	
Delta BCE	Mann-Whitney U	412	0.083	-0.07993	
Delta Basal	Mann-Whitney U	450	0.204	-25.06004	
Delta Complex I	Mann-Whitney U	436	0.150	-84.47995	
Delta Complex II	Mann-Whitney U	406	0.072	-201.42995	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	64	159.2238	130.1766	80.077	10.0096
	1	25	167.9995	125.765	105.295	21.0591
Complex I D1	0	64	328.2294	248.1481	226.307	28.2884
	1	25	338.4301	251.531	236.430	47.2861
Complex II D1	0	64	623.2679	546.6613	314.419	39.3024
	1	25	560.1665	482.054	289.384	57.8768
BCE D1	0	64	0.3022	0.3187	0.115	0.0144
	1	25	0.3109	0.316	0.109	0.0218
Basal D3	0	64	171.1329	166.6893	110.058	13.7573
	1	25	205.8815	221.124	128.030	25.6060
Complex I D3	0	64	368.1559	339.7060	268.650	33.5812
	1	25	528.5371	468.193	432.074	86.4148
Complex II D3	0	64	656.1737	644.4149	444.272	55.5340
	1	25	891.9828	775.274	690.098	138.0196
BCE D3	0	53	0.3500	0.2700	0.200	0.0275
	1	21	0.4201	0.441	0.205	0.0447
Delta BCE	0	53	0.0189	-0.0100	0.144	0.0198
	1	21	0.0862	0.100	0.150	0.0328

Delta Basal	0	53	39.9392	36.2800	91.771	12.6057
	1	21	67.6967	60.510	108.481	23.6724
Delta Complex I	0	53	89.8823	93.8900	248.924	34.1924
	1	21	260.5981	152.660	410.679	89.6176
Delta Complex II	0	53	143.1179	154.6200	433.168	59.5003
	1	21	480.3500	318.990	644.796	140.7059

Legend: 0, no polymyxin use; 1, polymyxin use; black square, mean of measurement; D1, first day of polymyxin use; D3, third day of polymyxin use.

ESM Figure 1. Meropenem mitochondrial parameteres

Legend: 0, no meropenem use; 1, meropenem use; black square, mean of measurement; D1, first day of meropenem use; D3, third day of meropenem use.

Figure 1.1 Meropenem Routine D1. Routine in graphic refers to Basal respiration.

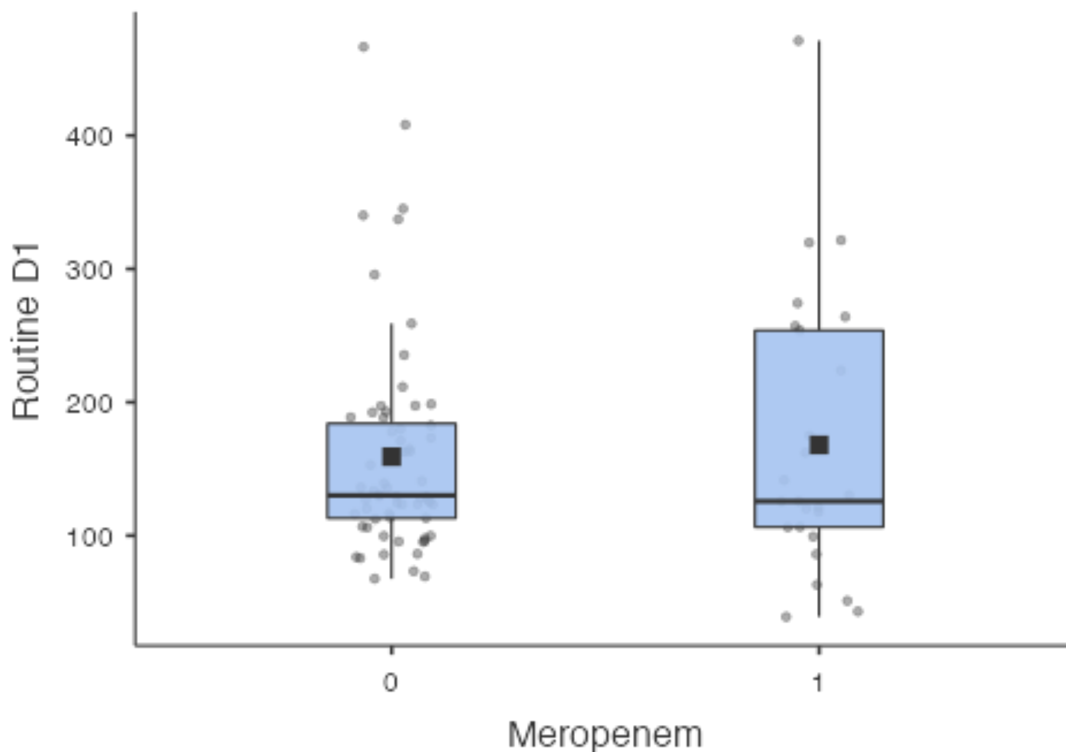


Figure 1.2 Meropenem Complex I D1

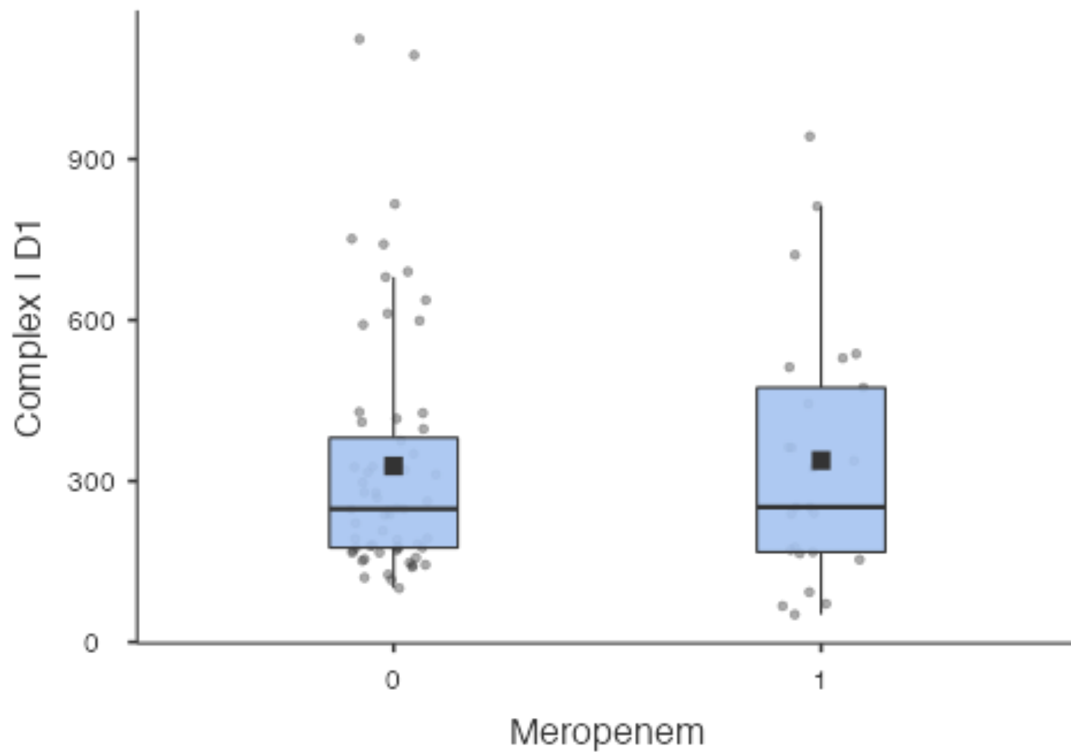


Figure 1.3 Meropenem Complex II D1

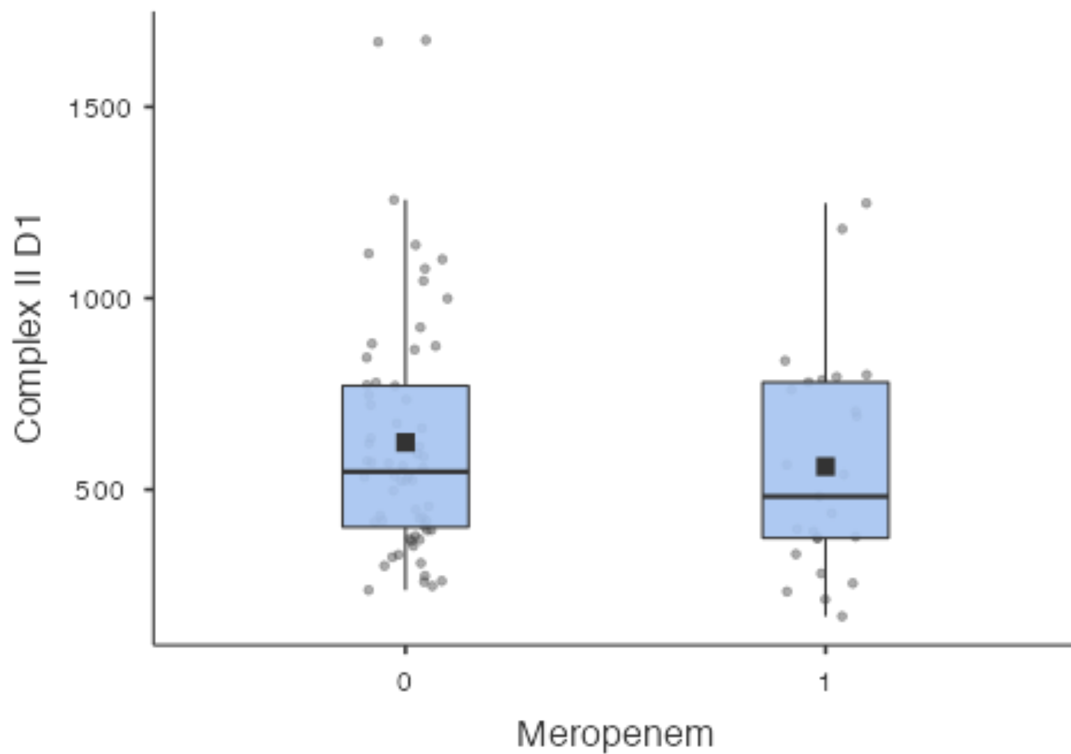


Figure 1.4 Meropenem BCE D1

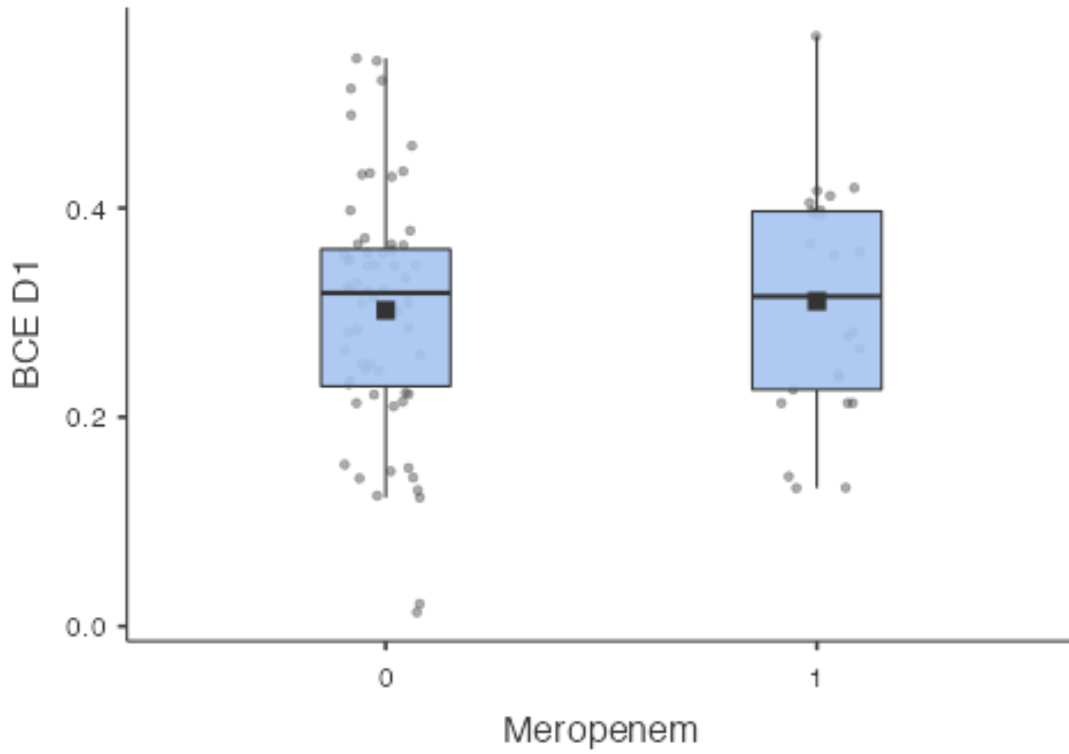


Figure 1.5 Meropenem Routine D3. Routine in graphic refers to Basal respiration.

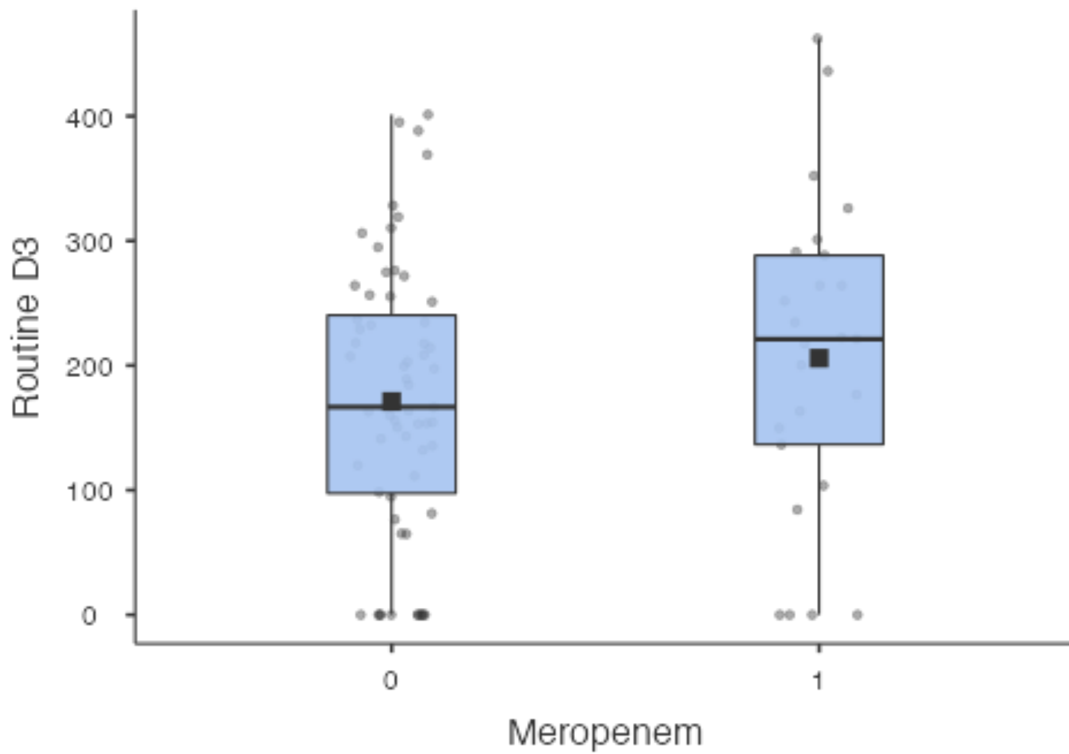


Figure 1.6 Meropenem Complex I D3

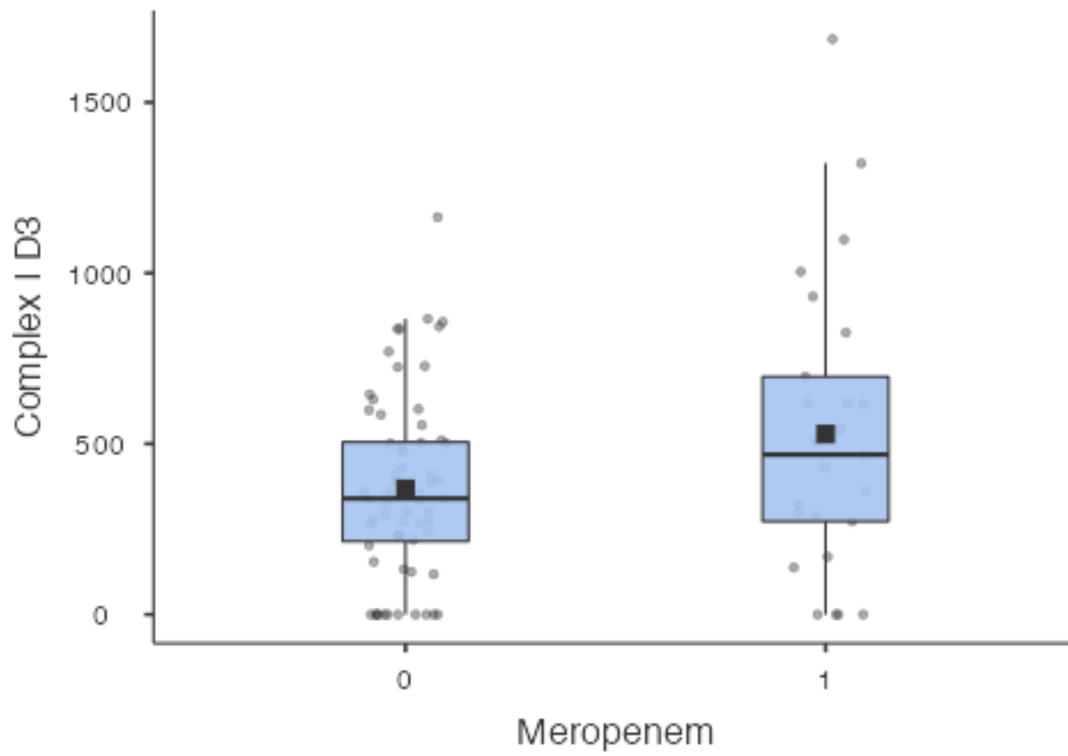


Figure 1.7 Meropenem Complex II D3

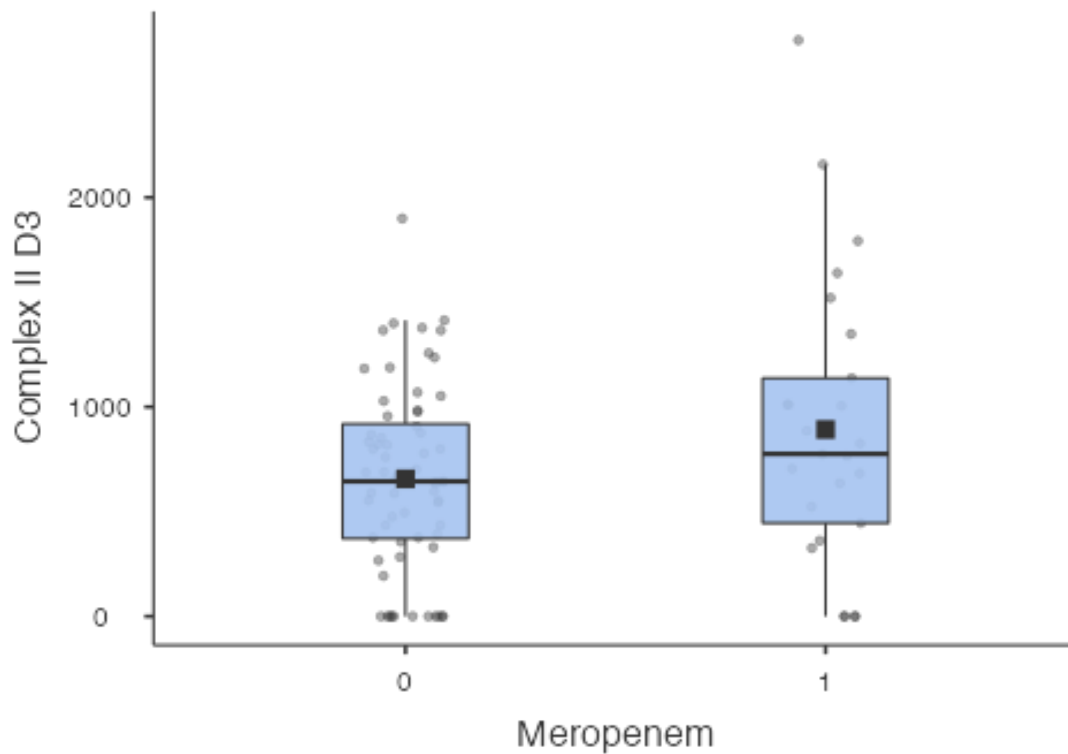


Figure 1.8 Meropenem BCE D3

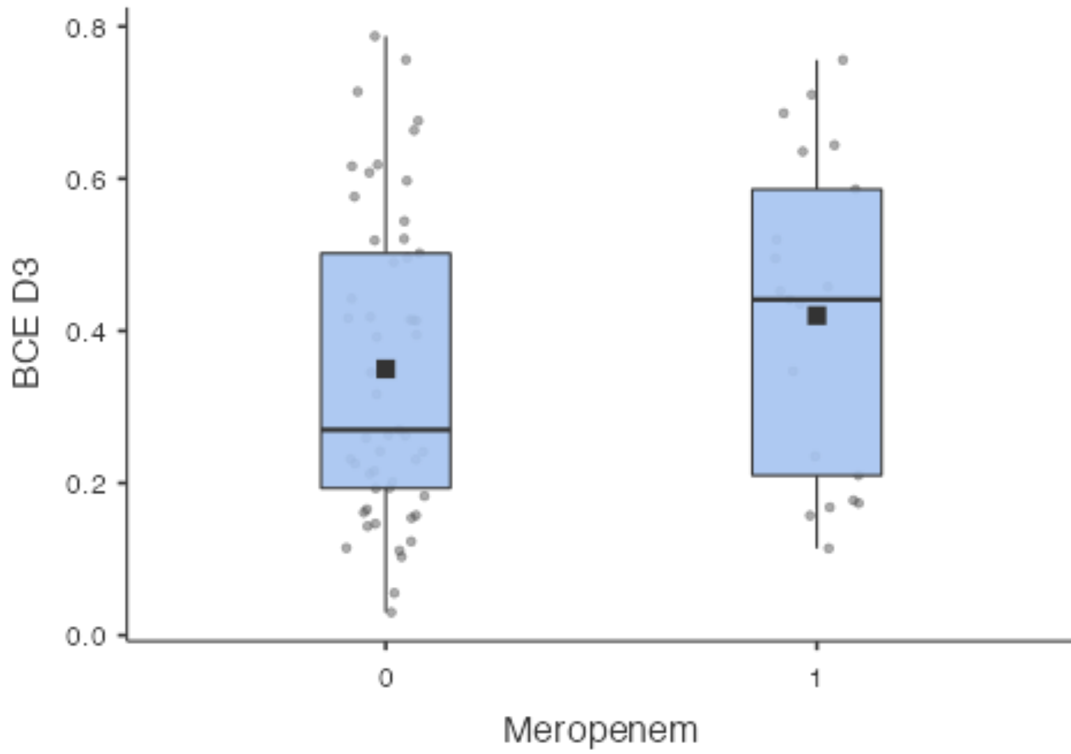


Figure 1.9 Meropenem Delta BCE

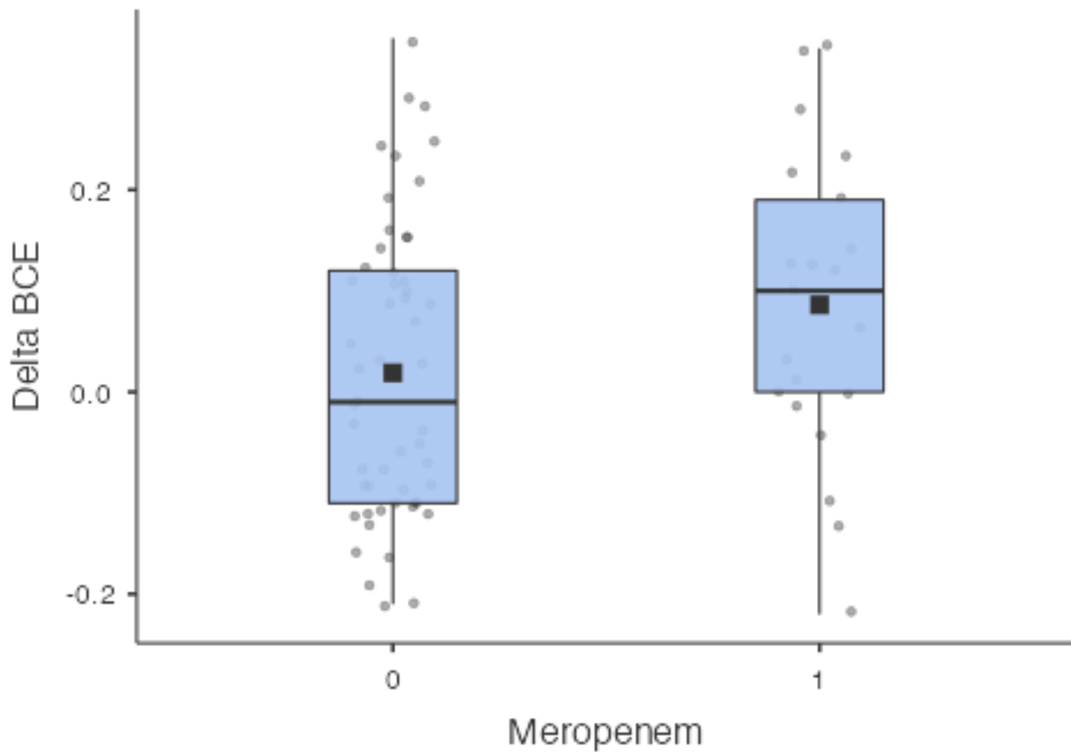


Figure 1.10 Meropenem Delta Routine. Routine in graphic refers to Basal respiration.

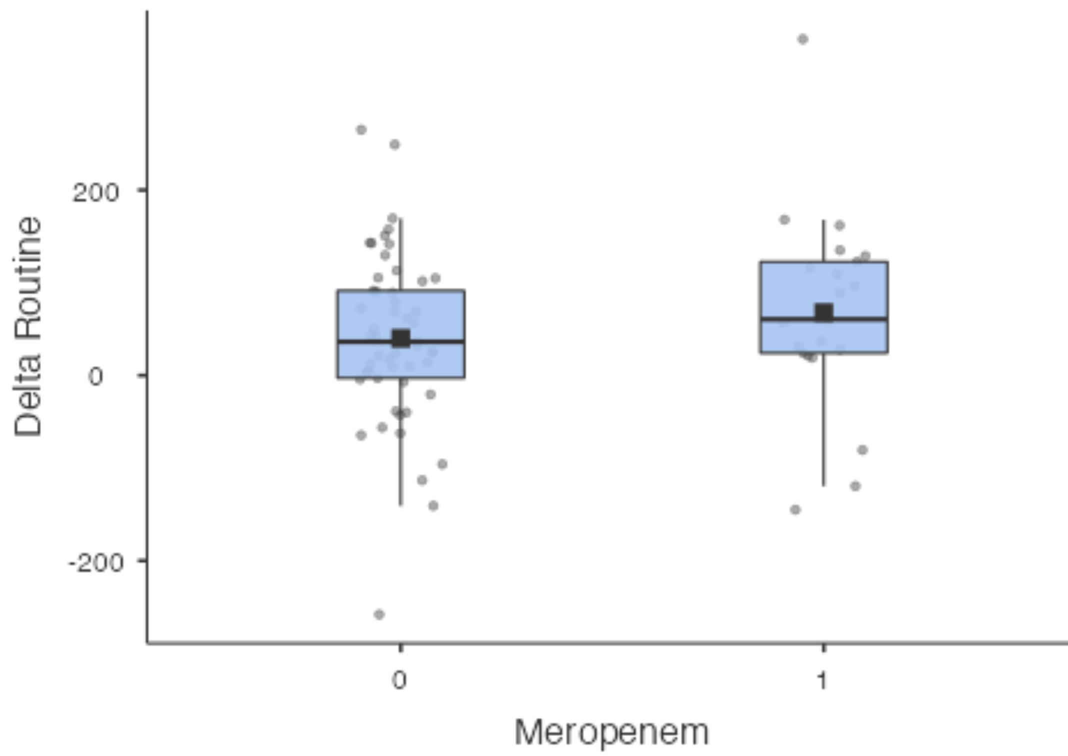


Figure 1.11 Meropenem Delta Complex I

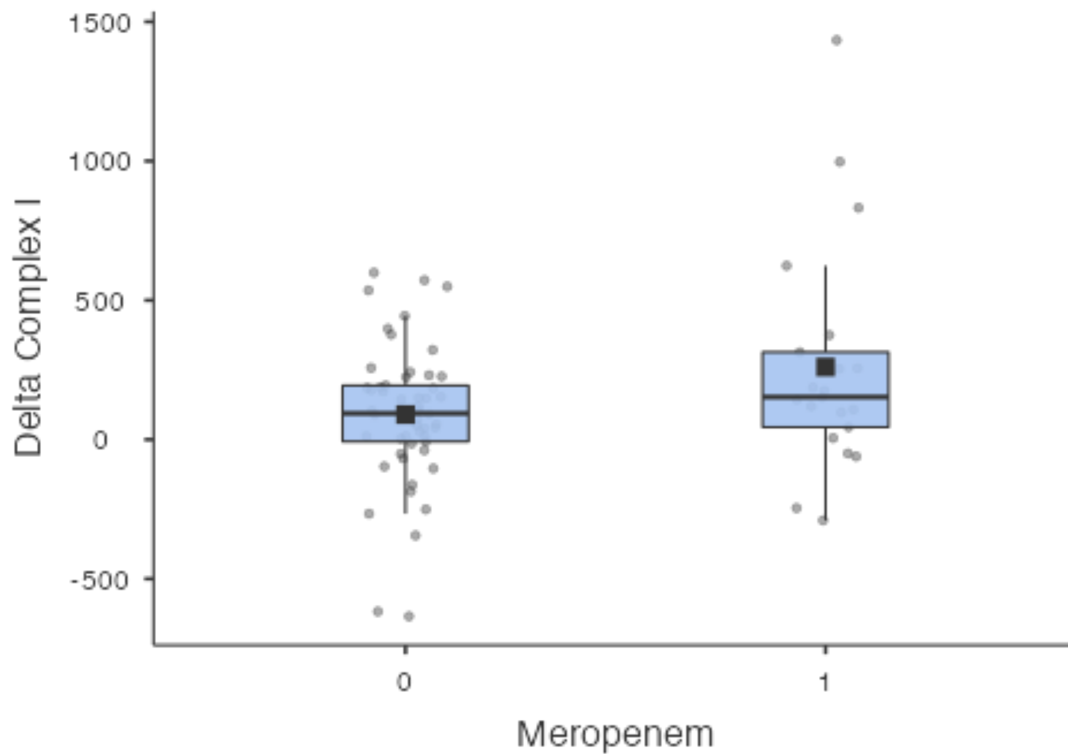
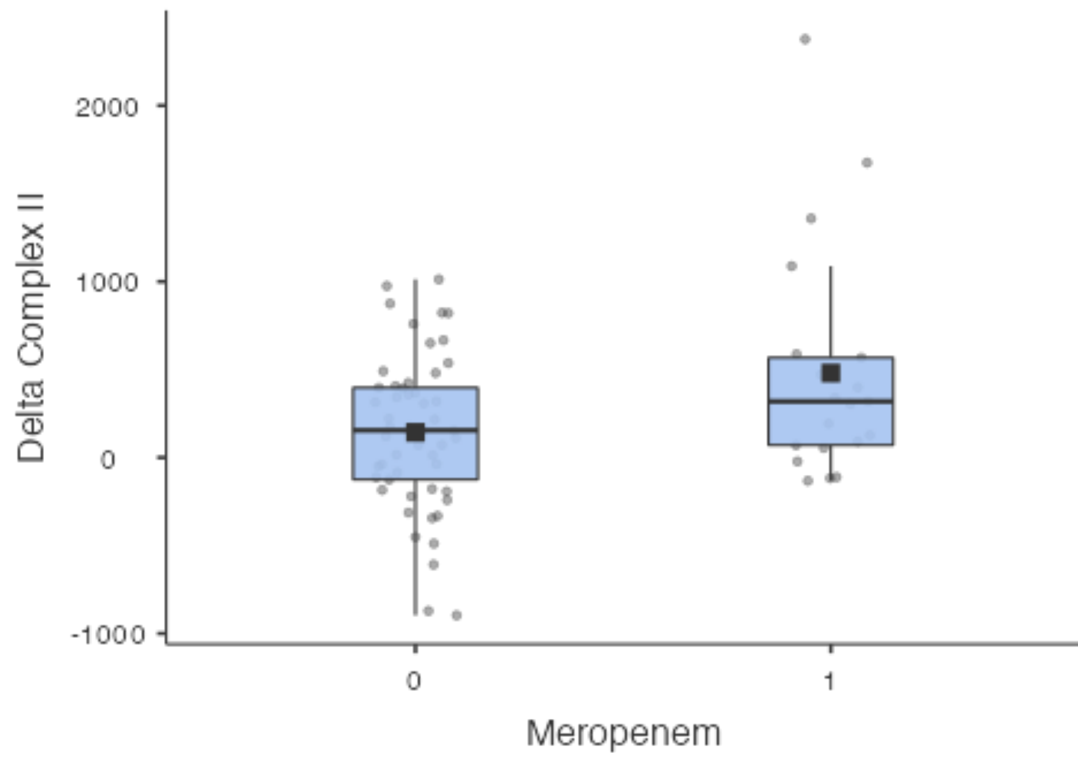


Figure 1.12 Meropenem Delta Complex II


Delta Complex II
2000
1000
0
-1000
0
1
Meropenem

ESM 3. Vancomycin use and its association with mitochondrial parameters in septic patients

Table 1. Summary statistics of patients that had use compared with those who did not use vancomycin.

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	606	0.558	10.1634	
Complex I D1	Mann-Whitney U	645	0.845	9.1037	
Complex II D1	Mann-Whitney U	560	0.295	68.3986	
BCE D1	Mann-Whitney U	618	0.641	-0.0130	
Basal D3	Mann-Whitney U	642	0.817	-3.1533	
Complex I D3	Mann-Whitney U	636	0.775	-14.9375	
Complex II D3	Mann-Whitney U	664	0.996	2.24e-5	
BCE D3	Mann-Whitney U	419	0.404	-0.0418	
Delta BCE	Mann-Whitney U	398	0.269	-0.0400	
Delta Basal	Mann-Whitney U	440	0.572	11.7700	
Delta Complex I	Mann-Whitney U	429	0.480	50.0000	
Delta Complex II	Mann-Whitney U	462	0.777	34.5400	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	70	162.2140	129.9964	80.132	9.5776
	1	19	159.7544	126.858	112.621	25.8370
Complex I D1	0	70	321.5126	249.9863	203.475	24.3199
	1	19	366.3975	251.531	305.791	70.1533
Complex II D1	0	70	612.0818	537.6019	285.152	34.0822
	1	19	581.4519	396.770	386.222	88.6055
BCE D1	0	70	0.3015	0.3187	0.111	0.0133
	1	19	0.3162	0.286	0.123	0.0282
Basal D3	0	70	178.7612	180.4832	118.207	14.1285
	1	19	188.7505	200.224	108.677	24.9322
Complex I D3	0	70	411.0055	357.9313	339.301	40.5543
	1	19	421.3169	363.058	292.933	67.2034
Complex II D3	0	70	722.1043	684.7698	552.272	66.0091
	1	19	723.5464	704.156	460.603	105.6695
BCE D3	0	57	0.3583	0.3453	0.192	0.0254
	1	17	0.4087	0.414	0.239	0.0580
Delta BCE	0	57	0.0282	0.0300	0.144	0.0190
	1	17	0.0706	0.120	0.162	0.0393
Delta Basal	0	57	50.8277	43.7200	99.212	13.1410

Group Descriptives

	Group	N	Mean	Median	SD	SE
	1	17	37.7194	37.010	90.633	21.9818
Delta Complex I	0	57	159.1958	119.2900	309.907	41.0481
	1	17	68.3624	97.780	310.965	75.4200
Delta Complex II	0	57	254.8460	173.1700	537.494	71.1928
	1	17	185.0812	126.400	470.413	114.0919

Legend: 0, no polymyxin use; 1, polymyxin use; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error

Figure 1 Vancomycin mitochondrial parameters

Legend: 0, no vancomycin use; 1, vancomycin use; black square, mean of measurement; D1, first day of vancomycin use; D3, third day of vancomycin use.

Figure 1.1 Vancomycin Basal D1. Routine in graphic refers to Basal respiration.

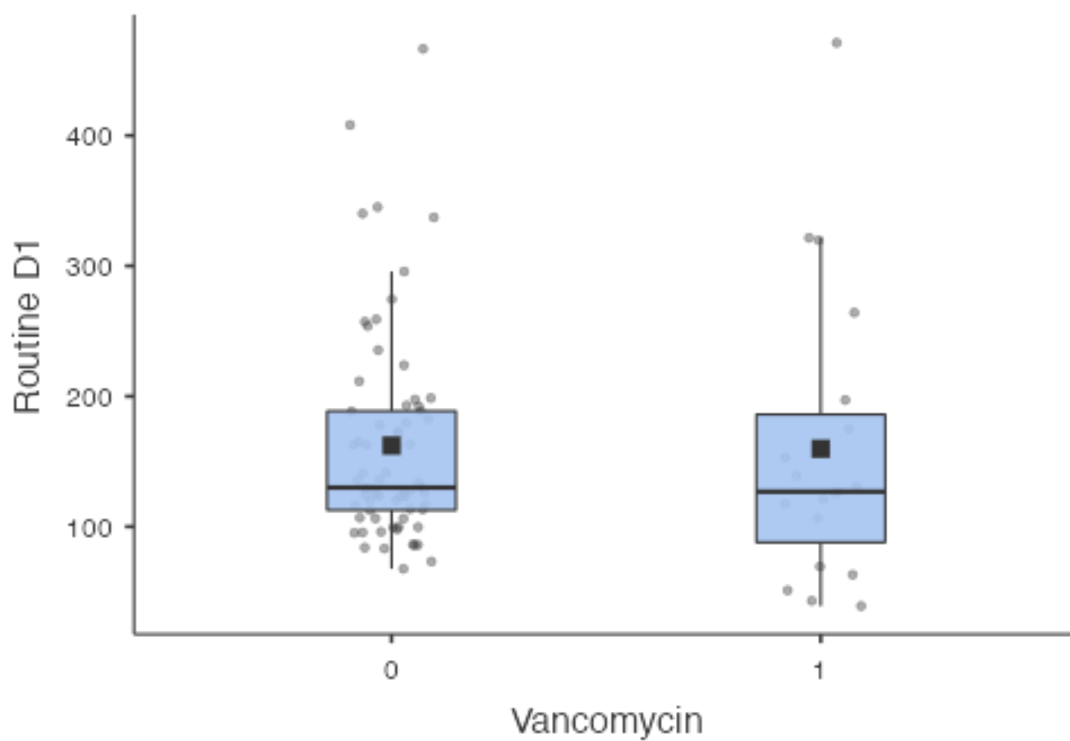


Figure 1.2 Vancomycin Complex I D1

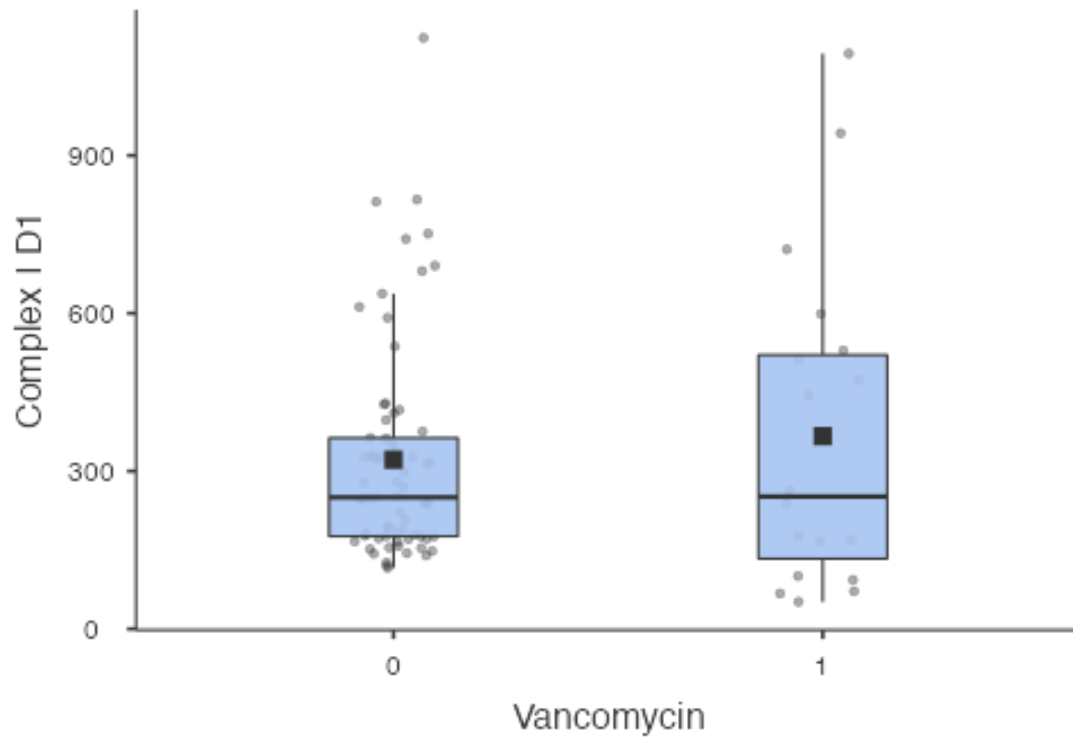


Figure 1.3 Vancomycin Complex II D1

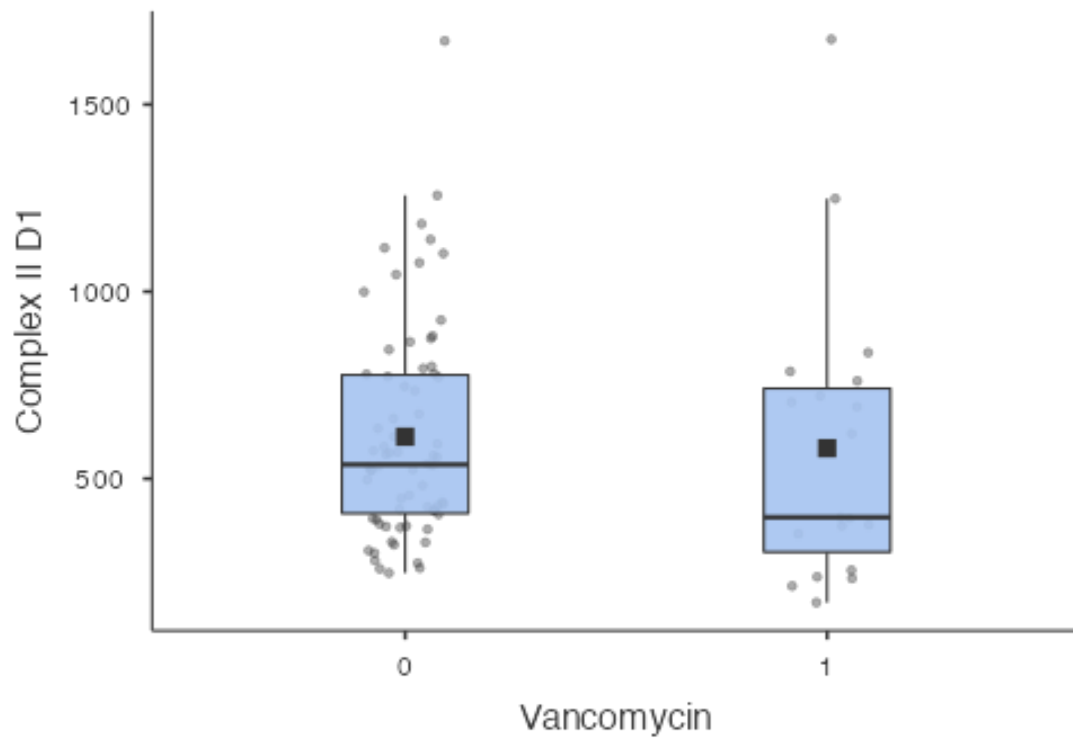


Figure 1.4 Vancomycin BCE D1

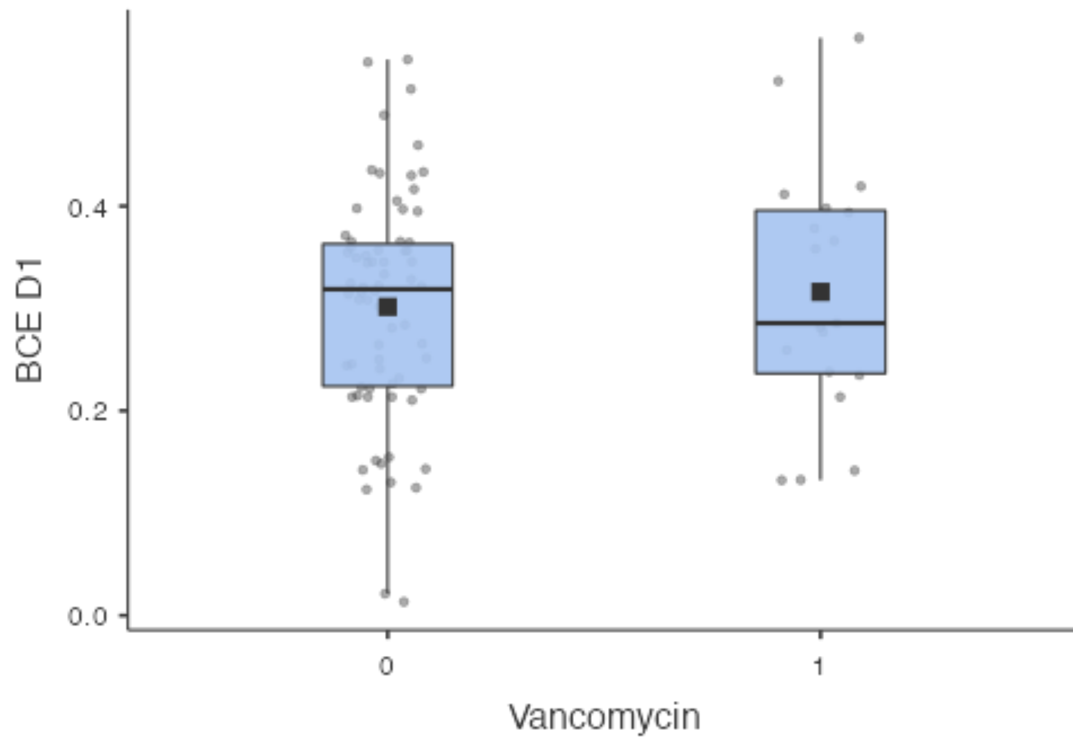


Figure 1.5 Vancomycin Basal D3. Routine in graphic refers to Basal respiration.

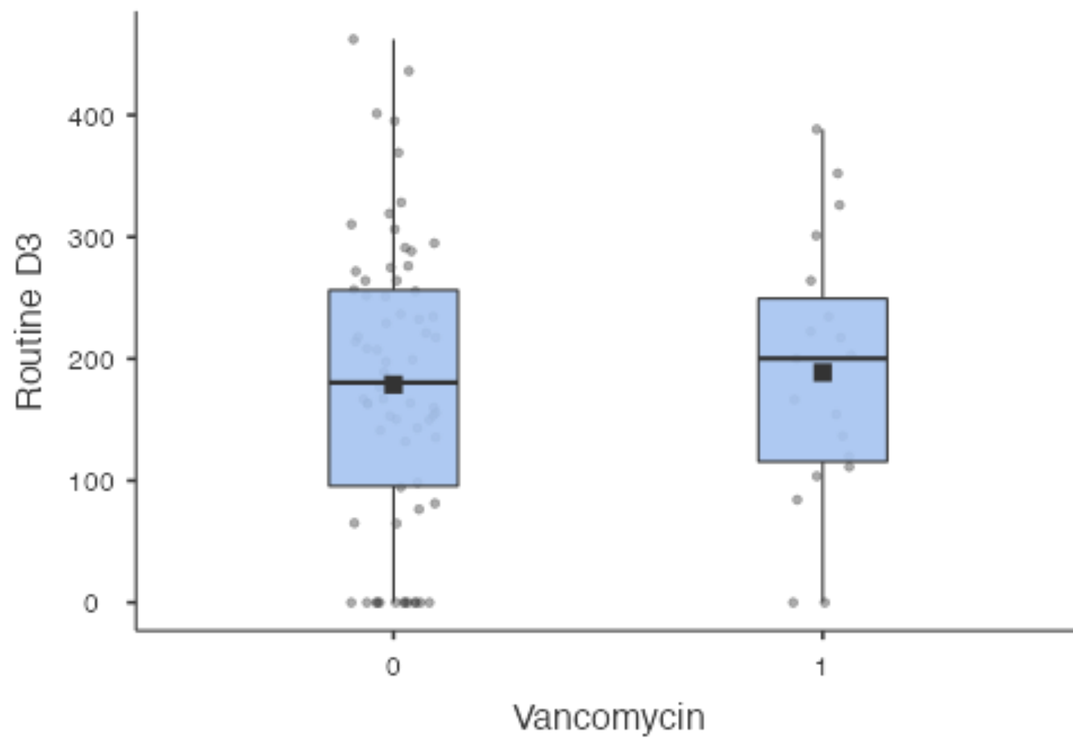


Figure 1.6 Vancomycin Complex I D3

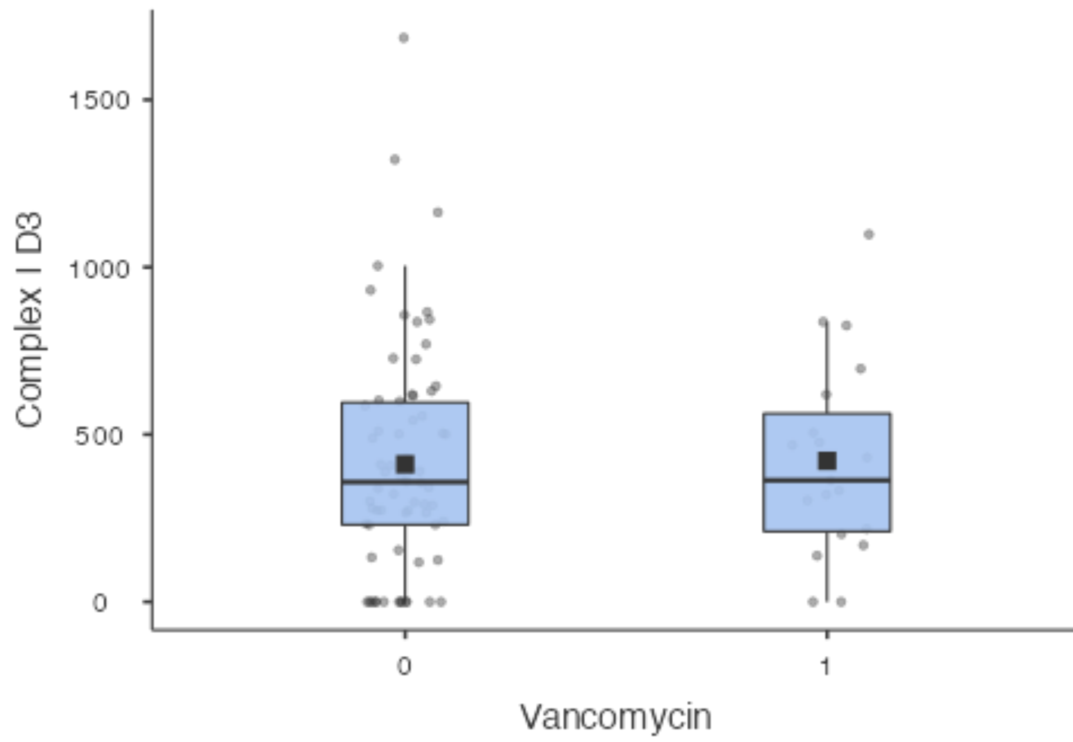


Figure 1.7 Vancomycin Complex II D3

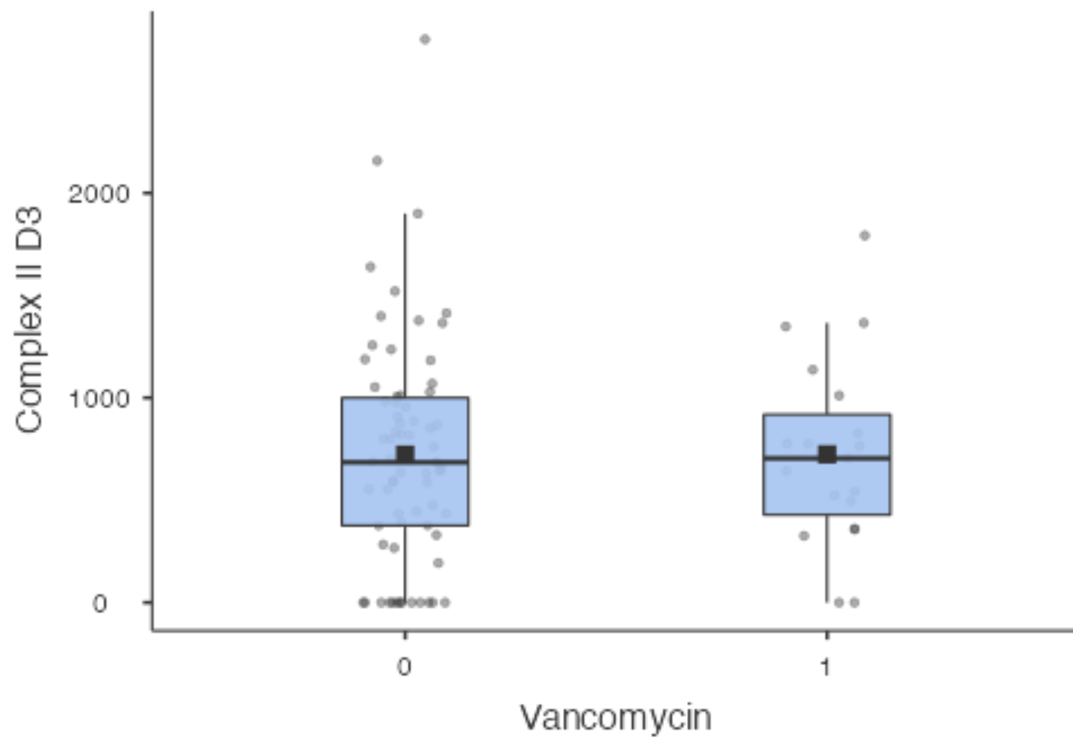


Figure 1.8 Vancomycin BCE D3

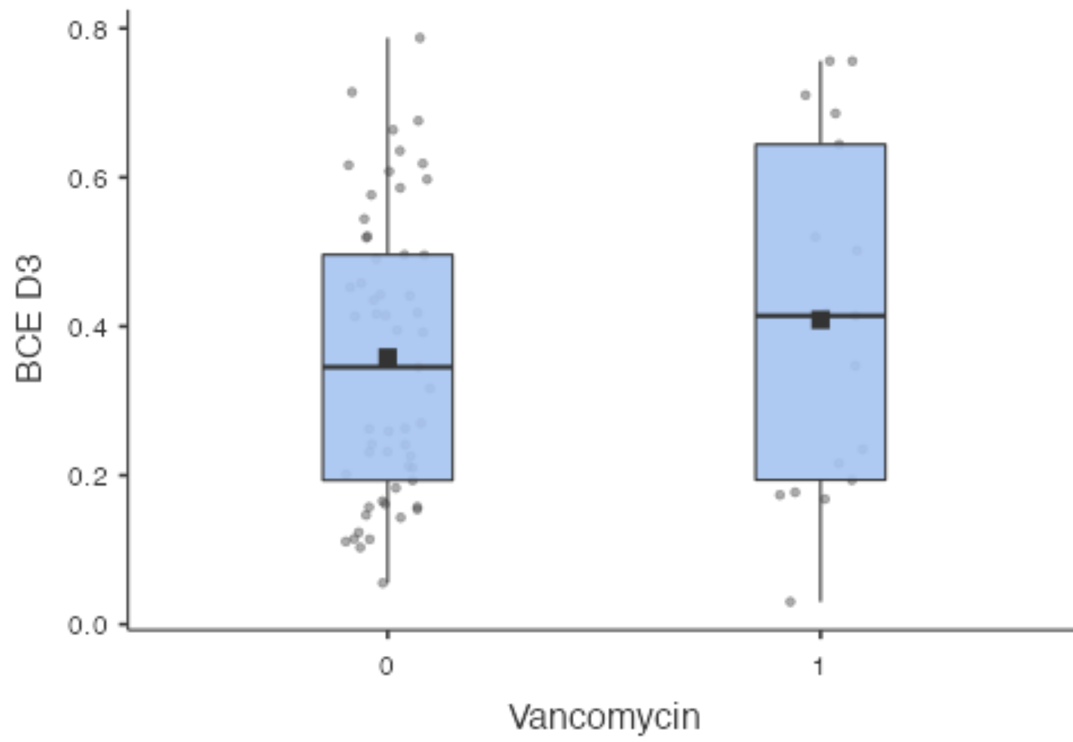


Figure 1.9 Vancomycin Delta BCE

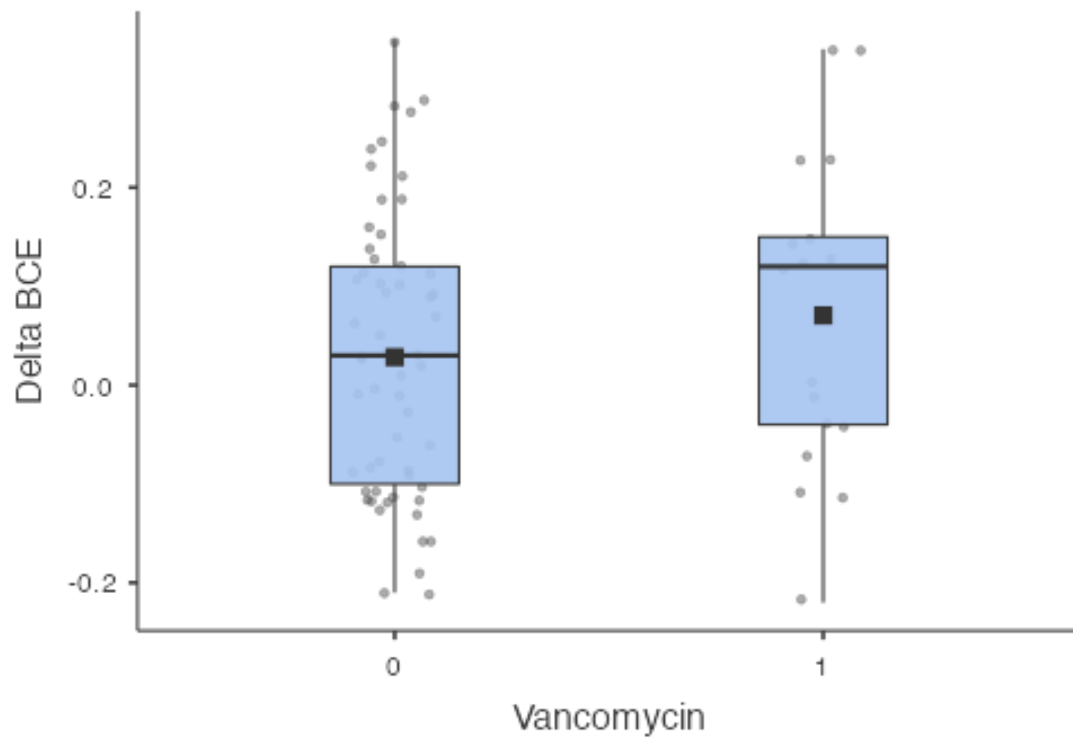


Figure 1.10 Vancomycin Delta Basal. Routine in graphic refers to Basal respiration.

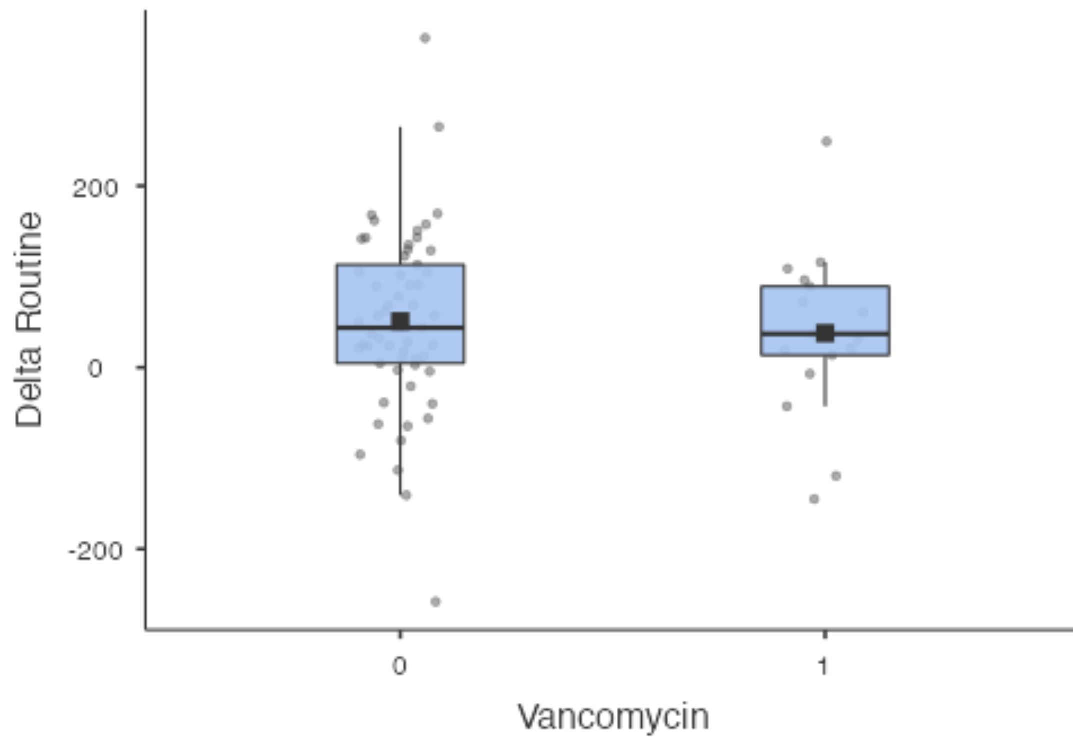


Figure 1.11 Vancomycin Delta Complex I

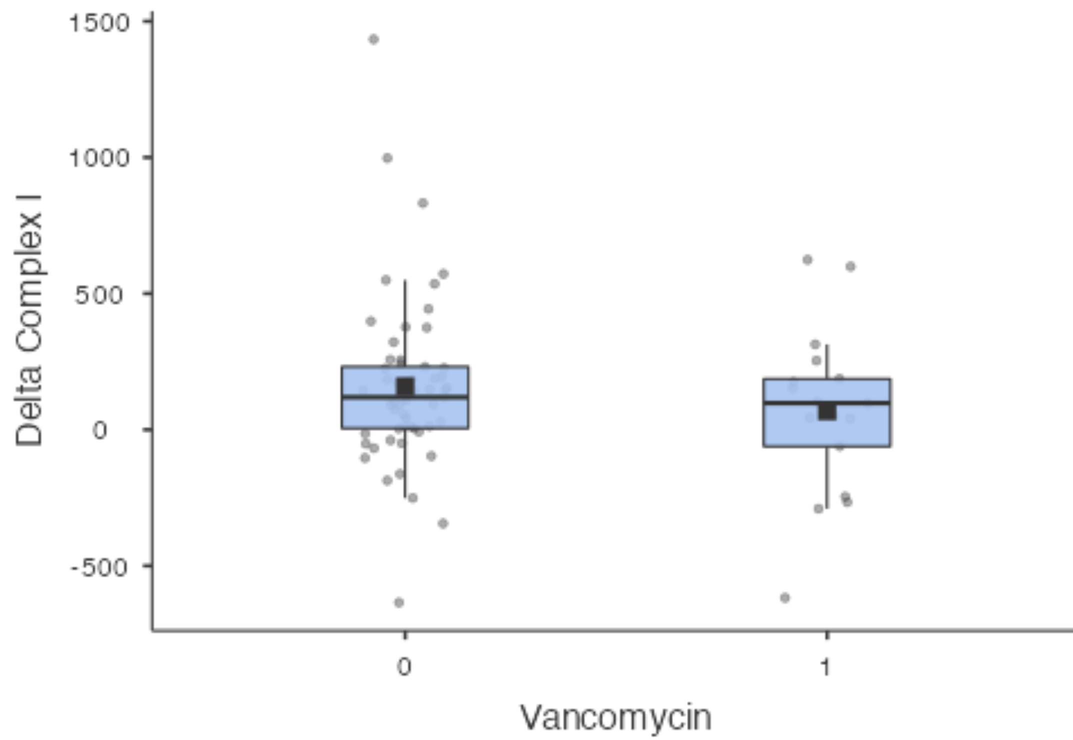
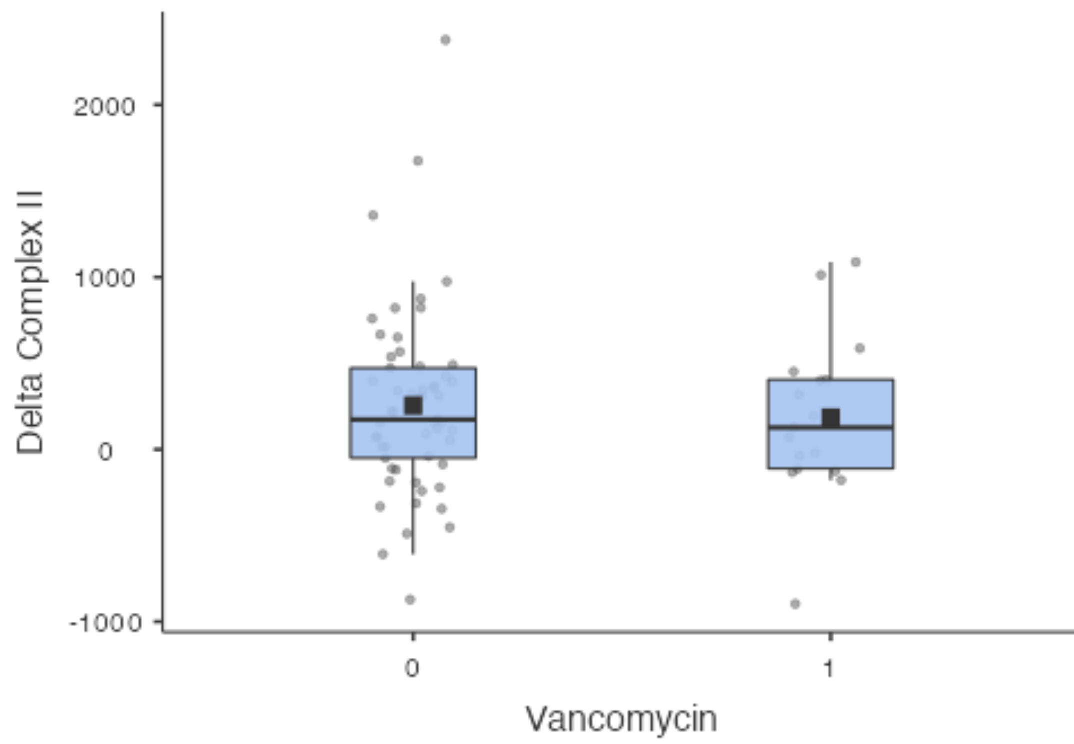


Figure 1.12 Vancomycin Delta Complex II


Delta Complex II
2000
1000
0
-1000
0
1
Vancomycin

ESM 4. Amoxicillin-clavulanate use and its association with mitochondrial parameters in septic patients
Table 1. Summary statistics of patients that had use compared with those who did not use ampicillin-sulbactam.

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	223	0.063	-46.7065	
Complex I D1	Mann-Whitney U	270	0.223	-66.1712	
Complex II D1	Mann-Whitney U	232	0.083	-159.9449	
BCE D1	Mann-Whitney U	266	0.203	-0.0474	
Basal D3	Mann-Whitney U	306	0.466	-31.8845	
Complex I D3	Mann-Whitney U	297	0.394	-86.9735	
Complex II D3	Mann-Whitney U	270	0.222	-187.8601	
BCE D3	Mann-Whitney U	235	0.346	0.0444	
Delta BCE	Mann-Whitney U	196	0.110	0.0801	
Delta Basal	Mann-Whitney U	218	0.221	40.7800	
Delta Complex I	Mann-Whitney U	273	0.753	36.0101	
Delta Complex II	Mann-Whitney U	253	0.519	120.1699	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	80	155.4723	128.0252	81.405	9.1014
	1	9	216.9474	182.5412	121.1195	40.3732
Complex I D1	0	80	320.7306	248.1481	216.951	24.2558
	1	9	423.2208	326.3080	309.5718	103.1906
Complex II D1	0	80	582.4828	528.7905	285.620	31.9333
	1	9	810.5211	672.9380	424.8752	141.6251
BCE D1	0	80	0.2998	0.3086	0.117	0.0131
	1	9	0.3480	0.3444	0.0506	0.0169
Basal D3	0	80	177.2675	180.4832	117.534	13.1407
	1	9	213.1273	208.4734	97.7851	32.5950
Complex I D3	0	80	407.5821	347.1218	339.949	38.0074
	1	9	463.2046	409.9480	204.6769	68.2256
Complex II D3	0	80	707.0573	673.1590	550.123	61.5057
	1	9	858.9002	852.1465	310.1070	103.3690
BCE D3	0	65	0.3781	0.4136	0.208	0.0258
	1	9	0.3101	0.2310	0.1525	0.0508
Delta BCE	0	65	0.0485	0.0500	0.149	0.0185
	1	9	-0.0378	-0.0900	0.1183	0.0394
Delta Basal	0	65	54.9662	43.4500	92.803	11.5108

Group Descriptives

	Group	N	Mean	Median	SD	SE
	1	9	-3.8211	25.3600	115.3124	38.4375
Delta Complex I	0	65	151.9455	107.6900	312.121	38.7138
	1	9	39.9844	153.5000	295.7286	98.5762
Delta Complex II	0	65	265.1875	173.1700	521.293	64.6585
	1	9	48.3789	155.4500	502.4121	167.4707

Legend: 0, no polymyxin use; 1, polymyxin use; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error

Figure 1. Amoxicillin-clavulanate mitochondrial parameters

Legend: 0, no amoxicillin-clavulanate use; 1, amoxicillin-clavulanate use; black square, mean of measurement; D1, first day of amoxicillin-clavulanate use; D3, third day of amoxicillin-clavulanate use.

Figure 1.1 Amoxicillin-clavulanate Basal D1. Routine in graphic refers to Basal respiration.

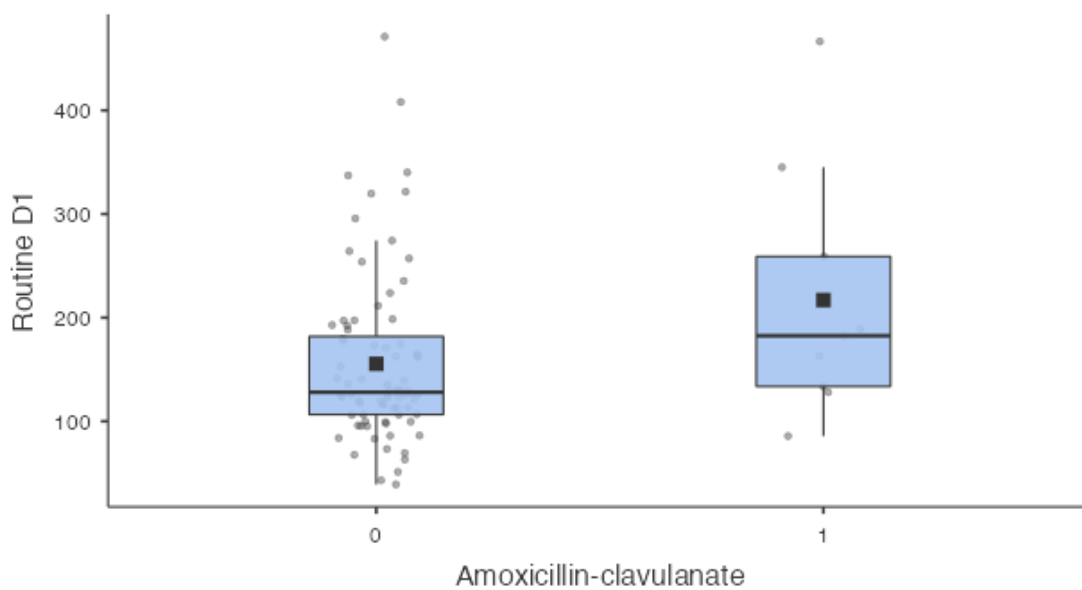


Figure 1.2 Amoxicillin-clavulanate Complex I D1

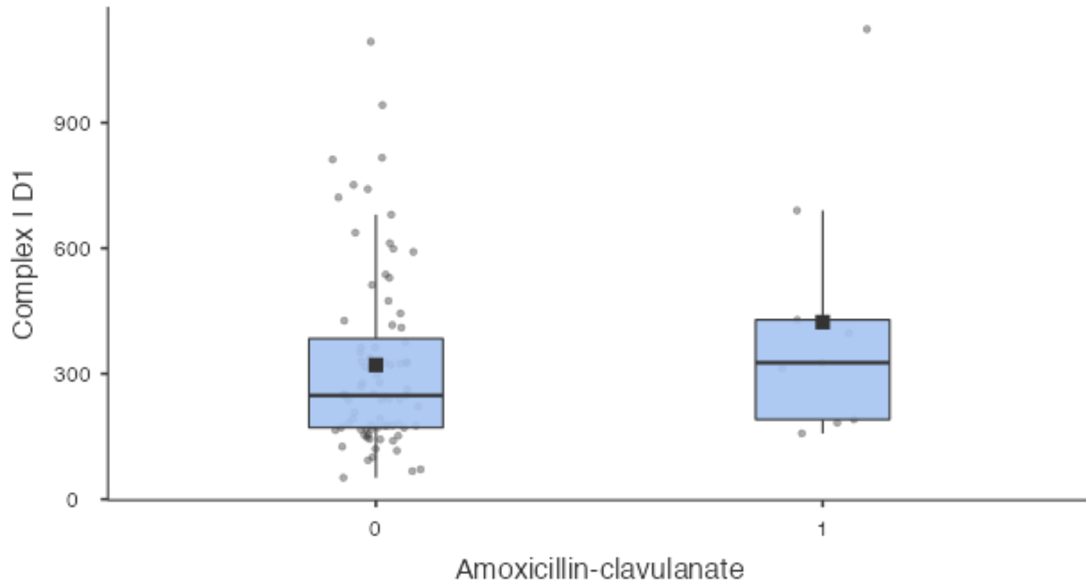


Figure 1.3 Amoxicillin-clavulanate Complex II D1

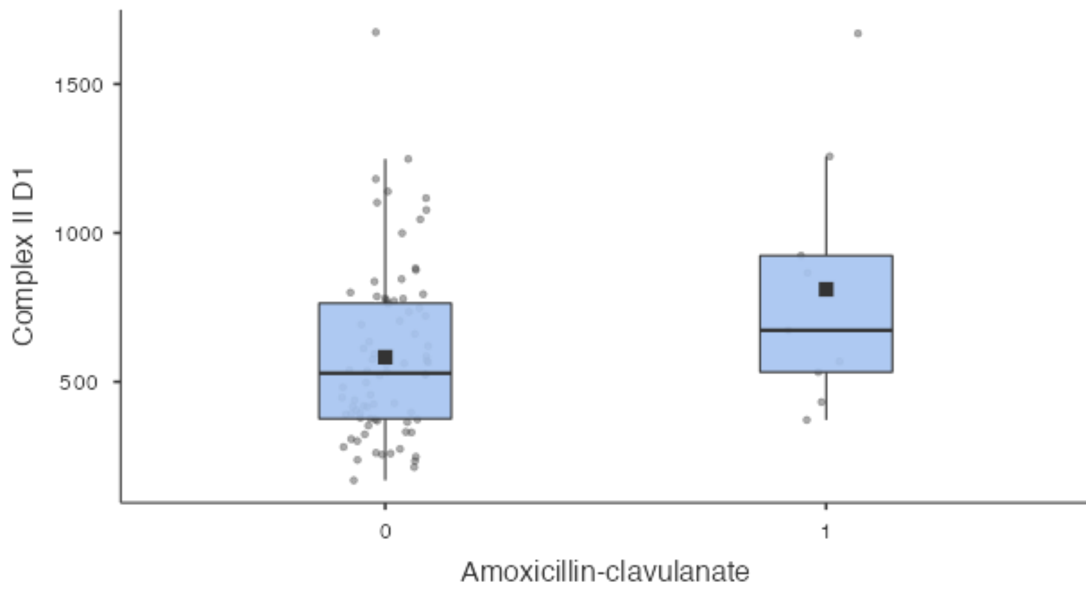


Figure 1.4 Amoxicillin-clavulanate BCE D1

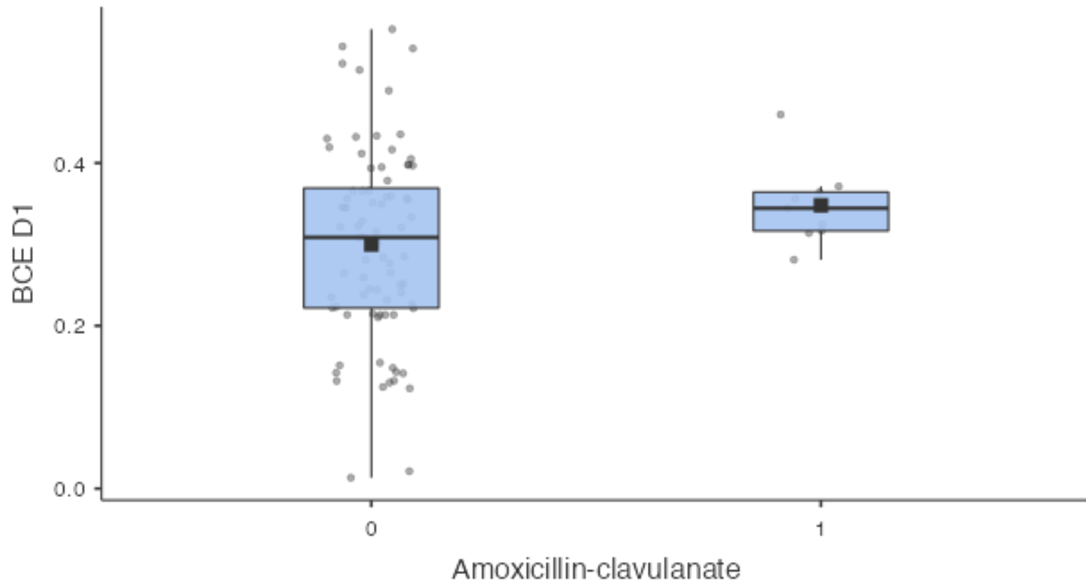


Figure 1.5 Amoxicillin-clavulanate Basal D3. Routine in graphic refers to Basal respiration.

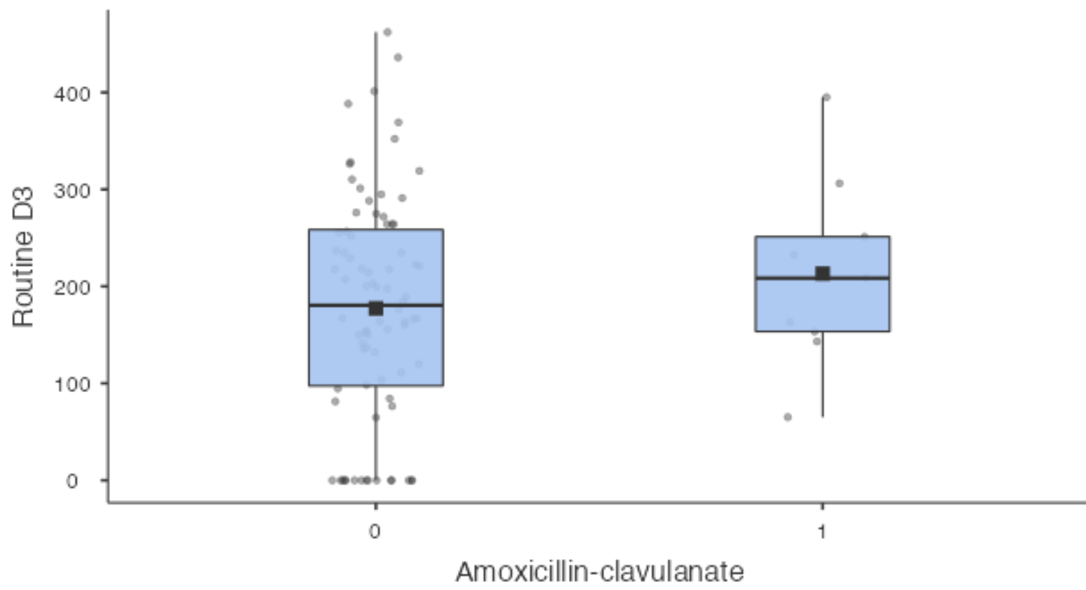


Figure 1.6 Amoxicillin-clavulanate Complex I D3

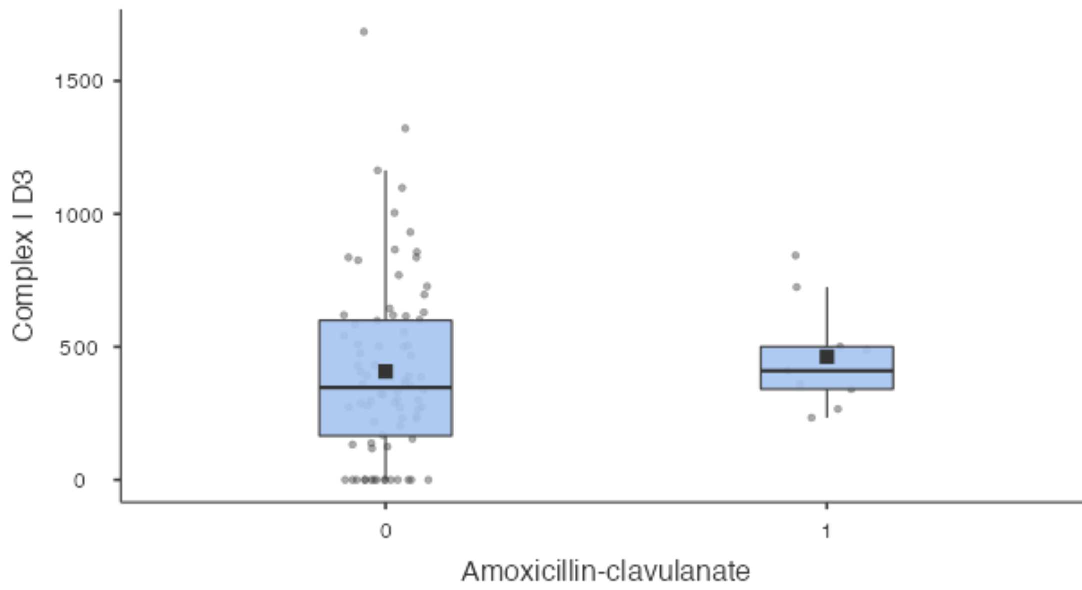


Figure 1.7 Amoxicillin-clavulanate Complex II D3

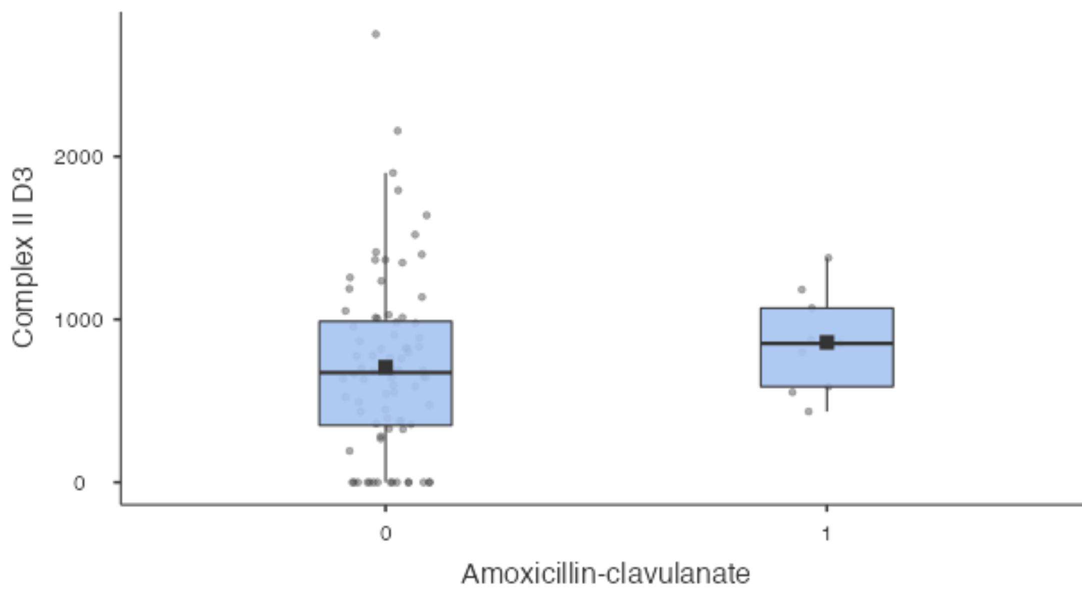


Figure 1.8 Amoxicillin-clavulanate BCE D3

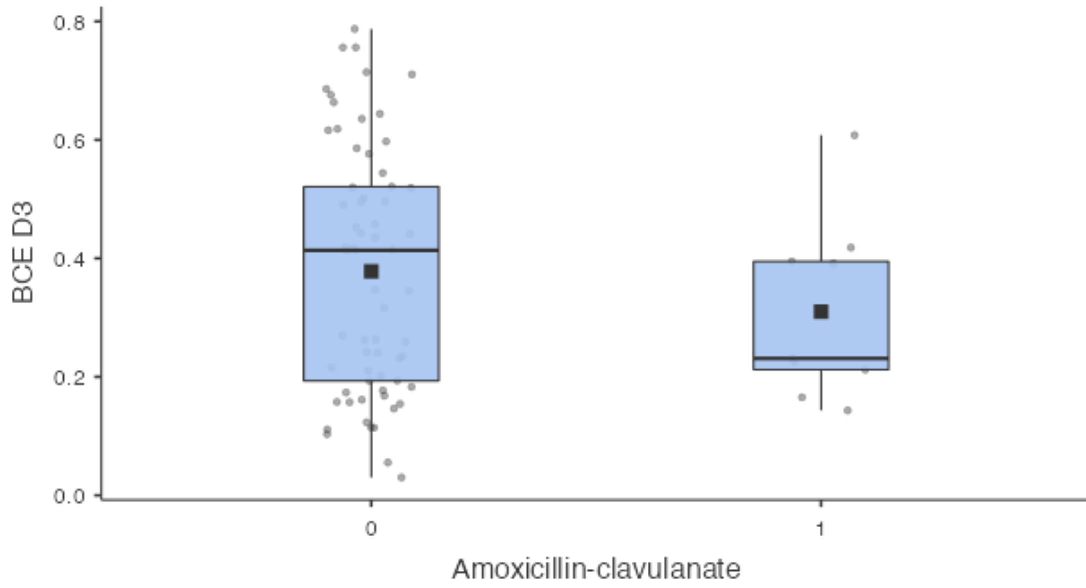


Figure 1.9 Amoxicillin-clavulanate Delta BCE

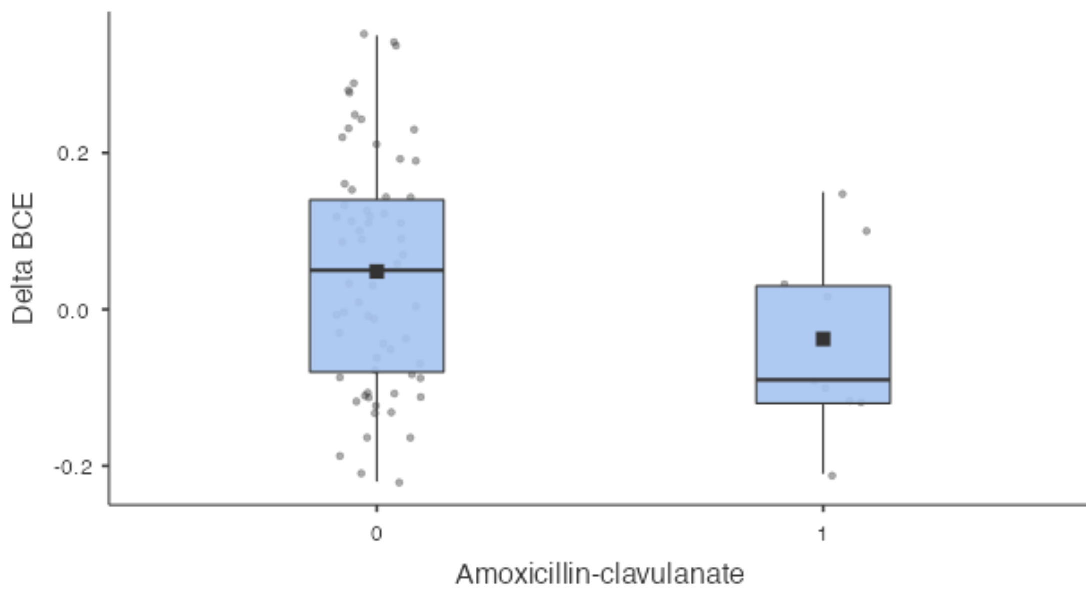


Figure 1.10 Amoxicillin-clavulanate Delta Basal. Routine in graphic refers to Basal respiration.

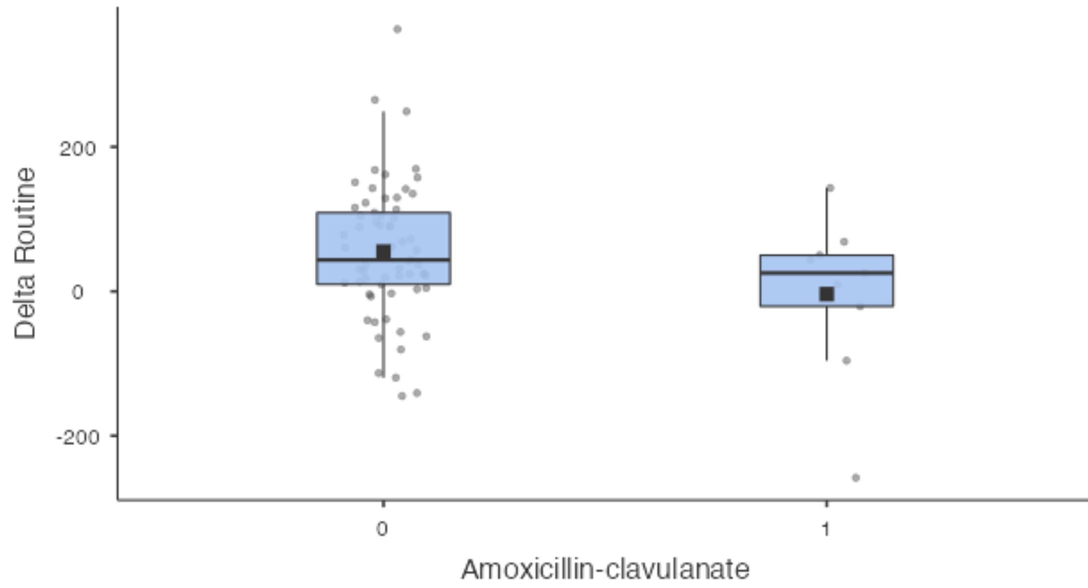


Figure 1.11 Amoxicillin-clavulanate Delta Complex I

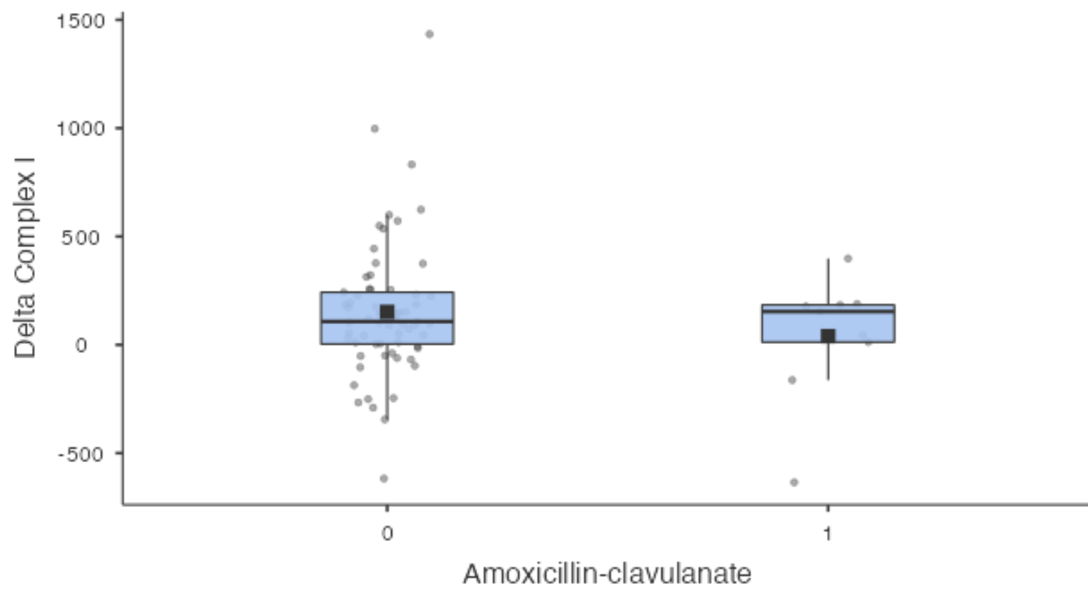
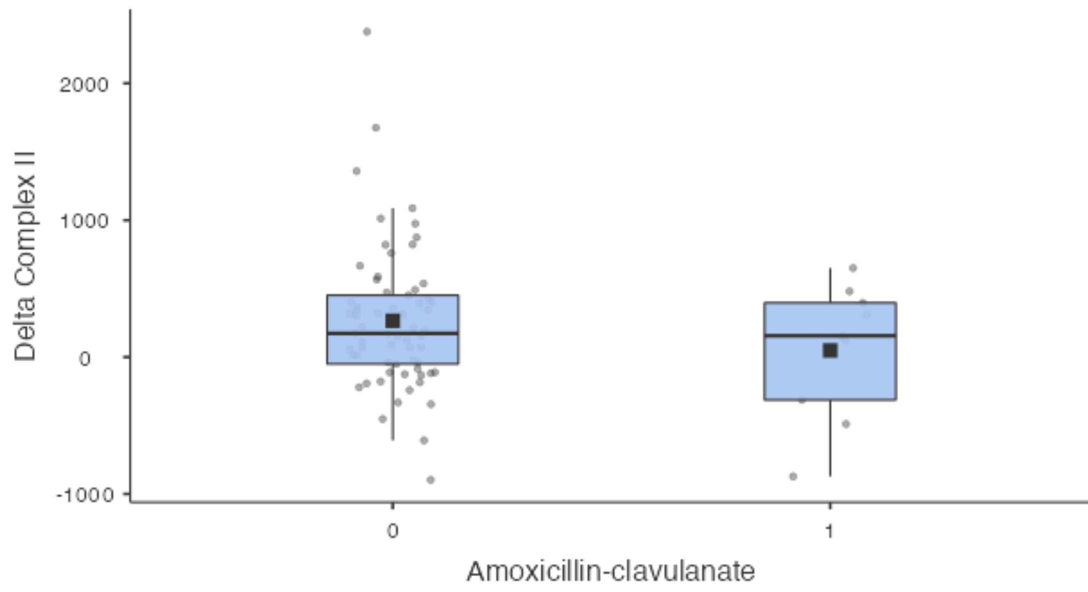


Figure 1.12 Amoxicillin-clavulanate Delta Complex II


Delta Complex II
2000
1000
0
-1000
0
1
Amoxicillin-clavulanate

ESM 5. Azitromycin use and its association with mitochondrial parameters in septic patients

Table 1. Summary statistics of patients that had use compared with those who did not use azitromycin.

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	256	0.032	-42.08049	
Complex I D1	Mann-Whitney U	293	0.091	-84.79207	
Complex II D1	Mann-Whitney U	281	0.066	-151.13604	
BCE D1	Mann-Whitney U	339	0.265	-0.04301	
Basal D3	Mann-Whitney U	375	0.504	-21.82278	
Complex I D3	Mann-Whitney U	384	0.578	-42.28976	
Complex II D3	Mann-Whitney U	399	0.712	-45.89838	
BCE D3	Mann-Whitney U	307	0.843	-0.01081	
Delta BCE	Mann-Whitney U	309	0.868	0.00999	
Delta Basal	Mann-Whitney U	254	0.300	27.90992	
Delta Complex I	Mann-Whitney U	272	0.453	56.67587	
Delta Complex II	Mann-Whitney U	241	0.215	197.77872	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	78	155.0215	126.3786	82.556	9.3476
	1	11	208.9669	165.2247	108.9061	32.8364
Complex I D1	0	78	319.0558	247.4986	219.464	24.8494
	1	11	416.4624	375.1647	277.4662	83.6592
Complex II D1	0	78	582.1640	524.4403	289.604	32.7912
	1	11	771.3196	612.1456	388.1477	117.0309
BCE D1	0	78	0.2989	0.3122	0.116	0.0131
	1	11	0.3454	0.3247	0.0813	0.0245
Basal D3	0	78	177.4981	180.4832	117.733	13.3306
	1	11	204.9718	208.4734	101.8033	30.6949

			Statistic	p	Mean difference	SE difference
Complex I D3	0	78	410.8360	348.5482	340.246	38.5253
	1	11	430.0183	409.9480	239.2037	72.1226
Complex II D3	0	78	720.4532	684.7698	551.285	62.4207
	1	11	736.3030	798.0758	382.1282	115.2160
BCE D3	0	64	0.3673	0.3463	0.205	0.0256
	1	10	0.3863	0.3934	0.1969	0.0623
Delta Basal	0	64	0.0395	0.0300	0.149	0.0187
	1	10	0.0280	0.0250	0.1467	0.0464
Delta Routine	0	64	54.4173	43.5850	93.558	11.6947
	1	10	5.5700	25.0000	111.9582	35.4043
Delta Complex I	0	64	154.9411	119.2850	313.913	39.2392
	1	10	32.0090	68.4550	277.8428	87.8616
Delta Complex II	0	64	275.8314	182.7350	522.786	65.3482
	1	10	1.9390	137.7850	461.3670	145.8971

Legend: 0, no azitromycin use; 1, azitromycin use; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error

Figure 1. Azitromycin mitochondrial parameters

Legend: 0, no azitromycin use; 1, azitromycin use; black square, mean of measurement; D1, first day of azitromycin use; D3, third day of azitromycin use.

Figure 1.1 Azitromycin Basal D1. Routine in graphic refers to Basal respiration.

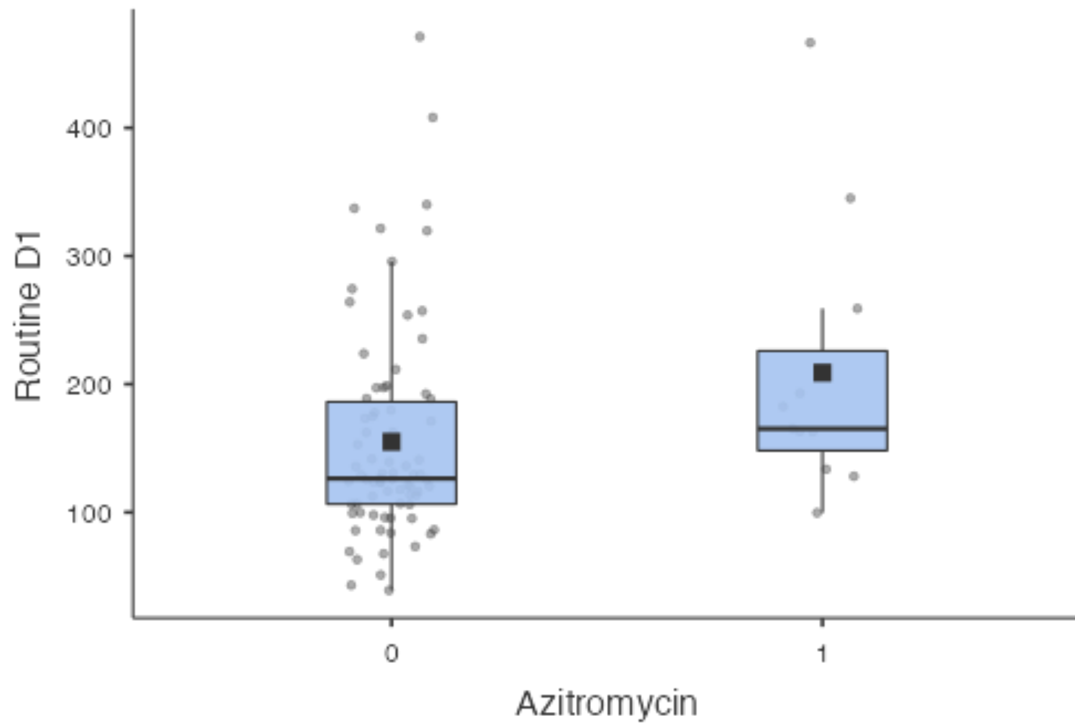


Figure 1.2 Azitromycin Complex I D1

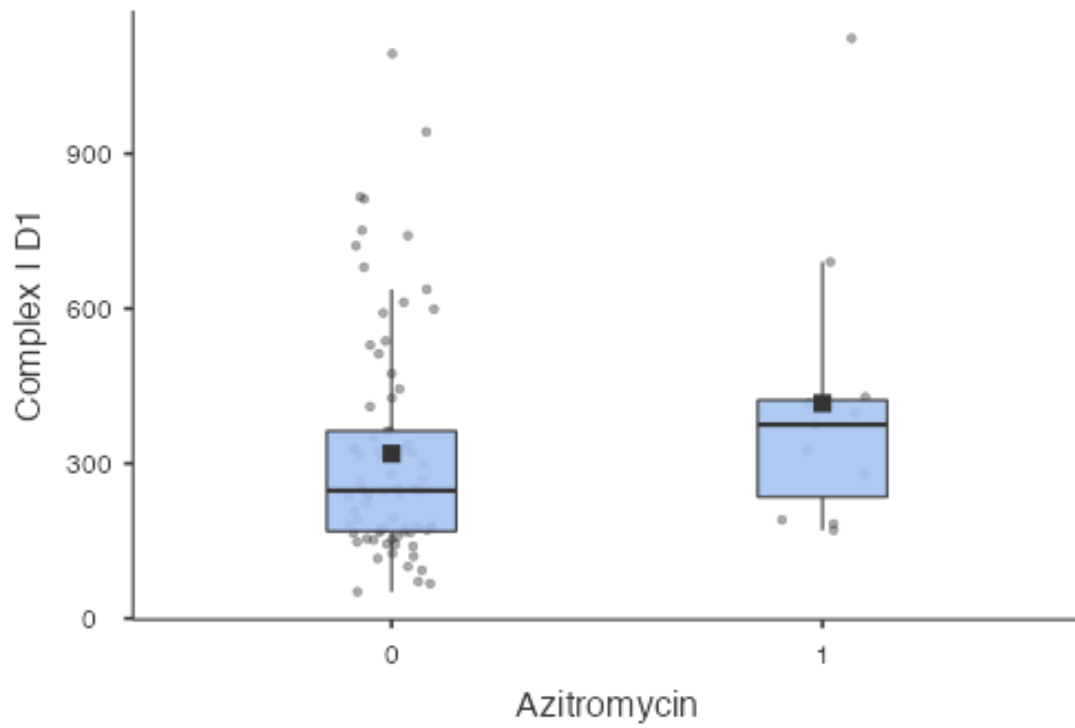


Figure 1.3 Azitromycin Complex II D1

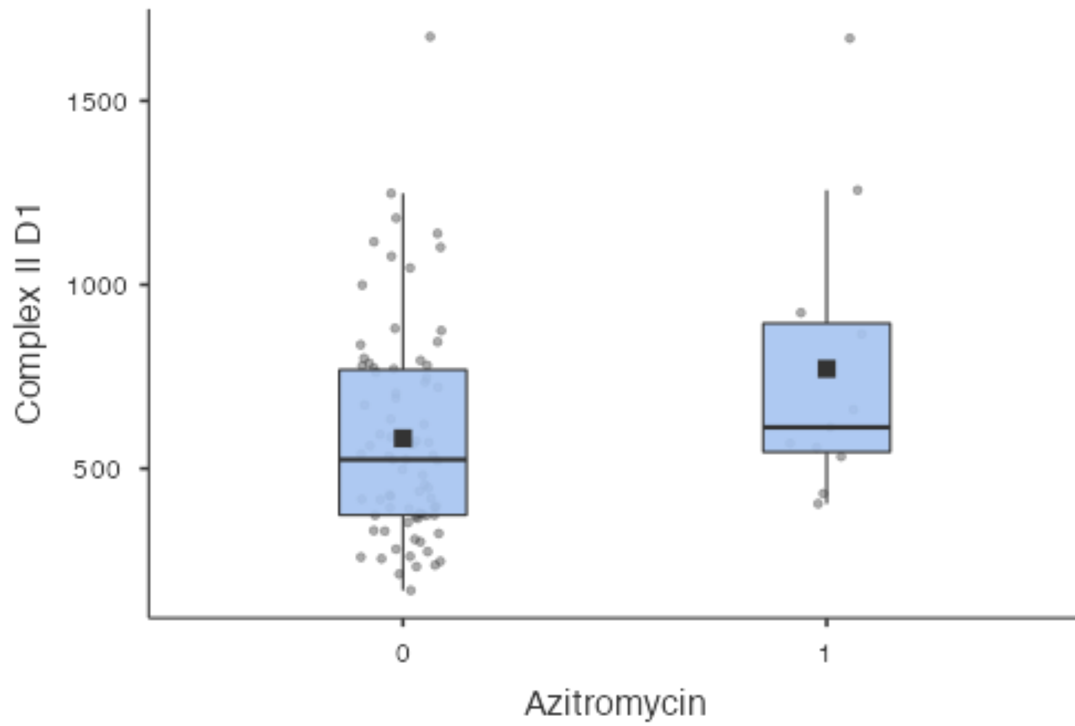


Figure 1.4 Azitromycin BCE D1

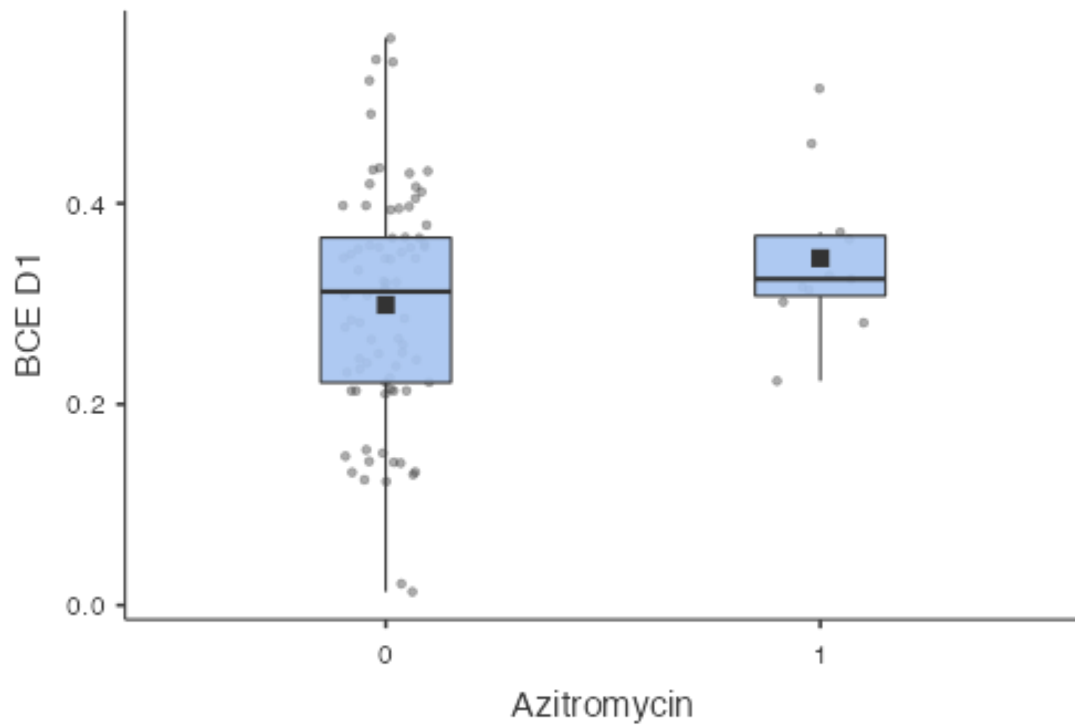


Figure 1.5 Azitromycin Basal D3. Routine in graphic refers to Basal respiration.

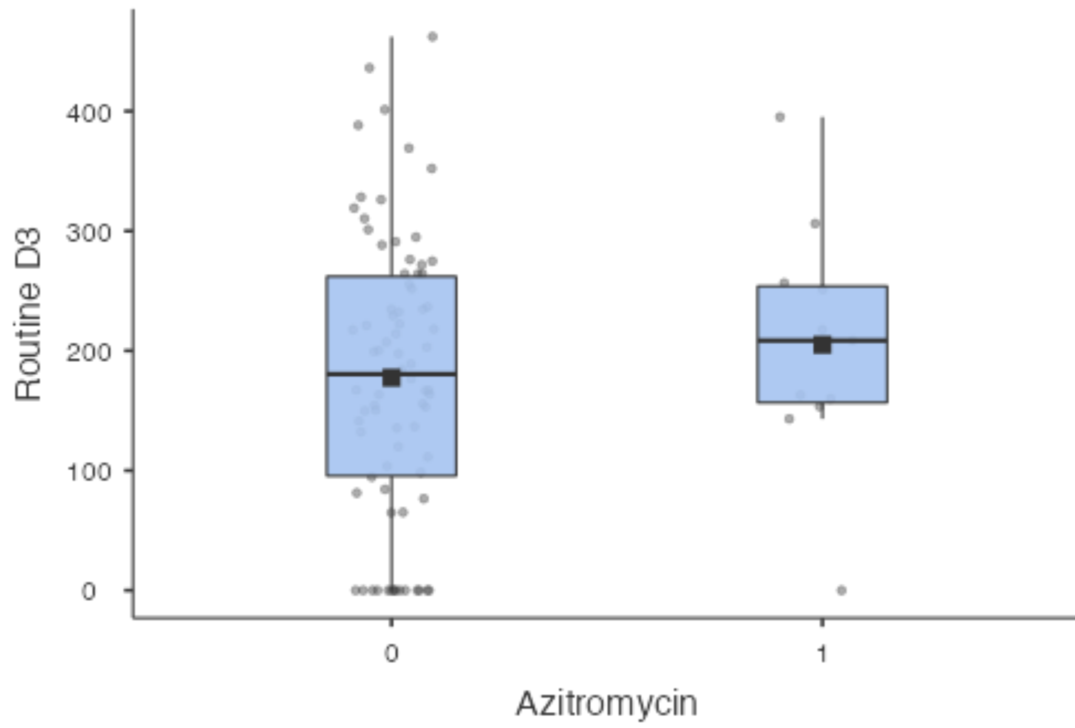


Figure 1.6 Azitromycin Complex I D3

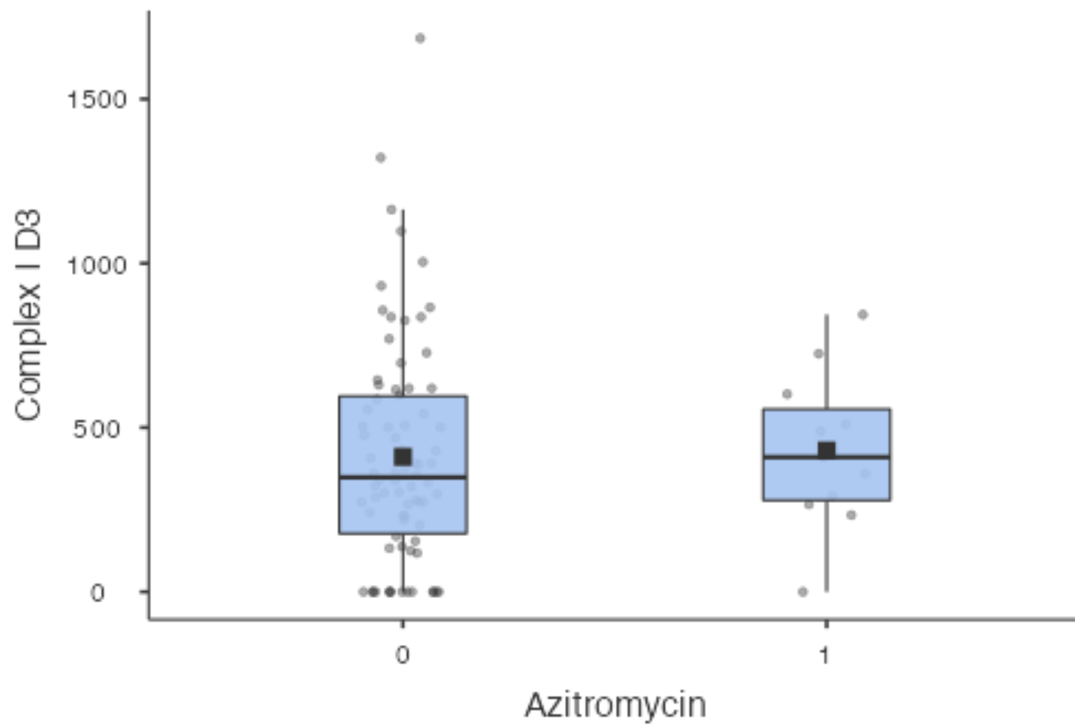


Figure 1.7 Azitromycin Complex II D3

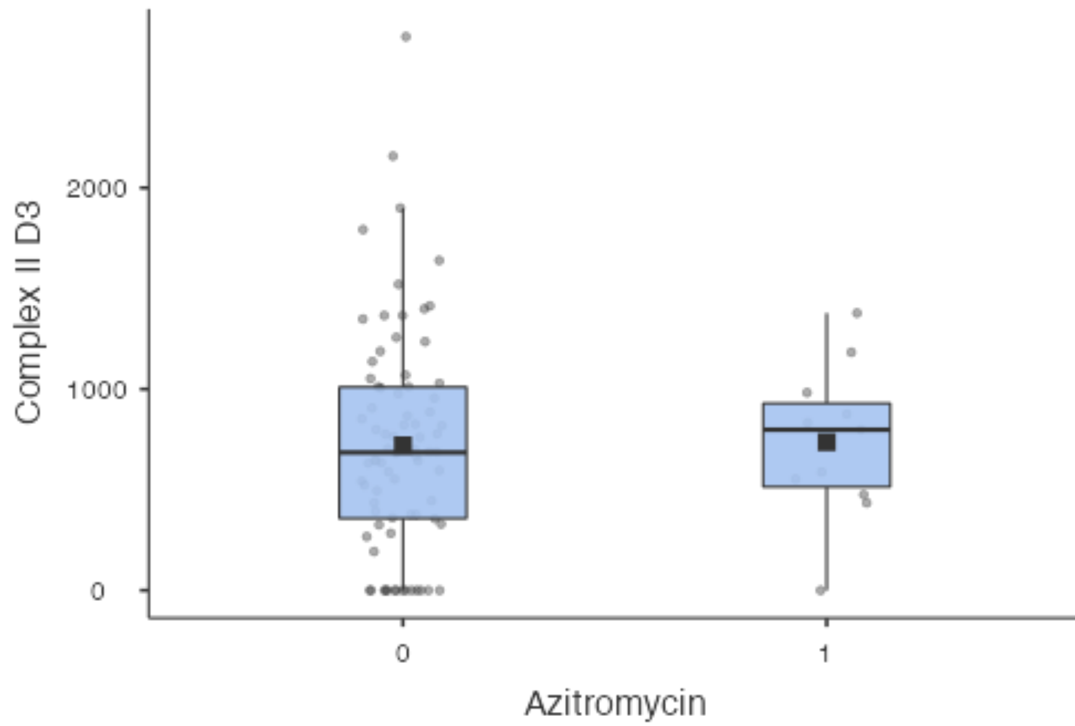


Figure 1.8 Azitromycin BCE D3

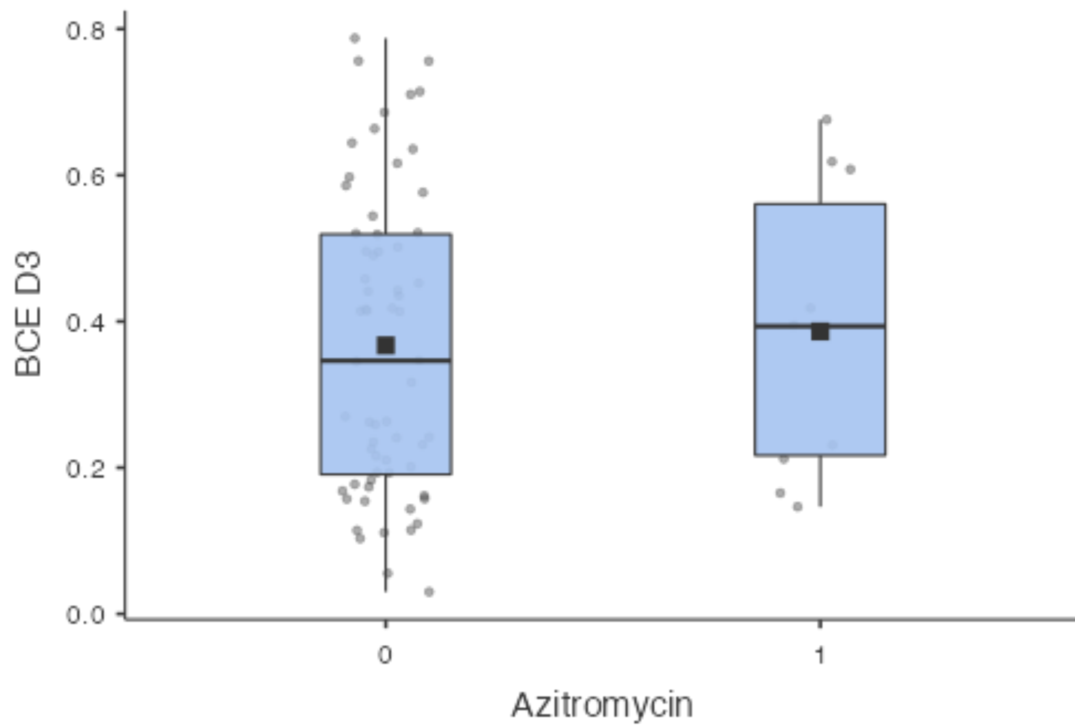


Figure 1.9 Azitromycin Delta BCE

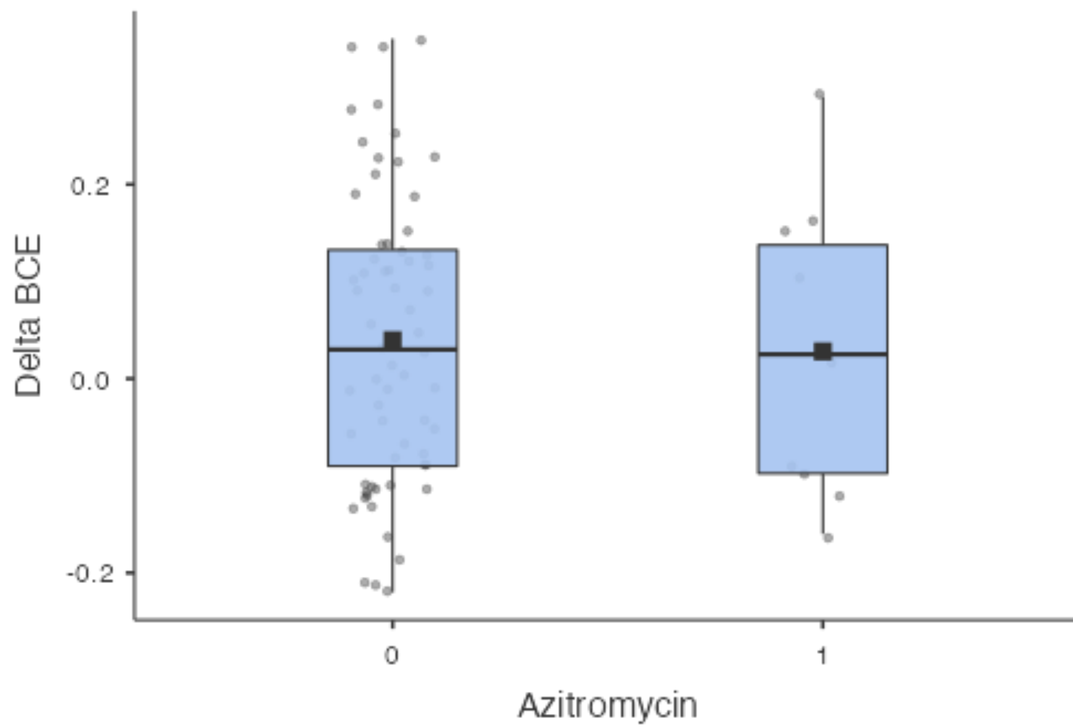


Figure 1.10 Azitromycin Delta Basal. Routine in graphic refers to Basal respiration.

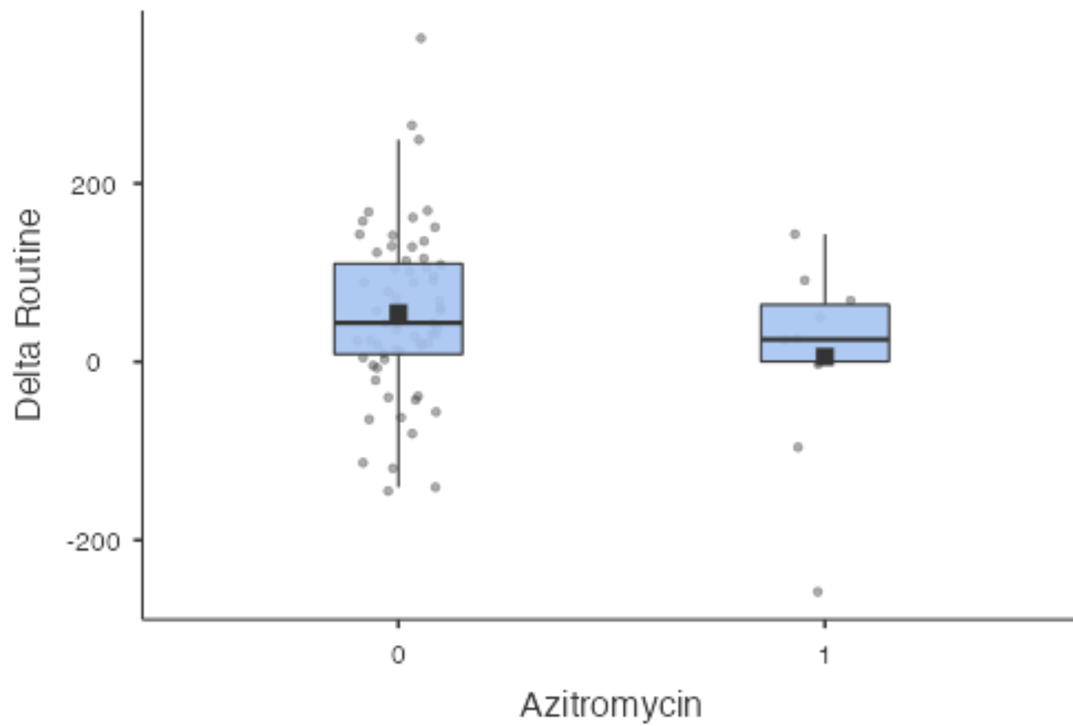


Figure 1.11 Azitromycin Delta Complex I

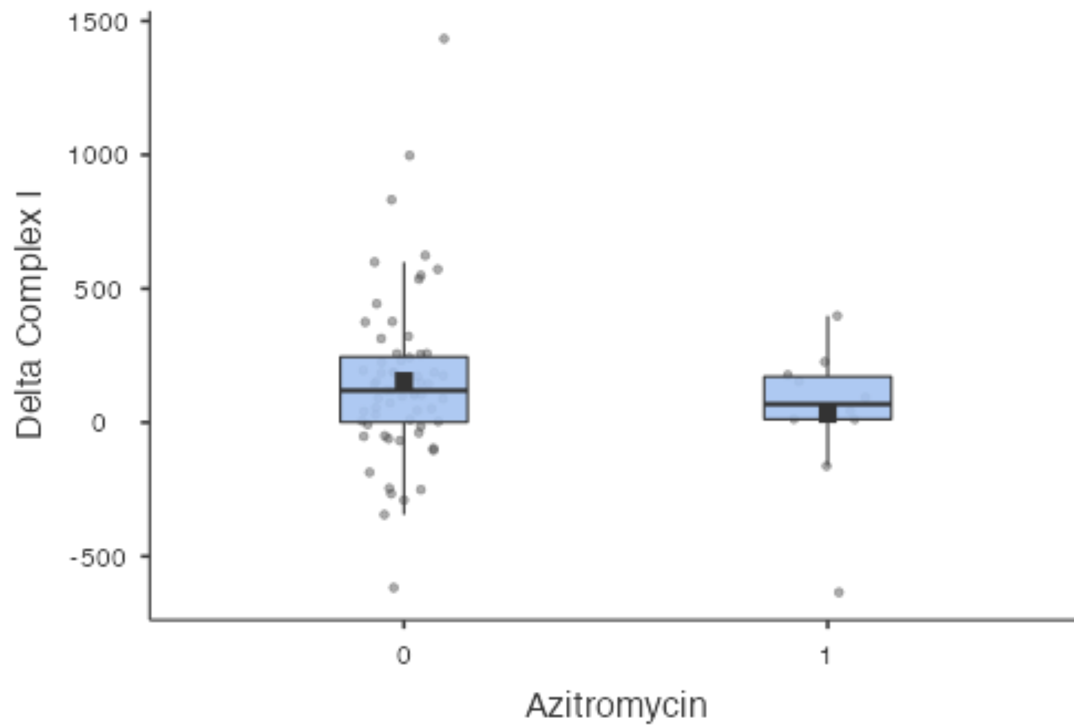
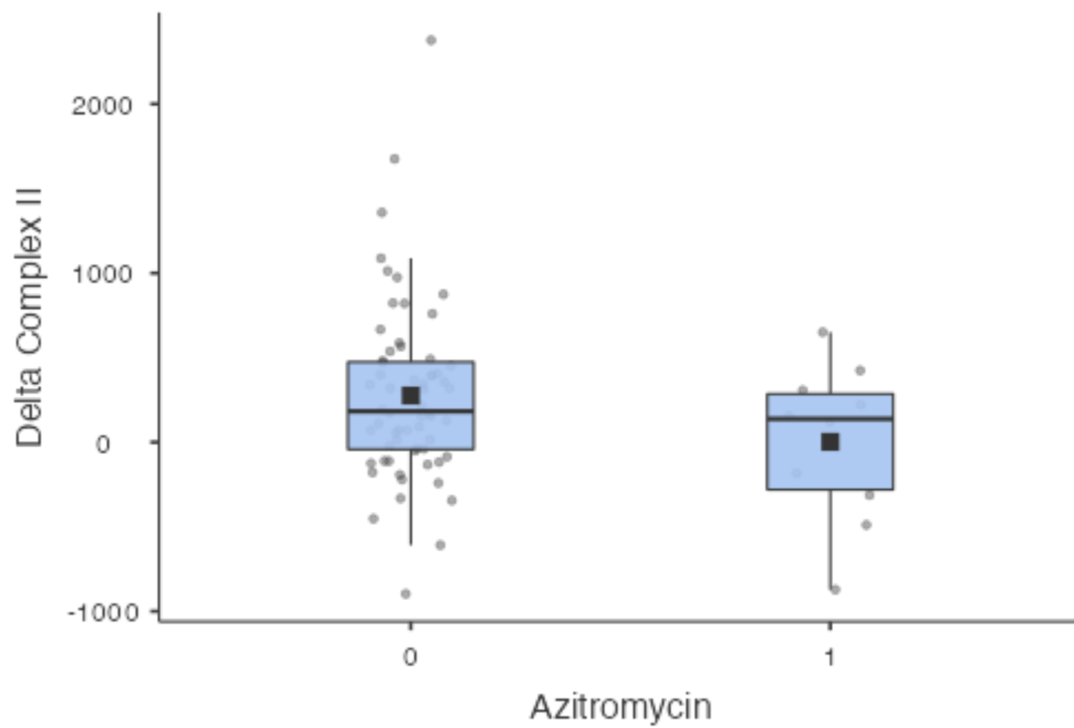


Figure 1.12 Azitromycin Delta Complex II



ESM 6. Ampicillin-sulbactam use and its association with mitochondrial parameters in septic patients

Table 1. Summary statistics of patients that had use compared with those who did not use ampicillin-sulbactam.

		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	348.0	0.876	-3.8797	
Complex I D1	Mann-Whitney U	358.0	0.984	0.4620	
Complex II D1	Mann-Whitney U	351.0	0.908	-11.2433	
BCE D1	Mann-Whitney U	248.5	0.131	0.0839	
BasalD3	Mann-Whitney U	357.0	0.973	-1.43e−5	
Complex I D3	Mann-Whitney U	341.0	0.801	0.0606	
Complex II D3	Mann-Whitney U	340.0	0.790	1.8846	
BCE D3	Mann-Whitney U	180.0	0.642	0.0548	
Delta BCE	Mann-Whitney U	191.5	0.812	0.0101	
Delta Basal	Mann-Whitney U	99.0	0.039	-78.3441	
Delta Complex I	Mann-Whitney U	123.0	0.111	-150.1812	
Delta Complex II	Mann-Whitney U	141.0	0.216	-220.6252	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	80	164.1554	129.2743	90.901	10.1630
	1	9	139.7644	136.1234	41.627	13.8757
Complex I D1	0	80	336.2861	249.4733	234.994	26.2731
	1	9	284.9498	278.2124	152.536	50.8453
Complex II D1	0	80	607.4511	548.5477	314.383	35.1491
	1	9	588.5804	524.2166	249.646	83.2154
BCE D1	0	80	0.3111	0.3212	0.110	0.0124
	1	9	0.2470	0.2135	0.126	0.0421
Basal D3	0	80	181.3618	180.4832	112.333	12.5592

			Statistic	p	Mean difference	SE difference
	1	9	176.7330	197.5617	150.482	50.1608
Complex I D3	0	80	416.2301	360.6101	327.096	36.5704
	1	9	386.3331	322.4998	358.856	119.6188
Complex II D3	0	80	730.7077	684.7698	528.609	59.1003
	1	9	648.6742	688.6660	584.685	194.8949
BCE D3	0	68	0.3737	0.3696	0.202	0.0245
	1	6	0.3266	0.3638	0.220	0.0900
Delta BCE	0	68	0.0388	0.0300	0.147	0.0179
	1	6	0.0283	0.0400	0.169	0.0688
Delta Basal	0	68	40.9176	36.6450	95.111	11.5339
	1	6	126.0017	120.8450	88.189	36.0028
Delta Complex I	0	68	127.1909	105.3100	317.596	38.5142
	1	6	264.5567	273.1300	185.169	75.5951
Delta Complex II	0	68	224.3954	163.1150	533.321	64.6747
	1	6	402.2850	379.6300	332.068	135.5662

Legend: 0, no polymyxin use; 1, polymyxin use; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error

Figure 1. Ampicillin-sulbactam mitochondrial parameters

Legend: 0, no ampicillin-sulbactam use; 1, ampicillin-sulbactam use; black square, mean of measurement; D1, first day of ampicillin-sulbactam use; D3, third day of ampicillin-sulbactam use.

Figure 1.1 Ampicillin-sulbactam Basal D1. Routine in graphic refers to Basal respiration.

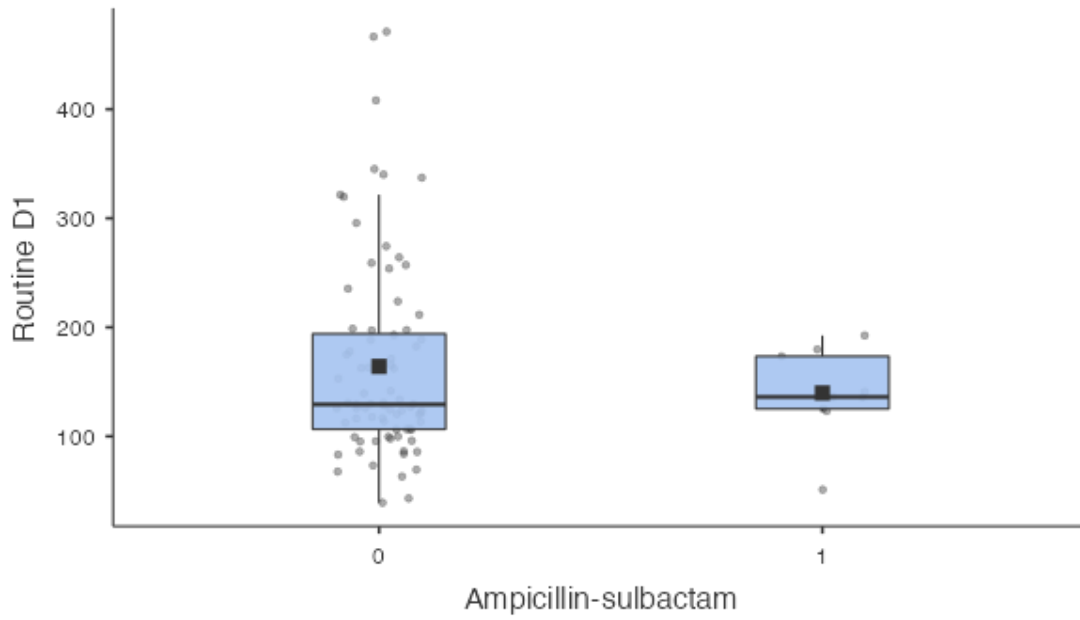


Figure 1.2 Ampicillin-sulbactam Complex I D1

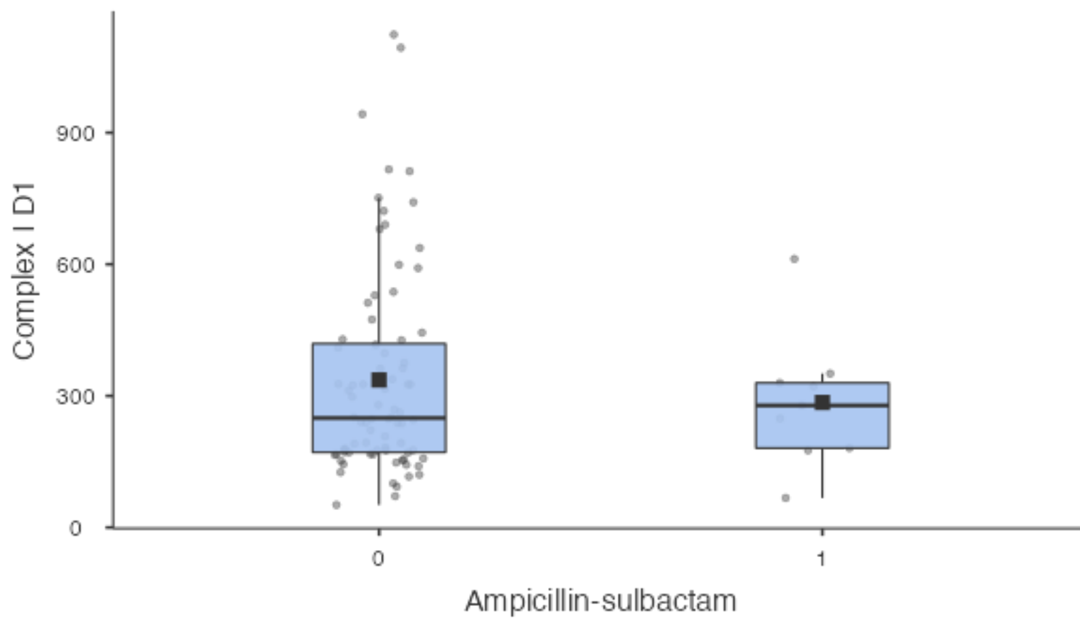


Figure 1.3 Ampicillin-sulbactam Complex II D1

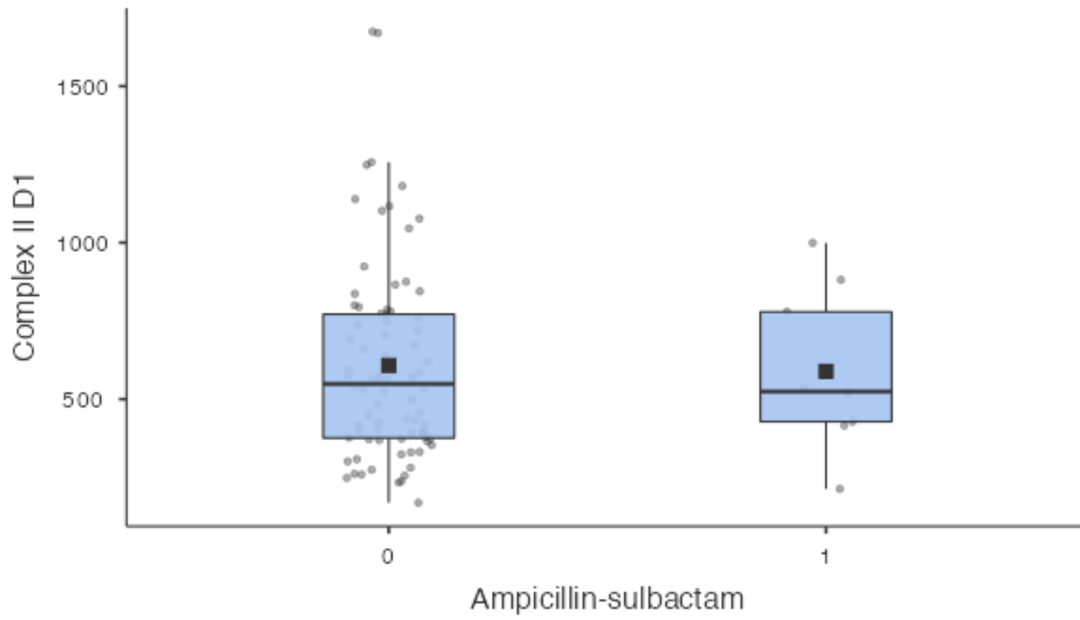


Figure 1.4 Ampicillin-sulbactam BCE D1

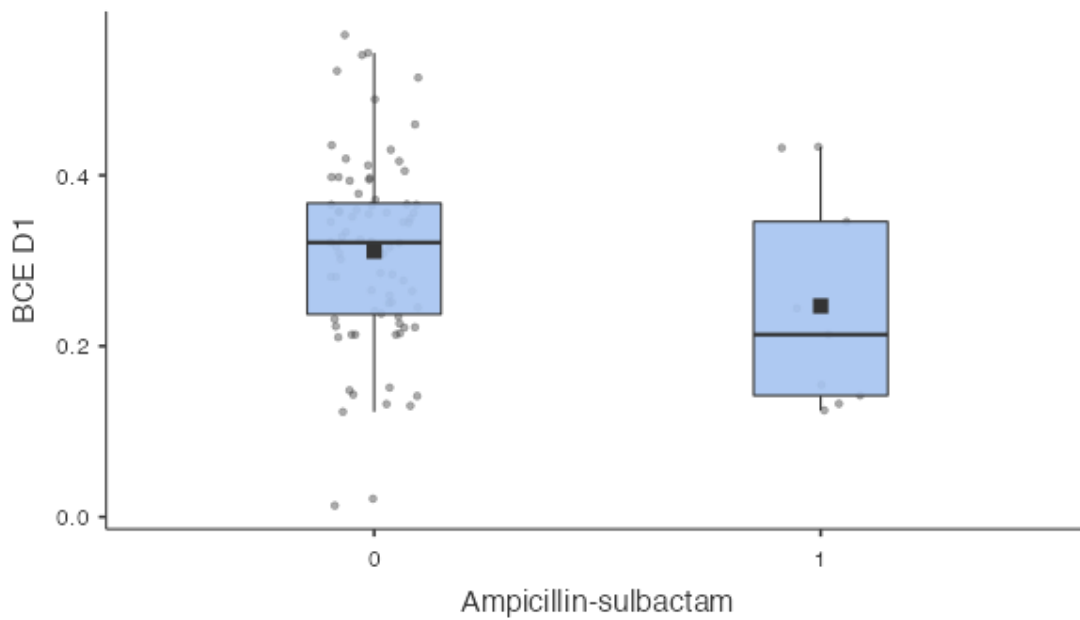


Figure 1.5 Ampicillin-sulbactam Routine D3. Routine in graphic refers to Basal respiration.

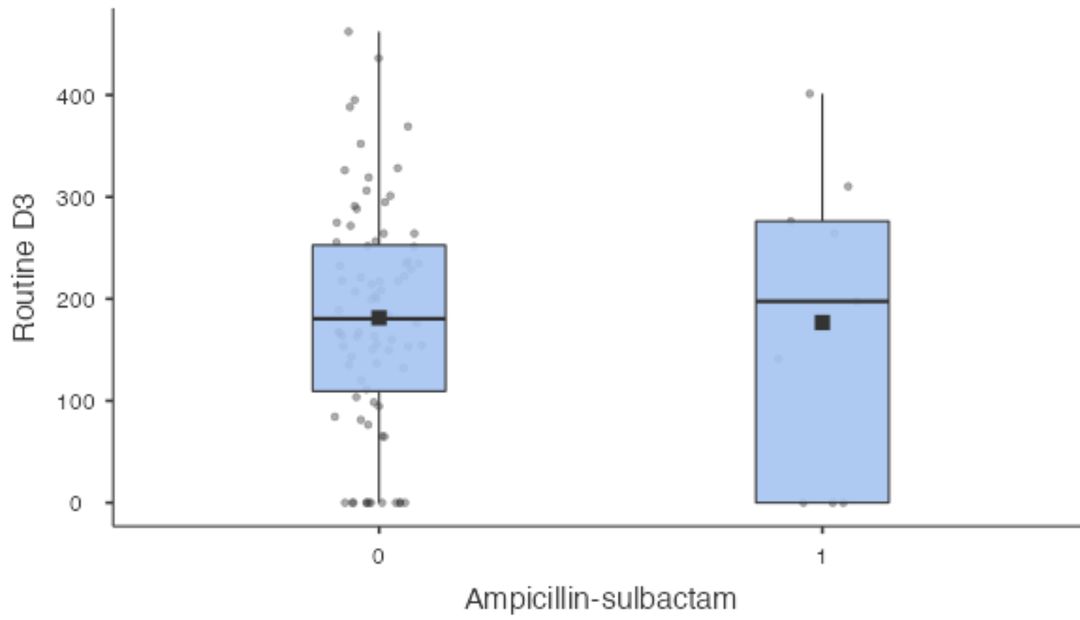


Figure 1.6 Ampicillin-sulbactam Complex I D3

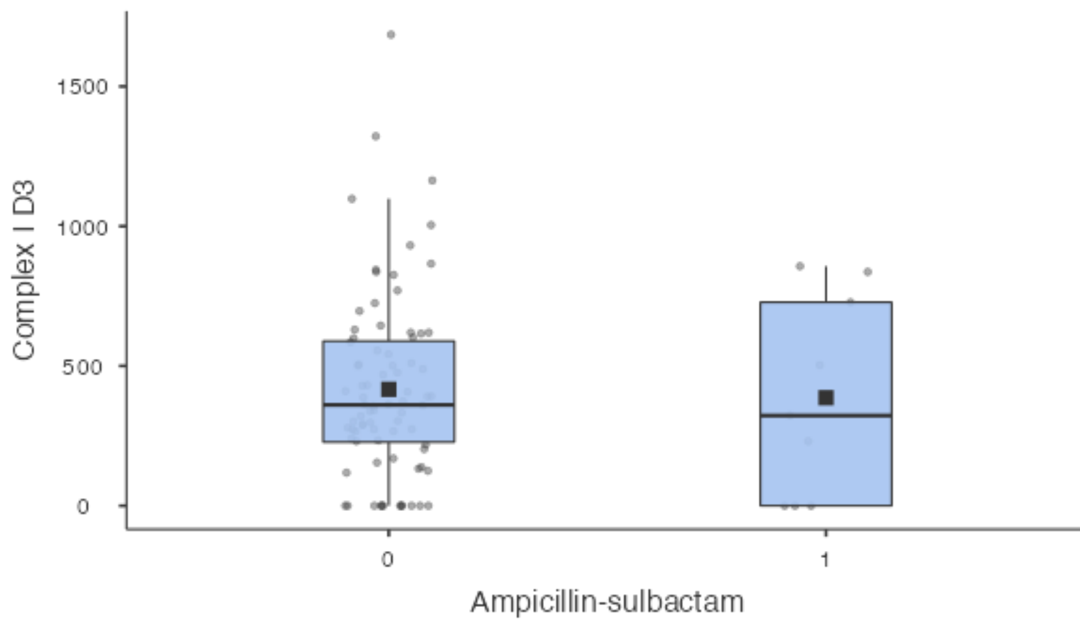


Figure 1.7 Ampicillin-sulbactam Complex II D3

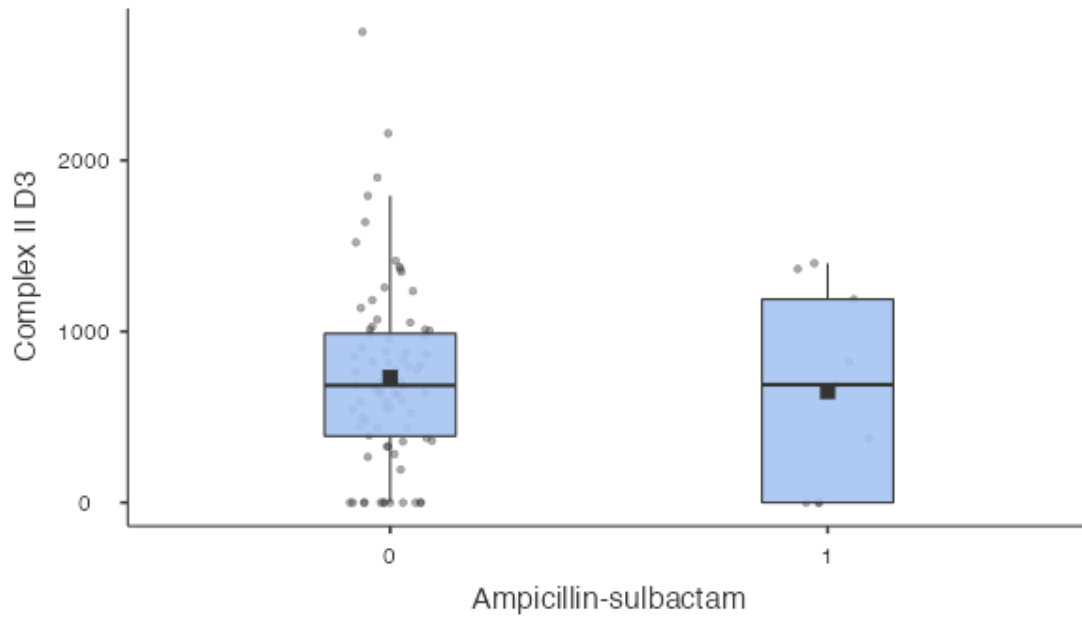


Figure 1.8 Ampicillin-sulbactam BCE D3

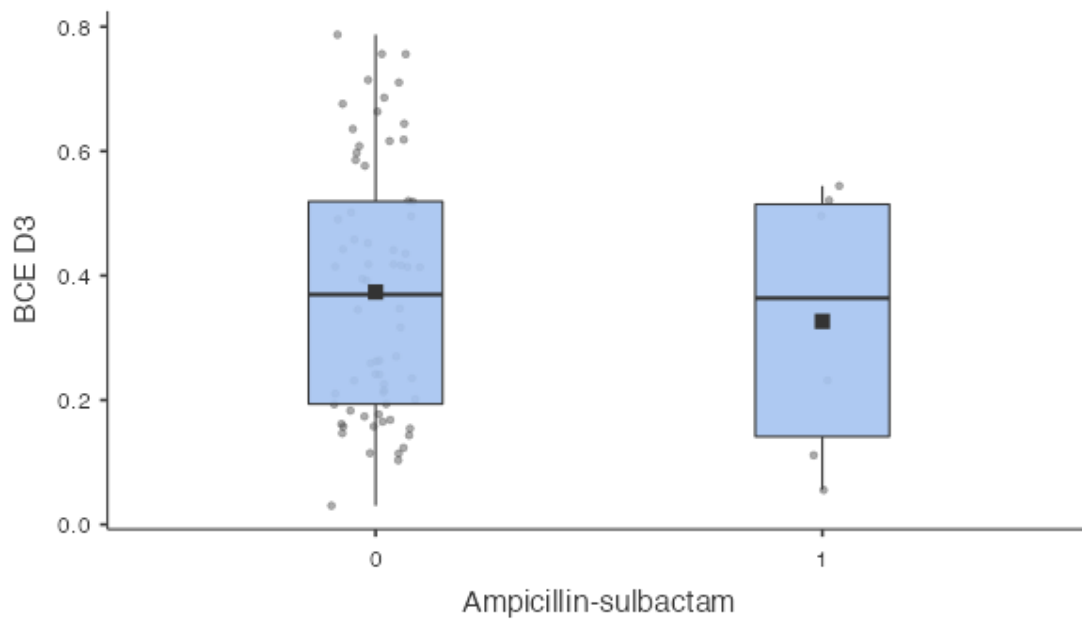


Figure 1.9 Ampicillin-sulbactam Delta BCE

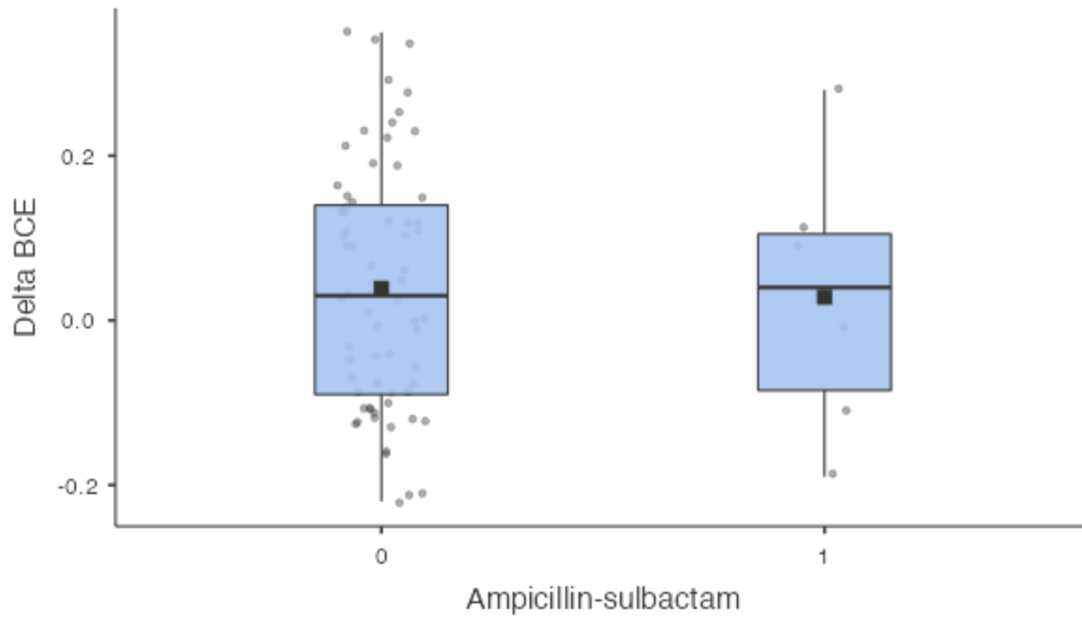


Figure 1.10 Ampicillin-sulbactam Delta Basal. Routine in graphic refers to Basal respiration.

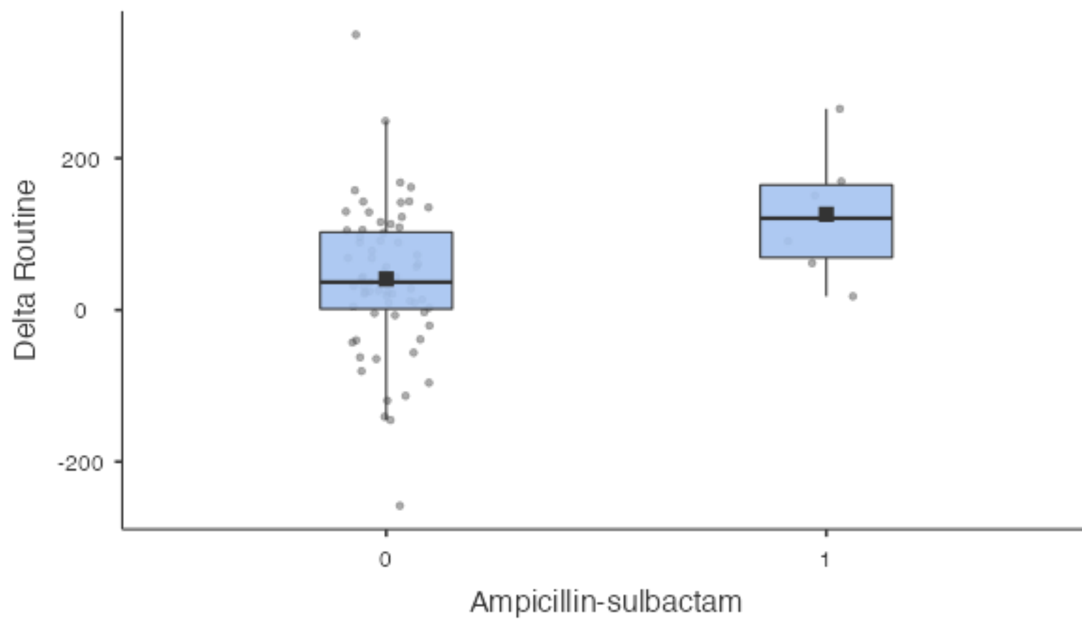


Figure 1.11 Ampicillin-sulbactam Delta Complex I

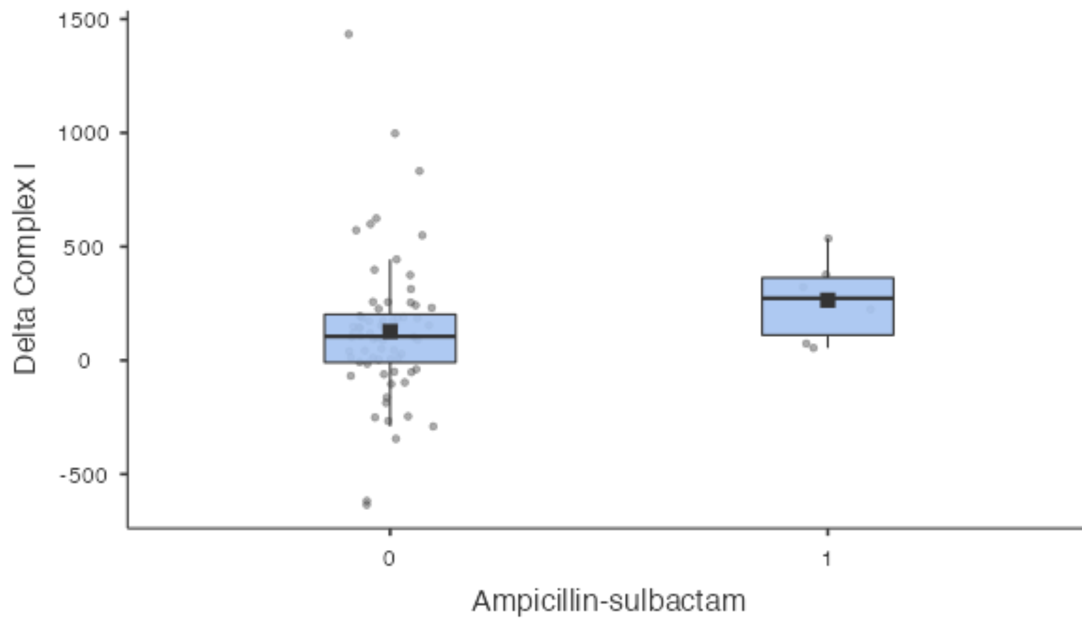
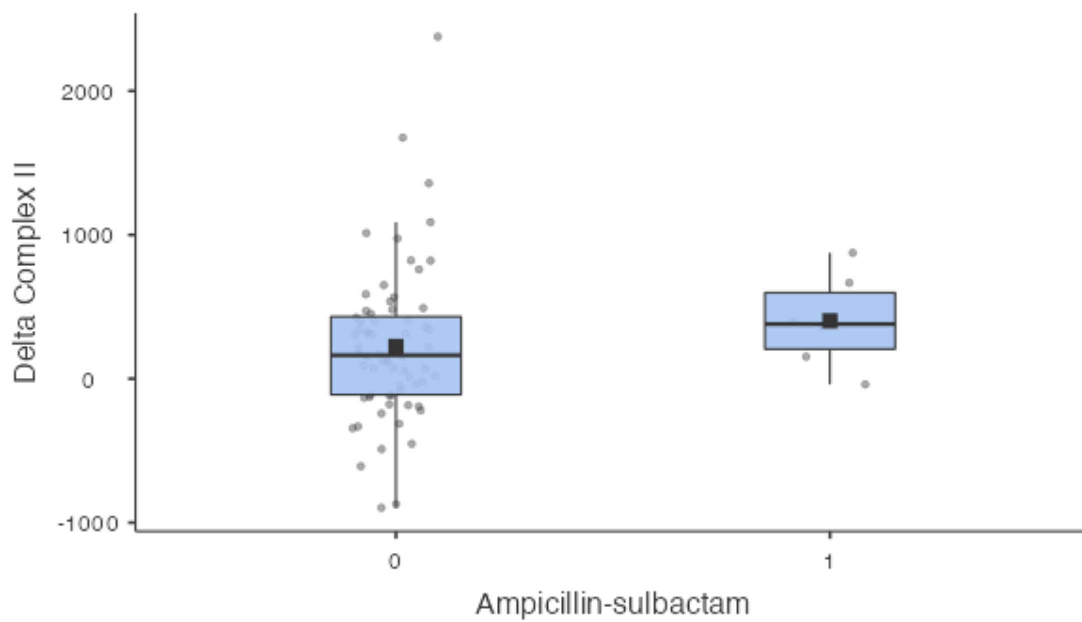


Figure 1.12 Ampicillin-sulbactam Delta Complex II



ESM 7. Piperacillin-tazobactam use and its association with mitochondrial parameters in septic patients

Table 1. Summary statistics of patients that had use compared with those who did not use piperacillin-tazobactam.

	Mann-Whitney U test between groups				
		Statistic	p	Mean difference	SE difference
Basal D1	Mann-Whitney U	871	0.392	10.4684	
Complex I D1	Mann-Whitney U	941	0.782	5.0650	
Complex II D1	Mann-Whitney U	960	0.905	-6.9773	
BCE D1	Mann-Whitney U	901	0.541	-0.0108	
Basal D3	Mann-Whitney U	827	0.220	28.6568	
Complex I D3	Mann-Whitney U	812	0.178	84.3640	
Complex II D3	Mann-Whitney U	773	0.095	174.4211	
BCE D3	Mann-Whitney U	629	0.645	-0.0224	
Delta BCE	Mann-Whitney U	665	0.939	4.00e-5	
Delta Basal	Mann-Whitney U	552	0.194	23.2550	
Delta Complex I	Mann-Whitney U	450	0.015	112.4500	
Delta Complex II	Mann-Whitney U	438	0.010	248.4250	

Group Descriptives

	Group	N	Mean	Median	SD	SE
Basal D1	0	50	170.4914	134.6876	96.5350	13.6521
	1	39	150.4037	129.1928	73.624	11.7892
Complex I D1	0	50	332.2138	264.8716	229.0131	32.3873
	1	39	329.6601	247.3621	229.413	36.7355
Complex II D1	0	50	605.3558	534.1903	318.4323	45.0331
	1	39	605.7826	557.6072	296.515	47.4804
BCE D1	0	50	0.2997	0.3147	0.0979	0.0138
	1	39	0.3111	0.3225	0.131	0.0210
Basal D3	0	50	195.2006	204.3486	123.9850	17.5341
	1	39	162.5516	166.8735	102.805	16.4620
Complex I D3	0	50	470.8027	388.7523	378.6929	53.5553
	1	39	339.3660	332.6894	234.200	37.5020
Complex II D3	0	50	824.4159	769.7757	607.2799	85.8823
	1	39	591.6381	642.8675	384.099	61.5051
BCE D3	0	42	0.3600	0.3934	0.1903	0.0294
	1	32	0.3828	0.3311	0.220	0.0390

Group Descriptives

	Group	N	Mean	Median	SD	SE
Delta BCE	0	42	0.0402	0.0300	0.1489	0.0230
	1	32	0.0350	0.0100	0.149	0.0264
Delta Basal	0	42	56.1824	53.4650	105.4198	16.2666
	1	32	36.8359	22.9650	84.709	14.9746
Delta Complex I	0	42	208.4286	164.3450	345.1366	53.2557
	1	32	46.3225	46.2700	232.270	41.0599
Delta Complex II	0	42	370.9586	329.1100	565.2265	87.2163
	1	32	65.3856	71.1300	401.132	70.9109

Legend: 0, non-piperacillin-tazobactam users; 1, piperacillin-tazobactam users; BCE, biochemical coupling efficiency; SD, standard deviation; SE, standard error.

Figure 1. Piperacillin-tazobactam mitochondrial parameters

Legend: 0, no piperacillin-tazobactam use; 1, piperacillin-tazobactam use; black square, mean of measurement; D1, first day of piperacillin-tazobactam use; D3, third day of piperacillin-tazobactam use.

Figure 1.1 Piperacillin-tazobactam Basal D1

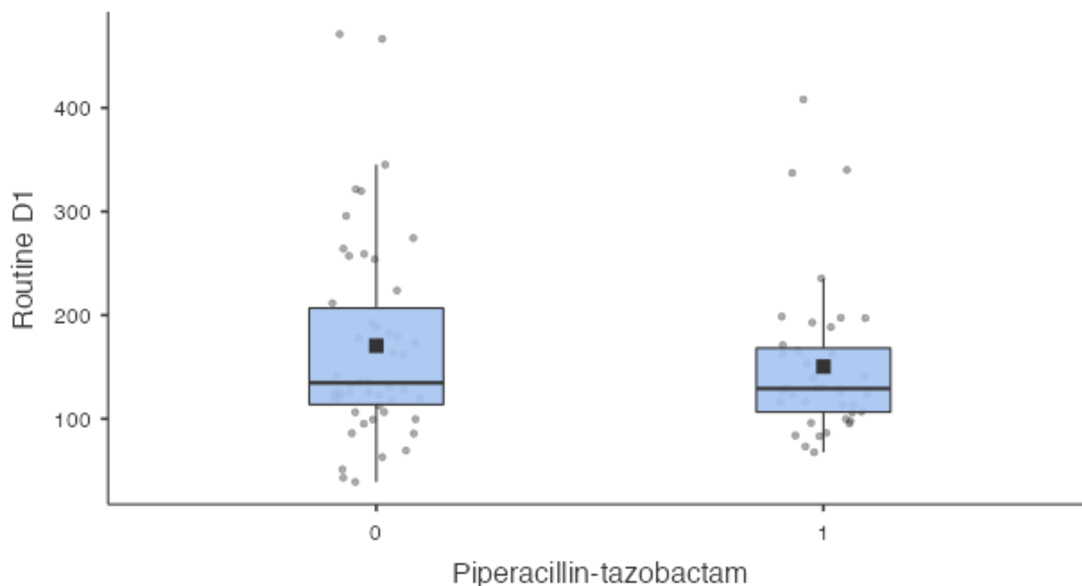


Figure 1.2 Piperacillin-tazobactam Complex I D1. Routine in graphic refers to Basal respiration.

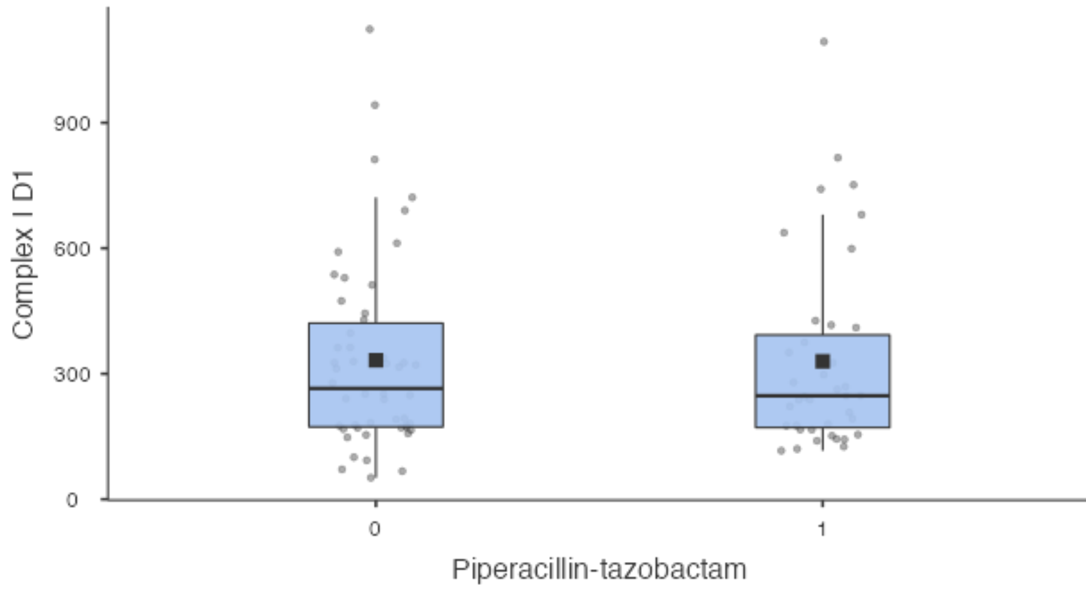


Figure 1.3 Piperacillin-tazobactam Complex II D1

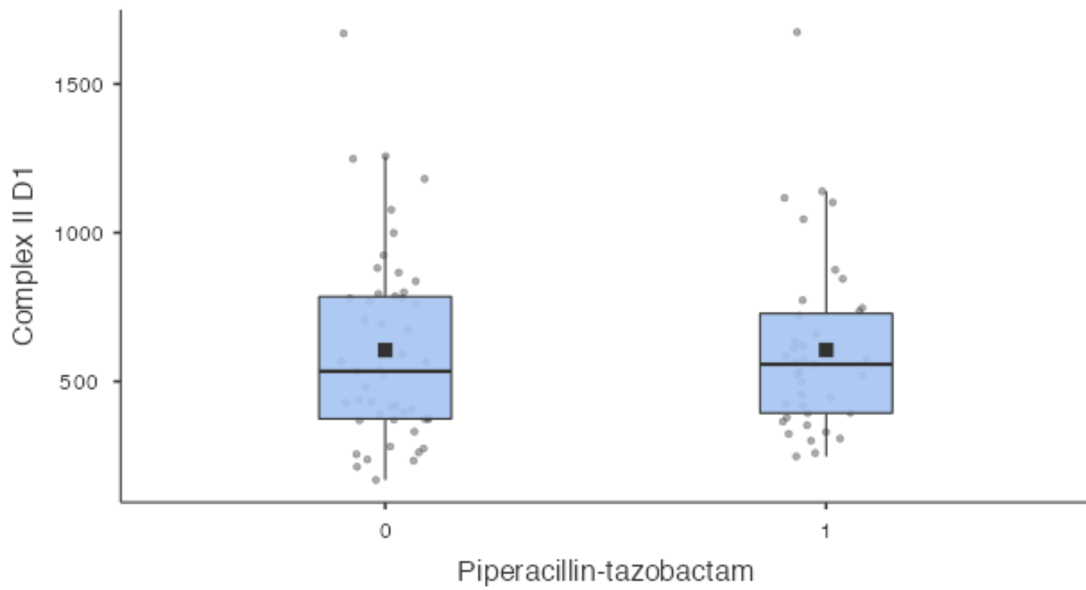


Figure 1.4 Piperacillin-tazobactam BCE D1

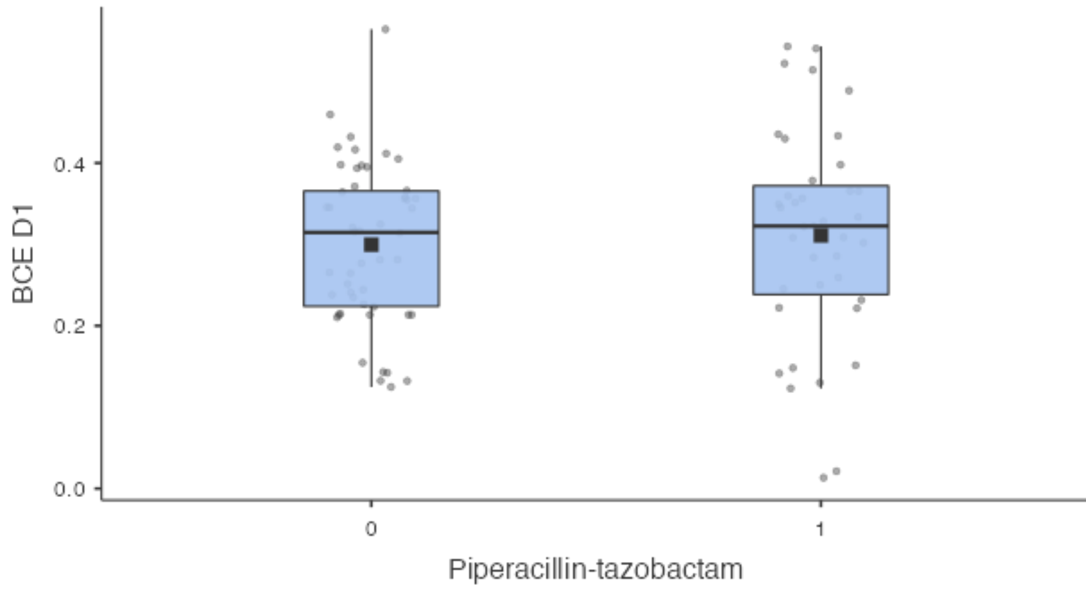


Figure 1.5 Piperacillin-tazobactam Basal D3. Routine in graphic refers to Basal respiration.

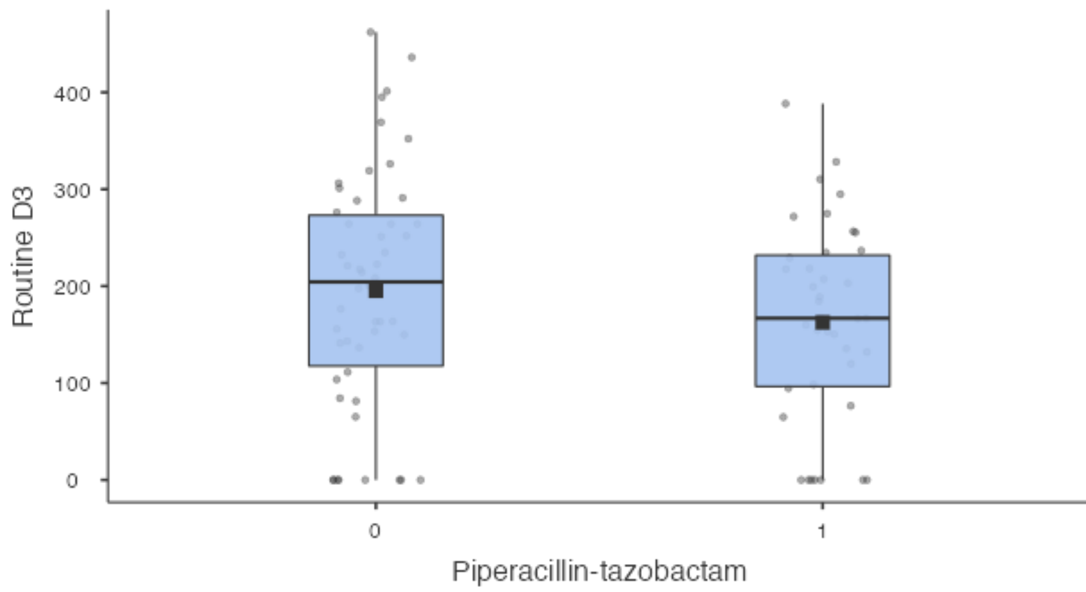


Figure 1.6 Piperacillin-tazobactam Complex I D3

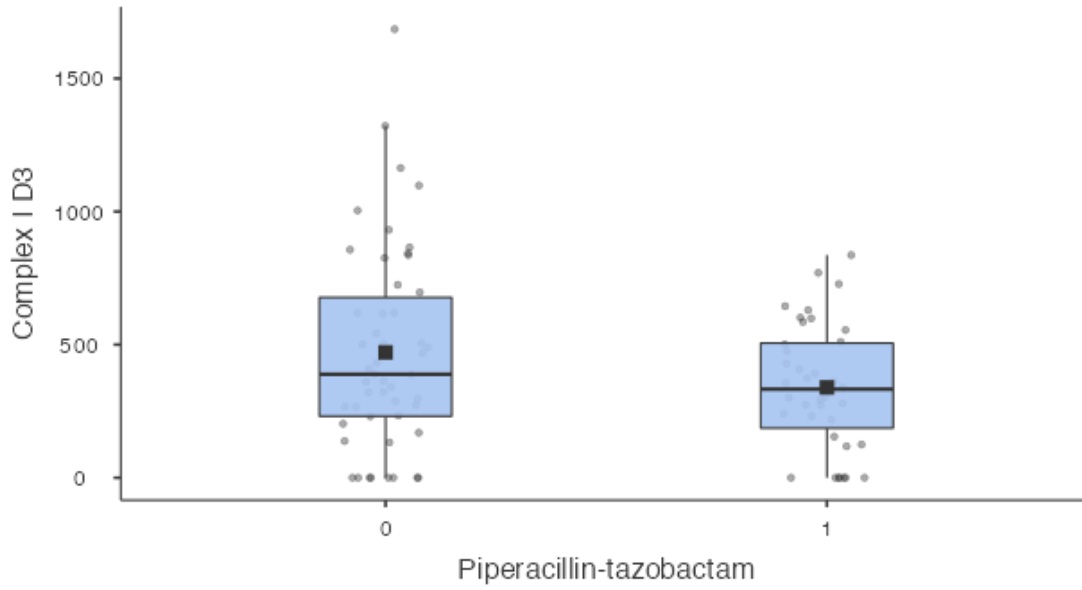


Figure 1.7 Piperacillin-tazobactam Complex II D3

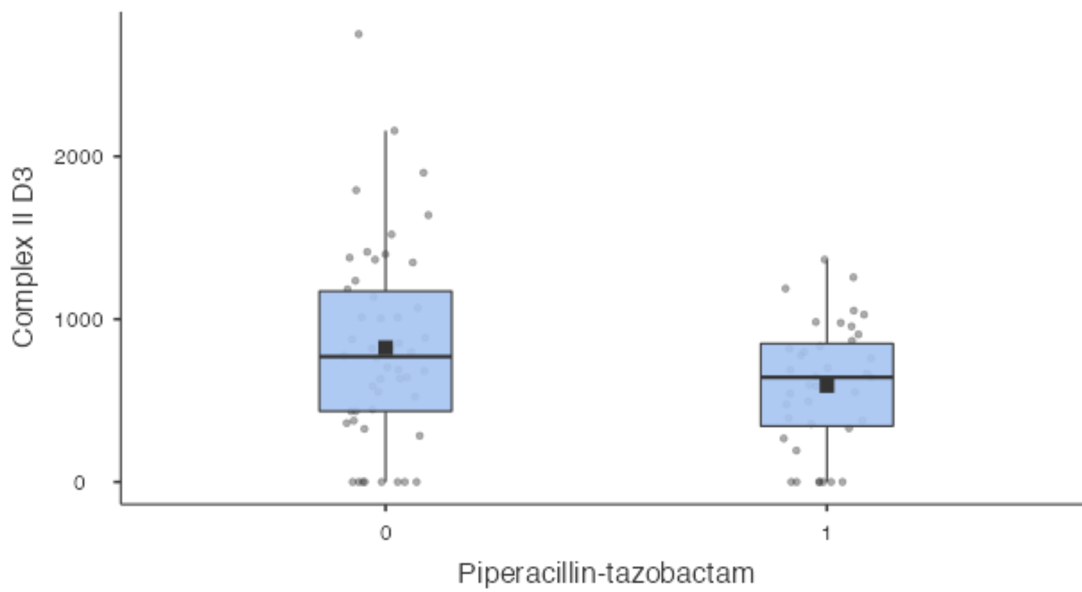


Figure 1.8 Piperacillin-tazobactam BCE D3

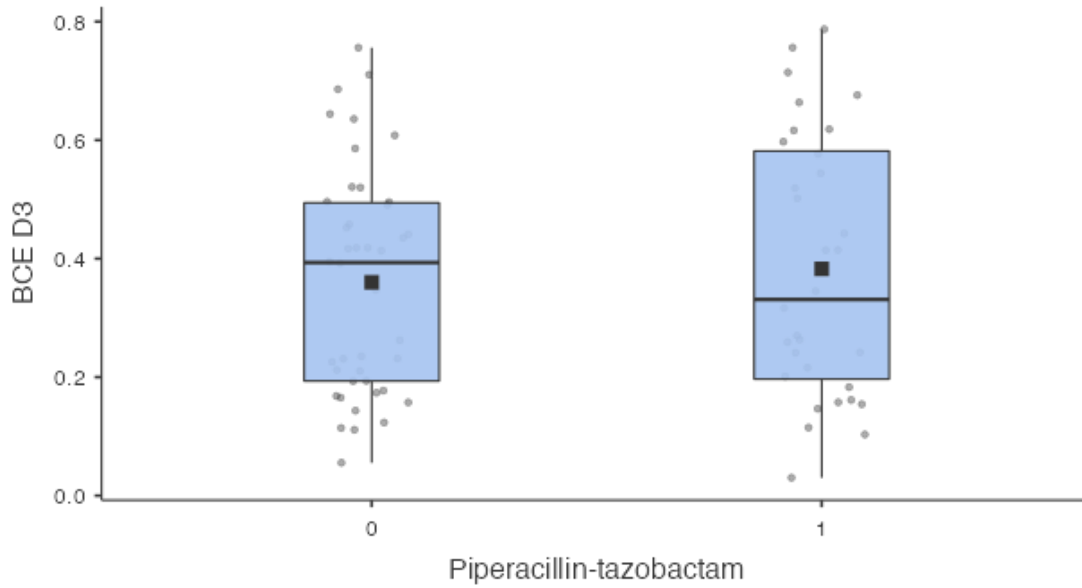


Figure 1.9 Piperacillin-tazobactam Delta BCE

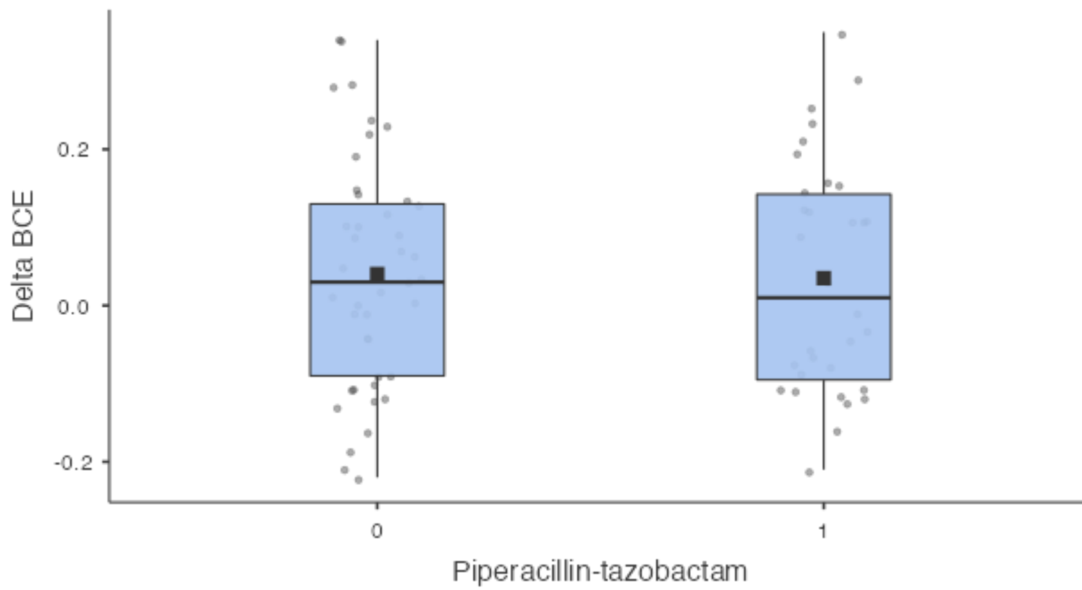


Figure 1.10 Piperacillin-tazobactam Delta Basal. Routine in graphic refers to Basal respiration.

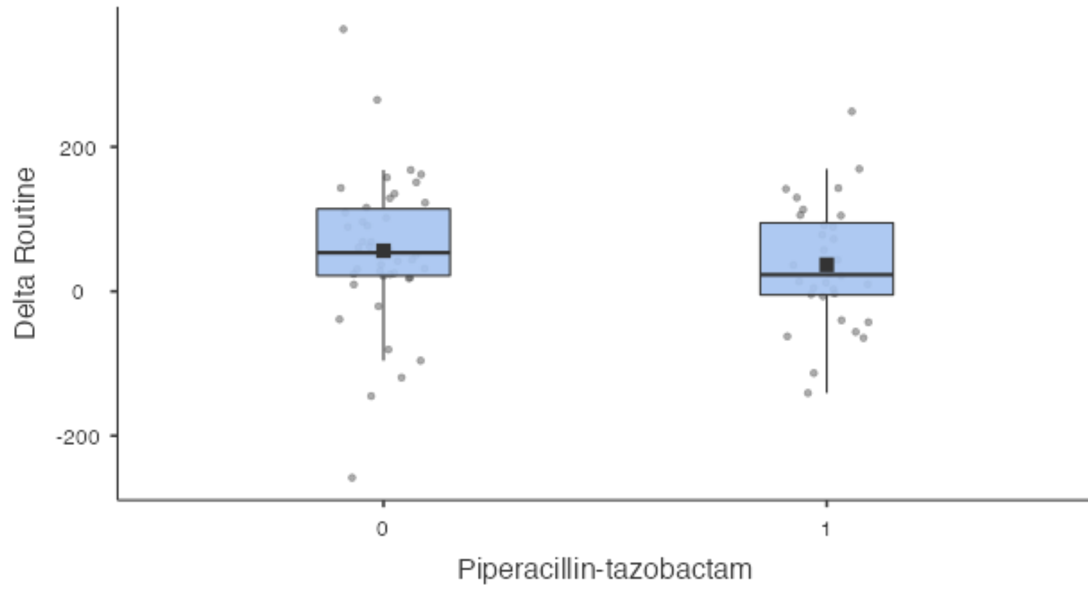


Figure 1.11 Piperacillin-tazobactam Delta Complex I

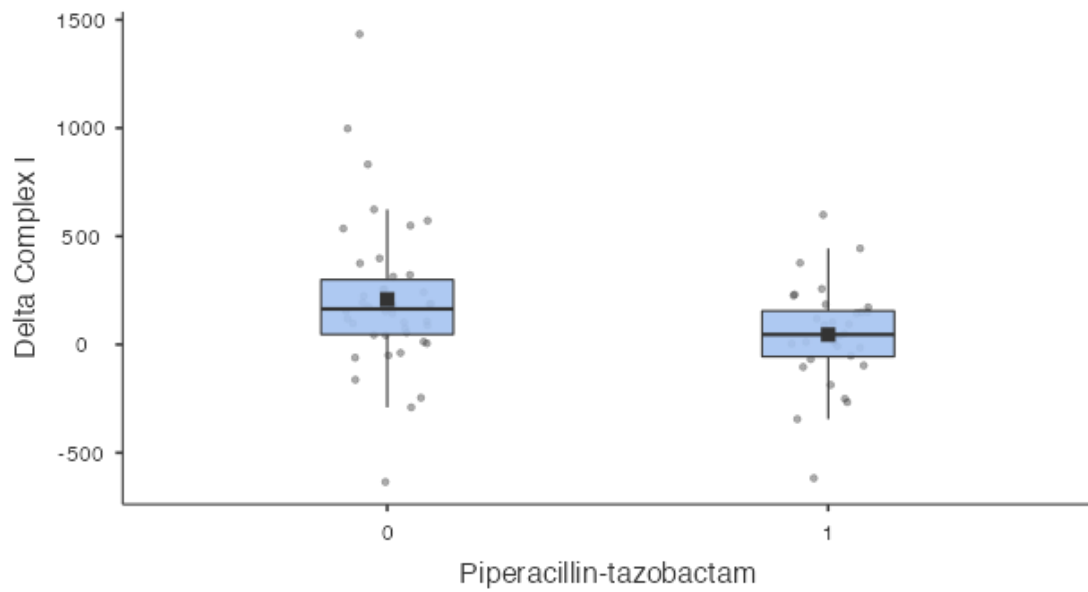
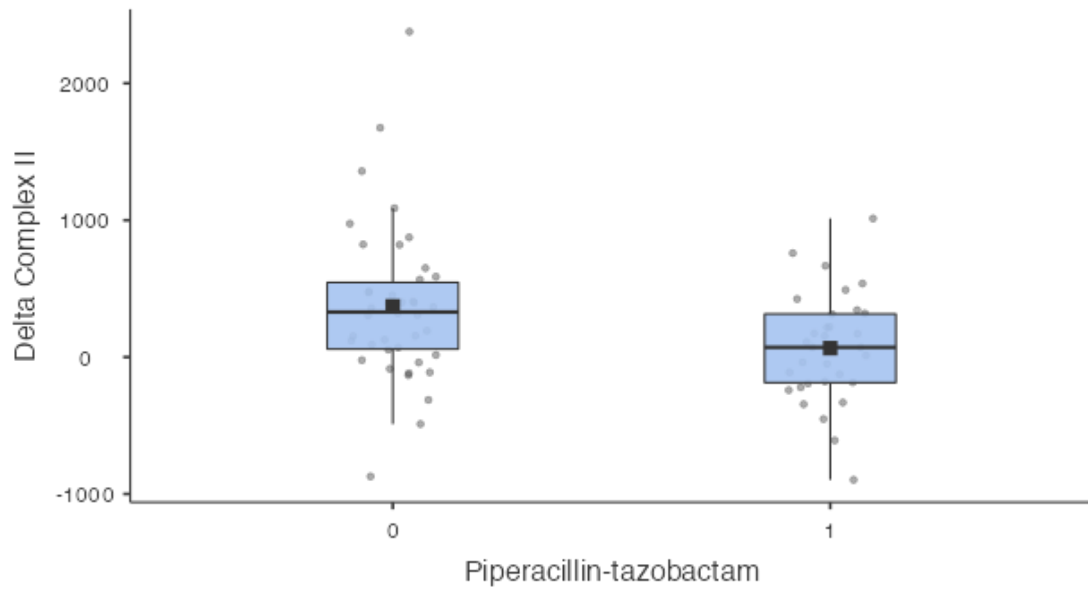


Figure 1.12 Piperacillin-tazobactam Delta Complex II


2000
1000
0
-1000
Delta Complex II
0
1
Piperacillin-tazobactam

Clinical study

Antibiotic therapy does not alter mitochondrial bioenergetics in lymphocytes of patients with septic shock - a prospective cohort study.

Wagner L. Nedel, MD, MSc. (1), Marcelo S. Rodolphi MSc. (2), Nathan R. Strogulski, MSc. (2), Afonso Kopczynski, UG. (2), Thiago H. M. Montes, MD (1), Jose Abruzzi Jr, MD (1), Luis V. Portela, Ph.D. *(2).

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Statistical analysis: association between antibiotic and mitochondrial variables

Meropenem ~ Delta BCE

	Coefficient (95% CI)	*P*
Intercept	*0.526 (0.199 – 0.852)*	*0.002*
Meropenem use (vs non-use)	*-0.082 (-0.209 to 0.044)*	*0.10*
Monotherapy (vs combined therapy)	*-0.082 (-0.209 to 0.044)*	*0.198*
SOFA at sepsis diagnosis	*-0.018 (-0.028 to -0.008)*	*<0.001*
Pulmonary sepsis (vs non-pulmonary sepsis)	*0.001 (-0.063 to 0.066)*	*0.959*
Nosocomial sepsis (vs community-acquired sepsis)	*0.023 (-0.043 to 0.091)*	*0.486*

Breusch Pagan test for homoscedasticity evaluation: p = 0.858
VIF values for multicollinearity: meropenem use (1.25), monotherapy (1.33), SOFA at sepsis diagnosis (1.09), pulmonary sepsis (1.03), nosocomial sepsis (1.07)
P value of F statistic = 0.0123; adjusted R squared = 0.148

Meropenem ~ Delta Routine

	Coefficient (95% CI)	*P*
Intercept	*116.929 (-20.058 to 446.85)*	*0.072*

Meropenem use (vs non-use)	*-94.890 (-300.79 to 111)*	*0.360*
Monotherapy (vs combined therapy)	*-9.826 (-100.68 to 81.03)*	*0.827*
SOFA at sepsis diagnosis	*-2.155 (-9.34 to 5.03)*	*0.551*
Pulmonary sepsis (vs non-pulmonary sepsis)	*21.302 (-25.08 to 67.69)*	*0.362*
Nosocomial sepsis (vs community-acquired sepsis)	*-20.259 (-68.36 to 27.85)*	*0.403*

Adjusted R square -0.014, P value for F statistic 0.551
Breusch Pagan test for collinearity evaluation p = 0.915
VIF values for collinearity: meropenem use (1.25), monotherapy (1.33), SOFA (1.09), pulmonary sepsis (1.03), nosocomial sepsis (1.07)

Meropenem ~ delta Complex I

	Coefficient (95% CI)	*P*
Intercept	*340.2 (-368. 05 to 1048.46)*	*0.34*
Meropenem use (vs non-use)	*181.42 (-443.24 to 806.08)*	*0.564*
Monotherapy (vs combined therapy)	*-117.03 (-392.67 to 158.6)*	*0.399*
SOFA at sepsis diagnosis	*1.688 (-20.11 to 23.48)*	*0.877*
Pulmonary sepsis (vs non-pulmonary sepsis)	*73.783 (-66.95 to 214.51)*	*0.299*
Nosocomial sepsis (vs community-acquired sepsis)	*-120.76 (-266.72 to 25.18)*	*0.103*

Adjust R squared 0.090, p value for F statistic = 0.063
Breush-Pagan test for homoscedasticity p = 0.782
VIF values for collinearity: meropenem use (1.25), monotherapy (1.33), SOFA (1.09), pulmonary sepsis (1.03), nosocomial sepsis (1.07)
Meropenem ~Complex II

	Coefficient (95% CI)	*P*
Intercept	*849.807 -368.05754 1048.46389*	*0.154*
Meropenem use (vs non-use)	*174.927 -443.24472 806.08460*	*0.737*
Monotherapy (vs combined therapy)	*-312.508 -392.67237 158.60864*	*0.177*
SOFA at sepsis diagnosis	*-8.426 -20.11437 23.48949*	*0.644*
Pulmonary sepsis (vs non-pulmonary sepsis)	*157.504 -66.95042 214.51663*	*0.183*
Nosocomial sepsis (vs community-acquired sepsis)	*-113.810 -266.72116 25.18735*	*0.352*

Adjusted R squared 0.10, p value for F statistic 0.041
Breush-Pagan test for homoscedasticity p = 0.765
VIF values for collinearity: meropenem use (1.25), monotherapy (1.33), SOFA (1.09), pulmonary sepsis (1.03), nosocomial sepsis (1.07)

Amoxicillin-clavulanate ~ delta BCE

	Coefficient (95% CI)	*P*
Intercept	*0.213734 0.05590646 0.371561438*	*0.0087*

Amoxicillin-clavulanate use (vs non-use)	*-0.097293 -0.19996670 0.005380506*	*0.0629*
Monotherapy (vs combined therapy)	*-0.056225 -0.14336532 0.116599353*	*0.3867*
SOFA at sepsis diagnosis	*-0.017553 -0.02773427 -0.007371806*	*0.0010*
Pulmonary sepsis (vs non-pulmonary sepsis)	*-0.002572 -0.06807101 0.062926373*	*0.9378*
Nosocomial sepsis (vs community-acquired sepsis)	*0.023068 -0.04579552 0.091930894*	*0.5060*

Adjusted R squared = 0.12, p value for F statistic. = 0.022
Breush-Pagan test for homoscedasticity p = 0.819
VIF values for collinearity: amoxicillin-clavulanate use (vs non-use) 1.085, monotherapy (vs combined therapy) 1.19, SOFA at sepsis diagnosis 1.087, pulmonary sepsis (vs non-pulmonary sepsis) 1.02, nosocomial sepsis (vs community-acquired sepsis) 1.089

Piperacillin-tazobactam vs Delta Complex I

	Coefficient (95% CI)	*P*
Intercept	*0.213734*	*0.0087*
Piperacillin-tazobactam use (vs non-use)	*-0.097293*	*0.0629 .*
Monotherapy (vs combined therapy)	*-0.056225*	*0.3867*
SOFA at sepsis diagnosis	*-0.017553*	*0.0010*
Pulmonary sepsis (vs non-pulmonary sepsis)	*-0.002572*	*0.9378*
Nosocomial sepsis (vs community-acquired sepsis)	*0.023068*	*0.5060*

Adjusted R squared 0.12, p value for F statistic 0.022
Breusch-Pagan test for homoscedasticity p = 0.787
VIF values for collinearity: piperacillin-tazobactam 1.26, monotherapy (vs combined therapy) 1.3, SOFA score at sepsis diagnosis 1.12, pulmonary sepsis (vs non-pulmonary sepsis) 1.02, nosocomial sepsis (vs community-acquired sepsis) 1.08.

Piperacillin-tazobactam vs Complex II

	Coefficient (95% CI)	*P*
Intercept	*574.85 3.736144 1145.96018*	*0.04856*
Piperacillin-tazobactam use (vs non-use)	*- 361.40 -624.637739 - 98.16183*	*0.00786*
Monotherapy (vs combined therapy)	*-57.99 -540.466333 424.48990*	*0.81114*
SOFA at sepsis diagnosis	*-13.78 -51.091951 23.53935*	*0.46376*
Pulmonary sepsis (vs non-pulmonary sepsis)	*199.64 -36.572863 435.84614*	*0.09626 .*
Nosocomial sepsis (vs community-acquired sepsis)	*-30.62 -278.650100 217.40115*	*0.80608*

Adjusted R squared 0.07, p value for F statistic: 0.074
Breusch-Pagan test for homoscedasticity p = 0.579
VIF values for collinearity: piperacillin-tazobactam 1.26, monotherapy (vs combined therapy) 1.3, SOFA score at sepsis diagnosis 1.12, pulmonary sepsis (vs non-pulmonary sepsis) 1.02, nosocomial sepsis (vs community-acquired sepsis) 1.08.

Ampicillin-sulbactan Delta Routine

	Coefficient (95% CI)	P
Intercept	83.003	0.1357
Ampicillin-sulbactam use (vs non-use)	95.204	0.0348
Monotherapy (vs combined therapy)	-23.074	0.6157
SOFA at sepsis diagnosis	-3.143	0.3866
Pulmonary sepsis (vs non-pulmonary sepsis)	28.056	0.2259
Nosocomial sepsis (vs community-acquired sepsis)	-4.543	0.8512

Adjusted R square = 0.015, p value for F statistic = 0.321
Breusch-Pagan test for homoscedasticity evaluation p = 0.874
VIF values for collinearity: ampicillin-sulbactam 1.16, monotherapy (vs combined therapy) 1.21, SOFA at sepsis diagnosis 1.13, pulmonary sepsis (vs non-pulmonary sepsis) 1.04, nosocomial sepsis (vs community-acquired sepsis) 1.11

Ampicillin-sulbactam Delta complex I

	Coefficient (95% CI)	P
Intercept	249.136 -105.92192 604.19397	0.166
Ampicillin-sulbactam use (vs non-use)	135.523 -149.90598 420.95105	0.347
Monotherapy (vs combined therapy)	-114.619 -410.24140 181.00347	0.442
SOFA at sepsis diagnosis	1.504 -21.79609 24.80507	0.898
Pulmonary sepsis (vs non-pulmonary sepsis)	99.296 -49.00009 247.59282	0.186
Nosocomial sepsis (vs community-acquired sepsis)	-90.523 -246.39762 65.35141	0.251

Adjusted R square = -0.0043, p value for F statistic = 0.467
Breusch-Pagan test for homoscedasticity evaluation p = 0.669
VIF values for collinearity: ampicillin-sulbactam 1.16, monotherapy (vs combined therapy) 1.21, SOFA at sepsis diagnosis 1.13, pulmonary sepsis (vs non-pulmonary sepsis) 1.04, nosocomial sepsis (vs community-acquired sepsis) 1.11

Ampicillin-sulbactam Dalta Complex II

	Coefficient (95% CI)	P
Intercept	526.304 -70.64774 1123.25647	0.083
Ampicillin-sulbactam use (vs non-use)	252.446 -227.43942 732.33153	0.297
Monotherapy (vs combined therapy)	-304.532 -801.55612 192.49253	0.226
SOFA at sepsis diagnosis	-8.509 -47.68351 30.66612	0.666
Pulmonary sepsis (vs non-pulmonary sepsis)	200.95 -48.42327 450.23269	0.112
Nosocomial sepsis (vs community-acquired sepsis)	-54.825 -316.89344 207.24422	0.678

Adjusted R square = 0.009, p value for F statistic = 0.509
Breusch-Pagan test for homoscedasticity evaluation p = 0.4299
VIF values for collinearity: ampicillin-sulbactam 1.16, monotherapy (vs combined therapy) 1.21, SOFA at sepsis diagnosis 1.13, pulmonary sepsis (vs non-pulmonary sepsis) 1.04, nosocomial sepsis (vs community-acquired sepsis) 1.11

Capítulo 5: *“Mortality of septic shock patients is associated with impaired mitochondrial oxidative coupling efficiency in lymphocytes: a prospective cohort study.”*

Estudo publicado na revista *Intensive Care Medicine Experimental*. Neste estudo, avaliamos 75 pacientes no primeiro e no terceiro dias do manejo do choque séptico. Quinze pacientes triados faleceram no período entre as coletas e não foram analisados. Avaliamos os marcadores de metabolismo mitocondrial (BCE, respiração basal, respiração de Complexo I e respiração de Complexo II) nos dois intervalos de tempo, com os seus respectivos deltas (dia 3 - dia 1), e investigamos a sua associação com a mortalidade dos pacientes, tanto em curto (internação na UTI e internação hospitalar) quanto em longo prazo (6 meses).

Encontramos o delta BCE, em análise multivariada, como um preditor independente de mortalidade nestes pacientes, tanto em curso quanto em longo prazo. Também estabelecemos um ponto de corte para o delta BCE como preditor de mortalidade a longo prazo nesta população.

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RESEARCH ARTICLES

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Mortality of septic shock patients is associated with impaired mitochondrial oxidative coupling efficiency in lymphocytes: a prospective cohort study

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Abstract

Background: Septic shock is a life-threatening condition that challenges immune cells to reprogram their mitochondrial metabolism towards to increase ATP synthesis for building an appropriate immunity. This could print metabolic signatures in mitochondria whose association with disease progression and clinical outcomes remain elusive.

Method: This is a single-center prospective cohort study performed in the ICU of one tertiary referral hospital in Brazil. Between November 2017 and July 2018, 90 consecutive patients, aged 18 years or older, admitted to the ICU with septic shock were enrolled. Seventy-five patients had Simplified Acute Physiology Score (SAPS 3) assessed at admission, and Sequential Organ Failure Assessment (SOFA) assessed on the first (D1) and third (D3) days after admission. Mitochondrial respiration linked to complexes I, II, V, and biochemical coupling efficiency (BCE) were assessed at D1 and D3 and Δ (D3–D1) in isolated lymphocytes. Clinical and mitochondrial endpoints were used to dichotomize the survival and death outcomes. Our primary outcome was 6-month mortality, and secondary outcomes were ICU and hospital ward mortality.

Results: The mean SAPS 3 and SOFA scores at septic shock diagnosis were 75.8 (± 12.9) and 8 (± 3) points, respectively. The cumulative ICU, hospital ward, and 6-month mortality were 32 (45%), 43 (57%), and 50 (66%), respectively. At the ICU, non-surviving patients presented elevated arterial lactate (2.8 mmol/L, IQR, 2–4), C-reactive protein (220 mg/L, IQR, 119–284), and capillary refill time (5.5 s, IQR, 3–8). Respiratory rates linked to CII at D1 and D3, and ΔCII were decreased in non-surviving patients. Also, the BCE at D1 and D3 and the ΔBCE discriminated patients who would evolve to death in the ICU, hospital ward, and 6 months after admission. After adjusting for possible confounders, the ΔBCE value but not SOFA scores was independently associated with 6-month mortality (RR 0.38, CI 95% 0.18–0.78; $P = 0.009$). At a cut-off of − 0.002, ΔBCE displayed 100% sensitivity and 73% specificity for predicting 6-month mortality

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Conclusions: The ΔBCE signature in lymphocytes provided an earlier recognition of septic shock patients in the ICU at risk of long-term deterioration of health status.

Keywords: Septic shock, Lymphocytes, Mitochondrial signatures, Mortality, Prognostic biomarker

Introduction

Sepsis is a disorder that develops as organ disfunction, and remains one of the leading causes of death globally. Although it may benefit from early diagnostic and prompt treatments to minimize mortality, the availability of diagnostic tools to better predict outcomes and support early clinical decisions are still limited. Septic shock, the most severe end of the spectrum of sepsis, represents a life-threatening condition, which requires vasopressor therapy to treat profound circulatory and metabolic abnormalities [1, 2]. Indeed, during septic shock the oxidative metabolism in several tissues may be limited by the oxygen saturation, delivery, and utilization by mitochondria. Therefore, an early antibiotic therapy, fluid resuscitation and vasopressor drugs alone may not overcome the respiratory deficits at the cellular level. This highlights that inherent or acquired mitochondrial defects associated with septic shock may hinder oxygen consumption coupled with ATP synthesis (OXPHOS), independent of standard therapeutic interventions, thereby contributing to mechanisms underlying organ failure and, eventually, to patient's death [3–5].

Current advances in the understanding of the physiopathology of sepsis have incorporated the concept of an existing crosstalk between immune cells metabolism and immunity [5–9]. The inflammatory course of septic shock challenges the immune cells to enhance their production of antibodies and signaling molecules over time, which relies on an increased energy consumption supported by the mitochondrial metabolic machinery [7, 10]. Based on this, mitochondria of lymphocytes are constantly reprogramming their metabolism, with the increased activity of oxidative complexes reflecting their higher requirements for ATP synthesis by working at high respiratory rates or the downregulation of oxidative complexes reflecting decreased ATP demands or mitochondrial metabolic exhaustion [9]. These unique features suggest that mitochondria might print metabolic signatures in immune cells of septic shock patients whose clinical significance as a biomarker remains elusive [4, 5, 11–14]. Remarkably, the rates of oxygen consumption associated with individual mitochondrial complexes can be profiled in lymphocytes by respirometry protocols allowing to derive a composite of metabolic signatures that may serve as candidate biomarkers of clinical outcomes. Particularly, the BCE is a parameter which scores how efficiently the mitochondrial machinery are synchronized towards ATP-synthesis [15, 16].

Early studies have analyzed OXPHOS metabolism in sepsis, with controversial results mainly regarding the capability of metabolic endpoints to predict organ failure and mortality [4, 5, 11–14, 17]. In general, they focused on sepsis and did not include a large number of patients with septic shock, which reduced the power of clinical associations between organ failure, mortality, the effectiveness of therapies and specific mitochondrial respiratory states. Also, few studies conducted a strategy of two sequential assessments in the ICU setting and following its impact on the short-, mid- and long-term prognosis.

Therefore, there are still gaps to fill regarding mitochondrial metabolic signatures in immune cells as feasible biomarkers for septic shock patients admitted to the ICU. Accordingly, we aimed to investigate mitochondrial bioenergetic signatures in lymphocytes associated with mortality for septic shock.

Material and methods

Participants and study design

This is a prospective cohort study designed to evaluate the mitochondrial metabolism of circulating lymphocytes in septic shock patients admitted to four different ICUs from one tertiary university public hospital in Brazil. This study was approved by the local ethics committee (Plataforma Brazil number 66240017.0.0000.5530). We prospectively enrolled 90 adult patients (> 18 years old, 45% were women) admitted to the ICU due to septic shock between November 2017 and July 2018 as indicated in Fig. 1. Patients were excluded if they presented with a known mitochondrial disease, pregnancy, refusal of the patient or the next of kin to sign the informed consent, patients with imminent death, and patients with withholding or withdrawing treatments. Septic shock was defined as the presence of persistent hypotension with the requirement of vasopressor therapy to maintain a mean arterial pressure of 65 mmHg or greater according to Sepsis 2001 definition [18]. The following demographic and clinical characteristics were prospectively recorded: gender, age, primary site of infection, community-acquired or hospital-acquired infection, comorbidities, noradrenaline maximum dose, lactate level,

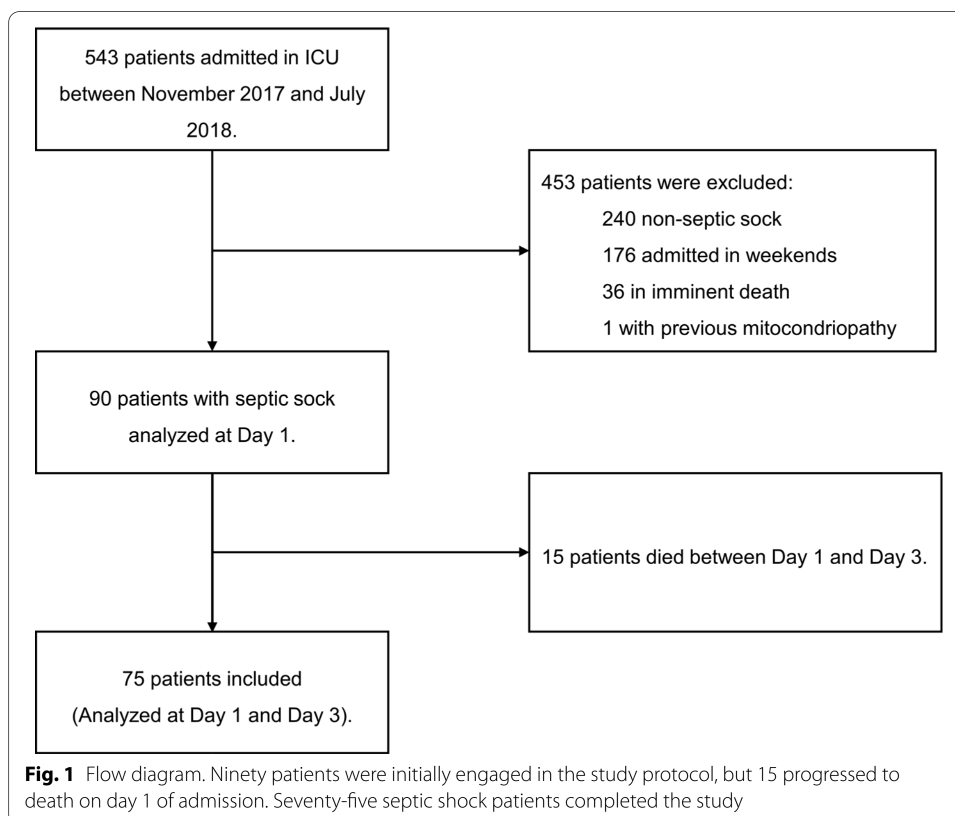

Fig. 1 Flow diagram. Ninety patients were initially engaged in the study protocol, but 15 progressed to death on day 1 of admission. Seventy-five septic shock patients completed the study

urine output, PaO_2:FiO_2 ratio, serum creatinine, total bilirubin, platelets, international normalized rate (INR), Glasgow Coma Scale, capillary refill time, central venous saturation (SvO_2), length of ICU and length of hospital stay. Also, Simplified Acute Physiology Score (SAPS 3) and the Sequential Organ Failure Assessment (SOFA) score were assessed [19, 20]. The SOFA score was used as a tool for defining both, the clinical condition of the individual patient and the response to therapies [21], as well as delta SOFA as an indicator of resolved or unresolved clinical condition [22]. This score is a gold standard for predicting hospital mortality, providing detailed information regarding the number and severity of organic failure in septic patients [23]. The management of septic shock in these patients was carried out as recommended by Surviving Sepsis Campaign [24] guidelines, especially with regard to early antibiotic therapy, culture collection, fluid replacement and preferential use of norepinephrine as first-line vasopressor drug.

Clinical and laboratory endpoints, including mitochondrial respirometry in permeabilized lymphocytes (digitonin 0.005% w/v), were evaluated at two timepoints: the first day of ICU admission (D1) and three days after admission (D3). The pairwise variations between D1 and D3 (Δ) were used to estimate the improvement or the worsening of the clinical, laboratory and mitochondrial endpoints. SOFA and mitochondrial endpoints were used to dichotomize the survival and death outcomes. The primary outcome was 6-month mortality for septic shock, and the secondary outcomes were ICU and hospital mortality.

Isolation of lymphocytes and mitochondrial respirometry

Six milliliters of blood were sampled in EDTA tubes at D1 and D3. Lymphocytes were isolated as described by Pecina et al. [25]. We assayed mitochondrial respiration in permeabilized lymphocytes isolated at D1 and D3. The cells counting showed 98% of the lymphocytes in the fraction.

The high-resolution respirometry measurements were performed within 3 h of blood sampling using oxygraphy (Oxygraph-2 k; Oroboros Instruments, Innsbruck, Austria) at 37 °C. Oxygen concentration (micromolar) and oxygen flux (expressed in pmol O_2.s^{-1}.mg of protein) were recorded with DatLab software 6.0. The basal oxygen consumption (oxygen flow per volume or per mass) was established without metabolic substrates. Subsequently, stepwise additions of pyruvate, malate and glutamate (10, 10 and 20 mM, respectively), followed by 2.5 mM ADP, 10 mM succinate, a second 2.5 mM ADP, and finally sodium azide plus antimycin A were performed. This protocol assesses the respiration linked to Complex I (CI), Complex II (CII) and maximal oxygen flow rate consumption coupled to ATP production (P), and nonmitochondrial oxygen consumption [26].

The BCE (also known as ≈ P control factor) was measured to estimate the mitochondrial oxygen flow coupled to ATP production. The BCE is calculated by the P–L/P fluxes; ($J \approx P = (P - L)/P$), as described previously [15, 16]. A representative image displaying the sequential addition of the substrates to the permeabilized lymphocytes, and the derived respiratory parameters obtained from a control subject, are shown in Additional file 1: Figure S1. The researchers involved in the mitochondrial analysis were blinded to the clinical outcomes, and the researchers involved in the clinical data collection were blinded to the mitochondrial outcomes. All chemicals used for high-resolution

respirometry analysis were analytical grade, purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

Descriptive statistics included frequencies and percentages for the categorical variables and means, and standard deviation, confidence intervals, medians, and interquartile ranges for continuous variables. Student's *t*-test or the Mann–Whitney *U* test were used to compare continuous variables according to normality, assessed by Shapiro–Wilk test. Chi-square test or Fisher's exact test were used to analyze categorical variables. Association between two continuous variables was measured with Spearman correlation coefficient. To access the impact of BCE improvement at D3 (ΔBCE > 0) on the outcomes, Poisson regression was performed with 6-month mortality as the dependent variable, and hematological neoplasia, chronic kidney disease, lactate at septic shock diagnosis, SOFA improvement at D3 (ΔSOFA < 0) and the SAPS 3 score at ICU admission as independent variables in the model. These variables were selected for the model because they presented a p value of less than 0.20 in the univariate analysis. In this statistical model Δbasal, ΔCI and ΔCII, were not included since they are partial components of the BCE. ΔSOFA score was dichotomized between improvement (ΔSOFA < 0) and worsening (ΔSOFA > 0) on D3, rather than using its value in D1 and D3. The variation between BCE in D3 and D1 (ΔBCE) was analyzed as a continuous variable, in an exploratory analysis. A receiver-operating characteristic (ROC) curve was performed to evaluate the accuracy of ΔBCE to predict 6-month mortality. The optimal cut-off point was mathematically defined using the Youden index [27–31].

Statistical tests were two-tailed with significance defined as a *P* value less than 0.05. All *P* values were two-tailed. We used SPSS version 21.0 (SPSS, Chicago, IL, USA) and R 4.1.0 (R Foundation for Statistical Computing) for all analyses.

Results

Clinical and epidemiological characteristics

A total of 90 patients were included in D1, but 15 deceased before D3. A total of 75 patients were included in the D1 and D3 analysis (Fig. 1). The mean age of the patients was 64.8 (± 15.9) years, 42 (56% were male), and 54 (61%) with clinical ICU admission. The most frequent foci of sepsis were the lung (*n* = 41; 46%) and abdominal (*n* = 36; 40%) infections. The mean SAPS 3 score was 75.8 (± 12.9) points and the mean SOFA score at sepsis diagnosis was 8.5 (± 3.2) points (Table 1). At study period, 32% of the patients were submitted to hemodialysis and 85% to mechanical ventilation. Median arterial lactate at septic shock diagnosis was 2.0 mmol/Dl (IQR 1.2–3.0), and the median volume of fluid resuscitation in septic shock was 44 ml/kg (IQR 30–65). Fluid balance in the first day of ICU admission was 2874 ml (IQR 1635–5493). Thirty-seven patients (49%) received hydrocortisone in the septic shock management. Maximum norepinephrine dose in the first day was 0.24 μg/kg/min (IQR 0.07–0.6124 μg/kg/min).

Table 1 Patient demographic and clinical variables at admission

Overall population	Distribution and values
Patients, *N*	90
Sex	
Male	50 (55%)
Female	40 (45%)
Age (years)	64.8 (15.9)
Clinical variables	
Surgical patients	35 (38%)
Sepsis foci	
Abdomen	36 (40%)
Cutaneous	2 (2%)
Blood	7 (8%)
Urinary	4 (4%)
Lung	41 (46%)
Capillary refill time (s)	4 (2–6)
Arterial lactate (mmol/L)	2 (1.2–3)
SvO2 (%)	70 (10.2)
$PaCO_2$—$PvCO_2$ (mmHg)	6.5 (3.9–10.9)
SOFA	8 (3.1)
SAPS 3	75.8 (12.8)
Clinical comorbidities	
Solid cancer	7 (8%)
Blood cancer	7 (8%)
HIV	2 (2%)
Cirrhosis	7 (8%)
Chronic kidney disease	10 (11%)
Diabetes	25 (27%)
Hypertension	32 (35%)

Data are *N* (%), mean (SD), or median (IQR). *SOFA* sequential organ failure assessment, *SAPS 3* simplified acute physiology score 3

Impact of bioenergetic and clinical variables on mortality

Overall, mitochondrial metabolic endpoints were associated with short- and long-term mortality. The variations (D3–D1) in the mitochondrial respiration sustained by the presence of endogenous intracellular substrates (ΔBasal), and the stimulated respiration linked to complex II (ΔCII) were significantly decreased in non-surviving patients in all timepoints evaluated (Fig. 2A and C, respectively). Further, stimulated respiration linked to complex I (ΔCI) was decreased in 6 months non-surviving patients relative to surviving (Fig. 2B). Individual values for mitochondrial oxygen consumption at D1 and D3 at basal, linked to CI and CII as well as ΔCII were associated with ICU, hospital and 6-month mortality after sepsis diagnosis (Additional file 3: Table S1). Patients that deceased in the ICU presented elevated arterial lactate, C-reactive protein, capillary refill time and SOFA scores at D1. These patients also had increased SOFA score at and D3 compared with those that survived (Table 2). BCE at D1 and D3 were reduced in non-surviving patients. The number of patients that improved SOFA ($\Delta SOFA < 0$; 11 [34%] versus 38 [88%], $P < 0.001$) and BCE ($\Delta BCE > 0$; 2 [6.3%] versus 33 [77%], $P < 0.001$) was lower in nonsurvivors (Table 2). Patients that deceased during hospital admission had increased arterial lactate, SOFA at D1 and D3, capillary refill time and SAPS 3. BCE

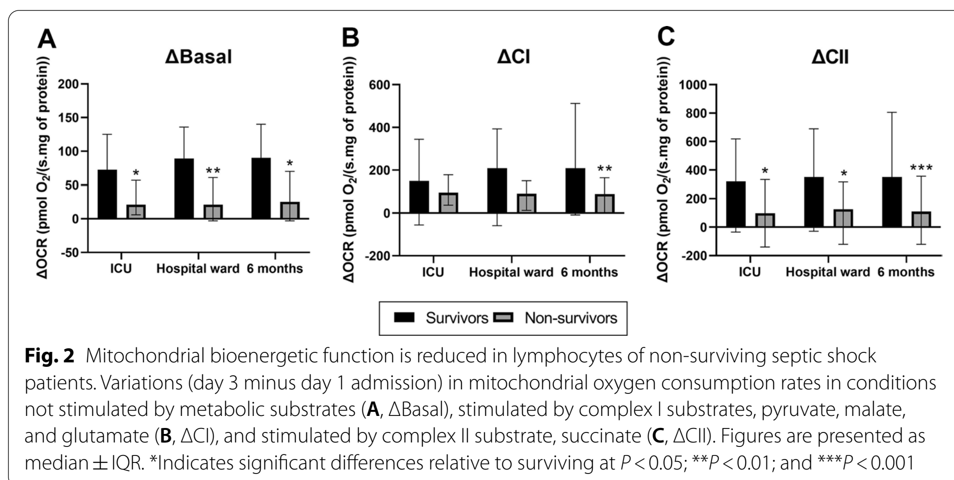

Fig. 2 Mitochondrial bioenergetic function is reduced in lymphocytes of non-surviving septic shock patients. Variations (day 3 minus day 1 admission) in mitochondrial oxygen consumption rates in conditions not stimulated by metabolic substrates (**A**, ΔBasal), stimulated by complex I substrates, pyruvate, malate, and glutamate (**B**, ΔCI), and stimulated by complex II substrate, succinate (**C**, ΔCII). Figures are presented as median ± IQR. *Indicates significant differences relative to surviving at $P < 0.05$; **$P < 0.01$; and ***$P < 0.001$

at D1 and D3 are lower in non-surviving than surviving patients. Survivors had greater incidence of improvement in SOFA (ΔSOFA < 0), as well as in BCE (ΔBCE > 0) than non-survivors (Table 2).

Patients who died within 6 months after septic shock presented elevated arterial lactate levels at ICU admission and increased SAPS 3, and had the highest SOFA scores at D1 and D3. Survivors also had greater incidence of improvement in SOFA (ΔSOFA < 0) and in BCE (ΔBCE > 0) than nonsurvivors (Table 2). Remarkably, after adjusting for possible confounders, the 6-month mortality rate was lower in patients who had an improved BCE at D3 (RR 0.38, CI 95% 0.18–0.78; $P < 0.001$), while classic laboratory and clinical variables such as lactate, CRP, SOFA and SAPS 3 were not associated with survival or death (Table 3).

BCE at D1 was inversely associated with SOFA (Spearman = − 0.28; $P = 0.005$), but had no associations with arterial lactate (Spearman = − 0.2; $P = 0.057$) or with plasma C-reactive protein levels (CRP) (Spearman = 0.007; $P = 0.947$). Six patients (8%) developed a need for a tracheostomy during ICU admission. These patients worsened in the ΔBCE when compared to those who did not need a tracheostomy (− 0.085 ± 0.1 versus 0.048 ± 0.14, respectively; $P = 0.11$), but without reaching statistical significance. Thirty-six patients developed a new infection after the initial injury, and these patients had no statistically significant difference in their median ΔBCE when compared to those who did not developed reinfection (− 0.008 ± 0.23 versus 0.089 ± 0.19, respectively, $P = 0.29$). Patients who needed MV had lower values of ΔBCE when compared with those who did not need it, reaching statistical significance: 0.006 ± 0.14 versus 0.1 ± 0.13, respectively; $P = 0.05$. Patients who needed hemodialysis had worsening in the ΔBCE, while those who did not need hemodialysis had an improvement in their values (− 0.029 ± 0.1 versus 0.07 ± 0.2; $P < 0.01$). Patients who improved ΔBCE (ΔBCE > 0) had a shorter ICU length of stay when compared with those who impaired ΔBCE (ΔBCE < 0): 6 ± 4 days versus 9.5 ± 14 days, respectively ($P = 0.02$). The AUROC for ΔBCE as prognostic biomarker of 6-month mortality was 0.90 (95% CI 0.83–0.91, $P < 0.01$) (see Additional file 2: Figure S2). A cut-off point of − 0.002 in ΔBCE had a 100% of sensitivity and 73% of specificity for the outcome (Youden's J Index = 0.73). Distinct cut-off values of ΔBCE relative to sensitivity and specificity, and Youden's J Index are shown in Table 4.

Table 2 Univariate analysis of variables associated with ICU, hospital and 6-month mortality

Variables	ICU		P	Hospital		P	Six months		P
	Survivors $n=43$	Non-survivors $n=32$		Survivors $n=32$	Non-survivors $n=43$		Survivors $n=25$	Non-survivors $n=50$	
Age	64.14 (18.1)	67.16 (14.7)	0.443	61.97 (16.5)	68.00 (16.6)	0.122	62.1 (14.0)	67.1 (17.8)	0.228
Clinical admission at ICU	25 (58%)	21 (66%)	0.51	21 (66%)	25 (58%)	0.51	16 (64%)	30 (60%)	0.737
Sex (male)	26 (60%)	16 (50%)	0.366	18 (57%)	24 (56%)	0.97	15 (60%)	27 (54%)	0.622
Pulmonary sepsis	21 (51%)	12 (38%)	0.24	18 (56%)	16 (37%)	0.101	13 (52%)	21 (42%)	0.412
Solid neoplasia	13 (30%)	4 (11%)	0.07	9 (28%)	8 (18%)	0.33	6 (24%)	11 (22%)	0.845
Hematological neoplasia	2 (4.6%)	3 (9.4%)	0.417	1 (3.1%)	4 (9.3%)	0.386	0 (0%)	5 (10%)	0.162
Cirrhosis	1 (2.3%)	4 (13%)	0.081	1 (3.1%)	4 (9.3%)	0.386	1 (4.0%)	4 (8.0%)	0.66
COPD	7 (16%)	4 (13%)	0.647	5 (16%)	6 (14%)	0.84	3 (12.0%)	8 (16%)	0.742
CKD	5 (12%)	2 (6.2%)	0.428	5 (16%)	2 (4.6%)	0.11	4 (16%)	3 (6%)	0.161
Chronic hypertension	11 (26%)	13 (41%)	0.167	9 (28%)	15 (35%)	0.534	7 (28%)	17 (34%)	0.6
Diabetes	11 (26%)	10 (31%)	0.589	10 (32%)	11 (26%)	0.589	9 (36%)	12 (24%)	0.275
SvO_2 (%)	68.01 (6.1)	71.36 (11.6)	0.215	67.71 (9.0)	70.70 (11.1)	0.266	68.18 (9.1)	70.06 (10.9)	0.504
Lactate D1 (mmol/L)	1.4 (1.1–2.1)	2.8 (2.0–4.0)	0.002	1.4 (1.1–1.9	2.55 (1.7–3.6	0.015	1.4 (0.9–1.6)	2.2 (1.6–3.5)	0.041
SOFA D1	7.63 (3.0)	1.0 (2.4)	< 0.0001	7.25 (2.55)	9.67 (2.95)	< 0.0001	7.40 (2.6)	9.26 (3.0)	0.011
SOFA D3	3 (2–5)	12.5 (7–19.5)	< 0.0001	3 (1.7–5)	9 (4–16.5)	< 0.0001	3 (1–4)	7.5 (3.25–15.5)	< 0.0001
ΔSOFA < 0	38 (88%)	11 (34%)	< 0.0001	28 (88%)	21 (49%)	< 0.0001	22 (88%)	27 (54%)	0.004
SAPS 3	73.98 (13.2)	78.60 (12.1)	0.1	72.16 (13.1)	78.77 (12.0)	0.026	70.68 (11.2)	78.58 (12.9)	0.011
CRP (mg/L)	153 (69.7–216)	220 (119–284)	0.02	153 (69–223)	196 (119–269)	0.062	153(72.6–209)	188 (98–258)	0.076
CRT D1 (s)	4 (2–4)	5.5 (3–8)	0.04	3.5 (2–4)	4 (3–7)	0.046	4 (3–4)	4 (2.25–7)	0.319
BCE D1	0.354 (0.1)	0.243 (0.1)	< 0.0001	0.378 (0.1)	0.254 (0.1)	< 0.0001	0.380 (0.1)	0.270 (0.1)	< 0.0001
BCE D3	0.456 (0.18)	0.210 (0.1)	< 0.0001	0.535 (0.1)	0.215 (0.1)	< 0.0001	0.543 (0.1)	0.267 (0.2)	< 0.0001
ΔBCE > 0	33 (77%)	2 (6.3%)	< 0.0001	31 (97%)	4 (9.3%)	< 0.0001	24 (96%)	11 (22%)	< 0.0001

Δ indicates the pairwise variation between values obtained at day 3 and day 1, mathematically represented as day 3–day 1 for the indicated variable. Data are mean (SD), median (IQR), or *n* (%).*CRP* C-reactive protein, *SOFA* sequential organ failure assessment, *ICU* intensive care unit, *SAPS 3* simplified acute physiology score 3, *BCE* biochemical coupling efficiency, *COPD* chronic obstructive pulmonary disease, *CKD* chronic kidney disease, *CRT* cardiac resynchronization therapy

Table 3 Multivariate analysis of variables associated with 6-month mortality

Variables	Multivariate analysis	P
Hematological neoplasia	RR 1.1 (0.37–3.26)	0.86
CKD	RR 0.75 (0.22–2.48)	0.64
Lactate—D1	RR 1.01 (0.88–1.14)	0.88
ΔSOFA < 0	RR 1.01 (0.49–2.08)	0.96
SAPS 3	RR 1.01 (0.98–1.03)	0.321
ΔBCE > 0	RR 0.38 (0.18–0.78)	0.009

Δ mathematically indicates the pairwise variation between values obtained at day 3 (D3) minus day 1 (D1), for the indicated variable. *SOFA* sequential organ failure assessment, *SAPS 3* simplified acute physiology score 3, *BCE* biochemical coupling efficiency, *CKD* chronic kidney disease, *RR* risk ratio

Table 4 Sensitivity, specificity and Youden's J index for measurements of ΔBCE

ΔBCE	Sensitivity	Specificity	Youden's J Index
− 0.220	100%	0%	0
− 0.111	98%	31%	0.31
− 0.002	100%	73%	0.73
0.11	70%	88%	0.58
0.219	33%	97%	0.31
0.354	3%	100%	0.03

Discussion

Our findings highlight a high short- and long-term mortality among septic shock patients. Mitochondrial metabolism in the lymphocytes of these patients provided a particular signature for the deterioration of clinical outcomes. Among the clinical and mitochondrial metabolic endpoints, ΔBCE was independently associated with long-term mortality in our cohort.

Although SOFA is a well-validated instrument to estimate mortality risk, the addition of new and more sensitive laboratory variables to this score may benefit its predictive validity. Indeed, in the "The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3)" [1], Kramer suggests the incorporation of new candidate biochemical markers to improve the SOFA score sensitivity. Such an approach is pertinent because it may provide earlier information to support clinical decisions, and thus, patients are more likely to achieve better clinical outcomes that reflect their prognosis beyond the ICU [1, 32]. In this context, our approach does not rule out the clinical relevance of SOFA or other clinical scores, but proposes "a look" to a promising complementary prognostic tool. Physiologically, the mitochondria are capable of adjusting their metabolic energy demands and signals to protect cells from insults [7, 9, 14]. This has been clinically investigated in immune cells of patients with sepsis and in septic shock, to estimate the influence of bioenergetics on inflammatory responsiveness, severity of symptoms and the deterioration of health status due to organ failure [9, 33, 34]. Such relevance is conceptually well-illustrated by proposals that activated immune cells can undergo complex plasticity phenomena, which is sustained by mitochondria, "the powerhouses of immunity" [6]. Accordingly, it was demonstrated in blood mononuclear cells that mitochondrial dysfunction and damage progress over time, along with the severity of symptoms, albeit strong conclusions were limited by the small number of patients [6, 9, 34].

Here, we showed an improved metabolism from D1 to D3 (ΔBCE) admission to the ICU which paralleled survival, implying that increments in mitochondrial bioenergetic function may reflect metabolic reprograming leading to improvements of the general health status in septic shock patients. Another study found that an early normalization of mitochondrial biogenesis genes expression profile accelerated sepsis recovery and shortened the time in the ICU [34], thus reinforcing the suggestion of clinical and functional relevance in approaching mitochondrial phenotypes. Although we do not address gene-profile, our study conciliates the mechanistic concept that an increased biogenesis governs plasticity mechanisms directed to renew damaged mitochondria, and as

consequence, increase energetic efficiency. On the other hand, some intrinsic features support the investigation of mitochondrial bioenergetic effectors in immune cells as biomarkers of outcomes. For instance, dysregulation of immune cells, including lymphocytes, are key components of inflammatory overreaction and organ failure, considered as hallmarks of septic shock; and as resident cells in the circulation, they survey whole body microenvironments, are cells easy to sample and to perform metabolic profiling in routine intensive care practice. The association between improved mitochondrial function with clinically relevant outcomes, such as length of stay in the ICU, the need for mechanical ventilation and the need for hemodialysis, reinforces this hypothesis.

The other clinically relevant spectrum of the sepsis, the compensatory anti-inflammatory response syndrome (CARS), is an event that occurs in a subgroup of septic patients that develop profound acquired immunosuppression. Such condition may cause difficulties to efficiently eradicate the primary infection despite patients are submitted to standardized treatment protocols [35]. Hence, it is important to take into consideration that sepsis leads to a complex immune response that evolves over time, with the simultaneous implication of both, proinflammatory and anti-inflammatory mechanisms for the clinical outcomes [36]. The intensity and duration of the mentioned exacerbated anti-inflammatory phase seem to be closely related with the development of nosocomial infections. Also, an immunometabolic reprogramming in response to sepsis may rely on the interplay between the energy demands and capacity of lymphocyte mitochondria to produce ATP [37]. However, whether or not a metabolic reprograming, here mirrored by BCE, exerts a significant influence in the state of immunosuppression remains to be explored [38]. It is known that the lymphocytes challenged by sepsis displayed an exhausted-like phenotype characterized by decreased functions including the capacity of proliferation, cytokine production and increased coinhibitory receptor expression, culminating in apoptotic cell death [35]. Considering that it is not completely known the exact extrinsic and intrinsic factors that determine the severity of lymphocytes exhaustion, and likely immunosuppression, we tend to suggest decreased mitochondrial bioenergetics as an active player. Accordingly, in physiological conditions lymphocytes are highly oxidative cells which implies in immunological processes highly dependent of an appropriate ATP support. Studies have shown a bidirectional interaction between mitochondria and molecular inflammatory effectors that drive cell responses to an infection. This crosstalk between immunogenic effectors and mitochondrial activity involves toll-like receptors, damage-associated molecular patterns (DAMP's), pathogen-associated molecular patterns (PAMP's); and NLRP3 inflammasome activation, all driving mitochondrial mechanisms such as increased reactive oxygen species production, and lymphocyte cytokines release [39, 40].

To expand the exploratory analysis of our study, we found that patients who did not develop a secondary infection, who theoretically would be less susceptible to immunosuppression, had greater increases in BCE when compared to those who had a secondary infection. However, this result was not statistically significant and the sample size was not designed for this analysis, being any conclusions based on these observations merely conjectures that deserve attention in future studies.

Moreover, in cases of unresolved inflammatory-related immunosuppression activity, this condition may progress to a persistent inflammation–immunosuppression

and catabolism syndrome (PICS), that often evolve to a chronic critical illness state [41]. A hallmark of this condition is the need for a tracheostomy during the course of septic shock, that is associated with impairment in adaptive immunity related to lymphocytes [42]. In our study, patients who needed a tracheostomy had a worsening in BCE (data no shown), when compared with those who did not. Despite this result did not reach statistical significance, we believe that the small number of tracheostomized patients in our sample could explain this fact.

Our study has the merits of enrolling a large number of septic shock patients in which the respiratory abnormalities in specific mitochondrial complexes represent immunometabolic targets associated with disease pathogenesis. Indeed, no previous study has integrated the activity of mitochondrial complexes CI, CII, and CV, and BCE in lymphocytes using two time-points, which allowed us to estimate metabolic reprogramming relative to the disease progression. The resulting improvement or worsening of the mitochondrial respiratory endpoints in the ICU highlight potential immunometabolic mechanistic targets, and also provide perspective regarding how metabolic reprogramming influences clinical outcomes. This approach outlined BCE as a candidate prognostic biomarker to be incorporated into clinical practice to support therapeutic management and decision-making. Although we provided promising insights regarding mitochondria bioenergetics as biomarker, this approach still requires advances to reach a clinical applicability. The development of ready-to-use assays may increase the potentialities of clinical utilization, nonetheless before pursuing this goal, it is imperative to identify specific mitochondrial functions implicated in the response to the disease.

Our study has a number of limitations that should be further explored. It is a unicentric study, which may limit the external validity of the main findings. Second, it is a population that presented with a high risk of mortality independent of the septic shock. Even so, the mortality reported here reflects the severity measured by the SAPS 3 score, and it is similar to previous studies performed in Brazil and worldwide [43–45]. Third, we evaluated lymphocytes as a whole, albeit different types of lymphocytes may have specific metabolic phenotypes; however, such specificity did not interfere with the predictive nature of ΔBCE. Indeed, the sensitivity and specificity ΔBCE assessed with a ROC curve further confirmed its potential prognostic value for 6- month mortality. As a future perspective, it is important to investigate associations with other clinically relevant outcomes, such as clinical frailty, quality of life, long-term functional status, secondary immunosuppression, incidence of PICS and chronic organ failure after septic shock.

Conclusions

Our work highlights that impaired mitochondrial capacity to improve BCE provided an earlier recognition of septic shock patients in the ICU at risk of deterioration of health status. On the contrary, improved BCE capacity was associated with favorable clinical outcomes. Further research is needed to outline BCE as a prognostic biomarker to be incorporated into intensive care setting.

Abbreviations
ICU: Intensive care unit; SOFA: Sequential organ failure assessment; SAPS 3: Simplified acute physiology score 3; D1: Day first admission ICU; D3: Day 3 admission ICU; Δ: Values obtained at day 3 minus day 1; CI: Mitochondrial complex I; CII: Mitochondrial complex II; BCE: Biochemical coupling efficiency; IQR: Interquartile range.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40635-021-00404-9.

Additional file 1: Figure S1. Respirometry curve profile. Representative image of a respirometry assay performed in lymphocytes from a control subject.

Additional file 2: Figure S2. Receiver-operating characteristic curve. The BCE at a cut-off value of − 0.002 (indicated by the black dot) represents 100% sensitivity and 73 % specificity for predicting 6-month mortality. The obtained area under the curve was 0.90.

Additional file 3: Table S1. Mitochondrial respiratory rates in lymphocytes of survival and nonsurvival patients.

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Authors' contributions
Dr. Portela, Dr. Nedel and Dr. Galina had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. WLN, TLH, LVP, and AG built the study concept. THMM, and JAJ were involved in the patients' clinical management, and collected the samples and data. LVP, M De B, and WLN designed the study and investigation. MSR, AK, and NRS prepared samples, performed the mitochondrial assays, analyzed the data and created the figures. WLN and LVP wrote the first draft of the manuscript, and all authors reviewed and edited the final manuscript. LVP acquired funding. All authors read and approved the final manuscript.

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Availability of data and materials
Individual participant data that underlie the results reported in this article, after deidentification, will be made available to researchers who provide a methodologically sound proposal for analyses to achieve aims in the approved proposal, immediately following article publication. Please address the proposals directly to roskaportela@gmail.com.

Declarations

Ethics approval and consent to participate
Ethics approval was obtained from the institutional ethics committees from the Hospital Conçeição, Porto Alegre, RS, Brazil. The study was registered at Plataforma Brazil (66240017.0.0000.5530). Consent waiver was granted by the ethics committee for the collection and use of participants' personal information.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Singer M, Deutschman CS, Seymour CW et al (2016) The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA 315:801. https://doi.org/10.1001/jama.2016.0287
2. Dellinger RP, Levy MM, Rhodes A et al (2013) Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 39:165–228. https://doi.org/10.1007/s00134-012-2769-8
3. Singer M (2017) Critical illness and flat batteries. Crit Care 21:309. https://doi.org/10.1186/s13054-017-1913-9
4. Carré JE, Orban J-C, Re L et al (2010) Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med 182:745–751. https://doi.org/10.1164/rccm.201003-0326OC
5. Brealey D, Brand M, Hargreaves I et al (2002) Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 360:219–223. https://doi.org/10.1016/S0140-6736(02)09459-X
6. Mills EL, Kelly B, O'Neill LAJ (2017) Mitochondria are the powerhouses of immunity. Nat Immunol 18:488–498. https://doi.org/10.1038/ni.3704
7. Cheng S-C, Scicluna BP, Arts RJW et al (2016) Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. Nat Immunol 17:406–413. https://doi.org/10.1038/ni.3398
8. Pearce EJ, Pearce EL (2018) Driving immunity: all roads lead to metabolism. Nat Rev Immunol 18:81–82. https://doi.org/10.1038/nri.2017.139
9. Kramer PA, Ravi S, Chacko B et al (2014) A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: Implications for their use as bioenergetic biomarkers. Redox Biol 2:206–210
10. Gotts JE, Matthay MA (2016) Sepsis: pathophysiology and clinical management. BMJ 353:i1585. https://doi.org/10.1136/bmj.i1585
11. Puskarich MA, Kline JA, Watts JA et al (2016) Early alterations in platelet mitochondrial function are associated with survival and organ failure in patients with septic shock. J Crit Care 31:63–67. https://doi.org/10.1016/j.jcrc.2015.10.005
12. Sjovall F, Morota S, Hansson MJ et al (2010) Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Crit Care 14:R214. https://doi.org/10.1186/cc9337
13. Sjövall F, Morota S, Persson J et al (2013) Patients with sepsis exhibit increased mitochondrial respiratory capacity in peripheral blood immune cells. Crit Care 17:R152. https://doi.org/10.1186/cc12831
14. Jang DH, Greenwood JC, Spyres MB, Eckmann DM (2017) Measurement of mitochondrial respiration and motility in acute care. J Intensive Care Med 32:86–94. https://doi.org/10.1177/0885066616658449
15. Makrecka-Kuka M, Krumschnabel G, Gnaiger E (2015) High-resolution respirometry for simultaneous measurement of oxygen and hydrogen peroxide fluxes in permeabilized cells, tissue homogenate and isolated mitochondria. Biomolecules 5:1319–1338. https://doi.org/10.3390/biom5031319
16. Gnaiger E (2014) Mitochondrial Pathways and Respiratory Control An Introduction to OXPHOS Analysis, 4th Ed. OROBOROS MiPNet Publications, Innsbruck
17. Japiassú AM, Santiago APSA, D'Avila JDCP et al (2011) Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5′-triphosphate synthase activity. Crit Care Med 39:1056–1063. https://doi.org/10.1097/CCM.0b013e31820eda5c
18. Levy MM, Fink MP, Marshall JC et al (2003) 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med 29:530–538. https://doi.org/10.1007/s00134-003-1662-x
19. Vincent JL, Moreno R, Takala J et al (1996) The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on sepsis-related problems of the European society of intensive care medicine. Intensive Care Med 22:707–710
20. Moreno RP, Metnitz PGH, Almeida E et al (2005) SAPS 3—from evaluation of the patient to evaluation of the intensive care unit. Part 2: development of a prognostic model for hospital mortality at ICU admission. Intensive Care Med 31:1345–1355. https://doi.org/10.1007/s00134-005-2763-5
21. Lambden S, Laterre PF, Levy MM, Francois B (2019) The SOFA score—development, utility and challenges of accurate assessment in clinical trials. Crit Care 23:374. https://doi.org/10.1186/s13054-019-2663-7
22. de Grooth H-J, Geenen IL, Girbes AR et al (2017) SOFA and mortality endpoints in randomized controlled trials: a systematic review and meta-regression analysis. Crit Care 21:38. https://doi.org/10.1186/s13054-017-1609-1
23. Karakike E, Kyriazopoulou E, Tsangaris I et al (2019) The early change of SOFA score as a prognostic marker of 28-day sepsis mortality: analysis through a derivation and a validation cohort. Crit Care 23:387. https://doi.org/10.1186/s13054-019-2665-5
24. Dellinger RP, Levy MM, Rhodes A et al (2013) Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 41:580–637. https://doi.org/10.1097/CCM.0b013e31827e83af
25. Pecina P, Houšťková H, Mráček T et al (2014) Noninvasive diagnostics of mitochondrial disorders in isolated lymphocytes with high resolution respirometry. BBA Clin 2:62–71. https://doi.org/10.1016/j.bbacli.2014.09.003
26. Carteri RB, Kopczynski A, Rodolphi MS et al (2019) Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. J Neurotrauma 36:2246–2259. https://doi.org/10.1089/neu.2018.6266
27. López-Ratón M, Rodríguez-Álvarez MX, Suárez CC, Sampedro FG (2014) OptimalCutpoints: an R package for selecting optimal cutpoints in diagnostic tests. J Stat Softw 61:1–36. https://doi.org/10.18637/jss.v061.i08
28. Aoki K, Misumi J, Kimura T et al (1997) Evaluation of cutoff levels for screening of gastric cancer using serum pepsinogens and distributions of levels of serum pepsinogen I, II and of PG I / PG II ratios in a gastric cancer case-control study. J Epidemiol 7:143–151. https://doi.org/10.2188/jea.7.143
29. Greiner M, Pfeiffer D, Smith R (2000) Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. Prev Vet Med 45:23–41. https://doi.org/10.1016/S0167-5877(00)00115-X
30. Youden WJ (1950) Index for rating diagnostic tests. Cancer 3:32–35. https://doi.org/10.1002/1097-0142(1950)3:1%3c32::AID-CNCR2820030106%3e3.0.CO;2-3
31. Shapiro DE (1999) The interpretation of diagnostic tests. Stat Methods Med Res 8:113–134. https://doi.org/10.1191/096228099666928387

32. Shankar-Hari M, Phillips GS, Levy ML et al (2016) Developing a new definition and assessing new clinical criteria for septic shock. JAMA 315:775. https://doi.org/10.1001/jama.2016.0289
33. Ganeshan K, Nikkanen J, Man K et al (2019) Energetic trade-offs and hypometabolic states promote disease tolerance. Cell 177:399-413.e12. https://doi.org/10.1016/j.cell.2019.01.050
34. Kraft BD, Chen L, Suliman HB et al (2019) Peripheral blood mononuclear cells demonstrate mitochondrial damage clearance during sepsis. Crit Care Med 47:651–658. https://doi.org/10.1097/CCM.0000000000003681
35. Venet F, Rimmelé T, Monneret G (2018) Management of sepsis-induced immunosuppression. Crit Care Clin 34:97–106
36. Hotchkiss RS, Monneret G, Payen D (2013) Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol 13:862–874
37. Pool R, Gomez H, Kellum JA (2018) Mechanisms of organ dysfunction in sepsis. Crit Care Clin 34:63–80. https://doi.org/10.1016/j.ccc.2017.08.003
38. Venet F, Davin F, Guignant C et al (2010) Early assessment of leukocyte alterations at diagnosis of septic shock. Shock 34:358–363. https://doi.org/10.1097/SHK.0b013e3181dc0977
39. Missiroli S, Genovese I, Perrone M et al (2020) The role of mitochondria in inflammation: from cancer to neurodegenerative disorders. J Clin Med 9:740. https://doi.org/10.3390/jcm9030740
40. Cloonan SM, Choi AMK (2013) Mitochondria: sensors and mediators of innate immune receptor signaling. Curr Opin Microbiol 16:327–338. https://doi.org/10.1016/j.mib.2013.05.005
41. Mira JC, Gentile LF, Mathias BJ et al (2017) Sepsis pathophysiology, chronic critical illness, and persistent inflammation-immunosuppression and catabolism syndrome. Crit Care Med 45:253–262. https://doi.org/10.1097/CCM.0000000000002074
42. Mira JC, Brakenridge SC, Moldawer LL, Moore FA (2017) Persistent inflammation, immunosuppression and catabolism syndrome. Crit Care Clin 33:245–258. https://doi.org/10.1016/j.ccc.2016.12.001
43. Buchman TG, Simpson SQ, Sciarretta KL et al (2020) Sepsis among medicare beneficiaries. Crit Care Med 48:276–288. https://doi.org/10.1097/CCM.0000000000004224
44. Machado FR, Cavalcanti AB, Bozza FA et al (2017) The epidemiology of sepsis in Brazilian intensive care units (the Sepsis PREvalence Assessment Database, SPREAD): an observational study. Lancet Infect Dis 17:1180–1189. https://doi.org/10.1016/S1473-3099(17)30322-5
45. Conde KAP, Silva E, Silva CO et al (2013) Differences in sepsis treatment and outcomes between public and private hospitals in Brazil: a multicenter observational study. PLoS ONE 8:e64790. https://doi.org/10.1371/journal.pone.0064790

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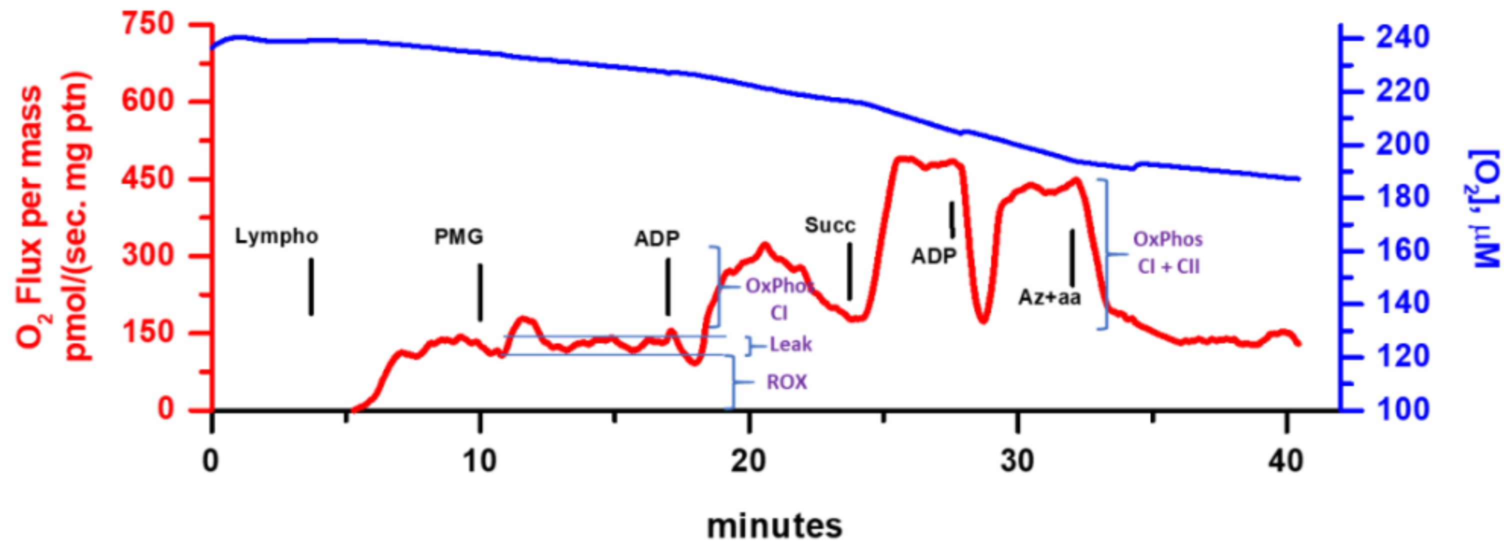
Nedel et al. Supplemental material Representative image of a respirometry assay performed in lymphocytes from a healthy subject.



Supplemental Figure 1. Representative image of a respirometry assay performed in lymphocytes from a healthy subject. After basal respiration was reached, pyruvate, malate and glutamate (10, 10 and 20 mM, respectively) were added, leading to corresponding leak respiration (L), followed by stepwise additions of 2.5 mM ADP, 10 mM succinate, and a second 2.5 mM ADP, allowing for the steady-state rate of oxygen

consumption in each step (oxygen flow per volume or per mass). This substrate titration protocol enables derivation of the Complex I (CI), Complex II (CII) and maximal oxygen flow rate consumption coupled to ATP production (State III). Sodium azide plus antimycin A was added to inhibit mitochondrial complex IV, allowing for the estimation of non-OXPHOS oxygen flow rate (ROX) and these fluxes were discounted to obtain an authentic flow rate from all of the abovementioned states, and tissue-mass specific oxygen fluxes were compared in different substrates and coupling states, and inhibitors (SUIT protocol) (ROX). Lymphocytes were permeabilized with 0.005%, digitonin w/v.

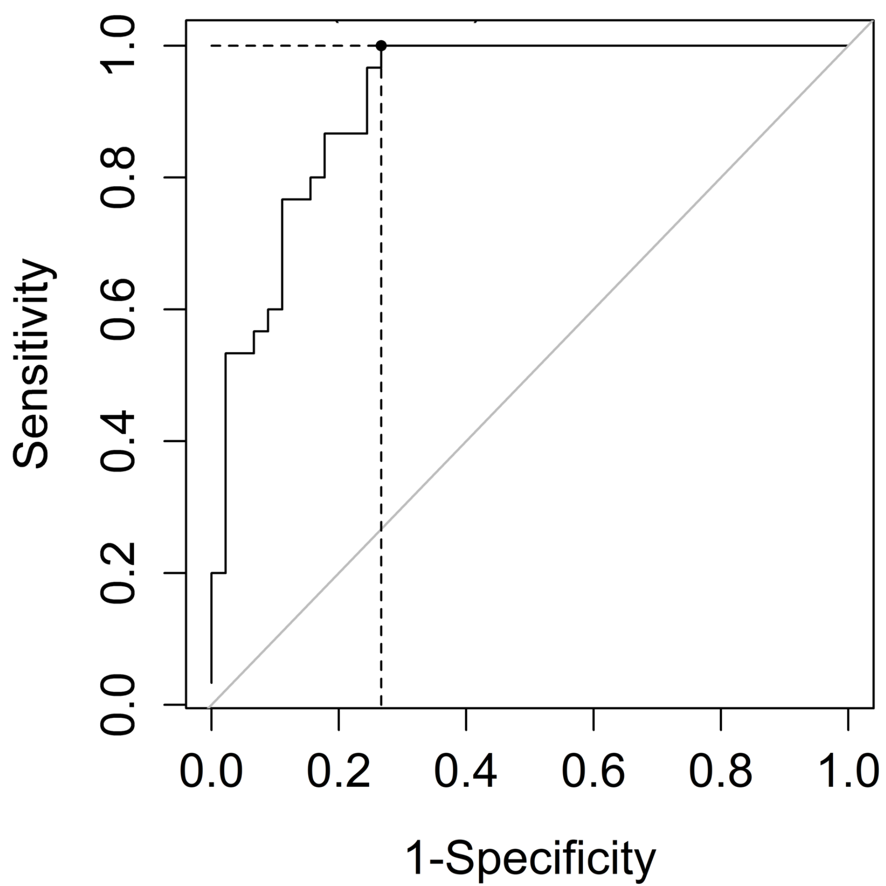

ROC Curve. Criterion: Youden
Sensitivity
1-Specificity
0.0
0.2
0.4
0.6
0.8
1.0

Table 2. Mitochondrial respiratory rates in lymphocytes of survival and non-survival patients.

Variables	ICU			Hospital ward			Six-months		
	Survivors n=43	Non-survivors n=32	*P*	Survivors n=32	Non-survivors n=43	*P*	Survivors n=25	Non-survivors n=50	*P*
Basal D1	192.3 (99.7)	128 (43.5)	<.001	208 (104.7)	131.1 (46.7)	<.001	214 (116.9)	128.7 (43.9)	.001
Basal D3	250.4 (88.7)	157.7 (51.9)	<.001	276 (79)	159 (53.6)	<.001	257.7 (51.2)	149.3 (68.8)	<.001
ΔBasal	72.3 (3 - 125)	21.2 (6 - 57)	.04	89.1 (24 - 136)	21.2 (-3 - 61)	.01	90 (23 - 140)	25.3 (-3 - 70)	.03
CI D1	445.1 (248.3)	197.1 (64.3)	<.001	503.7 (245.6)	210.7 (86.1)	<.001	525.2 (231.2)	235.3 (142.5)	<.001
CI D3	606.8 (308.5)	295.4 (92.6)	<.001	697.6 (285.9)	294.8 (100.5)	<.001	756.2 (386.5)	296.5 (146.9)	<.001
Δ CI	149.5 (-56 - 344)	95.7 (37 - 178)	.4	209.2 (-58.7 - 393)	90.9 (12.8 – 150)	.07	209.2 (-9 - 512)	88.9 (-1 - 164)	.01
CII D1	698.2 (348.2)	498.4 (182.2)	.01	760.2 (355.5)	494.1 (191.8)	<.001	715.9 (309.4)	555.2 (297.6)	.02
CII D3	1026.9 (481.3)	577.8 (193.2)	<.001	1138.8 (466.7)	596.4 (230.1)	<.001	1174.5 (513.7)	671.7 (296.2)	<.001
ΔCII	319 (-35 - 618)	98.5 (-140 to 333)	.03	350.6 (-29 - 689)	126.4 (-121 - 316)	.02	350.6 (32 – 805)	110.9 (-121 - 356)	<.001

Basal reflects respiratory rates without addition of exogenous substrates; D1: day first admission ICU; D3: day 3 admission ICU; CI: mitochondrial complex I; CII: mitochondrial complex II. Δ mathematically indicates the pairwise variation between values obtained at day 3 minus day 1, for the indicated variable. Respiratory rates of CI, CII, and the equivalent Δ values are expressed as pmol $O_2 \cdot s^{-1} \cdot$mg of protein. Data are mean (SD) or median (IQR). Lymphocytes were permeabilized with digitonin 0.005% (w/v).

Capítulo 6: *“Sepsis-induced mitochondrial dysfunction: A narrative review.”*

Artigo aceito na revista *World Journal of Critical Care Medicine*, em fase de edição e publicação. Inserimos neste capítulo a versão do manuscrito corrigida pelo Corpo Editorial da revista e a carta de aceite da publicação.

Trata-se de um artigo de revisão não-sistemática, abordando os aspectos fisiopatológicos da disfunção mitocondrial na sepse e suas diferentes formas de mensuração e interpretação. Realizamos uma análise dos estudos que abordaram este tópico, e apresentamos, também, uma avaliação propositiva para estudos posteriores nesta área.

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Dear Dr. Nedel,

We are pleased to inform you that your paper has successfully passed our very rigorous review process and has been accepted for publication in the *World Journal of Critical Care Medicine*. We are happy to tell you that this paper will be given priority for publishing, with all publishing fees waived.

1 BASIC INFORMATION OF THE MANUSCRIPT

Manuscript NO: 83391

Manuscript Type: Minireviews

Title: Sepsis-induced mitochondrial dysfunction: A narrative review

Authors: Wagner Nedel, Caroline Deutschendorf and Luis Valmor Cruz Portela

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2 COMPANY EDITOR-IN-CHIEF'S COMMENTS

All documents of the manuscript have met the publication requirements of the World Journal of Critical Care Medicine, and the manuscript is given final acceptance for publication. We will arrange the manuscript production. As the manuscript has been

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Sepsis-induced mitochondrial dysfunction: A narrative review

Nedel W *et al*. Sepsis-induced mitochondrial dysfunction

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Abstract

Sepsis represents a deranged and exaggerated systemic inflammatory response to infection, and is associated with vascular and metabolic abnormalities that trigger systemic organic dysfunction. Mitochondrial function has been shown to be severely impaired during the early phase of critical illness, with a reduction in biogenesis, increased generation of reactive oxygen species and a decrease in adenosine triphosphate synthesis of up to 50%. Mitochondrial dysfunction can be assessed using mitochondrial DNA concentration and respirometry assays, particularly in peripheral mononuclear cells. Isolation of monocytes and lymphocytes seems to be the most promising strategy for measuring mitochondrial activity in clinical settings because of the ease of collection, sample processing, and clinical relevance of the association between metabolic alterations and deficient immune responses in mononuclear cells. Studies have reported alterations in these variables in patients with sepsis compared with healthy controls and non-septic patients. However, few studies have explored the association between mitochondrial dysfunction in immune mononuclear cells and unfavorable clinical outcomes. An improvement in mitochondrial parameters in sepsis could theoretically serve as a biomarker of clinical recovery and response to oxygen and vasopressor therapies as well as reveal unexplored pathophysiological mechanistic targets. These features highlight the need for further studies on mitochondrial metabolism in immune cells as a feasible tool to evaluate patients in intensive care settings. The evaluation of mitochondrial metabolism is a promising tool for the evaluation and management of critically ill patients, especially those with sepsis. In this article, we explore the pathophysiological aspects, main methods of measurement, and the main studies in this field.

Key Words: Sepsis; Mitochondria; Mitochondrial dysfunction; Oxidative phosphorylation; Inflammation; Respirometry

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Core Tip: The Evaluation of mitochondrial metabolism is a promising tool for the evaluation and management of critically ill patients, particularly those with sepsis. In this article, we explore the pathophysiological aspects, main methods of measurement, and main studies in this field.

INTRODUCTION

Sepsis is a major health problem worldwide and can be characterized by a dysregulated host response to infection[1-3]. Particularly, an imbalanced systemic inflammatory response to infection contribute to the clinical progress to multi-organ dysfunction[2,4]. There is a great effort in the recognition and prompt treatment of sepsis, especially with regard to early antibiotic administration, hemodynamic resuscitation, and evacuation of septic foci[1]. The ultimate cause of death in patients with sepsis, however, remains unclear. Infections usually are eradicated through an intense, but localized, inflammatory response. Nonetheless, fatal infections are characterized by an inability to resolve the inflammatory response because cytokines released into the systemic circulation, activates inflammatory cells in remote locations[5]. This response leads to organ injury and dysfunction, which cannot be completely explained by a decrease in tissue oxygenation due to an impairment of blood flow[2,4,5] but likely to problems in oxygen utilization.

Mitochondria is a highly specialized organelle that is considered the power plant of cells thus supporting energy in the form of adenosine triphosphate (ATP) according to functional demands[6]. In the blood, except for erythrocytes, all cell types possess mitochondria which imply they are dependent on oxidative metabolism. Remarkably, mitochondria tightly connect ATP biosynthesis with oxygen consumption, and even minor changes in mitochondrial functional integrity affect many aspects of cellular homeostasis. Although, the main proposed function of mitochondria is to generate ATP *via* oxidative phosphorylation (OXPHOS) of adenosine diphosphate (ADP), additional functions include generation and detoxification of reactive oxygen species (ROS), calcium homeostasis, involvement in apoptosis, synthesis and catabolism of metabolites, and transport of organelles within the cell[6,7]. Any alteration in one of these processes can be defined as mitochondrial dysfunction[8]. Actually, we know that impaired mitochondrial

metabolism is an important mechanism that leads to organ dysfunction[2,5]. In acute diseases, such as sepsis, the measurement of mitochondrial metabolism, through cellular respiration, has the potential to identify those patients at risk of progressing in their organ failures. In addition, it can potentially help in monitoring the therapeutic response of these patients[6,9].

Considering that mitochondria interact with various other pathways involved in inflammation, Ca^{2+} balance, redox signaling, and apoptosis, it can be assumed that mitochondria are fundamental in cell survival and death[10]. Mitochondrial metabolism has been shown to be severely impaired during the early phase of an acute disease, with a reduction in biogenesis, increased generation of ROS, and a decrease in ATP synthesis[11,12]. The presence of an impaired mitochondrial metabolism is associated with the presence of a multiple organ failure syndrome, highlighting mitochondrial components as potential targets for therapeutic strategies[4,11,13]. There has been an increased interest in this field, with new studies exploring mitochondrial impairment and organ dysfunction and their relationship with prognosis in sepsis[14-17].

The main objective of this review is analyze the current state of the art in this field, explore potential methods for assessing mitochondrial metabolism in intensive care settings, and explore future perspectives on mitochondrial metabolism as a biomarker of clinical outcomes in sepsis.

PHYSIOPATHOLOGY

ATP is produced by mitochondria through OXPHOS by F1Fo-ATP synthase. ATP is generated to a greater extent by the oxidation of metabolic substrates in the tricarboxylic acid cycle leading to reduction of the electron acceptors NAD^+ and FAD to nicotinamide adenine dinucleotide (NADH) and 1,5-dihydroflavin adenine dinucleotide (FADH2)[18]. Both NADH and FADH2 are subsequently oxidized in the electron transport system of mitochondria. The electron transport chain is composed by enzyme complexes I to IV and the transporters ubiquinone and cytochrome c. With the electrons movement across the respiratory chain, protons are pumped across the inner mitochondrial membrane, generating an electrical potential. This proton-motive force provides the energy for F1Fo-

ATP synthase, as known as Complex V, to phosphorylate ADP to ATP (Figure 1). This mechanism is favored by the electrochemical gradient produced by the proton motive force. Oxygen is the terminal electron acceptor of the chain in Complex IV and is reduced to water[19]. An incomplete reduction of oxygen increases superoxide radical production in Complex III and at Complex I. As part of this process, ROS are generated as by-products of the incomplete four-electron reduction of molecular oxygen to water[20,21].

Under physiological conditions, mitochondria consume approximately 90% of the cellular O_2; however, 1%-4% of the respiratory chain reactions lead to a leak of electrons that directly react with O_2 to form O_2(*-), which can oxidize lipids, DNA, or proteins[22]. To avoid self-damage, mitochondria have intrinsic defense mechanisms that protect against ROS-induced damage through a large array of antioxidants[12]. In sepsis, not only ROS are generated, but also reactive nitrogen species (RNS), nitric oxide, and peroxynitrite[7,20,21]. Enzymatic defenses, such as the superoxide dismutase 2 (SOD2), convert O_2(*-) into hydrogen peroxide (H_2O_2), which can then be detoxified to water by catalase or selenium-containing glutathione peroxidase[23,24]. SOD2 expression is higher in survivors of critical illness[22]. Glutathione and Coenzyme Q10 (CoQ10) also have an important mitochondrial antioxidant function. CoQ10 Levels are lower in sepsis, suggesting a potential role in mitochondrial dysfunction[25].

Mitochondria are also essential for other cellular functions, such as calcium homeostasis, apoptosis, autophagy, and cellular signaling[6,9,12]. Mitochondrial DNA (mtDNA) is susceptible to mutations and deletions due to ROS increased levels and requires a set of self-regulated repair mechanisms[6]. mtDNA damage resulting from this phenomenon is associated with reduced mitochondrial respiratory capacity, which are potentially irreversible, depending on the intensity of oxidant "attack"[23]. Mitochondrial function is maintained by an equilibrium between fission, fusion, biogenesis, and autophagy[26]. In the case of an impairment in mitochondrial metabolism, various signaling routes allow an interaction between the mitochondria and nucleus, triggering mitochondrial biogenesis[10]. Defective mitochondria can become toxic by excessive ROS production, that can lead to apoptosis. Autophagy compensates for nutrient depletion or copes with cellular stress by recycling cellular components, to produce amino acids and

fatty acids that can be metabolized and used in OXPHOS[27]. Despite being important for critical illness recovery, excessive induction of autophagy can trigger apoptosis[28]. ROS is one of some signaling pathways that regulate autophagy, and, since mitochondria are the primary source of ROS, mitochondria themselves play a key role in regulating autophagy[10]. Disturbances in mitochondrial function leading to impaired ATP biosynthesis, increased ROS production, and oxidative stress are associated with skeletal muscle damage, which correlates with septic shock severity and is associated with impaired clinical outcomes[18].

Many inflammatory mediators are linked to the altered mitochondrial metabolism. Tumoral necrosis factor-alpha (TNF-α) is a major interleukin that participates in the host response to sepsis and is capable of causing mitochondrial impairment[29]. TNF-α binds to several TNF receptors, ultimately promoting the intracellular release of ceramides and production of ROS, which may lead to mitochondrial dysfunction. TNF receptor activation promotes pro-inflammatory responses in polymorphonuclear leukocytes and monocytes, that induce ROS formation, leading to mtDNA damage and inhibition of mitochondrial metabolism in these cells[30]. Inhibition of mitochondrial complexes causes deviation of electrons, also producing even more ROS. These phenomena can lead to an imbalance between ROS production and mitochondrial antioxidant capacity, through manganese superoxide dismutase and glutathione reductase. This imbalance can trigger mitochondrial uncoupling related to the opening of mitochondrial permeability transition pores (PTP)[31]. The resulting mitochondrial permeability transition leads to dissolution of the electrochemical gradient required to form ATP, and these dysfunctional mitochondria are targeted for removal *via* autophagy. The induction of mitochondrial PTP win this context can promote apoptosis. Therefore, the pro-inflammatory activity in sepsis, especially in its initial phase, can lead to both a reduction in mitochondrial mass and a decrease in its function.

MECHANISMS OF MITOCHONDRIAL DYSFUNCTION

Hypoxia has been assumed to be the main causative agent of mitochondrial dysfunction[32]. However, it was later shown that tissue oxygen levels are normal or even

elevated in sepsis[33]. Instead of a lower availability of oxygen, there is a lower use of it in septic patients[24]. Thus, OXPHOS dysfunction explain the inability to maintain ATP levels in this context, leading to increased glycolysis and increased lactate levels[6]. This imbalance between ROS production and antioxidant capacity leads to an oxidative stress that damage the electron transport chain and mtDNA, creating a vicious circle of mitochondrial damage and ROS production[20]. ROS and calcium overload cause an increased membrane permeability, and mitochondrial products such as mtDNA leak into the circulation, acting as danger-associated molecular patterns and contributing to multiorganic failure[13] in a vicious cycle (Figure 2). This cascade can trigger cell apoptosis[7], a component of tissue damage and organ failure.

Changes in mitochondrial form and function in critical illnesses suggest that mitochondria try to rescue mechanisms and adapt to harmful environments. Mitochondrial fission and fusion are upregulated during critical illness, although it appears to be insufficient for restoring mitochondrial function[34]. Mitochondrial biogenesis was observed in skeletal muscle taken on days 1 to 2 in intensive care unit (ICU) survivors, but not in non-survivors[35]. During extreme conditions, such as refractory shock, mitochondrial damage is disseminated, causing the induction of mitochondrial PTP in many mitochondria and a great decrease in ATP production[36]. In contrast, a reduction in mitochondrial density was observed after the onset of sepsis, suggesting that although upregulated, biogenesis may be insufficient to maintain homeostasis[10].

Mitochondrial metabolism and inflammatory activity in sepsis-what is the relationship?

The hallmark of sepsis inflammatory response is an imbalance between a systemic inflammatory response and compensatory anti-inflammatory response[37,38]. The imbalance of pro- and anti-inflammatory responses often results in immunoparalysis among critically ill patients, making them more vulnerable to additional infections, and is linked to higher mortality rates[39]. Actually, the cause of immune dysfunction is matter of debate[38], and immune response is dependent of metabolic pathways[40], that is known

as "immunometabolism". Mitochondria are a hub of the immune system, playing a crucial role in regulating the function of immune cells and shaping and modulating the response of the immune system to infection[7]. Evidence suggests that leukocytes from critically ill patients display mitochondrial dysfunction, which is thought to be the root cause of immunoparalysis and could be responsible for the onset of organ dysfunction[41,42]. Moreover, the recovery of mitochondrial function is associated with improved recovery in critically ill patients[14]. These changes are mostly detected in lymphocytes, monocytes, and macrophages[43].

Lymphocytes respond to cytokine stimuli induced by monocyte-macrophages, dendritic cells, and neutrophils, which are responsible for the innate immune response. Activated phagocytic cells, such as monocytes and macrophages, release the proinflammatory cytokines interleukin-1 (IL-1) and IL-6[44]. This phenomenon has been associated with energy deprivation[45]. Lymphocytes also attenuate the potentially harmful effects of the proinflammatory response, and this modulation of the immune response has a major impact on prognosis in septic patients[46]. On the other hand, IL-10 plays a major role in modulating the immune system by inhibiting monocyte-macrophage activation and suppressing the production of TNF-α, IL-1, and interferon-gamma (IFN-γ) from lymphocytes acting at the level of accessory cells[47]. In addition to cytokines, Krebs cycle intermediates, such as citrate, succinate, and itaconate, can activate pro-inflammatory gene expression[48]. Metabolites from the Krebs cycle impact the reprogramming of macrophages from the M1 phenotype (pro-inflammatory) to the M2 phenotype (anti-inflammatory). M1 macrophages have impaired OXPHOS, and M2 macrophages have an intact Krebs cycle, with OXPHOS as the main source of ATP generation[43].

Two major pathways are implicated in this interaction between mitochondrial function and inflammatory responses. The mammalian target of rapamycin (mTOR) pathway plays a pivotal role in metabolic regulation by modulating glycolysis. Furthermore, metabolic reprogramming and a transition to glycolysis for energy production in $CD4^+$ and $CD8^+$ T cells are also induced by the activation of mTOR and OXPHOS[30]. The nuclear factor kappabeta (NF-κβ) is a stress-induced pathway (*i.e.*, tissue damage,

cytokine, and PAMPs release) that promotes the expression of target genes involved in the immune response. Upon activation of the NF-κβ pathway and subsequent induction of cytokine expression, macrophages undergo differentiation into either M1 or M2 subtypes, contingent on the local cytokine milieu present at the site of infection. IFN-γ typically drives the differentiation of M1 macrophages, which in turn produce pro-inflammatory cytokines. Conversely, M2 cells refer to macrophages exposed to immune complexes, IL-4, IL-13, and IL-10[30].

MEASUREMENT OF MITOCHONDRIAL DYSFUNCTION

Regrettably, it is currently impractical to perform a thorough and accurate real-time analysis of the modified cells and tissues within malfunctioning organs of living human patients in a hospital setting. Therefore, mechanisms must be inferred from tissue specimens obtained from nonvital organs (*e.g.*, blood, skeletal muscle) or from postmortem examinations. In postmortem studies, septic patients exhibit mild to moderate mitochondrial swelling and autophagocytosis, with minimal cell death or indications of permanent damage, such as tissue fibrosis[49].

Mitochondrial biogenesis

The process of mitochondrial biogenesis encompasses the synthesis of mitochondrial proteins encoded by nuclear DNA, which are subsequently imported and integrated into the mitochondria. Additionally, biogenesis can also occur through mitochondrial DNA, which encodes 13 proteins primarily located within the OXPHOS pathway. In this way, biogenesis serves to replace damaged proteins and enhances the ability to generate energy if energy demand increases over time[4]. Increased mitochondrial biogenesis is detected in postmortem studies in critically ill patients, with increased expression of transcription factors[34]. A decrease in mitochondrial content has also been reported in the muscles of critically ill patients with sepsis-induced multiple organ failure[50]. Taken together, these data suggest that biogenesis activation may have a role in the recovery phase of critical illness[22], time when there was also an increase in RNA expression, participating in the restorative process[16]. These data point to compromised

mitochondrial biogenesis in critically ill patients; and an activation of the biogenesis pathway may represent a key prognostic factor in critically ill patients, associated with recovering of the initial injury[22].

From this point of view, it seems reasonable to imagine that the dynamic processes of mitochondrial fusion and fission must change during acute inflammatory injury observed in sepsis. This activity can be measured by assessing the concentrations of mitochondrial fusion proteins (mitofusins 1 and 2, optic atrophy 1 protein) and mitochondrial fission proteins (dynamin-related protein 1 and fission 1 protein)[22]. Currently, adequate characterization in relation to the pro-fusion or mitochondrial fission profile in sepsis is merely speculative, lacking clinical studies that may show some signs regarding its *in vivo* effects.

Mitochondrial DNA

Mitochondria experience various morphological changes during fusion and fission events, which help to sustain a healthy mitochondrial population by facilitating mitochondrial DNA exchange, preserving mitochondrial DNA integrity, and regulating the size, quantity, distribution, and upkeep of OXPHOS capacity[22]. These morphological changes also play a crucial role in cell division and proliferation, as well as in the selective elimination of damaged or excess mitochondria through a process referred to as mitophagy[4]. Proteins that facilitate fusion events (such as mitofusin-2) and fission events (such as dynamin related protein-1) have been linked to changes in mitochondrial membrane potentials and diminished oxygen consumption[51]. Fission and fusion processes become more prevalent under stressful conditions and play a crucial role in eliminating damaged mitochondria and enhancing repair mechanisms. Currently, there is insufficient data regarding these mitochondrial dynamics in septic patients, and the data may vary based on the tissue type. From a hypothetical perspective, the balance of mitochondrial dynamics in septic patients may shift in favor of mitochondrial fusion, which could represent a cellular response aimed at improving mitochondrial function and decreasing oxidative stress[52].

However, mitochondrial DNA levels in the serum should be interpreted as a potential damage-associated molecular pattern, propagating an inflammatory response through interactions with the immune system[12,53]. Thus, mtDNA damage can lead to a pathological cycle, resulting in metabolic dysfunction, especially in white blood cells[54]. A reduction in mtDNA content in the peripheral blood, observed in the acute phase of sepsis, could be due to an increased concentration of neutrophils in the peripheral blood[55]. Therefore, it remains uncertain whether there is an interaction between DNA concentration and changes in mitochondrial function, especially in immune cells. Mitochondria contain their own DNA, and the depletion of mtDNA in an injury process may theoretically cause a respiratory chain defect and compromise ATP synthesis[23,55].

Qualitative measurements of mitochondrial metabolism

In addition to changes in mitochondrial mass, the quality of mitochondrial function and a shift towards glycolytic pathways are regulated by several hormones, enzymes, and regulatory pathways within cells. Reactive derivatives of nitric oxide and superoxide anion (such as peroxynitrite), which cause oxidative stress, promote glycolysis by activating the rate-limiting step of the pentose pathway, glucose-6-phosphate dehydrogenase. The pentose pathway results in the formation of NADPH relative to NADH. While NADH is the substrate for mitochondrial OXPHOS of high-energy phosphates, NADPH is crucial for the formation and repair of proteins, DNA, and lipids. Thus, by diverting glycolytic intermediates from the Krebs cycle to suppress aerobic mitochondrial respiration, cells and tissues transition to a state of decreased oxygen consumption and ATP production. This phenomenon is commonly referred to as the "Warburg effect", particularly in the context of cancer[41]. In this context, cells are less dependent on oxidative metabolism, thus reducing oxidative stress and promoting the formation of reducing equivalents (*e.g.*, lactic acid and NADPH) that induce cell repair[56]. The Warburg effect and related mediators, such as HIF-1α, are induced under conditions that model sepsis, confer cytoprotection to vital organs, and inhibit inflammation under conditions of acute cell stress[57]. However, the HIF-1α activation in immune cells could perpetuate the activation of the pro-inflammatory pathway[58].

The proton pumps of the electron transport chain, in conjunction with F1Fo-ATP synthase, establish a proton gradient across the inner membrane, generating both an electrochemical potential (proton motive force, pmf, in mV) and a flux of protons (proton current in nmol of protons/min). The mitochondrial membrane potential is a critical component of healthy mitochondrial metabolism and contributes to determining the pmf[8].

Reductions in both the expression and activity of complexes I, II, III, and IV have been reported in critically ill patients[14,22]. However, it is still doubtful whether these alterations are determinants of the patient's prognosis, or if they are just epiphenomena in the acute context of critical illness. However, they are useful and commonly used measurements to assess mitochondrial activity[35,59-61], especially when normalized by enzymatic activity, protein, or DNA concentration.

Respirometry

Measurement of mitochondrial respiration is a cost-effective and time-efficient method compared to traditional methods of assessing mitochondrial function in biopsies, making it readily available for use. Advanced instruments equipped with highly sensitive micro-cathode oxygen electrodes enable high-resolution measurements of mitochondrial respiration and can be utilized in acute care settings. Mitochondrial respiration can be quantified by performing substrate-uncoupler-inhibitor titrations, commonly known as the SUIT protocol. This protocol involves titration with various combinations of substrates, uncouplers, and inhibitors to assess mitochondrial respiratory function[9]. This protocol allows the study of complex interactions of coupling and substrate control in a single assay, measuring multiple aspects of mitochondrial physiology[62].

Respirometry enables the real-time measurement of mitochondrial respiration, with key parameters obtainable through the use of established inhibitors and uncouplers that act as sensitive indicators of response to mitochondrial stress. Figure 3 depicts the mitochondrial respiration trace derived from the SUIT protocol, which was employed in the following procedures[9,62]: (1) Routine respiration: Routine respiration also known as basal respiration, measures the oxygen consumption resulting from ATP production and

proton leak. This represents energy demand under steady-state conditions. Changes in routine respiration in patients with disease compared to controls may indicate altered mitochondrial function and should be interpreted in the context of the following mitochondrial parameters; (2) proton leak: After measuring routine respiration, cells are exposed to oligomycin, an inhibitor of complex V. The remaining mitochondrial respiration after the addition of oligomycin is attributable to proton leak. While some proton leaks are expected under physiological conditions, significant proton leak may indicate damage to the mitochondrial membrane and/or complex damage. The use of oligomycin also allows for the estimation of oxygen consumption secondary to ATP production, often referred to as ATP-linked respiration; (3) maximal respiration: The addition of a mitochondrial uncoupler, such as dinitrophenol or carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone, stimulates maximal respiration by mimicking the physiological energy demand, leading to an increase in oxygen consumption. The difference between maximal respiration and routine respiration represents the spare respiratory capacity (SRC) of the cell. SRC indicates the ability of the cell to respond to energetic stress and is a measure of a cell’s fitness. A decrease in SRC may limit the cell's ability to handle stressors, resulting in mitochondrial dysfunction; and (4) residual oxygen consumption: The addition of mitochondrial inhibitors, such as the combination of rotenone (complex I) and antimycin (complex III), completely inhibits electron transport system. The remaining oxygen is consumed by non-mitochondrial respiration in the form of oxidases and other cellular enzymes that use oxygen. Residual oxygen consumption may increase in the presence of a stress response.

Under normal conditions with excess ADP and oxygen, mitochondrial respiration occurs rapidly, known as state 3 respiration. Conversely, when ADP is fully consumed, state 4 respiration occurs, which is significantly slower. This state 4 respiration can be induced by “uncoupling” oxygen consumption from OXPHOS, leading to proton leakage back into the mitochondrial matrix without the production of cellular energy. One of the most promising indicators of mitochondrial function is the biochemical coupling efficiency (BCE), which is calculated as the quotient between OXPHOS and proton leak. BCE reflects the true effectiveness of mitochondria in utilizing oxygen for ATP

production[62]. It is a useful way to gain more insight into the site of the dysfunction, namely, respiratory control decreases because of dysfunction in localized sites of substrate oxidation, ATP synthesis, proton conductance, or F1Fo-ATP synthase[8].

Although we understand that small clinical centers may have limited access to equipment for measuring real-time mitochondrial respiratory rates, this limitation could be easily overcome using simpler biochemical colorimetric methods that still maintain a reasonable level of sensitivity, the time to assay is usually short, and easy to implement in the laboratory hospital routine. For instance, the measurements of enzymatic activity of succinate dehydrogenase: Complex II (succinate: DCIP-oxidoreductase), complex, and complex V are routinely performed in research laboratories, and at the current state require standardization as a step forward to reach clinical settings[63,64]. However, the aforementioned colorimetric methods restrict the evaluation of metabolic activity to one complex each time, whereas in mitochondrial respirometry assays the metabolic activity of complexes can be assayed both, individually or more than one at the same time.

Which cells are ideal for measuring the mitochondrial activity? And what is the most suitable method?

Although it is logical that dysfunction in mitochondrial metabolism leads to a certain degree of organ failure, its measurement is often not feasible, because the ethical questions, costs, and viability of obtaining adequate mitochondrial samples from vital organs in critically ill patients[61]. Therefore, it is feasible to assess mitochondrial dysfunction in cells that are easy to collect, especially those that may reflect the "systemic" effect of sepsis on the body. Peripheral blood cells have been used to access bioenergetic functions in translational research. Peripheral blood mononuclear cells (PBMCs) are mitochondria-rich with high rates of respiration[16]; therefore, they are prime candidates in circulating blood to provide reliable estimation of global oxidative metabolism, particularly the metabolism linked to immune response. Lymphocytes comprise the majority of PBMCs and are traditionally used to measure defects in mitochondrial OXPHOS[30]. Exhaustion of lymphocytes, especially T cells, leads to an increased risk of secondary infections, which is correlated with mortality[65]. Circulating

immune cells play an important role in the pathophysiology of sepsis because their activation may remotely induce inflammation in non-infected organs[66]. The measurement of mitochondrial respiration in PBMCs of septic patients has the potential to identify those at risk of negative outcomes, and also can monitor the clinical course and response to treatment, making it a useful marker for acute care settings[9]. Thus, it is a candidate biomarker in sepsis.

In addition, this approach allows us to unravel mechanist readouts associated with impaired metabolism that follow this syndrome and also challenges how or whether this could be improved by therapeutic intervention. These primary observations collected from the literature are encouraging, and more clinical studies will help to advance methodological issues, clinical validation, and the level of reproducibility and sensitivity.

SEPSIS AND MULTI-ORGANIC FAILURE

Various systemic inflammatory processes can exert different effects on mitochondria. In the early stages of sepsis, reduced perfusion resulting from intrinsic and extrinsic fluid losses, decreased intake, myocardial depression, microcirculatory redistribution of blood flow, and loss of vascular tone, can lead to tissue hypoxia. This condition, characterized by insufficient oxygen levels at the mitochondrial level, impedes the ability of mitochondria to carry out OXPHOS, leading to a deficit in ATP production[12]. Although Complex IV exhibits distinctive enzyme properties that facilitate its functionality under hypoxic conditions, severely diminished oxygen concentrations may compromise ATP generation and activate cell death pathways, thereby adversely impacting cellular homeostasis[12,67,68]. Hormonal alterations in sepsis also affect mitochondrial function and efficiency. For example, thyroid hormones are believed to work predominantly through the modulation of mitochondrial activity[4,33]. Thirdly, genes that transcribe mitochondrial proteins are downregulated early in the inflammatory response. This was first recognized in human volunteers receiving endotoxins[69] and subsequently described in critically ill patients[35].

Impairment of mitochondrial metabolism in sepsis

Different studies, in different contexts, have evaluated mitochondrial activity in septic compared to nonseptic or control patients. Muscular cells are prone to impaired mitochondrial metabolism during critical illness[70] and are an important research field. Carré *et al*[35] used muscle tissue biopsies from critically ill patients, comparing them to those of controls subjected to hip surgery. They found a decrease in mitochondrial density in critically ill patients, without a decrease in Complex I and Complex IV activity. In a study evaluating patients with ICU-acquired weakness, comparing ATP synthesis in this population with metabolically healthy controls, ICU patients had an approximately 50% reduction in the ability of skeletal muscle to synthesize ATP in mitochondria, with a depletion of complex III and IV concentrations[71]. A similar loss of mitochondrial activity was detected in a previous study in a population with sepsis and multiorgan failure[72]. Complex I and complex IV activity was reduced in the intercostal and leg muscles, respectively, compared to controls.

Belikova demonstrated a higher baseline PBMC oxygen consumption and attenuated response to ADP stimulation in patients with sepsis than in healthy volunteers[66]. In blood mononuclear cells, Kraft *et al*[16] demonstrated an early sepsis-mediated disruption of mitochondrial quality control in septic patients, with a later activation of mitochondrial biogenesis in this population. These patients also showed increased mitochondrial damage (measured by mtDNA levels) during the early phase of sepsis management. In a cohort of septic and non-septic ICU patients with measurement of mitochondrial function in isolated lymphocytes, critically ill patients had increased mitochondrial oxygen consumption but no significant difference in mitochondrial membrane potential[17]. Jang *et al*[15] also found a lower routine, uncoupled Complex I, and maximal respiration in septic patients, when compared to controls in the early sepsis management. A respirometric study in PBMC developed by Japiassú *et al*[73] reported reduced F1Fo-ATP synthase activity, thereby reducing ATP production. This may contribute to the energetic failure reported in these cells during the course of septic insult. In addition, septic shock PBMC have reduced O_2 consumption, ADP-induced state 3 respiration, and respiratory control ratio compared to control PBMC. Inhibition of complexes I, III, and IV in PBMCs from septic patients compared with controls was also detected in another study[74]. In a

pediatric population, Weiss *et al*[75] detected a decrease in spare respiratory capacity on days 1-2 of sepsis compared with controls. Spare respiratory capacity normalized on days 5-7. Patients with sepsis also had a higher ratio of leak to maximal respiration than controls, with normalization in the later phase of sepsis. Patients with sepsis did not show differences in basal or ATP-linked oxygen consumption or membrane potential.

In patients admitted to the emergency department with and without sepsis, Puskarich[76] did not find differences in plasma levels of cytochrome B, NADH, and Cox-III mtDNA between groups. Pyle *et al*[55], in a protocol that evaluated mononuclear cell mtDNA content, found that these levels were lower in patients with sepsis, with depletion of monocyte and lymphocyte mtDNA. Platelet studies have also evaluated mitochondrial metabolism in patients with sepsis. Sjövall *et al*[61] found an increase in state 3 and a decrease in RCR in patients with sepsis compared to controls during sequential evaluations in the first week of sepsis diagnosis. Additionally, patients with sepsis had increased rates of complex I and complex II respiration compared to controls. However, the mtDNA concentration did not differ between the platelets of patients with sepsis and controls.

Are mitochondrial metabolisms associated with mortality?

A criticism can be made of the differences in mitochondrial measurements between survivors and non-survivors observed in studies. Whether they are pathological or just another measure of disease severity requires further investigation[77]. In human subjects, a significant constraint of this research approach is the uncertainty surrounding whether mitochondria sourced from PBMCs, platelets, or muscle cells can accurately serve as proxies for the mitochondria present in essential organs like the liver, kidneys, and heart[77]. The establishment of a workable, all-encompassing strategy to investigate the complete energy production pathway in human beings would signify a more significant achievement, as it would facilitate a deeper comprehension of the origin of lactate in distinct patients and across time, thereby enabling more targeted clinical trials for novel treatments for bioenergetic dysfunction.

Defects in leukocyte energy metabolism[78], particularly in T lymphocyte cells[79], are intrinsically associated with the state of immunoparalysis in sepsis. Metabolic events in the mitochondria of macrophages, dendritic cells, and T-lymphocytes have profound effects on immunity. When exposed to infectious injury, OXPHOS levels decrease, with a concomitant increase in glycolysis. An outcome of the reduced ATP production *via* OXPHOS is the redirection of mitochondria towards generating mitochondrial ROS, which function as signaling molecules essential for eliciting an appropriate immune response[54]. Therefore, mitochondrial respiration is essential for the functioning of these cells.

Despite the fact that the current knowledge suggests a potential role of mitochondrial metabolism impairment in septic patients (when compared with controls) with a potential impact on prognosis, the literature is quite heterogeneous with regard to the findings of mitochondrial dysfunction (Table 2). It is still necessary to define the most practical way of measuring, with the greatest clinical applicability, the greatest prognostic impact, and, above all, the most accurate in predicting the clinical course of the disease.

Are mitochondrial metabolisms associated with recovery?

Therefore, mitochondrial biogenesis is critical for recovery, and the recovery from organ dysfunction is preceded by an increased mitochondrial biogenesis[33]. In our study of patients with multi-organ failure in intensive care, we found that those who ultimately survived had higher levels of PGC-1α and better-preserved levels of Complex protein, along with a more robust antioxidant response (specifically, manganese superoxide) in the early stages of their disease progression[35]. The ability to clear damaged mitochondria is another important phenomenon[80]. Mitophagy (autophagic degradation) and mitoptosis (programmed destruction) are the processes by which cells deal with impaired mitochondria[12]. The efficiency of these processes may be an important contributing factor to the pathogenesis of various states of the disease. The process of mitophagy entails the targeted sequestration and subsequent degradation of damaged mitochondria, which occurs prior to their ability to activate cell death pathways and potentially jeopardize the viability of the entire cell. Thus, mitophagy operates as an initial protective

response. Conversely, heightened levels of oxidative stress and apoptotic proteases can impede the function of mitophagy and stimulate additional inflammatory responses[81].

CONCLUSION

Alterations caused by acute inflammatory conditions, such as sepsis, is associated with impaired function of mitochondrial components including protein content, mtDNA concentration, oxidative complexes activity, and F1Fo-ATP synthase. Often, these alterations are associated with clinical outcomes. Given these features mitochondria deserve to be better explored regarding its role as potential biomarker in prognosis, sepsis rehabilitation, and its association with different spectrum of organ failure.

REFERENCES

1 **Evans L**, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC, Schorr C, Simpson S, Wiersinga WJ, Alshamsi F, Angus DC, Arabi Y, Azevedo L, Beale R, Beilman G, Belley-Cote E, Burry L, Cecconi M, Centofanti J, Coz Yataco A, De Waele J, Dellinger RP, Doi K, Du B, Estenssoro E, Ferrer R, Gomersall C, Hodgson C, Hylander Møller M, Iwashyna T, Jacob S, Kleinpell R, Klompas M, Koh Y, Kumar A, Kwizera A, Lobo S, Masur H, McGloughlin S, Mehta S, Mehta Y, Mer M, Nunnally M, Oczkowski S, Osborn T, Papathanassoglou E, Perner A, Puskarich M, Roberts J, Schweickert W, Seckel M, Sevransky J, Sprung CL, Welte T, Zimmerman J, Levy M. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock 2021. *Crit Care Med* 2021; **49**: e1063-e1143 [PMID: 34605781 DOI: 10.1097/CCM.0000000000005337]

2 **Cecconi M**, Evans L, Levy M, Rhodes A. Sepsis and septic shock. *Lancet* 2018; **392**: 75-87 [PMID: 29937192 DOI: 10.1016/S0140-6736(18)30696-2]

3 **Angus DC**, van der Poll T. Severe sepsis and septic shock. *N Engl J Med* 2013; **369**: 840-851 [PMID: 23984731 DOI: 10.1056/NEJMra1208623]

4 **Singer M**. The role of mitochondrial dysfunction in sepsis-induced multi-organ failure. *Virulence* 2014; **5**: 66-72 [PMID: 24185508 DOI: 10.4161/viru.26907]

5 **Leite HP**, de Lima LF. Metabolic resuscitation in sepsis: a necessary step beyond the hemodynamic? *J Thorac Dis* 2016; **8**: E552-E557 [PMID: 27501325 DOI: 10.21037/jtd.2016.05.37]
6 **Supinski GS**, Schroder EA, Callahan LA. Mitochondria and Critical Illness. *Chest* 2020; **157**: 310-322 [PMID: 31494084 DOI: 10.1016/j.chest.2019.08.2182]
7 **Arulkumaran N**, Deutschman CS, Pinsky MR, Zuckerbraun B, Schumacker PT, Gomez H, Gomez A, Murray P, Kellum JA; ADQI XIV Workgroup. MITOCHONDRIAL FUNCTION IN SEPSIS. *Shock* 2016; **45**: 271-281 [PMID: 26871665 DOI: 10.1097/SHK.0000000000000463]
8 **Brand MD**, Nicholls DG. Assessing mitochondrial dysfunction in cells. *Biochem J* 2011; **435**: 297-312 [PMID: 21726199 DOI: 10.1042/BJ20110162]
9 **Jang DH**, Greenwood JC, Spyres MB, Eckmann DM. Measurement of Mitochondrial Respiration and Motility in Acute Care: Sepsis, Trauma, and Poisoning. *J Intensive Care Med* 2017; **32**: 86-94 [PMID: 27443317 DOI: 10.1177/0885066616658449]
10 **Moonen HPFX**, Van Zanten ARH. Mitochondrial dysfunction in critical illness during acute metabolic stress and convalescence: consequences for nutrition therapy. *Curr Opin Crit Care* 2020; **26**: 346-354 [PMID: 32487844 DOI: 10.1097/MCC.0000000000000741]
11 **Maestraggi Q**, Lebas B, Clere-Jehl R, Ludes PO, Chamaraux-Tran TN, Schneider F, Diemunsch P, Geny B, Pottecher J. Skeletal Muscle and Lymphocyte Mitochondrial Dysfunctions in Septic Shock Trigger ICU-Acquired Weakness and Sepsis-Induced Immunoparalysis. *Biomed Res Int* 2017; **2017**: 7897325 [PMID: 28589148 DOI: 10.1155/2017/7897325]
12 **Kozlov AV**, Bahrami S, Calzia E, Dungel P, Gille L, Kuznetsov AV, Troppmair J. Mitochondrial dysfunction and biogenesis: do ICU patients die from mitochondrial failure? *Ann Intensive Care* 2011; **1**: 41 [PMID: 21942988 DOI: 10.1186/2110-5820-1-41]
13 **McClave SA**, Wischmeyer PE, Miller KR, van Zanten ARH. Mitochondrial Dysfunction in Critical Illness: Implications for Nutritional Therapy. *Curr Nutr Rep* 2019; **8**: 363-373 [PMID: 31713718 DOI: 10.1007/s13668-019-00296-y]
14 **Nedel WL**, Kopczynski A, Rodolphi MS, Strogulski NR, De Bastiani M, Montes THM, Abruzzi J Jr, Galina A, Horvath TL, Portela LV. Mortality of septic shock patients is

associated with impaired mitochondrial oxidative coupling efficiency in lymphocytes: a prospective cohort study. *Intensive Care Med Exp* 2021; **9**: 39 [PMID: 34304333 DOI: 10.1186/s40635-021-00404-9]

15 **Jang DH**, Orloski CJ, Owiredu S, Shofer FS, Greenwood JC, Eckmann DM. Alterations in Mitochondrial Function in Blood Cells Obtained From Patients With Sepsis Presenting to an Emergency Department. *Shock* 2019; **51**: 580-584 [PMID: 29905672 DOI: 10.1097/SHK.0000000000001208]

16 **Kraft BD**, Chen L, Suliman HB, Piantadosi CA, Welty-Wolf KE. Peripheral Blood Mononuclear Cells Demonstrate Mitochondrial Damage Clearance During Sepsis. *Crit Care Med* 2019; **47**: 651-658 [PMID: 30730439 DOI: 10.1097/CCM.0000000000003681]

17 **Ayala JC**, Grismaldo A, Aristizabal-Pachon AF, Mikhaylenko EV, Nikolenko VN, Mikhaleva LM, Somasundaram SG, Kirkland CE, Aliev G, Morales L. Mitochondrial Dysfunction in Intensive Care Unit Patients. *Curr Pharm Des* 2021; **27**: 3074-3081 [PMID: 33292115 DOI: 10.2174/1381612826666201207112931]

18 **Brealey D**, Singer M. Mitochondrial Dysfunction in Sepsis. *Curr Infect Dis Rep* 2003; **5**: 365-371 [PMID: 13678565 DOI: 10.1007/s11908-003-0015-9]

19 **Hsiao CP**, Hoppel C. Analyzing mitochondrial function in human peripheral blood mononuclear cells. *Anal Biochem* 2018; **549**: 12-20 [PMID: 29505781 DOI: 10.1016/j.ab.2018.03.003]

20 **Galley HF**. Oxidative stress and mitochondrial dysfunction in sepsis. *Br J Anaesth* 2011; **107**: 57-64 [PMID: 21596843 DOI: 10.1093/bja/aer093]

21 **Mantzarlis K**, Tsolaki V, Zakynthinos E. Role of Oxidative Stress and Mitochondrial Dysfunction in Sepsis and Potential Therapies. *Oxid Med Cell Longev* 2017; **2017**: 5985209 [PMID: 28904739 DOI: 10.1155/2017/5985209]

22 **Preau S**, Vodovar D, Jung B, Lancel S, Zafrani L, Flatres A, Oualha M, Voiriot G, Jouan Y, Joffre J, Uhel F, De Prost N, Silva S, Azabou E, Radermacher P. Energetic dysfunction in sepsis: a narrative review. *Ann Intensive Care* 2021; **11**: 104 [PMID: 34216304 DOI: 10.1186/s13613-021-00893-7]

23 **Crouser ED**. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion* 2004; **4**: 729-741 [PMID: 16120428 DOI: 10.1016/j.mito.2004.07.023]

24 **Nagar H**, Piao S, Kim CS. Role of Mitochondrial Oxidative Stress in Sepsis. *Acute Crit Care* 2018; **33**: 65-72 [PMID: 31723865 DOI: 10.4266/acc.2018.00157]

25 **Vassiliou AG**, Mastora Z, Jahaj E, Keskinidou C, Pratikaki ME, Kampisiouli E, Orfanos SE, Kotanidou A, Dimopoulou I. Serum Coenzyme Q10 Levels are Decreased in Critically-Ill Septic Patients: Results From a Preliminary Study. *Biol Res Nurs* 2021; **23**: 198-207 [PMID: 32705879 DOI: 10.1177/1099800420944489]

26 **Zamponi N**, Zamponi E, Cannas SA, Billoni OV, Helguera PR, Chialvo DR. Mitochondrial network complexity emerges from fission/fusion dynamics. *Sci Rep* 2018; **8**: 363 [PMID: 29321534 DOI: 10.1038/s41598-017-18351-5]

27 **Roca-Agujetas V**, de Dios C, Lestón L, Marí M, Morales A, Colell A. Recent Insights into the Mitochondrial Role in Autophagy and Its Regulation by Oxidative Stress. *Oxid Med Cell Longev* 2019; **2019**: 3809308 [PMID: 31781334 DOI: 10.1155/2019/3809308]

28 **Gunst J**, Derese I, Aertgeerts A, Ververs EJ, Wauters A, Van den Berghe G, Vanhorebeek I. Insufficient autophagy contributes to mitochondrial dysfunction, organ failure, and adverse outcome in an animal model of critical illness. *Crit Care Med* 2013; **41**: 182-194 [PMID: 23222264 DOI: 10.1097/CCM.0b013e3182676657]

29 **Pool R**, Gomez H, Kellum JA. Mechanisms of Organ Dysfunction in Sepsis. *Crit Care Clin* 2018; **34**: 63-80 [PMID: 29149942 DOI: 10.1016/j.ccc.2017.08.003]

30 **Koutroulis I**, Batabyal R, McNamara B, Ledda M, Hoptay C, Freishtat RJ. Sepsis Immunometabolism: From Defining Sepsis to Understanding How Energy Production Affects Immune Response. *Crit Care Explor* 2019; **1**: e0061 [PMID: 32166242 DOI: 10.1097/CCE.0000000000000061]

31 **Exline MC**, Crouser ED. Mitochondrial dysfunction during sepsis: still more questions than answers. *Crit Care Med* 2011; **39**: 1216-1217 [PMID: 21603080 DOI: 10.1097/CCM.0b013e31821487cb]

32 **Wesselink E**, Koekkoek WAC, Grefte S, Witkamp RF, van Zanten ARH. Feeding mitochondria: Potential role of nutritional components to improve critical illness

convalescence. *Clin Nutr* 2019; **38**: 982-995 [PMID: 30201141 DOI: 10.1016/j.clnu.2018.08.032]

33 **Singer M**. Critical illness and flat batteries. *Crit Care* 2017; **21**: 309 [PMID: 29297363 DOI: 10.1186/s13054-017-1913-9]

34 **Vanhorebeek I**, Gunst J, Derde S, Derese I, Boussemaere M, D'Hoore A, Wouters PJ, Van den Berghe G. Mitochondrial fusion, fission, and biogenesis in prolonged critically ill patients. *J Clin Endocrinol Metab* 2012; **97**: E59-E64 [PMID: 22013100 DOI: 10.1210/jc.2011-1760]

35 **Carré JE**, Orban JC, Re L, Felsmann K, Iffert W, Bauer M, Suliman HB, Piantadosi CA, Mayhew TM, Breen P, Stotz M, Singer M. Survival in critical illness is associated with early activation of mitochondrial biogenesis. *Am J Respir Crit Care Med* 2010; **182**: 745-751 [PMID: 20538956 DOI: 10.1164/rccm.201003-0326OC]

36 **Exline MC**, Crouser ED. Mitochondrial mechanisms of sepsis-induced organ failure. *Front Biosci* 2008; **13**: 5030-5041 [PMID: 18508567 DOI: 10.2741/3061]

37 **Duggal NA**, Snelson C, Shaheen U, Pearce V, Lord JM. Innate and adaptive immune dysregulation in critically ill ICU patients. *Sci Rep* 2018; **8**: 10186 [PMID: 29976949 DOI: 10.1038/s41598-018-28409-7]

38 **Surbatovic M**, Vojvodic D, Khan W. Immune Response in Critically Ill Patients. *Mediators Inflamm* 2018; **2018**: 9524315 [PMID: 30116156 DOI: 10.1155/2018/9524315]

39 **Frazier WJ**, Hall MW. Immunoparalysis and adverse outcomes from critical illness. *Pediatr Clin North Am* 2008; **55**: 647-668, xi [PMID: 18501759 DOI: 10.1016/j.pcl.2008.02.009]

40 **Faas MM**, de Vos P. Mitochondrial function in immune cells in health and disease. *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**: 165845 [PMID: 32473386 DOI: 10.1016/j.bbadis.2020.165845]

41 **McBride MA**, Owen AM, Stothers CL, Hernandez A, Luan L, Burelbach KR, Patil TK, Bohannon JK, Sherwood ER, Patil NK. The Metabolic Basis of Immune Dysfunction Following Sepsis and Trauma. *Front Immunol* 2020; **11**: 1043 [PMID: 32547553 DOI: 10.3389/fimmu.2020.01043]

42 **Angajala A**, Lim S, Phillips JB, Kim JH, Yates C, You Z, Tan M. Diverse Roles of Mitochondria in Immune Responses: Novel Insights Into Immuno-Metabolism. *Front Immunol* 2018; **9**: 1605 [PMID: 30050539 DOI: 10.3389/fimmu.2018.01605]
43 **Iwasaki Y**, Takeshima Y, Fujio K. Basic mechanism of immune system activation by mitochondria. *Immunol Med* 2020; **43**: 142-147 [PMID: 32393116 DOI: 10.1080/25785826.2020.1756609]
44 **de Pablo R**, Monserrat J, Prieto A, Alvarez-Mon M. Role of circulating lymphocytes in patients with sepsis. *Biomed Res Int* 2014; **2014**: 671087 [PMID: 25302303 DOI: 10.1155/2014/671087]
45 **Kelly B**, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 2015; **25**: 771-784 [PMID: 26045163 DOI: 10.1038/cr.2015.68]
46 **de Pablo R**, Monserrat J, Reyes E, Diaz-Martin D, Rodriguez Zapata M, Carballo F, de la Hera A, Prieto A, Alvarez-Mon M. Mortality in patients with septic shock correlates with anti-inflammatory but not proinflammatory immunomodulatory molecules. *J Intensive Care Med* 2011; **26**: 125-132 [PMID: 21464065 DOI: 10.1177/0885066610384465]
47 **Andrea AD'**, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) Inhibits Human Lymphocyte Interferon 3,-Production by Suppressing Natural Killer Cell Stimulatory Factor/IL-12 Synthesis in Accessory Cells. *J Exp Med* 1993; **178**: 1041-1048
48 **Murphy MP**, O'Neill LAJ. Krebs Cycle Reimagined: The Emerging Roles of Succinate and Itaconate as Signal Transducers. *Cell* 2018; **174**: 780-784 [PMID: 30096309 DOI: 10.1016/j.cell.2018.07.030]
49 **Takasu O**, Gaut JP, Watanabe E, To K, Fagley RE, Sato B, Jarman S, Efimov IR, Janks DL, Srivastava A, Bhayani SB, Drewry A, Swanson PE, Hotchkiss RS. Mechanisms of cardiac and renal dysfunction in patients dying of sepsis. *Am J Respir Crit Care Med* 2013; **187**: 509-517 [PMID: 23348975 DOI: 10.1164/rccm.201211-1983OC]
50 **Fredriksson K**, Tjäder I, Keller P, Petrovic N, Ahlman B, Schéele C, Wernerman J, Timmons JA, Rooyackers O. Dysregulation of mitochondrial dynamics and the muscle transcriptome in ICU patients suffering from sepsis induced multiple organ failure. *PLoS One* 2008; **3**: e3686 [PMID: 18997871 DOI: 10.1371/journal.pone.0003686]

51 **Liesa M**, Palacín M, Zorzano A. Mitochondrial dynamics in mammalian health and disease. *Physiol Rev* 2009; **89**: 799-845 [PMID: 19584314 DOI: 10.1152/physrev.00030.2008]
52 **Willems PH**, Rossignol R, Dieteren CE, Murphy MP, Koopman WJ. Redox Homeostasis and Mitochondrial Dynamics. *Cell Metab* 2015; **22**: 207-218 [PMID: 26166745 DOI: 10.1016/j.cmet.2015.06.006]
53 **Harrington JS**, Choi AMK, Nakahira K. Mitochondrial DNA in Sepsis. *Curr Opin Crit Care* 2017; **23**: 284-290 [PMID: 28562385 DOI: 10.1097/MCC.0000000000000427]
54 **Mills EL**, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. *Nat Immunol* 2017; **18**: 488-498 [PMID: 28418387 DOI: 10.1038/ni.3704]
55 **Pyle A**, Burn DJ, Gordon C, Swan C, Chinnery PF, Baudouin SV. Fall in circulating mononuclear cell mitochondrial DNA content in human sepsis. *Intensive Care Med* 2010; **36**: 956-962 [PMID: 20224905 DOI: 10.1007/s00134-010-1823-7]
56 **Meloche J**, Pflieger A, Vaillancourt M, Paulin R, Potus F, Zervopoulos S, Graydon C, Courboulin A, Breuils-Bonnet S, Tremblay E, Couture C, Michelakis ED, Provencher S, Bonnet S. Role for DNA damage signaling in pulmonary arterial hypertension. *Circulation* 2014; **129**: 786-797 [PMID: 24270264 DOI: 10.1161/CIRCULATIONAHA.113.006167]
57 **Cicchillitti L**, Di Stefano V, Isaia E, Crimaldi L, Fasanaro P, Ambrosino V, Antonini A, Capogrossi MC, Gaetano C, Piaggio G, Martelli F. Hypoxia-inducible factor 1-α induces miR-210 in normoxic differentiating myoblasts. *J Biol Chem* 2012; **287**: 44761-44771 [PMID: 23148210 DOI: 10.1074/jbc.M112.421255]
58 **Tannahill GM**, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 2013; **496**: 238-242 [PMID: 23535595 DOI: 10.1038/nature11986]
59 **Svistunenko DA**, Davies N, Brealey D, Singer M, Cooper CE. Mitochondrial dysfunction in patients with severe sepsis: an EPR interrogation of individual respiratory chain components. *Biochim Biophys Acta* 2006; **1757**: 262-272 [PMID: 16626626 DOI: 10.1016/j.bbabio.2006.03.007]

60 **Brealey D**, Brand M, Hargreaves I, Heales S, Land J, Smolenski R, Davies NA, Cooper CE, Singer M. Association between mitochondrial dysfunction and severity and outcome of septic shock. *Lancet* 2002; **360**: 219-223 [PMID: 12133657 DOI: 10.1016/S0140-6736(02)09459-X]

61 **Sjövall F**, Morota S, Hansson MJ, Friberg H, Gnaiger E, Elmér E. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. *Crit Care* 2010; **14**: R214 [PMID: 21106065 DOI: 10.1186/cc9337]

62 **Makrecka-Kuka M**, Krumschnabel G, Gnaiger E. High-Resolution Respirometry for Simultaneous Measurement of Oxygen and Hydrogen Peroxide Fluxes in Permeabilized Cells, Tissue Homogenate and Isolated Mitochondria. *Biomolecules* 2015; **5**: 1319-1338 [PMID: 26131977 DOI: 10.3390/biom5031319]

63 **Rustin P**, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, Munnich A. Biochemical and molecular investigations in respiratory chain deficiencies. *Clin Chim Acta* 1994; **228**: 35-51 [PMID: 7955428 DOI: 10.1016/0009-8981(94)90055-8]

64 **Fischer JC**, Ruitenbeek W, Berden JA, Trijbels JM, Veerkamp JH, Stadhouders AM, Sengers RC, Janssen AJ. Differential investigation of the capacity of succinate oxidation in human skeletal muscle. *Clin Chim Acta* 1985; **153**: 23-36 [PMID: 3000647 DOI: 10.1016/0009-8981(85)90135-4]

65 **Brady J**, Horie S, Laffey JG. Role of the adaptive immune response in sepsis. *Intensive Care Med Exp* 2020; **8**: 20 [PMID: 33336293 DOI: 10.1186/s40635-020-00309-z]

66 **Belikova I**, Lukaszewicz AC, Faivre V, Damoisel C, Singer M, Payen D. Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis. *Crit Care Med* 2007; **35**: 2702-2708 [PMID: 18074472 DOI: 10.1097/01.ccm.0000295593.25106.c4]

67 **Srinivasan S**, Avadhani NG. Cytochrome c oxidase dysfunction in oxidative stress. *Free Radic Biol Med* 2012; **53**: 1252-1263 [PMID: 22841758 DOI: 10.1016/j.freeradbiomed.2012.07.021]

68 **Szabó C**, Módis K. Pathophysiological roles of peroxynitrite in circulatory shock. *Shock* 2010; **34 Suppl 1**: 4-14 [PMID: 20523270 DOI: 10.1097/SHK.0b013e3181e7e9ba]

69 **Calvano SE**, Xiao W, Richards DR, Felciano RM, Baker HV, Cho RJ, Chen RO, Brownstein BH, Cobb JP, Tschoeke SK, Miller-Graziano C, Moldawer LL, Mindrinos MN,

Davis RW, Tompkins RG, Lowry SF; Inflamm and Host Response to Injury Large Scale Collab. Res. Program. A network-based analysis of systemic inflammation in humans. *Nature* 2005; **437**: 1032-1037 [PMID: 16136080 DOI: 10.1038/nature03985]

70 **De Jonghe B**, Sharshar T, Lefaucheur JP, Authier FJ, Durand-Zaleski I, Boussarsar M, Cerf C, Renaud E, Mesrati F, Carlet J, Raphaël JC, Outin H, Bastuji-Garin S; Groupe de Réflexion et d'Etude des Neuromyopathies en Réanimation. Paresis acquired in the intensive care unit: a prospective multicenter study. *JAMA* 2002; **288**: 2859-2867 [PMID: 12472328 DOI: 10.1001/jama.288.22.2859]

71 **Jiroutková K**, Krajčová A, Ziak J, Fric M, Waldauf P, Džupa V, Gojda J, Němcova-Fürstová V, Kovář J, Elkalaf M, Trnka J, Duška F. Mitochondrial function in skeletal muscle of patients with protracted critical illness and ICU-acquired weakness. *Crit Care* 2015; **19**: 448 [PMID: 26699134 DOI: 10.1186/s13054-015-1160-x]

72 **Fredriksson K**, Hammarqvist F, Strigård K, Hultenby K, Ljungqvist O, Wernerman J, Rooyackers O. Derangements in mitochondrial metabolism in intercostal and leg muscle of critically ill patients with sepsis-induced multiple organ failure. *Am J Physiol Endocrinol Metab* 2006; **291**: E1044-E1050 [PMID: 16803854 DOI: 10.1152/ajpendo.00218.2006]

73 **Japiassú AM**, Santiago AP, d'Avila JC, Garcia-Souza LF, Galina A, Castro Faria-Neto HC, Bozza FA, Oliveira MF. Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5'-triphosphate synthase activity. *Crit Care Med* 2011; **39**: 1056-1063 [PMID: 21336129 DOI: 10.1097/CCM.0b013e31820eda5c]

74 **Garrabou G**, Morén C, López S, Tobías E, Cardellach F, Miró O, Casademont J. The effects of sepsis on mitochondria. *J Infect Dis* 2012; **205**: 392-400 [PMID: 22180620 DOI: 10.1093/infdis/jir764]

75 **Weiss SL**, Selak MA, Tuluc F, Perales Villarroel J, Nadkarni VM, Deutschman CS, Becker LB. Mitochondrial dysfunction in peripheral blood mononuclear cells in pediatric septic shock. *Pediatr Crit Care Med* 2015; **16**: e4-e12 [PMID: 25251517 DOI: 10.1097/PCC.0000000000000277]

76 **Puskarich MA**, Shapiro NI, Trzeciak S, Kline JA, Jones AE. Plasma levels of mitochondrial DNA in patients presenting to the emergency department with sepsis. *Shock* 2012; **38**: 337-340 [PMID: 22777124 DOI: 10.1097/SHK.0b013e318266a169]

77 **Puskarich MA**. The Challenge and the Promise of Studying Mitochondrial Dysfunction in Humans with Sepsis. *Ann Am Thorac Soc* 2015; **12**: 1595-1596 [PMID: 26540415 DOI: 10.1513/AnnalsATS.201509-592ED]

78 **Cheng SC**, Scicluna BP, Arts RJ, Gresnigt MS, Lachmandas E, Giamarellos-Bourboulis EJ, Kox M, Manjeri GR, Wagenaars JA, Cremer OL, Leentjens J, van der Meer AJ, van de Veerdonk FL, Bonten MJ, Schultz MJ, Willems PH, Pickkers P, Joosten LA, van der Poll T, Netea MG. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat Immunol* 2016; **17**: 406-413 [PMID: 26950237 DOI: 10.1038/ni.3398]

79 **Luperto M**, Zafrani L. T cell dysregulation in inflammatory diseases in ICU. *Intensive Care Med Exp* 2022; **10**: 43 [PMID: 36279072 DOI: 10.1186/s40635-022-00471-6]

80 **Kubli DA**, Gustafsson ÅB. Mitochondria and mitophagy: the yin and yang of cell death control. *Circ Res* 2012; **111**: 1208-1221 [PMID: 23065344 DOI: 10.1161/CIRCRESAHA.112.265819]

81 **Zhou R**, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* 2011; **469**: 221-225 [PMID: 21124315 DOI: 10.1038/nature09663]

82 **Sjövall F**, Morota S, Persson J, Hansson MJ, Elmér E. Patients with sepsis exhibit increased mitochondrial respiratory capacity in peripheral blood immune cells. *Crit Care* 2013; **17**: R152 [PMID: 23883738 DOI: 10.1186/cc12831]

Footnotes

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Figure Legends

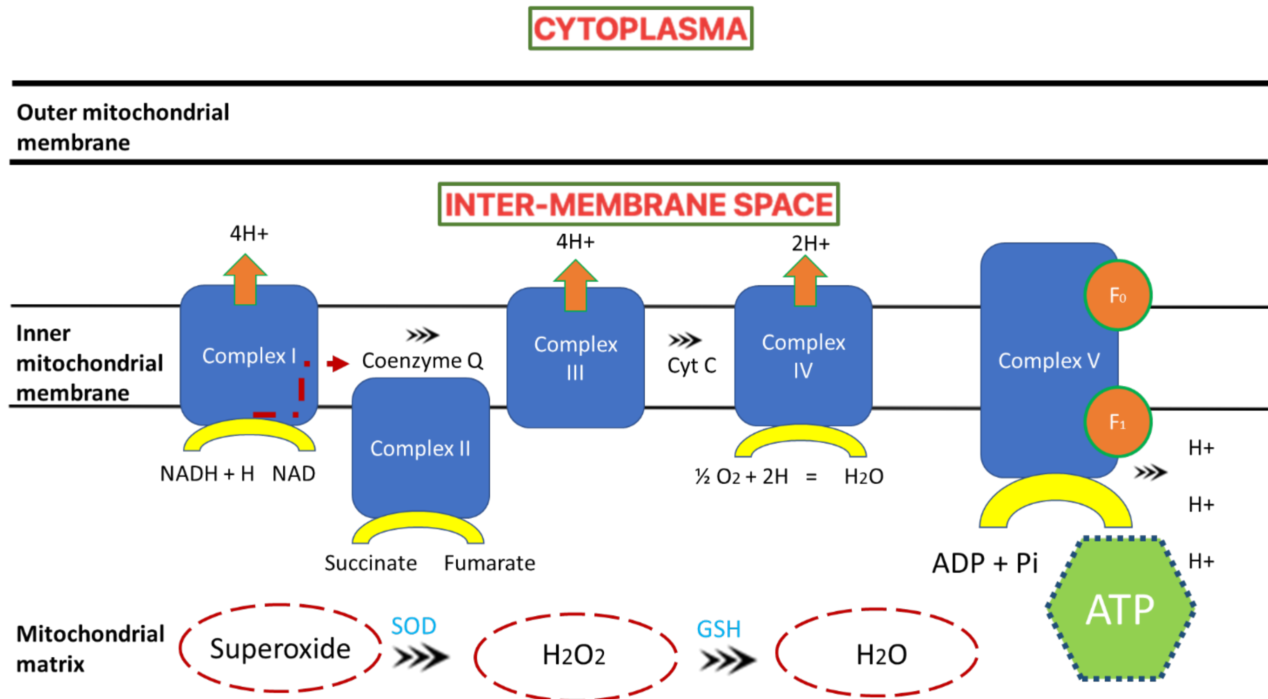

Figure 1 Electron transport chain through mitocondrial complexes and oxidative phosphorylation. ADP: Adenosine diphosphtate; ATP: Adenosine triphosphate; Cyt c: Cytochrome c; GSH: Gluthatione synthetase; NAD: Nicotinamide adenine dinucleotide; NADH: Nicotinamide adenine dinucleotide (reduced); SOD: Superoxide dismuthase.

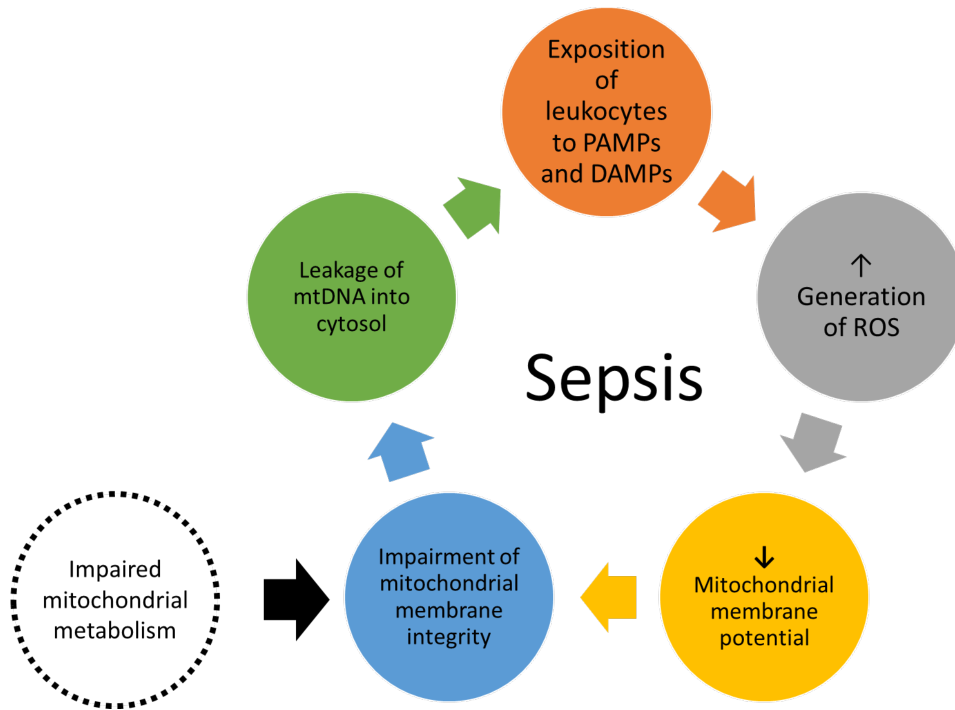

Figure 2 Mitochondrial DNA damage as a potential trigger for the inflammatory response. DAMP: Damage associated molecular patterns; mtDNA: Mitochondrial deoxyribonucleic acid; PAMP: Pathogen associated molecular patterns; ROS: Reactive oxygen species.

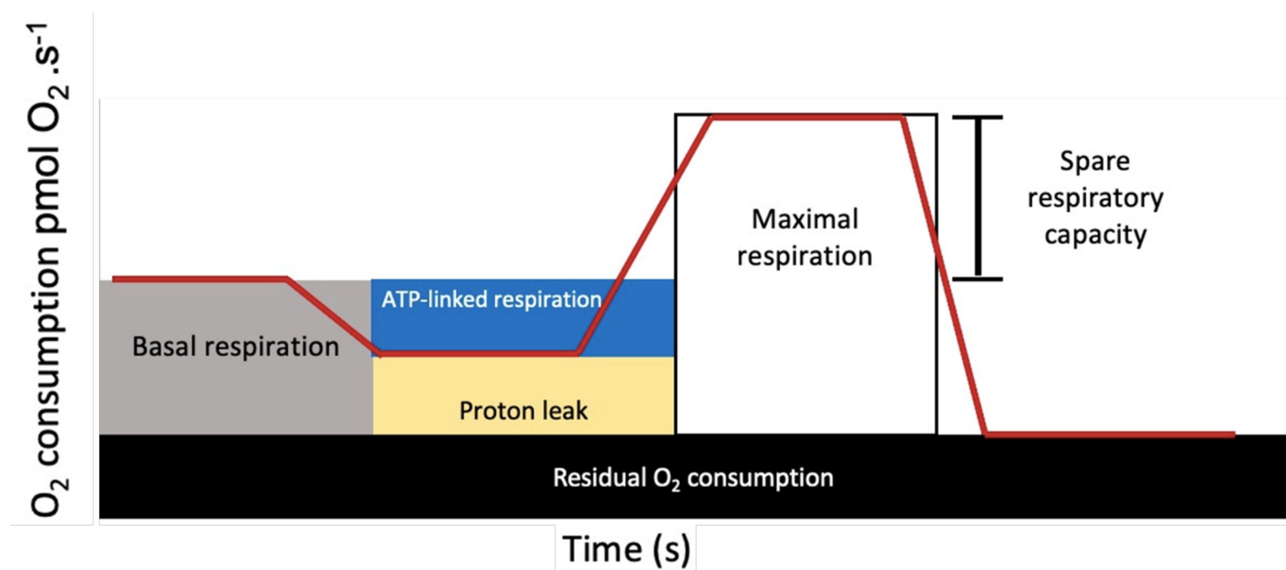

Figure 3 Example of a respirometry assay. ATP: Adenosine triphosphate.

Table 1 Different methods of mensuration of mitochondrial damage and recovery

Item	Description
Mitochondrial biogenesis	PPAR-γ coactivator 1-α, nuclear respiratory factor 1, mitochondrial transcription factor A. Material obtained from tissue biopsy or from peripheral PBMC
Mitochondrial content	Mitochondrial mass, concentration and area
Mitochondrial DNA content	PGC-1α, NRF-1, absolute DNA number of copies
Mitochondrial fusion	Mitofusins 1 and 2, optic atrophy 1 protein
Mitochondrial fission	Dynamin-related protein 1 and fission 1 protein

PPAR-γ: Peroxisome proliferator-activated receptor gamma; PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator-1 alpha; NRF-1: Nuclear respiratory factor-1; PBMC: Peripheral blood mononuclear cells.

Table 2 Studies that explored association of mitochondrial dysfunction and prognosis in sepsis

Ref.	***N* of critically ill septic patients**	**Mitochondrial measurement**	**Main findings (survivors *vs* nonsurvivors)**
Belikova *et al*[66], 2007			
Brealey *et al*[18], 2003	28	ATP concentration, Complex I, II, and IV activities in biopsied muscle cells	Sepsis survivors had an increased level of ATP and Complex I and IV activity
Carré *et al*[35], 2010	16	Mitochondrial morphology (surface density and volume), RT-PCR of mitochondrial biogenesis factors and concentration of Complex I and Complex IV mitochondrial proteins and OXPHOS transcripts in muscle biopsy specimens	Nonsurvivors had an increased decline in mitochondrial surface density, with a similar mitochondrial volume in these groups; OXPHOS transcripts were more abundant in survivors; increased ATP content in survivors; no difference between groups in Complex I and Complex IV activity
Kraft *et al*[16], 2019	37	qRT-PCR for genes that regulate mitochondrial biogenesis in PBMCs	Increased genetic activation of mitochondrial biogenesis in day 3 compared with day 1; decrease in mtDNA in septic patients compared

			with controls, with a recovery on day 5; early activation of mitochondrial biogenesis by day 1 associated with ICU discharge; increased mRNA levels in survivors
Japiassú *et al*[73], 2011	20	Respirometry of PBMC evaluating state 3, 4 and respiratory control ratio	No difference in ADP-stimulated respiration in nonsurvivors, when compared with survivors
Nedel *et al*[14], 2021	90 patients	Respirometry of permeabilized lymphocytes	Improvement in Complex I, Complex II, basal and in BCE in day 3, compared with day 1, were associated with lower mortality. In multivariate analysis, BCE improvement was associated with lower 6-mo mortality
Puskarich *et al*[77], 2015	28 patients	Respirometry of platelets	Routine and state 3 respiration were significantly higher in non-survivors compared to survivors; state 4 respiration had a non-significant increase in non-survivors

Pyle *et al*[55], 2010	Not reported (147 patients, including septic)	mtDNA content from mononuclear cells	No relationship between mtDNA content and survival outcome at 180 d
Sjövall *et al*[61], 2010	18 patients	Respirometry of isolated platelets evaluating Complex I, state 3, 4 and respiratory control ratio	Non-survivors had an increased Complex I, Complex II, state 3 respiration and an increased respiratory control ratio at day 6-7 of sepsis when compared with survivors
Sjövall *et al*[82], 2013	20 patients	Respirometry of permeabilized peripheral blood immune cells	Survivors and non-survivors at 90 d after sepsis did not have difference in Complex I plus Complex II respiration normalized to citrate synthase, mtDNA, and cytochrome c

ATP: Adenosine triphosphate; BCE: Biochemical coupling efficiency; ICU: Intensive care unit; qRT-PCR: Quantitative reverse transcriptase-polymerase chain reaction; mtDNA: Mitochondrion desoxyribonucleic acid; OXPHOS: Oxidative phosphorylation; PBMC: Peripheral blood mononuclear cells; ADP: Adenosine diphosphtate.

Capítulo 7 – Discussão

Associação entre disfunção mitocondrial e variáveis hemodinâmicas

Choque é definida como uma condição ameaçadora a vida, sendo uma forma generalizada de insuficiência circulatória aguda associada com a utilização inadequada de oxigênio pelas células (Cecconi M, de Backer D, Antonelli M, 2014). A circulação é incapaz de fornecer oxigênio suficiente para suprir as demandas tissulares, resultado em disfunção celular. O choque é clinicamente caracterizado por um nível sérico aumentado de lactato e sinais de hipoperfusão tissular (Singer M, Deutschman CS, Seymour CW, 2016). Um estado no qual a oferta de oxigênio tissular é inadequada para suprir as demandas metabólicas irá resultar em dano tissular e disfunção orgânica (Bakker J, Postelnicu R, Mukherjee V, 2020) – nessas condições, um dos eventos que surge é um aumento nos níveis de lactato (Ronco JJ, Fenwick JC, Tweeddale MG, 1993).

Tradicionalmente, um nível aumentado de lactato acarreta uma intervenção terapêutica guiada para otimizar a perfusão microcirculatória, tanto com o emprego de ressuscitação volêmica quanto com o emprego de vasopressores (Hernández G, Ospina-Tascón G, Damiani LP, 2019) (van Genderen ME, Engels N, van der Valk RJP, 2015). Uma disfunção microcirculatória, na sepse, pode acarretar numa oferta insuficiente de oxigênio para as células, consequentemente, elevando os níveis de lactato (Jacob M, Chappell D, Becker BF, 2016). No entanto, um contexto clínico no qual haja um metabolismo de glicose aumentado acarreta num aumento nos níveis de lactato,

uma vez que a capacidade do ciclo de Krebs é limitada (Bakker J, Postelnicu R, Mukherjee V; 2020). Na sepse, mais particularmente no choque séptico, há um aumento na ativação do sistema nervoso simpático, que pode acarretar neste desbalanço metabólico (Jansen TC, van Bommel J, Bakker J; 2009), não necessariamente relacionado a uma menor oferta de oxigênio, em decorrência de um déficit perfusional. A hipóxia tissular na sepse, inicialmente acreditada como sendo secundária às alterações microcirculatórias, não justifica o aumento nas tensões de oxigênio tissulares, associada a baixa incidência de apoptose celular observada em diversos órgãos afetados pela sepse, como também pela recuperação quase completa de falências orgânicas em sobreviventes (Singer M, 2017). Nas fases precoces do choque séptico, no entanto, o papel da hipoperfusão como um fator contribuinte a disfunção mitocondrial é ainda incerta; mesmo em tecidos com oxigenação adequada o metabolismo anaeróbico ocorre, com a produção aumentada de lactato em decorrência do aumento na concentração de piruvato (Suetrong B, Walley KR; 2016). Na fase pós-ressuscitação, como na nossa coorte, o aumento na oferta de oxigênio não causa um aumento no consumo de oxigênio, não havendo, portanto, uma relação consistente entre a oferta de oxigênio e a hiperlactatemia nesta população (Astiz ME, Rackow EC, Kaufman B, 1988).

Neste trabalho, planejamos a avaliação dos níveis de lactato após a ressuscitação volêmica inicial, deslocando-se do momento no qual os níveis de lactato são mais caracteristicamente dependentes do balanço entre oferta e consumo de oxigênio (Friedman G, de Backer D, Shahla M, 1998). O momento da nossa avaliação do lactato foi, posteriormente, chancelado pelas definições de choque séptico das diretrizes atuais (Singer M, Deutschman CS, Seymour

CW, 2016). Reforçam os nossos dados a sua correlação com marcadores perfusionais sabidamente correlacionados com um prejuízo na microcirculação, como é o caso do enchimento capilar (Hariri G, Joffre J, Leblanc G, 2019), como também com o gradiente arterio-venoso de CO_2, sabidamente correlacionado com o fluxo sanguíneo periférico (Araujo DT, Felice VB, Meregalli AF, 2019).

Nós encontramos uma associação fraca entre as variáveis de metabolismo mitocondrial, mais notoriamente a respiração de Complexo I, e a dosagem sérica de lactato. A produção de energia na forma de ATP se dá através do fluxo pela cadeia transportadora de elétrons; logo, a fosforilação oxidativa torna-se intimamente associada com o funcionamento adequado da cadeia (Galley HF, 2011). O fluxo de elétrons via NADH é processado no Complexo I, e a concentração de NADH é intimamente associada a concentração de lactato (Takahashi K, Tamura Y, Kitaoka Y, 2022). Logo, justifica-se avaliar especificamente esta associação, como realizamos neste estudo.

Em modelos experimentais de sepse, uma diminuição nos níveis de lactato durante as primeiras horas de ressuscitação se associa a alterações na respiração mitocondrial que são órgão-específicas (Corrêa TD, Pereira AJ, Brandt S, 2017). Em hepatócitos, por exemplo, num modelo de sepse experimental (Tapia P, Soto D, Bruhn A, 2015), o clareamento dos níveis de lactato não se associou nem com achados de hipoperfusão hepática nem com um déficit no metabolismo mitocondrial. Posto que uma avaliação em tempo real de diferentes tecidos é impraticável clinicamente, a avaliação de células "sistêmicas", como é o caso do linfócito, acarretam uma maior aplicabilidade dos resultados. Sabe-se que as células imunes ativadas, no caso da sepse, podem

apresentar uma taxa elevada de produção de lactato, que, teoricamente, podem estar associadas ao seu estado de ativação (Haji-Michael PG, Ladrière L, Sener A, 1999). Logo, pode-se interpretar o lactato como um produto da inflamação sistêmica, considerando-se, assim, uma contribuição relevante do lactato produzido pelos leucócitos em geral nos níveis séricos deste biomarcador.

No nosso estudo, no entanto, não foi possível avaliar o papel da produção de lactato como substrato energético, sob a teoria dos diferentes “shuttles” de lactato entre os órgãos (Hernandez G, Bellomo R, Bakker J, 2019). Um aumento na sua produção, que não é correspondido por um aumento no seu consumo ou na sua depuração, também podem ser causa da hiperlactatemia (Masyuk M, Wernly B, Lichtenauer M, 2019). A depuração do lactato, especialmente por via hepática, também não foi uma variável possível de ser mensurada, a qual também pode justificar os seus níveis séricos neste estudo.

Associação entre metabolismo mitocondrial e o uso de medicamentos em doentes críticos

A linha-mestra do tratamento de pacientes sépticos é a administração precoce de antimicrobianos, preferencialmente na primeira hora de detecção da sepse (Evans L, Rhodes A, Alhazzani W, 2021); estando associada a uma melhora no prognóstico desta população (Liu VX, Fielding-Singh V, Greene JD, 2017). No entanto, a administração de diferentes classes de antibióticos pode ocasionar uma miríade de efeitos adversos em diversos órgãos-alvo. Um dos principais mecanismos causadores destes eventos é através da disfunção mitocondrial (Arulkumaran N, Routledge M, Schlebusch S, 2020). Modelos

experimentais encontram uma redução na produção de ATP e uma produção excessiva de ROS com o uso de drogas bactericidas (Duewelhenke N, Krut O, Eysel P; 2007) (Kalghatgi S, Spina CS, Costello JC, 2013) (Tune BM, Hsu CY, 1990). Drogas de uso rotineiro no manejo da sepse, como é o caso da ampicilina, inibe os Complexos I e III da cadeia transportadora de elétrons (Kalghatgi S, Spina CS, Costello JC, 2013). Tanto os betalactâmicos quanto as cefalosporinas podem inibir a fosforilação oxidativa, diminuindo a produção de ATP e contribuindo para a falência orgânica da sepse (Duewelhenke N, Krut O, Eysel P; 2007).

O nosso estudo tem a vantagem de avaliar antibióticos de emprego usual na prática clínica (Metlay JP, Waterer GW, Long AC, 2019) (Kalil AC, Metersky ML, Klompas M, 2016) (Solomkin JS, Mazuski JE, Bradley JS, 2010) – em especial por analisar diferentes betalactâmicos - em um estudo clínico, na intenção de testar as hipóteses previamente aventadas em estudos experimentais. Não encontramos associação relevante entre o uso de diferentes classes de antimicrobianos – e de moléculas – com uma menor atividade mitocondrial nos seus diferentes segmentos (respiração basal, de Complexo I, de Complexo II e BCE), em um conjunto extenso de análises exploratórias, para cada tipo de antimicrobiano empregado. Este dado pode sugerir que as alterações encontradas em modelos experimentais não refletem em alterações clínicas relevantes, uma vez que os resultados com valor de $p<0,05$ não foram corrigidos para as múltiplas análises empregadas, sendo, portanto, dados geradores de hipóteses. Também estes resultados são sujeitos ao erro tipo II, posto que o tamanho amostral das análises para cada molécula de antimicrobiano, pode, a princípio, ser insuficiente para uma maior robustez dos

dados. No entanto, as análises secundárias, avaliando a atividade mitocondrial na sepse adquirida na comunidade (em comparação a atividade mitocondrial na sepse nosocomial) e a atividade mitocondrial no uso de antibioticoterapia combinada (em comparação a monoterapia) não encontraram diferenças nestes subgrupos; o que reforça a nossa hipótese de não haver um impacto clinicamente relevante dos antibióticos de uso comum na atividade mitocondrial.

Associação entre disfunção mitocondrial e atividade inflamatória na sepse

A resposta imune adaptativa é essencial para a eliminação dos patógenos, e ela é orquestrada pela interação entre os marcadores imunológicos e o metabolismo celular. Os linfócitos não ativados são altamente dependentes da OXPHOS (Chapman NM, Chi H; 2022) (Griffiths HR, Gao D, Pararasa C; 2017). As células T são altamente anabólicas, havendo uma transição da OXPHOS para a glicólise aeróbica, que é importante para a sua ativação e a sua proliferação induzida pela ativação (Pearce EL, Poffenberger MC, Chang CH, 2013). Essa transição para a glicólise aeróbica induz a secreção de citocinas pró-inflamatórias, contribuindo para a resposta imune. Esta transição se dá após ativação linfocitária, em especial dos linfócitos T, mediante a estimulação induzida pela IL-1β e pela IL-6, enquanto uma maior atividade de OXPHOS se associa a um perfil antinflamatório, induzido pela IL-10 (Toro J, Manrique-Caballero CL, Gómez H; 2021). Os linfócitos atenuam os efeitos potencialmente deletérios da resposta pro-inflamatória através da reprogramação metabólica, sendo que esta reprogramação impacta no prognóstico destes pacientes (de Pablo R, Monserrat J, Prieto A, et al; 2014).

Nossos resultados reiteram este fenômeno, ao observarmos uma melhora na atividade de OXPHOS linfocitária, quando mensurada através da BCE, da atividade de Complexo I e da atividade de Complexo II em associação a uma diminuição dos níveis séricos de IL-6, sugerindo uma atenuação dos efeitos pró-inflamatórios mediada por linfócitos. A IL-6 promove a diferenciação de linfócitos T e linfócitos B (del Giudice M, Gangestad SW; 2018), potencializando a resposta imune, reiterando, em uma coorte de doentes sépticos, os achados previamente descritos em modelos experimentais (Venet F, Demaret J, Blaise BJ, 2017) (McCall CE, Zabalawi M, Liu T, 2018). Neste cenário, o aumento da atividade metabólica linfocitária no terceiro dia, associada a diminuição nos níveis de IL-6, pode sugerir que esta mudança na respiração celular se dá no momento da diminuição da atividade pró-inflamatória. Este resultado não foi acompanhado de um aumento de igual magnitude nos níveis de IL-10; possivelmente em decorrência do tamanho amostral insuficiente para demonstrar o efeito em questão.

Analisando-se estas variáveis em conjunto, presume-se que uma maior atividade de IL-6, no estado “pro-inflamatório” da sepse, media uma menor eficiência bioenergética. Por outro lado, uma maior atividade da IL-10, parece se conectar com uma melhor atividade metabólica, quando analisamos a biossíntese de ATP.

A dinâmica do metabolismo linfocitário não encontrou associação com as concentrações de proteína C reativa nos mesmos intervalos de tempo. A síntese de proteína C reativa é induzida pela atividade da IL-6 e, embora seja um biomarcador de fácil aplicabilidade, de baixo custo e de ampla experiência de uso (Póvoa P, Teixeira-Pinto AM, Carneiro AH, 2011) (Póvoa P, Coelho L, Dal-

Pizzol F, 2023) a PCR não pode servir como um “modulador” do metabolismo mitocondrial em linfócitos.

Associação entre disfunção mitocondrial e mortalidade

Encontramos uma elevada mortalidade a curto e a longo prazo em pacientes portadores de choque séptico, condizente com dados da literatura, em especial no Brasil (Machado FR, Cavalcanti AB, Bozza FA, 2017). O metabolismo mitocondrial dos linfócitos, neste estudo, demonstrou ser um preditor independente de mortalidade. Este fenômeno parece ser um sinalizador de deterioração clínica, impactando nos desfechos clínicos. A disfunção quantitativa de linfócitos é historicamente reconhecida na doença crítica em geral e na sepse especificamente (de Pablo R, Monserrat J, Prieto A, 2014), reconhecida mais comumente através do déficit quantitativo de linfócitos T CD4+, T CD8+ e células NK (Finfer S, Venkatesh B, Hotchkiss RS, 2022). A linfopenia mostra associação com piores desfechos tanto na fase aguda (Vahedi HSM, Bagheri A, Jahanshir A, 2019) quanto na fase crônica da sepse (Adrie C, Lugosi M, Sonneville R, 2017). O nosso estudo acrescenta aspectos qualitativos na disfunção de linfócitos, apresentando uma associação com mortalidade tanto em curto quanto em longo prazo, especialmente naqueles pacientes que apresentaram uma piora do metabolismo celular no terceiro dia, em comparação ao primeiro dia do choque séptico. Deste fenômeno, pode-se compreender que uma melhora no metabolismo energético dos linfócitos pode refletir em uma reprogramação metabólica, previamente detectada através da expressão gênica em leucócitos, a qual se associou com desfechos clínicos favoráveis (Kraft BD,

Chen L, Suliman HB, et al; 2019). Não realizamos a análise de expressão gênica, porém uma mensuração funcional de atividade metabólica, como é o caso do BCE, parece trazer maior aplicabilidade clínica.

Esta associação permite-nos inferir que a mensuração da BCE pode, futuramente, apresentar um papel como biomarcador no estabelecimento do prognóstico dos doentes sépticos, complementando as informações clínicas disponíveis como também as de escores clínicos, como é o caso do escore SOFA, como também como uma ferramenta capaz de identificar subtipos de doentes sépticos, com características peculiares referentes a diferentes estratégias terapêuticas (Nunnally ME, Ferrer R, Martin GS, 2021). Idealmente, em estudos posteriores, o BCE pode se tornar um biomarcador de utilidade clínica, especialmente no que tange a identificação de pacientes que apresentem um risco maior de evoluir para uma trajetória clínica desfavorável. A sua mensuração em adição a de outros biomarcadores, em tese, pode acrescentar acurácia preditiva (Póvoa P, Coelho L, Dal-Pizzol F, 2023), orientando, ao menos teoricamente, a estratégia terapêutica.

O BCE é uma estimativa do fluxo de oxigênio mitocondrial acoplado a produção de ATP, logo, é um marcador global de “eficiência” da respiração mitocondrial. No nosso estudo, a melhora da BCE foi um preditor independente de sobrevida, a curto e a médio prazo. Não encontramos a mesma associação ao avaliar a respiração basal, a respiração acoplada a Complexo I e a respiração acoplada a Complexo II. Estes dados sugerem que, ao se avaliar o metabolismo mitocondrial como um potencial marcador de prognóstico, novos estudos devem ter o foco em uma avaliação global do metabolismo, como é proposto pela BCE, ao invés de avaliar diferentes intervalos da cadeia respiratória.

Nossos resultados apontam também para uma melhora em outros desfechos clinicamente relevantes, que não a mortalidade: pacientes com melhora da disfunção mitocondrial no D3 obtiveram um menor tempo de internação na UTI e uma menor incidência de evolução para a necessidade de ventilação mecânica e de terapia dialítica, o que reforça a relevância dos nossos achados. Sabe-se que a linfopenia persistente se associa a uma maior incidência de infecções nosocomiais em doentes críticos (Adrie C, Lugosi M, Sonneville R, 2017), no entanto, nosso estudo não foi planejado para avaliar este desfecho, que também apresenta uma grande relevância clínica. A contribuição da linfopenia, em associação ou não a uma produção prejudicada de ATP pelo linfócito, deve gerar uma perspectiva futura de pesquisa na área.

Uma das respostas inadequadas do curso da sepse, que pode se associar a desfechos clínicos deletérios, é em casos de manutenção de um estado de imunossupressão relacionado a atividade antinflamatória; progredindo para um quadro de síndrome de PIICS, a qual pode acarretar num estado de doença crítica crônica (Mira JC, Gentile LF, Mathias BJ, 2017). Durante a sepse, há uma expansão de células mieloides, em detrimento da linfo e da hematopoiese, promovendo anemia e linfopenia (Ueda Y, Kondo M, Kelsoe G, 2005). Um dos principais sinalizadores clínicos de evolução para doença crítica crônica é a necessidade de traqueostomia nestes pacientes (Mira JC, Brakenridge SC, Moldawer LL, 2017). Embora no nosso estudo não tenhamos encontrado uma associação entre a evolução para traqueostomia – que é um marcador clínico de doença crítica crônica - e atividade pelo BCE, nosso tamanho amostral não apresenta poder estatístico para avaliar tal associação, assim como não foi desenhado, inicialmente, uma avaliação sistemática da incidência de PIICS.

Limitações e perspectivas futuras

Este trabalho apresenta diversas limitações, que devem ser devidamente ressaltadas. Trata-se de um estudo unicêntrico, em uma população diversa de doentes sépticos, que, no entanto, pode limitar a generalização dos dados. Sob uma perspectiva futura, a realização de uma coorte multicêntrica tende a contemplar uma maior diversidade populacional e de espectros da síndrome séptica, aumentado a validade externa dos dados.

Trata-se de uma população com uma mortalidade a curto e a longo prazo elevadas, compatíveis com a sua mortalidade predita pelo escore SAPS 3. Embora a mortalidade encontrada seja semelhante à de outros estudos (Buchman TG, Simpson SQ, Sciarretta KL, 2020) (Machado FR, Cavalcanti AB, Bozza FA, 2017) (Conde KAP, Silva E, Silva CO, 2013) é uma população cuja magnitude das suas falências orgânicas, quantificada pelo escore SOFA, é discretamente menor que a encontrada em estudos contemporâneos sobre sepse (Fowler III AA, Truwit JD, Hite RD, 2019) (Lamontagne F, Masse MH, Menard J, 2022). Logo, inferimos que seja uma população com doenças de base de grande relevância, porém com falências orgânicas de menor magnitude. Tentamos analisar os nossos dados comparando a atividade mitocondrial em pacientes com sepse adquirida na comunidade e com sepse nosocomial, no entanto, sem encontrar diferença neste grau de estratificação. Utilizamos os critérios diagnósticos para choque séptico de consensos anteriores (Bone RC, Balk RA, Cerra FB, 1992), tendo havido uma atualização dos critérios ao longo da realização do estudo (Singer M, Deutschman CS, Seymour CW, 2016). A

nossa análise posterior, comparando a atividade mitocondrial de pacientes portadores de choque séptico pelo critério Sepse 2 e os pacientes perfazendo critérios através do Sepse 3 não encontrou diferença entre os grupos. Uma avaliação mais heterogênea, em uma população de menor gravidade, baseada nos critérios diagnósticos atuais de choque séptico, poderia conferir maior relevância e aplicabilidade aos nossos resultados.

Nossos dados sugerem uma maior relevância clínica na análise da BCE, em comparação a respiração basal, a respiração de Complexo I e a respiração de Complexo II. Logo, este parece ser o biomarcador de eleição para a mensuração da atividade mitocondrial em linfócitos. Estes resultados apresentam uma razoabilidade fisiopatológica, como previamente descrito. Possivelmente, em estudos posteriores, uma análise exclusiva da BCE seja pertinente, com vistas a validação dos resultados obtidos. Uma análise da BCE através da respirometria pode ser impraticável em pequenos centros, devido ao consumo de insumos e ao tempo empregado na análise. Esta limitação, no entanto, pode ser ultrapassada através de métodos colorimétricos, que ainda carecem de validação.

Em outras etiologias de choque, como no caso do choque cardiogênico, busca-se também compreender a interação entre as variáveis hemodinâmicas e perfusionais com o metabolismo celular, especialmente através da avaliação da relação lactato:piruvato (O’Brien C, Beaubien-Souligny W, Amsallem M, 2020). O nosso modelo de avaliação em tempo real da respiração celular é de mais fácil execução, com maior aplicabilidade clínica. Desta maneira, acreditamos que o nosso modelo de mensuração possa ser expandido para áreas de pesquisa em choques de outras etiologias.

A nossa análise do perfil inflamatório da sepse e a sua interação com o metabolismo linfocitário foi bastante limitada, em virtude das poucas interleucinas avaliadas (IL-1, IL-6, IL-10). Embora sejam as interleucinas mais relevantes para a compreensão da sepse, seria pertinente realizar uma análise de interleucinas de produção linfocitária, como a IL-17, a IL-21, o TNF-$\propto$ e o IFN-γ (de Pablo R, Monserrat J, Prieto A, 2014) e a sua interação com a OXPHOS. Uma linha de estudo posterior a ser considerada é a de também se avaliar, concomitantemente, a resposta da OXPHOS em monócitos, e a sua resposta a injúria quando comparada a de linfócitos. Em virtude do papel destas células na resposta a sepse (Ferreira da Mota NV, Brunialti MKC, Santos SS, 2018), esta seria uma análise pertinente, inclusive como forma de se avaliar se analisar-se adicionalmente os monócitos pode ou não conferir uma avaliação prognóstica e de resposta clínica adicional àquela encontrada nos linfócitos. Como também seria relevante, do ponto de vista fisiopatológico, termos realizado citometria de fluxo, a fim de compreender a resposta de cada subpopulação linfocitária – em especial os subtipos de linfócitos T – ao estímulo inflamatório, e o seu metabolismo em específico, como forma de resposta a injúria. Deste modo, seria possível uma melhor compreensão da progressão destes pacientes, sobreviventes a fase inicial de injúria da sepse, a um quadro de PIICS, e a sua correlação a resposta metabólica linfocitária. Assim, uma análise através de novo estudo, abordando com maior ênfase o quadro de imunossupressão, seria de grande relevância, considerando-se a interação entre o metabolismo linfocitário, a incidência de infecções posteriores à sepse (tanto durante a internação hospitalar como no pós-alta) e, possivelmente, a qualidade de vida e funcionalidade posterior, com foco em desfechos centrados no paciente.

Como já salientamos anteriormente, seria impossível realizar este tipo de estudo em órgãos-alvo, como o cérebro, coração, fígado e rins, que são fundamentais na compreensão da falência multiorgânica secundária a sepse. No entanto, uma possibilidade a ser considerada é a avaliação destes órgãos-alvo em estudos animais, comparando-se a resposta destas células em comparação a resposta encontrada em linfócitos. Dados semelhantes já foram avaliados em modelos de choque hemorrágico, demonstrando comportamentos distintos órgão-dependente (Karamercan MA, Weiss SL, Villaroel JPP, et al, 2013).

Considerações finais

A disfunção mitocondrial apresenta uma correlação relevante com a evolução das falências multiorgânicas na sepse, sendo, possivelmente, um dos principais fenômenos causadores desta complicação. A mensuração da atividade mitocondrial, especialmente em linfócitos, é um método de fácil execução e potencialmente aplicável no cenário clínico, podendo ser um potencial biomarcador a ter o seu impacto medido em estudos posteriores.

A atividade mitocondrial se associa, em curto prazo, a respostas inflamatórias específicas, relacionadas a ativação de linfócitos e expressão de interleucinas. Ela aparenta apresentar um curso clínico dissociado dos marcadores perfusionais, sendo uma avaliação complementar a primeira numa compreensão dos níveis séricos de lactato nesta população. Não parece ser influenciada pela administração de diferentes antimicrobianos, de diferentes classes, em monoterapia ou em uso combinado. O impacto da utilização de outros fármacos comumente usados nestes pacientes, no entanto, deve ser melhor estudado posteriormente.

Neste trabalho, conseguimos avaliar a interação entre o metabolismo mitocondrial e as variáveis perfusionais no choque séptico, demonstrando a interação de ambos com os níveis de lactato. Compreendemos uma revisão ampla dos estudos clínicos que atualmente justificam a avaliação da disfunção mitocondrial, e do metabolismo mitocondrial em específico, como uma ferramenta potencial para acompanhamento em estudos posteriores. Foi possível empreender uma avaliação básica do perfil de resposta de citocinas e sua associação com o metabolismo mitocondrial de linfócitos. A avaliação dos

diferentes antimicrobianos com o metabolismo mitocondrial foi prejudicada pelo tamanho amostral insuficiente para se refutar plenamente uma ausência de associação entre as variáveis. Também não foi possível realizar uma avaliação robusta de outros desfechos clínicos relevantes, que não a mortalidade: incidência de reinfecções, necessidade de terapias de suporte, qualidade de vida a longo prazo.

Deve-se também levar em consideração, futuramente, o impacto da disfunção do metabolismo linfocitário para um estado de imunossupressão e de catabolismo persistente, que não é incomum nestes pacientes.

Bibliografia

Adrie C, Lugosi M, Sonneville R, et al. Persistent lymphopenia is a risk factor for ICU-acquired infections and for death in ICU patients with sustained hypotension at admission. Annals Intensive Care 2017;30:7.

Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369:840-51.

Araujo DT, Felice VB, Meregalli AF, et al. Value of Central Venous to Arterial CO_2 Difference after Early Goal-directed Therapy in Septic Shock Patients. Indian J Crit Care 2019;23:449-453.

Arulkumaran N, Routledge M, Schlebusch S, et al. Antimicrobial-associated harm in critical care: a narrative review. Intensive Care Med 2020,46:225-35.

Astiz ME, Rackow EC, Kaufman B, et al. Relationship of oxygen delivery and mixed venous oxygenation to lactic acidosis in patients with sepsis and acute myocardial infarction. Crit Care Med 1988;16:655–58.

Bakker J, Postelnicu R, Mukherjee V. Lactate: Where are we now? Crit Care Clin 2020;36:115-124.

Belikova I, Lukaszewicz AC, Faivre V, et al. Oxygen consumption of human peripheral blood mononuclear cells in severe human sepsis. Crit Care Med 2007;35:2702–28.

Bone RC, Balk RA, Cerra FB, et al. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864-874.

Brealey D, Brand M, Hargreaves I, et al. Association between mitochondrial dysfunction and severity and outcome of septic shock. Lancet 2002;360:219-23.

Buchman TG, Simpson SQ, Sciarretta KL, et al. Sepsis Among Medicare Beneficiaries.Crit Care Med 2020;48:276–288.

Carré JE, Orban J-C, Re L, et al. Survival in critical illness is associated with early activation of mitochondrial biogenesis. Am J Respir Crit Care Med 2010;182:745–51.

Carta A, de Lucca MG, Pires MD, et al. Sepsis-associated organ dysfunction and increased supportive care are associated with high serum interleukin-6 levels. J Bras Patol Med Lab 2016;52:367–373.

Carteri RB, Kopczynski A, Rodolphi MS, et al. Testosterone administration after traumatic brain injury reduces mitochondrial dysfunction and neurodegeneration. J Neurotrauma 2019;36:2246-59.

Cecconi M, de Backer D, Antonelli M, et al. Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med 2014;40:1795-1815.

Cecconi M, Evans L, Levy M, et al. Sepsis and septic shock. Lancet 2018;392:75-87.

Certo M, Tsai CH, Pucino, V, et al. Lactate modulation of immune responses in inflammatory versus tumour micro-environments. Nature Rev Immunol 2021;21:151–161.

Chapman NM, Chi H. Metabolic adaptation of lymphocytes in immunity and disease. Immunity 2022;55:14-30.

Cheng SC, Scicluna BP, Arts RJW, et al. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. Nat Immunol 2016;17:406–13.

Conde KAP, Silva E, Silva CO, et al. Differences in Sepsis Treatment and Outcomes between Public and Private Hospitals in Brazil: A Multicenter Observational Study. PLoS One 8:e64790.

Coopersmith CM, De Backer D, Deutschman CS, et al. Surviving sepsis campaign: research priorities for sepsis and septic shock. Intensive Care Med 2018;44:1400-1426.

Conway-Morris A, Wilson J, Shankar-Hari M. Immune Activation in Sepsis. Crit Care Clin 2018;34:29–42.

Corrêa TD, Pereira AJ, Brandt S, et al. Time course of blood lactate levels, inflammation, and mitochondrial function in experimental sepsis. Crit Care 2017;21:1–12.

de Grooth H, Geenen IL, Girbes AR, et al. SOFA and mortality endpoints in randomized controlled trials: a systematic review and meta-regression analysis. Crit Care 2017;21:38.

Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun 2018;70:61-75.

de Pablo R, Monserrat J, Prieto A, et al. Role of circulating lymphocytes in patients with sepsis. Biomed Res Int 2014;2014:671087.

Duewelhenke N, Krut O, Eysel P. Influence on mitochondria and cytotoxicity of different antibiotics administered in high concentrations on primary human osteoblasts and cell lines. Antimicrob Agents Chemother 2007;51:54-63.

Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: international guidelines for management of sepsis and septic shock 2021. Crit Care Med 2021;49:e1063-e1143.

Ferreira da Mota NV, Brunialti MKC, Santos SS, et al. Immunophenotyping of Monocytes During Human Sepsis Shows Impairment in Antigen Presentation: A Shift Toward Nonclassical Differentiation and Upregulation of FCγRi-Receptor. Shock 2018;50:293-300.

Finfer S, Venkatesh B, Hotchkiss RS, et al. Lymphopenia in sepsis – an acquired immunodeficiency? Immunol Cell Biol 2022;1-10.

Fowler III AA, Truwit JD, Hite RD, et al. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure. The CITRIS-ALI Randomized Clinical Trial. J Am Med Assoc 2019;322:1261-1270.

Friedman G, de Backer D, Shahla M, et al. Oxygen supply dependency can characterize septic shock. Intensive Care Med 1998;24:118-23.

Galley HF. Oxidative stress and mitochondrial dysfunction in sepsis. Br J Anaesth 2011;107:57-64.

Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. Crit Care 2014;18:503.

Gleeson LE, Sheedy FJ. Metabolic reprogramming & inflammation: Fuelling the host response to pathogens. Semin Immunol 2016;28:450–468.

Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. BMJ 2016;353:i1585.

Gnaiger E. Mitochondrial Pathways and Respiratory Control An Introduction to OXPHOS Analysis The Blue Book Mitochondrial Pathways and Respiratory Control An Introduction to OXPHOS Analysis. 4th Ed. OROBOROS MiPNet Publications; 2014.

Griffiths HR, Gao D, Pararasa D. Redox regulation in metabolic programming and inflammation. Redox Biol 2017;12:50-57.

Haji-Michael PG, Ladrière L, Sener A, et al. Leukocyte glycolysis and lactate output in animal sepsis and ex vivo human blood. Metabolism 1999;48:779-85.

Hariri G, Joffre J, Leblanc G, et al. Narrative review: clinical assessment of peripheral tissue perfusion in septic shock. Ann Intensive Care 2019;9:37.

Hartmann C, Radermacher P, Wepler M, et al. Non-hemodynamic effects of cathecolamines. Shock 2017;48:390-400.

Hernandez G, Bellomo R, Bakker J. The ten pitfalls of lactate clearance in sepsis. Intensive Care Med 2019;45:82-85.

Hernández G, Ospina-Tascón GA, Damiani LP, et al. Effect of a Resuscitation Strategy Targeting Peripheral Perfusion Status vs Serum Lactate Levels on 28-Day Mortality Among Patients With Septic Shock. The ANDROMEDA-SHOCK Randomized Clinical Trial. J Am Soc Assoc 2019;321:654-664.

Ho KM, Lipman J: An update on C-reactive protein for intensivists. Anaesth Intensive Care 2009; 37:234–241.

Hsiao CP, Hoppel C. Analyzing mitochondrial function in human peripheral blood mononuclear cells. Anal Biochem 2018;549:12–20.

Iepsen UW, Plovsing RR, Tjelle K, et al. The role of lactate in sepsis and COVID-19: Perspective from contracting skeletal muscle metabolism. Exp Phisiol 2022;107:665-673.

Jacob M, Chappell D, Becker BF. Regulation of blood flow and volume exchange across the microcirculation. Crit Care 2016;20:319.

Jang DH, Greenwood JC, Spyres MB, et al. Measurement of Mitochondrial Respiration and Motility in Acute Care: Sepsis, Trauma, and Poisoning. J Intensive Care Med 2017;32:86–94.

Jansen TC, van Bommel J, Bakker J. Blood lactate monitoring in critically ill patients: a systematic health technology assessment. Crit Care Med 2009;37: 2827–39.

Japiassú AM, Santiago APSA, D'Avila JDCP, et al. Bioenergetic failure of human peripheral blood monocytes in patients with septic shock is mediated by reduced F1Fo adenosine-5′-triphosphate synthase activity. Crit. Care Med 2011;39:1056-63.

Jones AE, Shapiro NI, Trzeciak S, et al. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. JAMA. 2010,303:739-746.

Kalghatgi S, Spina CS, Costello JC, et al. Bactericidal Antibiotics Induce Mitochondrial Dysfunction and Oxidative Damage in Mammalian Cells. Sci Transl Med 2013;5:192ra85.

Kalil AC, Metersky ML, Klompas M, et al. Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clinical Infectious Diseases 2016;63:e61-e111.

Karakike E, Kyriazopoulou E, Tsangaris I, et al. The early change of SOFA score as a prognostic marker of 28-day sepsis mortality: analysis through a derivation and validation cohort. Crit Care 2019;23:387.

Karamercan MA, Weiss SL, Villaroel JPP, et al. Can peripheral blood mononuclear cells be used as a proxy for mitochondrial dysfunction in vital organs during hemorrhagic shock and resuscitation? Shock 2013;40:476-84.

Koutroulis I, Batabyal R, McNamara B, et al. Sepsis Immunometabolism: From Defining Sepsis to Understanding How Energy Production Affects Immune Response. Crit Care Explor 2019; 1:e0061.

Kozlov AV, Bahrami S, Calzia E. Mitochondrial dysfunction and biogenesis: do ICU patients die from mitochondrial failure? Ann Intensive Care 2011;1:41.

Kraft BD, Chen L, Suliman HB, et al. Peripheral Blood Mononuclear Cells Demonstrate Mitochondrial Damage Clearance During Sepsis. Crit Care Med 2009;47:651–658.

Kramer PA, Ravi S, Chacko B, et al. A review of the mitochondrial and glycolytic metabolism in human platelets and leukocytes: implications for their use as bioenergetic biomarkers. Redox Biol 2014;2:206-210.

Kushimoto S, Akaishi S, Sato T, et al. Lactate, a useful marker for disease mortality and severity but an unreliable marker of tissue hypoxia/hypoperfusion in critically ill patients. Acute Med Surg 2016;3:293-297.

Lambden S, Laterre PF, Levy MM, et al. The SOFA score – development, utility and challenges of accurate assessment in clinical trials. Crit Care 2019;23:374.

Lamontagne F, Masse MH, Menard J, et al. Intravenous Vitamin C in Adults with Sepsis in the Intensive Care Unit. N Engl J Med 2022;386:2387-2398.

Liu H, Yang H, Chen X, et al. Cellular metabolism modulation in T lymphocyte immunity. Immunology 2014; doi: 10.1111/imm.12321.

Liu VX, Fielding-Singh V, Greene JD, et al. The Timing of Early Antibiotics and Hospital Mortality in Sepsis. Am J Respir Crit Care Med 2017;196:856-863.

Lunemann JD, Buttgereit F, Tripmacher R, et al. Norepinephrine inhibits energy metabolism of human peripheral blood mononuclear cells via adrenergic receptors. Biosci Rep 2001;21:627-635.

Machado FR, Cavalcanti AB, Bozza FA, et al. The epidemiology of sepsis in Brazilian intensive care units (the Sepsis Prevalence Assessment Database, SPREAD): an observational study. Lancet Infect Dis 2017;17:1180-1189.

Macintyre AN, Rathmell JC. Activated lymphocytes as a metabolic model for carcinogenesis. Cancer Metab 2013;1:5.

Makrecka-Kuka M, Krumschnabel G, Gnaiger E. High-Resolution Respirometry for Simultaneous Measurement of Oxygen and Hydrogen Peroxide Fluxes in Permeabilized Cells, Tissue Homogenate and Isolated Mitochondria. Biomolecules 2015;5:1319–38.

Martínez-García JJ, Martínez-Banaclocha H, Angosto-Bazarra D, et al. P2X7 receptor induces mitochondrial failure in monocytes and compromises NLRP3 inflammasome activation during sepsis. Nat Commun 2019;10:1–14.

Masyuk M, Wernly B, Lichtenauer M, 2019. Prognostic relevance of serum lactate kinetics in critically ill patients. Intensive Care Med 2019;45:55-61.

McCall CE, Zabalawi M, Liu T, et al. Pyruvate dehydrogenase complex stimulation promotes immunometabolic homeostasis and sepsis survival. JCI Insight 2018;3:e99292.

Merz TM, Pereira AJ, Schürch R, et al. Mitochondrial function of immune cells in septic shock: A prospective observational cohort study. PLoS One 2017;12:e0178946.

Messina A, Greco M, Cecconi M. What should I use next if clinical evaluation and echocardiographic haemodynamic assessment is not enough? Curr Opin Crit Care 2019;25:259–65.

Metlay JP, Waterer GW, Long AC, et al. Diagnosis and Treatment of Adults with Community-acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. American Journal of Respiratory and Critical Care Medicine 2019;200:e45-e67.

Mikkelsen ME, Miltiades AN, Gaieski DF, et al. Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock. Crit Care Med 2009;37:1670-1677.

Mira JC, Brakenridge SC, Moldawer LL, et al. Persistent Inflammation, Immunosuppression and Catabolism Syndrome. Crit Care Clin 2017;33:245-258.

Mira JC, Gentile LF, Mathias BJ, et al. Sepsis pathophysiology, chronic critical illness, and persistent inflammation-immunosuppression and catabolism syndrome. Crit Care Med 2017;45:253–262.

Nguyen HB, Loomba M, Yang JJ, et al. Early lactate clearance is associated with biomarkers of inflammation, coagulation, apoptosis, organ dysfunction and mortality in severe sepsis and septic shock. J Inflamm 2010;7:6.

Nolt B, Tu F, Wang X, et al.: Lactate and Immunosuppression in Sepsis. Shock 2018;49:120–125.

Nunnally ME, Ferrer R, Martin GS, et al. The Surviving Sepsis Campaign: research priorities for the administration, epidemiology, scoring and identification of sepsis. Intensive Care Med Exp 2021;9:34.

O'Brien C, Beaubien-Souligny W, Amsallem M, et al. Cardiogenic Shock: Reflections at the Crossroad Between Perfusion, Tissue Hypoxia, and Mitochondrial Function. Can J Cardiol 2020;36:184-196.

Park DW, Zmijewski JW. Mitochondrial dysfunction and immune cell metabolism in sepsis. Infect Chemother 2017;49:10-21.

Pearce EJ, Pearce EL. Immunometabolism in 2017: Driving immunity: All roads lead to metabolism. Nat Rev Immunol 2018;18:81–2.

Pearce EL, Poffenberger MC, Chang CH, et al. Fueling immunity: insights into metabolism and lymphocyte function. Science 2013;342:1242454.

Pecina P, Houšťková H, Mráček T, et al. Noninvasive diagnostics of mitochondrial disorders in isolated lymphocytes with high resolution respirometry. BBA Clin 2014;2:62–71.

Pène F, Ait-Oufella H, Taccone FS, et al. Insights and limits of translational research in critical care medicine. Ann Intensive Care 2015;5:8.

Piel DA, Gruber PJ, Weinheimer CJ, et al. Mitochondrial resuscitation with exogenous cytochrome c in septic heart. Crit Care Med 2007;35:2120-7.

Pool R, Gomez H, Kellum JA. Mechanisms of Organ Dysfunction in Sepsis. Crit Care Clin 2018;34:63-80.

Póvoa P, Coelho L, Dal-Pizzol F, et al. How to use biomarkers of infection or sepsis at the bedside: guide to clinicians. Intensive Care Med 2023;49:142-153.

Póvoa P, Teixeira-Pinto AM, Carneiro AH. C-reactive protein, an early marker of community-acquired sepsis resolution: a multi-center prospective observational study. Crit Care 2011;15:R169.

Pyle A, Burn DJ, Gordon C, et al. Fall in circulating mononuclear cell mitochondrial DNA content in human sepsis. Intensive Care Med. 2010;36:956–62.

Puskarich MA, Kline JA, Watts JA, et al. Early alterations in platelet mitochondrial function are associated with survival and organ failure in patients with septic shock. J Crit Care 2016;31:63–7.

Rudd KE, Kisson N, Limmathurotsakul D, et al. The global burden of sepsis: barriers and potential solutions. Crit Care 2018;22:232.

Ryoo SM, Han KS, Ahn S, et al. The usefulness of C-reactive protein and procalcitonin to predict prognosis in septic shock patients: A multicenter prospective registry-based observational study. Sci Rep 2019;9:6579.

Silva E, Pedro MA, Sogayar ACB, et al. Brazilian Sepsis Epidemiological Study (BASES study). Crit Care 2004;8:R251-R260.

Singer M. Critical illness and flat batteries. Crit Care 2017;21:309.

Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). J Am Med Soc 2016;315:801-810.

Sjovall F, Morota S, Hansson MJ, et al. Temporal increase of platelet mitochondrial respiration is negatively associated with clinical outcome in patients with sepsis. Crit Care 2010;14:R214.

Sjövall F, Morota S, Persson J, et al. Patients with sepsis exhibit increased mitochondrial respiratory capacity in peripheral blood immune cells. Crit Care 2013;17:R152.

Sogayar AMC, Machado FR, Rea-Neto A, et al. A Multicentre, Prospective Study to Evaluate Costs of Septic Patients in Brazilian Intensive Care Units. Pharmacoeconomics 2008;26:425-434.

Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: Guidelines by the surgical infection society and the infectious diseases society of america. Clinical Infectious Diseases 2010;50:133-164.

Song J, Park DW, Moon S, et al. Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: A prospective controlled study according to the Sepsis-3 definitions. BMC Infect Dis 2019;19:968.

Sterling SA, Puskarich MA, Shapiro NI, et al. Characteristics and outcomes of patients with vasoplegic versus tissue dysoxic septic shock. Shock. 2013,40:11-14.

Suetrong B, Walley KR. Lactic acidosis in sepsis: It's Not All anaerobic: Implications for diagnosis and management. Chest 2016;149:252–61.

Takahashi K, Tamura Y, Kitaoka Y, et al. Effects of Lactate Administration on Mitochondrial Respiratory Function in Mouse Skeletal Muscle. Front Physiol 2022;13:920034.

Tapia P, Soto D, Bruhn A, et al. Impairment of exogenous lactate clearance in experimental hyperdynamic septic shock is not related to total liver hypoperfusion. Crit Care 2015;19:188.

Toro J, Manrique-Caballero CL, Gómez H: Metabolic Reprogramming and Host Tolerance: A Novel Concept to Understand Sepsis-Associated AKI. J Clin Med 2021;10:4184.

Tune BM, Hsu CY. The renal mitochondrial toxicity of beta-lactam antibiotics: in vitro effects of cephaloglycin and imipenem. J Am Soc Nephrol 1990;1:815-21.

Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, et al. Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: Implications for a minimally invasive biomarker of mitochondrial health. Redox Biol 2016;10:65–77.

Ueda Y, Kondo M, Kelsoe G. Inflammation and the reciprocal production of granulocytes and lymphocytes in bone marrow. J Exp Med 2005;201:1771-80.

Vahedi HSM, Bagheri A, Jahanshir A, et al. Association of Lymphopenia with Short Term Outcomes of Sepsis Patients; a Brief Report. Arch Acad Emerg Med 2019;7:e14.

van Genderen ME, Engels N, van der Valk RJP, et al. Early peripheral perfusion-guided fluid therapy in patients with septic shock. Am J Respir Crit Care Med 2015;191:477-80.

Venet F, Demaret J, Blaise BJ, et al. IL-7 Restores T Lymphocyte Immunometabolic Failure in Septic Shock Patients through mTOR Activation. J Immunol 2017;199:1606-1615.

Vincent JL, Quintairos e Silva A, Couto Jr L, et al. The value of blood lactate kinetics in critically ill patients: a systematic review. Crit Care 2016;20:257.

Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. Intensive Care Med 1996;22:707-710.

Vivas MC, Villamarin Guerrero HF, Tascon AJ, et al.: Plasma interleukin-6 levels correlate with survival in patients with bacterial sepsis and septic shock. Interv Med Appl Sci 2021;11:224–230.

Weiss SL, Zhang D, Bush J, et al. Persistent Mitochondrial Dysfunction Linked to Prolonged Organ Dysfunction in Pediatric Sepsis. Crit Care Med 2019;47:1433–41.

Williams NC, O'Neill LAJ. A Role for the Krebs Cycle Intermediate Citrate in Metabolic Reprogramming in Innate Immunity and Inflammation. Front Immunol 2018;9:141.

Yang Y, Xie J, Guo F, et al. Combination of C-reactive protein, procalcitonin and sepsis-related organ failure score for the diagnosis of sepsis in critical patients. Ann Intensive Care 2016;6:51.

Yang L, Xie M, Yang M, et al. PKM2 regulates the Warburg effect and promotes HMGB1 release in sepsis. Nat Commun 2014;5:4436.