Universidade Federal do Rio Grande do Sul

Faculdade de Medicina

Programa de Pós-graduação em Ciências Médicas: Endocrinologia

Complicações endocrinológicas da doença crítica: da hiperglicemia de estresse à

fraqueza muscular adquirida na unidade de terapia intensiva

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Esta tese de Doutorado segue o formato proposto pelo Programa de Pós-graduação em Ciências Médicas: Endocrinologia da Faculdade de Medicina da Universidade de Federal do Rio Grande do Sul, sendo apresentada na forma de uma breve introdução sobre o assunto, seguida de três artigos (dois artigos originais e um de revisão sistemática e matanálise) sobre o tema da tese.

Artigo 1: "Diabetes associates with mortality in critically ill patients with SARS-CoV-2 pneumonia: no diabetes paradox in COVID-19"

Artigo 2: "Association between diabetes and stress-induced hyperglycemia with skeletal muscle gene expression of *IRS1*, *IRS2*, *INSR*, *SLC2A1,* and *SLC2A4* in critically ill patients: a prospective cohort study"

Artigo 3: "Association between neuromuscular blocking agents and the development of intensive care unit-acquired weakness (ICU-AW): A systematic review with meta-analysis and trial sequential analysis"

SUMÁRIO

ÍNDICE DE ABREVIATURAS

1. INTRODUÇÃO

ATP: adenosina trifosfato

DM: diabetes *mellitus*

ECA-2: enzima conversora de angiotensina 2

FA-UTI: fraqueza muscular adquirida na unidade de terapia intensiva

GLUT: transportadores de glicose

GLUT-1: transportador de glicose 1

GLUT-4: transportador de glicose 4

HbA1c: hemoglobina glicada

IL-1: interleucina-1

IL-6: interleucina-6

INSR: gene receptor da insulina

PICS: *pos-intensive care syndrome* – síndrome pós-cuidados intensivos

SARS-CoV-2: coronavírus do tipo 2

SDRA: síndrome do desconforto respiratório agudo

TNF-α: fator de necrose tumoral alfa

UTI: unidade de terapia intensiva

2. ARTIGOS

ADA: American Diabetes Association

aOR: adjusted odds ratio

ARDS: acute respiratory distress syndrome

CI: confidence intervals

BMI: body mass index

DM: diabetes *mellitus*

GLUT-1: glucose transporter-1

GLUT-4: glucose transporter-4

HbA1c: glycated hemoglobin

HR: hazard ratio

ICU: intensive care unit

ICU-AW: intensive care unit-acquired weakness

IRS1: insulin receptor substrate 1 gene

IRS2: insulin receptor substrate 2 gene

ISNR: insulin receptor gene

LOS: length of stay

MD: mean differences

MRC: Medical Research Council

MV: mechanical ventilation

NMBA: neuromuscular blocking agents

OR: odds ratio

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT: randomized clinical trial

ROB: risk of bias

RRT: renal replacement therapy

RT-qPCR: quantitative real-time polymerase chain reaction method

SAPS 3: Simplified Acute Physiology Score 3

SD: standard deviation

SLC2A1: solute carrier family 2, member 1 gene

SLC2A4: solute carrier family 2, member 4 gene

TSA: trial sequential analysis

RESUMO

A hiperglicemia de estresse refere-se à hiperglicemia e à intolerância à glicose, decorrentes da resistência à ação da insulina, que ocorrem como resposta metabólica à doença aguda. Se, por um lado, a hiperglicemia é vista como resposta adaptativa e benéfica, por outro, ela tem sido apontada como mal adaptativa. Inúmeros mecanismos envolvendo o eixo hipotálamo-hipófise-adrenal, a liberação de hormônios contrarreguladores de insulina e a liberação de citocinas inflamatórias interagem de forma complexa aumentando a disponibilidade de glicose durante a doença aguda grave, o que garante uma oferta de glicose suficiente a tecidos vitais. A hiperglicemia é, dessa forma, vista como uma resposta evolutiva atuando como combustível necessário para a necessidade de o organismo "lutar ou fugir". Porém, estudos têm demonstrado piores desfechos, incluindo aumento de mortalidade, em pacientes com hiperglicemia de estresse. A relação causal entre hiperglicemia e mortalidade não é clara, podendo ser apenas um marcador de gravidade da doença. Postula-se que a hiperglicemia leve a disfunções mitocondrial, endotelial e imunológica, culminando em uma resposta imune insatisfatória do organismo.

A hiperglicemia de estresse não parece ter a mesma magnitude em subgrupos diferentes de pacientes. Em pacientes com diabetes *mellitus* (DM), por exemplo, que são cronicamente expostos aos efeitos pró-inflamatórios, pró-trombóticos e pró-oxidativos de níveis cronicamente elevados de glicose, a correção intensiva da hiperglicemia por meio do uso de insulina endovenosa parece relacionar-se a piores desfechos. Sendo assim, dentro da uma perspectiva de medicina de precisão, o estado metabólico prévio pode ser importante para a individualização de alvos de controle glicêmico.

Outro subgrupo particular de pacientes criticamente doentes é o de pacientes com DM e infecção por COVID-19, que tem sido acometido pela forma mais grave da doença viral. Acredita-se que os pacientes com DM, que se apresentam num estado basal de inflamação crônica, possam ter um sistema imune debilitado e sejam mais suscetíveis à "tempestade de citocinas" que ocorre durante a replicação viral. Em pacientes com DM e COVID-19 internados na unidade de terapia intensiva (UTI), um dos tantos desafios do tratamento é manter o controle glicêmico adequado.

Seguindo a linha da medicina de precisão, é plausível que subgrupos de pacientes com diferentes assinaturas genéticas respondam de forma diferente à exposição à hiperglicemia e, nesse contexto, a avaliação genética dos pacientes criticamente doentes pode ser muito interessante. Estudos têm demonstrado uma redução da translocação de transportadores de glicose para a membrana celular em contexto de estresse agudo e intensa resposta inflamatória. Alterações no gene receptor da insulina também têm sido identificadas e podem associar-se à resistência à ação da insulina e à hiperglicemia crônica.

Com o avanço da terapia intensiva, um número cada vez maior de sobreviventes à doença crítica aguda tem levado a novas condições clínicas, como a síndrome pós-cuidados intensivos (PICS). A PICS é definida como o surgimento ou agravamento de danos à saúde física, mental e cognitiva após uma internação prolongada na UTI e que persistem após a alta hospitalar, levando a uma piora significativa na qualidade de vida dos sobreviventes e seus familiares. Entre as complicações mais graves da cronificação da doença crítica aguda, encontra-se a fraqueza muscular adquirida na UTI (FA-UTI). A FA-UTI corresponde à fraqueza muscular generalizada que se desenvolve no curso da internação na UTI e para qual nenhuma outra causa pode ser identificada além da própria

doença aguda e do seu tratamento. Sua patogênese é complexa, mas sabe-se que a hiperglicemia desempenha um papel fundamental no seu desenvolvimento.

Assim, o intrincado mecanismo que associa hiperglicemia, inflamação e FA-UTI deve ser melhor estudado a fim de se compreender os mecanismos fisiopatológicos e moleculares pelos quais a lesão muscular esquelética se desenvolve. Dessa forma, uma medicina individualizada e de precisão poderia ser implementada, melhorando desfechos clínicos dos pacientes criticamente doentes a curto e longo prazos.

Nessa tese, estudamos diferentes aspectos dos efeitos da hiperglicemia na doença crítica, passando pelos efeitos do controle glicêmico em pacientes criticamente doentes com COVID-19 por meio de um estudo retrospectivo, pelo impacto da hiperglicemia de estresse na expressão de genes relacionados ao transporte da glicose no tecido muscular esquelético por meio de um estudo prospectivo, até uma metanálise dos fatores de risco para desenvolvimento de FA-UTI.

ABSTRACT

Stress-induced hyperglycemia refers to the increase in blood glucose levels in the face of acute disease. While it has been recognized as adaptive and beneficial, some deleterious effects are also well described. Several mechanisms involving the hypothalamic-pituitary-adrenal axis, the secretion of counter-regulatory hormones, and the release of inflammatory cytokines contribute to increased glucose availability during severe acute illness. This response ensures an adequate supply of glucose to vital organs during critical illness, representing an evolutionary response acting as the necessary fuel for the need of the organism to "fight or flee". However, studies have reported increased mortality rates in patients with stress-induced hyperglycemia. The causal relationship between hyperglycemia and mortality has not been definitively established, and stress-induced hyperglycemia may simply serve as a marker of disease severity. It has been postulated that hyperglycemia leads to mitochondria, endothelial cells, and the immune system dysfunction, resulting in suboptimal immune response.

The relevance of stress-induced hyperglycemia varies among different patient subgroups. For instance, in patients with diabetes melitus (DM), who are chronically exposed to a low grade inflammatory, pro-thrombotic, and pro-oxidative effects of elevated blood glucose levels, the intensive correction of hyperglycemia with insulin therapy appears to be associated with worse outcomes. Therefore, the previous metabolic state is crucial for individualizing glycemic targets.

Another subgroup of critically ill patients is those with DM and COVID-19 infection, who have been afflicted by the most severe form of the viral disease. It is believed that patients with DM, who present with a chronic state of inflammation, may have a compromised immune system and be more susceptible to the "cytokine storm"

that occurs during viral replication. In patients with DM and COVID-19 admitted to the intensive care unit (ICU), one of the many treatment challenges is to maintain proper glycemic control.

In line with the principles of precision medicine, it is plausible that a subset of patients with diferent genetic signature may exhibit diferent responses to hyperglycemia. Studies have shown a reduced translocation of glucose transporters to the cell membrane during acute stress and a higher inflammatory response. Additionally, changes in the insulin receptor gene have been identified, potentially contributing to insulin resistance and chronic hyperglycemia.

The growing number of survivors of critical illness has given rise to new clinical conditions such as the post-intensive care syndrome (PICS). This condition involves the onset or worsening of physical, mental, and cognitive health problems after a prolonged ICU stay, persisting after hospital discharge and significantly impairing the quality of life of ICU survivors and their family. One of the most important components of PICS is the ICU-acquired weakness (ICU-AW), a generalized muscle weakness that develops during ICU stay, with no identifiable cause other than the acute illness and its treatment. The pathophysiology of ICU-AW is complex, but hyperglycemia is known to play a significant role in its development.

Therefore, it is crucial to further investigate the intricate interplay among hyperglycemia, inflammation, and ICU-AW. A better understanding of the pathophysiological and molecular mechanisms underlying skeletal muscle injury can pave the way for individualized approaches, ultimately improving the short and long-term clinical outcomes of critically ill patients.

This tesis was dedicated to study several aspects of the effects of hyperglycemia in critical illness. Our investigation encompassed an evaluation of the effects of glycemic

control in critically ill patients with COVID-19, using a retrospective study. Additionally, we examined the impact of stress-induced hyperglycemia on the expression of genes associated with glucose transport in skeletal muscle, employing a prospective study. Finally, we conducted a comprehensive meta-analysis to identify risk factors contributing to the development of ICU-AW.

1. INTRODUÇÃO

1.1. Cenário histórico, atual e futuro da doença crítica

A medicina intensiva como especialidade médica teve início em 1953 durante a epidemia de poliomielite, na Dinamarca, quando pacientes com insuficiência ventilatória aguda receberam ventilação mecânica invasiva pela primeira vez na história (1), embora já houvesse áreas designadas para tratar pacientes em recuperação pós-anestésica cerca de um século antes (2). Os pacientes com poliomielite passaram a receber cuidados intensivos de enfermagem e ventilação com pressão positiva por estudantes de medicina, que se revezavam 24 horas por dia. Essa abordagem inovadora para a época resultou numa significativa redução da mortalidade neste grupo de pacientes, levando à criação de unidades de terapia intensiva (UTI), locais especializados no tratamento de pacientes com condições agudas ameaçadoras à vida. Os pacientes tratados nessas unidades passaram então a ser chamados de pacientes criticamente doentes. Desde então, a expansão das UTIs não para, existindo uma tendência global de aumentar proporcionalmente o número de leitos de terapia intensiva dentro dos hospitais (3).

Há inúmeros desafios dentro da medicina intensiva atualmente (4), como, por exemplo, o fato de as síndromes tratadas nas UTIs não terem um diagnóstico simples com base em um único exame. A medicina intensiva engloba o tratamento de condições clínicas muito diversas, como sepse, choque circulatório, insuficiência respiratória, disfunções neurológicas, com suas mais diversas apresentações clínicas, laboratoriais, radiológicas e fisiológicas. Os tratamentos dessas condições também estão sob contínua revisão e debate. Além disso, o curso da doença crítica costuma ser rápido e com altos índices de mortalidade, dependendo do tipo de pacientes e dos recursos locais. No Hospital de Clínicas de Porto Alegre, por exemplo, a mortalidade é muito elevada, em

torno de 40%, porém de acordo com a mortalidade predita pelos seus escores prognósticos (5,6). Essa variabilidade de definições diagnósticas, terapêuticas e prognósticas torna a epidemiologia comparativa desafiadora no âmbito da pesquisa em medicina intensiva.

Recentemente, um termo amplamente debatido tem sido a "medicina de precisão" (7), a qual objetiva entender os fatores de saúde individuais como centrais para definir um tratamento personalizado (7). Especialmente em medicina intensiva, dada a grande heterogeneidade dos pacientes, o conceito de medicina de precisão é interessante, mas extremamente desafiador. Inicialmente, é necessário encontrar grupos e subgrupos de pacientes semelhantes (8). Além disso, é necessário considerar as múltiplas comorbidades dos pacientes, as quais tornam o processo de adoecimento complexo (9). Sabe-se que tanto fatores genéticos, quanto a a fisiopatologia da resposta à doença crítica aguda e a sua interação com as comorbidades prévias do paciente, influenciam no curso e na recuperação da doença crítica. Dessa forma, as doenças prévias e fatores ambientais podem ter um papel em alterações epigenéticas, as quais se referem a modificações químicas no DNA que regulam a expressão gênica, podendo ativar ou inativar genes (10). Tal fenômeno tem sido estudado em vias imunossupressoras da doença crítica (11). Outra dificuldade para a implementação da medicina de precisão em medicina intensiva é a indisponibilidade de análises genéticas e de biomarcadores em larga escala e em tempo real, reduzindo a aplicabilidade clínica até o momento, dada a necessidade de condutas imediatas em muito casos. Provavelmente, a medicina de precisão em medicina intensiva será uma realidade futura (8), especialmente quando for possível combinar avaliações fisiopatológicas, genéticas e prognósticas individualizadas no tratamento dos pacientes.

1.2. Hiperglicemia em resposta à doença crítica

Em 1878, Claude Bernard descreveu a ocorrência de hiperglicemia durante o choque hemorrágico (12). Desde então, muito se tem estudado sobre as alterações glicêmicas que ocorrem como resposta a doenças críticas. A resposta metabólica à lesão aguda leva à hiperglicemia ou tolerância diminuída à glicose, decorrentes da resistência à ação da insulina que, em conjunto, são denominados de hiperglicemia de estresse (13), a qual pode ser considerada uma resposta adaptativa e benéfica nesse cenário. No entanto, tal resposta fisiológica também tem sido associada a prognóstico desfavorável em pacientes criticamente doentes (14), com aumento de mortalidade, tanto em pacientes clínicos quanto cirúrgicos (5,15,16).

1.2.1. *Hiperglicemia de estresse como resposta adaptativa*

Na doença crítica, a hiperglicemia ocorre como uma resposta metabólica compensatória ao estresse agudo em consequência do aumento de hormônios contrarreguladores da insulina, mediado pelo eixo hipotálamo-hipófise-adrenal, liberação de catecolaminas endógenas e hormônio do crescimento. O cortisol desempenha um papel chave na ativação de enzimas envolvidas na gliconeogênese e glicogenólise, culminando na inibição da captação de glicose pelos tecidos periféricos, como o músculo, por exemplo (17). Além dessa via, a produção de citocinas inflamatórias age em sinergismo para induzir hiperglicemia de estresse. As interleucinas inflamatórias, que também são produzidas como resposta ao estresse, especialmente o fator de necrose tumoral alfa (TNF-α), a interleucina-1 (IL-1) e a interleucina-6 (IL-6), também contribuem para o aumento da resistência à ação da insulina, aumentando os níveis plasmáticos de glicose. Dessa forma, nota-se que os altos níveis de glicose parecem associar-se mais ao aumento da produção hepática de glicose do que à dificuldade de extração por parte dos tecidos. A

Figura 1 (13) demonstra as vias implicadas no desencadeamento da hiperglicemia de estresse.

Figura 1. A resposta neuroendócrina ao estresse é caracterizada por gliconeogênese e glicogenólise, resultando em hiperglicemia de estresse fornecendo ao sistema imune e ao cérebro uma fonte de combustível imediato. ACTH: hormônio corticotrópico; CRH: hormônio liberador de corticotrofina; LC/NE: sistema norepinefrina/locus ceruleus; NPV: núcleo paraventricular; SNS: sistema nervoso simpático. Adaptado de: Marik PE et al, Critical Care, 2013.

Assim, a hiperglicemia como consequência neuroendócrina durante a doença crítica grave pode ser vista como uma resposta adaptativa evolutiva e conservada nas espécies, que aumenta as chances de sobrevida (18), uma vez que garante uma oferta suficiente de glicose a tecidos vitais, como o cérebro, por exemplo. Estudos demonstram que o grau dessa resposta ao estresse e a severidade da hiperglicemia relacionam-se tanto com a capacidade de liberação de hormônios contrarreguladores da insulina como com a intensidade do fator estressor, com sepse, hipoxemia e hemorragia figurando entre os principais (19).

Fisiopatologicamente, a hiperglicemia de estresse resulta em um balanço glicêmico alterado, permitindo um gradiente de difusão sanguíneo maior da glicose, o

que maximiza sua captação pelos tecidos que estão sofrendo com fluxo microvascular anormal, como em casos de isquemia e sepse (20). Esses dados sugerem que níveis moderados de hiperglicemia (140 – 220 mg/dL) maximizam a captação de glicose pelos tecidos sem cursar com hiperosmolaridade. Tal fato poderia explicar por que estudos com controle glicêmico estrito associaram-se a piores desfechos em pacientes criticamente doentes, como foi o caso do NICE-SUGAR (21). Esse estudo foi um ensaio clínico randomizado, publicado em 2009, que incluiu 6.104 pacientes de UTIs clínicas e cirúrgicas para receber controle glicêmico intensivo (80 – 108 mg/dL) ou controle glicêmico convencional (144 – 180 mg/dL), onde os resultados não demonstraram benefício no grupo de controle intensivo em comparação ao grupo de controle convencional, que foi submetido a níveis glicêmicos moderados. Porém, houve maior incidência de hipoglicemia no grupo intervenção (6,8%) em comparação com o grupo controle (0,5%). Além disso, houve aumento na mortalidade no grupo de controle intensivo da glicemia (OR 1,14; IC 1,02-1,28; p=0,02).

Dessa forma, a hiperglicemia de estresse pode ser vista como um mecanismo protetor necessário para o sistema imune e para o tecido cerebral em um momento crítico, resultando em melhor plasticidade e resistência celulares aos insultos hipóxico-isquêmicos. Assim, tentativas de interferir nessa resposta adaptativa – como por meio de controle glicêmico estrito – podem ser prejudiciais no curso da doença aguda grave.

1.2.2. *Hiperglicemia de stress como resposta mal adaptativa*

Se por um lado a hiperglicemia parece ser uma resposta fisiológica ao estresse, por outro lado, há diferentes estudos demonstrando uma associação entre hiperglicemia de estresse e pior prognóstico em diversos cenários de doença crítica (5,15,16). No entanto, até o momento, não foi possível comprovar uma relação causal entre a sua

ocorrência e o aumento da mortalidade, sugerindo que ela seja apenas um marcador da gravidade de doença.

Greet Van den Bergh demonstrou em estudos com coelhos expostos a doenças críticas que a hiperglicemia desencadeia uma sobrecarga sobre o fígado e sobre o miocárdio, induzindo disfunção mitocondrial, o que poderia explicar o dano aos órgãos causado pelos níveis elevados de glicose (22). Em nível celular, estudos demonstram que a hiperglicemia pode se associar à disfunção imunológica e endotelial, influenciando tanto a migração e a capacidade fagocítica de macrófagos quanto alterando o sistema do complemento e a produção de citocinas (23). A disfunção quimiotáxica das células de defesa do organismo pode levar a uma resposta subótima do organismo à infecção. Se por um lado a produção de citocinas inflamatórias pode contribuir para a resposta imune inicial frente ao insulto agudo, por outro lado, ela exacerba o estresse oxidativo, levando ao aumento da permeabilidade vascular, disfunção mitocondrial e prejuízo à autofagia (24). Além disso, a hiperglicemia prejudica a função endotelial por inibir a formação de óxido nítrico, coibindo seu efeito vasodilatador vascular (25). A hiperglicemia também cursa com diurese osmótica por glicosúria, a qual pode levar à hipovolemia, redução da taxa de filtração glomerular e azotemia pré-renal (26). A **Figura 2** (26) resume os principais desencadeadores e as principais consequências sistêmicas deletérias da hiperglicemia de estresse.

Figura 2. Fisiopatologia da hiperglicemia e suas complicações em adultos. TG: triglicerídeos. Adaptado de: Umpierrez GE et al, Diabetes Care, 2017.
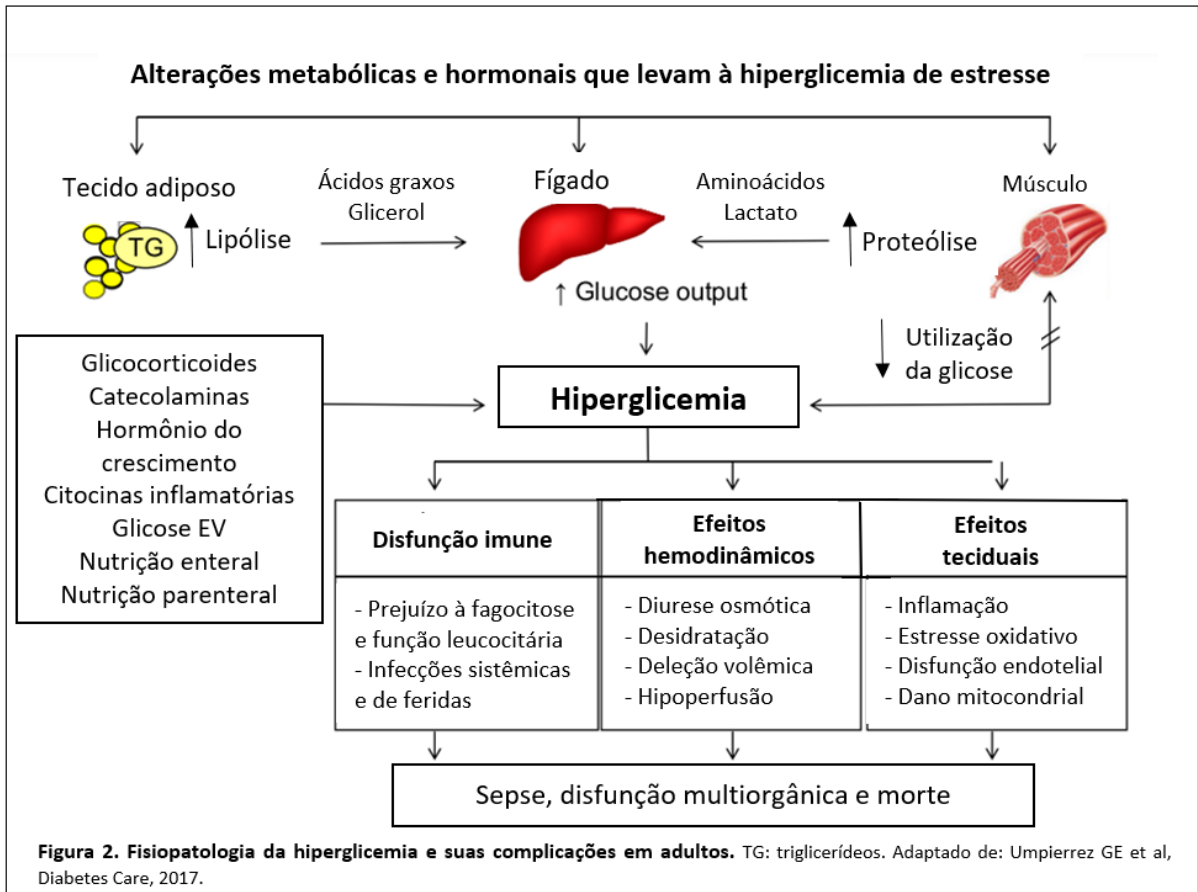
1.2.3. *O subgrupo de pacientes com diabetes mellitus (DM)*

Estudos publicados na década de 1990, como o DDCT (27) e o UKPDS (28) já demonstraram que os efeitos deletérios da hiperglicemia crônica levam a múltiplas complicações orgânicas (doença renal do diabetes, retinopatia, neuropatia e doença cardiovascular, entre outras) em pacientes ambulatoriais. Esse fato deve-se ao efeito pró-inflamatório, pró-trombótico e pró-oxidativo dos níveis persistentemente elevados de glicose (13). Ao contrário do que seria intuitivo pensar com base nas complicações e riscos associados ao DM em outros ambientes, seu impacto sobre a mortalidade de pacientes internados na UTI parece ser diferente, uma vez que em pacientes críticos o DM não se associa de forma independente ao aumento de mortalidade, condição conhecida como "paradoxo do DM" (29).

Além disso, pacientes com DM parecem apresentar piores desfechos quando submetidos a controle glicêmico intensivo durante o curso da doença crítica, ou seja, a correção da hiperglicemia de estresse parece deletéria para pacientes cronicamente expostos a níveis basais elevados de glicose. Em um estudo retrospectivo publicado em 2013, Lanspa et al. (30) analisaram 3.529 pacientes e compararam um protocolo de controle glicêmico intensivo (80 – 110 mg/dL) com um protocolo de controle moderado (90 – 140 mg/dL), sugerindo que o controle moderado da glicemia se associava a um menor risco de morte em pacientes com DM. Além disso, um estudo do tipo antes e depois, que incluiu apenas pacientes criticamente doentes com diabetes, não identificou malefício de utilizar controle liberal (180 – 252 mg/dL) nessa população (31).

Por esse motivo, parâmetros glicêmicos que levam em consideração o controle metabólico prévio à internação na UTI têm sido estudados, uma vez que podem influenciar o efeito do controle glicêmico durante a doença crítica aguda. Nesse cenário, a hemoglobina glicada (HbA1c) é capaz de separar a hiperglicemia crônica da hiperglicemia aguda induzida pelo estresse. Isso é possível porque a glicação é um processo irreversível e representa as glicemias médias das últimas 12 semanas (32). A partir da HbA1c pode-se calcular o *gap* glicêmico, que é definido pela diferença entre a glicemia na admissão na UTI e a glicemia média estimada, determinada a partir do valor de HbA1c (glicemia média estimada= 28,7 x HbA1c – 46,7 mg/dL) (33), cujo objetivo é isolar o efeito da hiperglicemia aguda. Um estudo recente do nosso grupo de pesquisa desmonstrou que que o *gap* glicêmico é um preditor independente de desfechos desfavoráveis em paciente criticamente doentes (5).

Dessa forma, nota-se que a duração e a magnitude da hiperglicemia de estresse parecem ser cruciais para determinar se seu efeito será protetor ou deletério. Além disso, o estado metabólico prévio do paciente é importante para a individualização das metas de

controle glicêmico em diferentes cenários de doença crítica e pode ter implicação no desfecho desses pacientes.

1.2.4. *O subgrupo de pacientes com COVID-19*

Em dezembro de 2019, na província de Wuhan, na China, falou-se pela primeira vez sobre uma pneumonia grave de origem desconhecida. Após pesquisas, descobriu-se que se tratava do coronavírus do tipo 2 (SARS-CoV-2), um vírus que pertence à família Coronaviridae. Quando esse vírus invade a célula do hospedeiro, ele utiliza o maquinário genético da célula para se replicar, causando uma doença chamada COVID-19. Após a replicação, ele sai da célula provocando a sua morte (34). Nesse momento, uma resposta inflamatória é desencadeada a partir da liberação de citocinas inflamatórias produzidas a partir da apoptose celular, culminando na conhecida "tempestade de citocinas" (35). Esses fatores levam às manifestações clínicas da doença, que podem ser leves (como tosse seca, coriza, dor de garganta) ou graves (dispneia, insuficiência respiratória), podendo evoluir para síndrome do desconforto respiratório agudo (SDRA), com elevada mortalidade.

Pessoas com doenças crônicas, incluindo DM, são desproporcionalmente afetadas pela COVID-19, com aumento do risco de hospitalização e morte (36). Uma revisão sistemática que incluiu 18 estudos demonstrou um aumento no risco de COVID-19 grave (RR 2,11; 1,40 – 3,19) em pacientes com DM quando comparados a pacientes sem DM (37). Neste mesmo sentido, uma metanálise de Kumar et al. (38), que incluiu 33 estudos, demonstrou um risco aumentado do desfecho composto por doença grave e morte em 2,49 vezes (95% CI: 1,98–3,14; $p < 0.01$).

Há algumas explicações fisiopatológicas plausíveis para a conexão entre DM e COVID-19 na forma mais grave. Inicialmente, acredita-se que os pacientes com DM não

controlado apresentem um sistema imune deficiente (39). Além disso, o DM é classicamente uma doença pró-inflamatória, caracterizada por uma quantidade cronicamente elevada de citocinas inflamatórias na corrente sanguínea, demonstrada por níveis séricos mais elevados de IL-6, ferritina e proteína C reativa (40). Esse fato demonstra que pacientes com níveis de glicose mais elevados são mais vulneráveis a surtos de produção de citocinas, podendo cursar com rápida exacerbação da doença, devido ao desenvolvimento de choque e SDRA (41). Adicionalmente, uma proliferação reduzida de linfócitos e uma resposta fagocitária débil têm sido vistas em pacientes com DM (42). Experimentos *in vitro* demonstraram que altos níveis de glicose promovem a infecção e replicação do vírus Influenza nas células epiteliais pulmonares, indicando que o alto conteúdo de glicose pode aumentar a replicação viral in vivo (43). Experimentos em animais também indicam que alterações pulmonares estruturais, como aumento da permeabilidade vascular e colapso do epitélio pulmonar associam-se com DM (44).

Outro mecanismo que pode estar implicado na inter-relação entre DM e COVID-19 refere-se à diminuição da enzima conversora de angiotensina 2 (ECA-2) em pacientes com DM. Essa enzima é encontrada em células epiteliais dos alvéolos, intestino delgado e endotélio vascular, entre vários outros órgãos. Em condições normais, essa enzima degrada a angiotensina 2 e, em menor grau, a angiotensina 1 em peptídeos menores denominados angiotensina 1-7 e angiotensina 1-9, respectivamente. Esses peptídeos têm efeito benéfico em reduzir a inflamação e o estresse oxidativo. Embora a ECA-2 seja reconhecida como o receptor da célula hospedeira através do qual ocorre a ligação e a entrada das partículas de SARS-CoV-2 nas células, essa enzima também é apontada como tendo um importante papel protetor contra danos pulmonares (45). Portanto, por mais paradoxal que possa parecer, a diminuição basal da expressão dessa enzima em pacientes com DM está diretamente ligada a uma perda mais expressiva do efeito protetor da ECA-

2, o que pode explicar o aumento da prevalência de SDRA e danos pulmonares graves nesses pacientes (46). Assim, o balanço entre todos esses fatores pode explicar o pior desfecho respiratório dos pacientes com DM quando acometidos por COVID-19.

Em pacientes com DM e COVID-19 internados na UTI, um dos tantos desafios urgentes é manter o controle glicêmico adequado e a dose ideal de insulina (41). Novas pesquisas em controle glicêmico serão necessárias também para entender o papel da predisposição genética e os principais processos fisiopatológicos implicados na evolução clínica de pacientes com DM e infecção por SARS-CoV-2, visando a alvos terapêuticos mais precisos e individualizados.

Além dos efeitos agudos da infecção por COVID-19, tem sido descrita a cronificação desta doença. Se os sintomas continuam além de quatro semanas após a infecção, o termo COVID longa tem sido utilizado, enquanto a persistência dos sintomas por mais de doze semanas define a síndrome pós-COVID (47). Os sintomas podem variar desde tosse, cefaleia, mialgia e dispneia, até dor articular, disfunção neurológica e cognitiva e fadiga crônica (48), implicando enormemente na piora da qualidade de vida desses pacientes (49).

Considerando que o desenvolvimento de DM tipo 1 tem sido associado com infecções virais prévias (50,51), pode-se destacar o aumento nos casos de DM relacionado à pandemia de COVID-19, sugerindo um efeito diabetogênico da infecção por COVID-19 (52). Se por um lado há evidências de que a presença de DM seja um fator de risco para o desenvolvimento da forma grave de COVID-19, há estudos que sugerem que a infecção por SARS-CoV-2 desencadeie estados hiperglicêmicos levando a dano pancreático (45). O mecanismo preciso pelo qual ocorre o desenvolvimento de DM (denominado "*new-onset*" DM) em pacientes com COVID-19 ainda é incerto, mas é provável que processos complexos interligados estejam envolvidos, incluindo estado pré-

diabético, hiperglicemia de estresse, hiperglicemia induzida por corticoterapia e efeitos diretos e indiretos do vírus sobre as células beta pancreáticas (53). Alguns estudos demonstraram que o SARS-CoV-2 pode infectar o tecido pancreático através da expressão da ECA-2 nas ilhotas pancreáticas, levando à lesão das células beta em 17% dos casos de COVID-19 grave (54), podendo evoluir com DM tipo 1 (55). No entanto, estudos com tecido pancreático humano sugerem que seja pouco provável que a infecção de células pancreáticas pelo SARS-CoV-2 tenha um papel central no desenvolvimento de DM (56). Alternativamente, sugere-se que proteínas de fase aguda e citocinas inflamatórias liberadas pela infecção por COVID-19 possam causar inflamação e dano direto às células beta pancreáticas (57). Postula-se que a *downregulation* da ECA-2 localizada nas células beta ocasionada pelo vírus aumente a intensidade da tempestade de citocinas e a fibrose do pâncreas, o que pode contribuir para a alteração do funcionamento pancreático com redução da liberação de insulina e progressão para doença mais severa (41). De fato, ainda há muitas questões a serem compreendidas, entre elas qual tipo de DM é desencadeado (tipo 1, tipo 2 ou até mesmo um novo subtipo complexo), se esses pacientes realmente estão sob maior risco de desenvolver DM e se a infecção por COVID-19 em pacientes com DM altera seu curso fisiopatológico natural (52).

1.3. Hiperglicemia e alterações gênicas

A glicose é amplamente utilizada pelos tecidos como fonte de energia. A sua captação celular é mediada por transportadores de glicose presentes na membrana plasmática das células, denominados GLUTs, que facilitam o transporte da glicose para o interior da célula (58). O GLUT-1 é amplamente distribuído pelos tecidos, sendo responsável pelos níveis basais de glicose (59) e tem uma alta afinidade com a molécula de glicose de forma independente da insulina (60). O GLUT-4, por sua vez, é regulado

pela insulina e expressa-se de forma abundante nos tecidos adiposo e musculoesquelético (61). O estresse agudo e a resposta inflamatória parecem resultar em redução da translocação do GLUT-4 do sarcolema para a membrana celular, devido à ação de citocinas inflamatórias, como TNF-α e IL-6 (17), culminando em resistência muscular à captação da glicose.

O gene *INSR* (do inglês, *insulin receptor*), por sua vez, codifica uma proteína da família das tirosina-cinases. A ligação de insulina a este receptor ativa uma via de sinalização intracelular, culminando com a ativação de segundos mensageiros, como o *IRS1* e *IRS2*, que regulam a absorção e a liberação da glicose, bem como a síntese e o armazenamento de carboidratos, lipídeos e proteínas (62). Alterações no gene *INSR* podem levar à resistência à ação da insulina e à hiperglicemia crônica. A análise da expressão desses genes pode esclarecer, em parte, o mecanismo relacionado às alterações da glicemia e do controle glicêmico em pacientes criticamente doentes.

1.4. Consequências em longo prazo da doença crítica

O número cada vez maior de pacientes que sobrevivem à doença crítica aguda tem levado ao aparecimento de novas condições clínicas. Os sobreviventes de uma internação prolongada em UTI têm apresentado danos físicos, cognitivos e mentais em longo prazo após a alta hospitalar (63). Em 2010, a Society of Critical Care Medicine criou o termo *pos-intensive care syndrome* (PICS) para englobar tais alterações, sendo definida como o "surgimento ou agravamento de danos em saúde física, mental e/ou cognitiva após internação na UTI e que persistem após a alta hospitalar" (63). Um estudo holandês encontrou uma incidência de PICS em torno de 43% em pacientes internados por cirurgias eletivas, 58% por condições clínicas e 64% por cirurgias de urgência (64). Os principais sintomas incluem fadiga, dor crônica, sintomas depressivos e de ansiedade, fragilidade e

síndrome do estresse pós-traumático. Uma revisão sistemática identificou cerca de 60 fatores de risco para o seu desenvolvimento (65), incluindo idade avançada, sexo feminino, história prévia de doença mental, delirium e experiências negativas na UTI. Mais estudos são necessários para avaliar potenciais intervenções que possam melhorar e reduzir a ocorrência de PICS (66).

Um dos componentes principais da PICS e uma das consequências mais graves em curto e longo prazo da doença crítica é a fraqueza muscular adquirida na UTI (FA-UTI), a qual consiste na fraqueza muscular generalizada que se desenvolve no curso da internação na UTI e para qual nenhuma outra causa pode ser identificada além da doença aguda e dos seus tratamentos. Ela pode ocorrer tanto por polineuropatia quanto por miopatia, ou pela combinação de ambas (67). Sua incidência é alta, variando de 26 a 67% dependendo do momento da avaliação e da gravidade da população estudada. No momento da alta hospitalar, cerca de 36% dos pacientes apresenta FA-UTI (68). A polineuropatia e a perda de musculatura esquelética, que acometem tanto a musculatura apendicular quanto a axial, têm impacto negativo no tempo de ventilação mecânica (69-71), na mortalidade em cinco anos de pacientes com SDRA (72) e nos custos hospitalares (67).

A patogênese da FA-UTI é complexa e pouco compreendida. A hiperglicemia parece desempenhar um papel importante, principalmente quando relacionada à inflamação sistêmica. Efeitos tóxicos diretos (glicotoxicidade), disfunção mitocondrial e prejuízos à autofagia evocados pela hiperglicemia podem contribuir para o dano muscular (70,73,74). A glicotoxicidade leva à apoptose das células beta, um dos mecanismos de morte celular no DM (75). Além disso, altas concentrações de glicose resultam em estresse oxidativo, disfunção mitocondrial com redução da capacidade de síntese aeróbica de adenosina trifosfato (ATP), ativação de caspases e apoptose em neurônios (76). A

hiperglicemia também foi descrita como um fator de risco independente para a identificação de sinais clínicos (77) e eletrofisiológicos (78) de FA-UTI, enquanto doses maiores de insulina relacionaram-se à sua redução (79). Tais achados sugerem que o controle glicêmico por meio do uso da insulina pode contribuir para a redução da FA-UTI. Outro fator de risco conhecido para o desenvolvimento de FA-UTI é a presença de inflamação sistêmica (80) que, por sua vez, predispõe à hiperglicemia, produzindo um ciclo vicioso entre hiperglicemia e inflamação, que promove a perda proteica músculo-esquelética, potencializando o risco de fraqueza muscular (67).

Além da severidade da doença crítica, outros fatores de risco têm sido implicados no desenvolvimento de FA-UTI. Além da hiperglicemia, a presença de sepse (81), a ocorrência de disfunção de múltiplos órgãos e a imobilização prolongada parecem ser bem estabelecidos. Por outro lado, o uso de medicamentos, como corticoides, aminoglicosídeos e bloqueadores neuromusculares, ainda é controverso. Tais achados sugerem que a relação entre a doença e as medicações utilizadas no seu tratamento e alterações neuromusculares são extremamente complexas e dependem de muitos fatores, como dose, tempo de uso e controle glicêmico concomitante (70).

Especificamente com relação ao uso de bloqueadores neuromusculares, seu uso é indicado na UTI para facilitar a intubação orotraqueal por sequência rápida e manter os pacientes em ventilação mecânica prolongada, reduzindo as assincronias ventilatórias e o trabalho respiratório, especialmente em pacientes com graves prejuízos às trocas gasosas, como na SDRA e na COVID-19 grave, por exemplo (82-84). O uso de tais medicações tem sido sugerido como um fator preditor independente para FA-UTI em diferentes patologias (81,85-89), mas não há evidências definitivas que corroborem a associação entre o uso de bloqueadores neuromusculares e o desenvolvimento de FA-UTI (90-93).

Dessa forma, o intrincado mecanismo que associa hiperglicemia, inflamação, uso de medicações e FA-UTI deve ser melhor estudado, a fim de se compreender os mecanismos fisiopatológicos e moleculares pelos quais a lesão muscular esquelética se desenvolve. Como não há opções específicas de tratamento da FA-UTI, esforços devem ser feitos para identificar, controlar e prevenir os seus fatores de risco, a fim de reduzir sua incidência e suas consequências em longo prazo.

2. JUSTIFICATIVA

A doença crítica reúne inúmeras síndromes clínicas com aspectos em comum. Entre esses aspectos, pode-se incluir a hiperglicemia de estresse e suas ações potencialmente benéficas e deletérias. A compreensão das bases fisiopatológicas e genéticas pelas quais a hiperglicemia pode levar a danos celulares na doença crítica é de suma importância para definir estratégias adequadas para o manejo de pacientes que apresentam essa condição. Populações específicas de pacientes criticamente doentes parecem responder de forma diferente frente à hiperglicemia de estresse. Nesse cenário, o estudo de pacientes criticamente doentes com pneumonia por SARS-CoV2 faz-se necessário, tendo em vista que, conforme aponta a literatura, essa população parece não apresentar o "paradoxo do diabetes", como acontece em outros pacientes internados na UTI. Ainda, o estudo de expressão gênica do tecido muscular de pacientes criticamente doentes com e sem DM ou hiperglicemia de estresse contribui para o entendimento dos mecanismos moleculares envolvidos no desenvolvimento dessa condição clínica. Por fim, a identificação de fatores associados à FA-UTI, uma síndrome multifatorial associada a graves consequências a curto e longo prazos em sobreviventes da doença crítica, tem o potencial de influenciar o manejo mais adequado desses pacientes.

Na presente tese, procuramos avaliar as particularidades de pacientes criticamente doentes com insuficiência respiratória aguda por COVID-19 e se há alguma diferença prognóstica de acordo com a presença ou não de DM, pesquisamos a expressão de genes relacionados ao controle glicêmico no músculo esquelético de pacientes críticos e avaliamos a relação entre o uso de bloqueadores neuromusculares e o desenvolvimento de FA-UTI. Acreditamos que a intrincada inter-relação entre a hiperglicemia de estresse, a inflamação, a presença ou não de DM e outros fatores de risco deva ser melhor estudada,

a fim de focar na prevenção dessa patologia tão incidente e ainda sem tratamento específico que é a FA-UTI. Dessa forma, uma medicina de precisão pode ser implementada, melhorando os desfechos clínicos nessas populações.

OBJETIVOS

2.1. Investigar a associação entre DM e hiperglicemia de estresse com mortalidade e desfechos clínicos em pacientes criticamente doentes internados por pneumonia por COVID-19.

2.2. Investigar a associação entre DM e hiperglicemia de estresse com a expressão dos genes *IRS1*, *IRS2*, *INSR*, *SLC2A1* e *SLC2A4* na musculatura esquelética de pacientes criticamente doentes.

2.3. Realizar uma revisão sistemática da literatura e sintetizar as evidências disponíveis relacionadas ao uso de bloqueadores neuromusculares e sua associação com o desenvolvimento de FA-UTI em pacientes criticamente doentes.

REFERÊNCIAS DA INTRODUÇÃO

1. Ibsen B. The anaesthetist's viewpoint on the treatment of respiratory complications in poliomyelitis during the epidemic in Copenhagen, 1952. Proc R Soc Med. 1954;47:72–74.
2. Grenvik A, Pinsky MR. Evolution of the intensive care unit as a clinical center and critical care medicine as a discipline. Crit Care Clin. 2009;25:239–50.
3. Marshall JC, Bosco L, Adhikari NK, Connolly B, Diaz JV, Dorman T, Fowler RA, Meyfroidt G, Nakagawa S, Pelosi P, Vincent JL, Vollman K, Zimmerman J. What is an intensive care unit? A report of the task force of the World Federation of Societies of Intensive and Critical Care Medicine. J Crit Care. 2017;37:270-276.
4. Neill K J Adhikari, Robert A Fowler. Critical care and the global burden of critical illness in adults. Lancet. 2010;375:1339-46.
5. Bellaver P, Schaeffer AF, Dullius DP, Viana MV, Leitão CB, Rech TH. Association of multiple glycemic parameters at intensive care unit admission with mortality and clinical outcomes in critically ill patients. Sci Rep. 2019; 6;9(1):18498.
6. Fialkow L, Farenzena M, Wawrzeniak IC, Brauner JS, Vieira SR, Vigo A, Bozzetti MC. Mechanical ventilation in patients in the intensive care unit of a general university hospital in southern Brazil: an epidemiological study. Clinics (Sao Paulo). 2016;71(3):144-51.
7. Mirnezami R, Nicholson J, Darzi A. Preparing for precision medicine. N Engl J Med. 2012;366(6):489–91.
8. Seymour CW, Gomez H. Precision medicine for all? Challenges and opportunities for a precision medicine approach to critical illness. Seymour et al. Critical Care. 2017;21:257.
9. Bierman AS, Tinetti ME. Precision medicine to precision care: managing multimorbidity. Lancet. 2016;3;388(10061):2721-2723.
10. Esteller M. Epigenetics in cancer. N Engl J Med. 2008;358(11):1148–59.
11. Saeed S, Quintin J, Kerstens HH, Rao NA, Aghajanirefah A, Matarese F, Cheng SC, Ratter J, Berentsen K, van der Ent MA, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science. 2014;345(6204):1251086.
12. Bernard C. Lecons sur les Phenomenes de la Vie Communs aux Animaux et aux Vegetaux. Paris, France: JB Bailliere et fi ls; 1878.
13. Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response! Critical Care. 2013;17:305.
14. Ellger B, Debaveye J, Van den Berghe G. Endocrine Interventions in the ICU. European Journ of Intern Medicine. 2005;16:71-82.
15. Preiser JC, Devos P, Ruiz-Santana S, et al. A prospective randomized multicentre controlled trial on tight glucose control by intensive insulin therapy in adult intensive care units: the Glucontrol study. Intensive Care Medicine. 2009;35(10):1738-48.

16. Badawi O, Waite MD, Fuhrman SA, Zuckerman IH: Association between intensive care unit-acquired dysglycemia and in-hospital mortality. Crit Care Med. 2012;40:3180-3188.
17. Dungan K, Braithwaite SS, Preiser JC. Stress hyperglycemia. Lancet. 2009;373:1798-1807.
18. Soeters MR, Soeters PB. The evolutionary benefit of insulin resistance. Clin Nutrition. 2012;31:1002-1007.
19. Hart BB, Stanford GG, Ziegler MG, Lake CR, Chernow B. Catecholamines: study of interspecies variation. Crit Care Med. 1989;17:1203-1218.
20. Losser MR, Damoisel C, Payen D. Bench-to-bedside review: Glucose and stress conditions in the intensive care unit. Crit Care. 2010;14:231.
21. NICE-SUGAR Study Investigators, Finfer S, Chittock DR, et al. Intensive versus conventional glucose control in critically ill patients. N Engl J Med. 2009;26;360(13):1283-97.
22. Vanhorebeek I, Ellger B, De VR, Boussemaere M, Debaveye Y, Perre SV, Rabbani N, et al. Tissue-specific glucose toxicity induces mitochondrial damage in a burn injury model of critical illness. Crit Care Med. 2009; 37:1355-1364
23. Cantuaria APC. Efeito da hiperglicemia crônica na resposta imunológica em humanos. Monografia (Biomedicina) - Universidade Católica de Brasília, 49, 2014.
24. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
25. Dandona P. Vascular reactivity in diabetes mellitus. Endocrinol Nutr. 2009;56(Suppl. 4):12–14.
26. Umpierrez GE, Pasquel F. Management of Inpatient Hyperglycemia and Diabetes in Older Adults. Diabetes Care. 2017;40:509–517.
27. The DCCT Research Group. Diabetes Control and Complications Trial (DCCT): results of feasibility study. Diabetes Care. 1987;10(1):1-19.
28. UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet. 1998;352(9131):854-65. Erratum in: Lancet 1998 Nov 7;352(9139):1558.
29. Krinsley JS, Fisher M. The diabetes paradox: diabetes is not independently associated with mortality in critically ill patients. Hosp Pract (1995). 2012;40(2):31-5.
30. Lanspa MJ, Hirshberg EL, Phillips GD, et al. Moderate glucose control is associated with increased mortality compared with tight glucose control in critically ill patients without diabetes. Chest. 2013;143:1226-34.
31. Luethi N, Cioccari L, Biesenbach P, Lucchetta L, Kagaya H, Morgan R, et al. Liberal Glucose Control in ICU Patients With Diabetes: A Before-and-After Study. Crit Care Med. 2018;46(6):935-942.
32. Netto AP, Andriolo A, Fraige Filho F, et al. Update on glycated hemoglobin (HbA1C) for assessment of glycemic control and the diagnosis of diabetes: clinical and laboratory aspects. Bras Patol Med Lab. 2009;45(1):31-48.

33. Liao W, Sheng CHL, Hsing CHL, et al. An elevated Glycemic Gap is associated with adverse outcomes in diabetic patients with acute myocardial infarction. Sci Rep. 2016;6:27770.

34. Li YC, Bai WZ, Hashikawa T. Response to Commentary on "The neuroinvasive potential of SARS-CoV-2 may play a role in the respiratory failure of COVID-19 patients". J. Med Virol. 2020;92:707–709.

35. Palm NW, Medzhitov R. Not so fast: adaptive suppression of innate immunity. Nat. Med. 2007;13:1142–1144.

36. Khunti K, Valabhji J, Misra S. Diabetes and the COVID-19 pandemic. Diabetologia. 2023;66(2):255-266.

37. Singh AK, Gillies CL, Singh R, et al. Prevalence of comorbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis. Diabetes Obes Metab. 2020;22(10):1915–1924.

38. Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis. Diabetes Metab Syndr. 2020;14(4):535-545.

39. Jafar N, Edriss H, Nugent K. The effect of short-term hyperglycemia on the innate immune system. Am. J. Med Sci. 2016;351:201–211.

40. Guo W, Li M, Dong Y, Zhou H, Zhang Z, Tian C, Qin R, Wang H, Shen Y, DuK, Zhao L, et al. Diabetes is a risk factor for the progression and prognosis of COVID-19. Diabetes Metab. 2020;31:e3319.

41. Sharma P, Behl T, Sharma N, Singh S, Grewal AS, Albarrati A, Albratty M, Meraya AM, Bungau S. COVID-19 and diabetes: Association intensify risk factors for morbidity and mortality. Biomed Pharmacother. 2022;151:113089.

42. MP Moutschen, AJ Scheen, PJ Lefebvre. Impaired immune responses in diabetes mellitus: analysis of the factors and mechanisms involved. Relevance to the increased susceptibility of diabetic patients to specific infections. DiabeteMetab. 1992;18:187-201.

43. HP Kohio, AL Adamson. Glycolytic control of vacuolar-type ATPase activity: a mechanism to regulate influenza viral infection. Virology. 2013;444:301–309.

44. Popov D, Simionescu M. Alterations of lung structure in experimental diabetes, and diabetes associated with hyperlipidaemia in hamsters. Eur. Respir. J. 1997;10:1850–1858.

45. da Silva PHA, Garcia AS, Alves FA, Dos Santos ALS, Sodré CL. COVID-19 and Diabetes Mellitus: Potential Metabolic Associations. Curr Top Med Chem. 2021;21(11):929-936.

46. Tikellis C, Thomas MC. Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease. Int J. Pept. 2012;256294.

47. Davis HE, Assaf GS, McCorkell L, et al. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. EClinicalMedicine. 2021;38:101019.

48. Crook H, Raza S, Nowell J, Young M, Edison P. Long COVID: mechanisms, risk factors, and management. BMJ. 2021;374, n1648.

49. Han Q, Zheng B, Daines L, Sheikh A. Long-term sequelae of COVID-19: a systematic review and meta-analysis of one-year follow-up studies on post-COVID symptoms. Pathogens. 2022;11:269.

50. Ruiz PLD, Tapia G, Bakken IJ, Håberg SE, Hungnes O, Gulseth HL, Stene LC. Pandemic influenza and subsequent risk of type 1 diabetes: A nationwide cohort study. Diabetologia. 2018;61(9):1996-2004.

51. Lönnrot M, Lynch KF, Elding Larsson, H, Lernmark Å, Rewers MJ, Törn C, Burkhardt BR, Briese T, et al. TEDDY Study Group. Respiratory infections are temporally associated with initiation of type 1 diabetes autoimmunity: The TEDDY study. Diabetologia. 2017;60(10):1931-1940.

52. Rubino F, Amiel AS, Zimmet P, Alberti G, Bornstein S, Eckel RH, Mingrone G, Boehm B, Cooper ME, Chai Z, et al. New-onset diabetes in Covid-19. N. Engl. J. Med. 2020;383(8):789-790.

53. Khunti K, Del Prato S, Mathieu C, Kahn SE, Gabbay RA, Buse JB. COVID-19, Hyperglycemia, and New-Onset Diabetes. Diabetes Care. 2021;44(12):2645-2655.

54. Liu F, Long X, Zhang B, Zhang W, Chen X, Zhang Z. ACE2 expression in pancreas may cause pancreatic damage after SARS-CoV-2 infection. Clin. Gastroenterol. Hepatol. 2020;18(9):2128-2130.

55. Yang JK, Lin SS, Ji XJ, Guo LM. Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes. Acta Diabetol. 2010;47(3):193-199.

56. Kusmartseva I, Wu W, Syed F, et al. Expression of SARS-CoV-2 entry factors in the pancreas of normal organ donors and individuals with COVID19. Cell Metab. 2020;32:1041-1051.

57. Ahlqvist E, Storm P, K€ar€aj€am€aki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. Lancet Diabetes Endocrinol. 2018;6:361-369.

58. Shepherd PR, Kahn BB. Glucose transporters and insulin action- implications for insulin resistance and diabetes mellitus. N Engl J Med. 1999;341:248-257.

59. SLC2A1 solute carrier family 2 member 1 [*Homo sapiens* (human)] - Gene ID: 6513, PubMed, updated on 2-Jul-2017.

60. Brown, GK. Glucose transporter: Structure, function and consequences of deficiency. J. Inherit. Metab. Dis. 2000;23:237-246.

61. SLC2A4 solute carrier family 2 member 4 [*Homo sapiens* (human)] - Gene ID: 6517, PubMed, updated on 2-Jul-2017.

62. INSR insulin receptor [Homo sapiens (human)] - Gene ID: 3643, PubMed, updated on 2-Jul-2017.

63. Needham DM, Davidson J, Cohen H, et al. Improving long-term outcomes after discharge from intensive care unit. Crit Care Med. 2012;40(2):502–9.

64. Geense WW, Zegers M, Peters MAA, et al. New physical, mental, and cognitive problems 1 year after ICU admission. Am J Respir Crit Care Med. 2021;203(12):1512–21.

65. Lee M, Kang J, Jeong Y. Risk factors for post-intensive care syndrome: a systematic review and meta-analysis. Aust Crit Care. 2020;33(3):287–94.

66. Hiser SL, Fatima A, Ali M, Needham DM. Post-intensive care syndrome (PICS): recent updates. J Intensive Care. 2023;11(1):23.

67. Hermans G, Van den Berghe G. Clinical review: intensive care unit acquired weakness. Crit Care. 2015;274.

68. Fan E, Dowdy DW, Colantuoni E, et al. Physical Complications in Acute Lung Injury Survivors: A Two-Years Longitudinal Prospective Study. Crit Care Med. 2014;42:849-59.

69. De Jonghe B, Bastuji-Garin S, Durand MC, et al. Respiratory weakness is associated with limb weakness and delayed weaning in critical illness. Crit Care Med. 2007;39.

70. Hermans G, Wilmer A, Meersseman W, et al. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. Am J Respir Crit Care Med. 2007;175:480-9.

71. Weber-Carstens S, Koch S, Spuler S, et al. Nonexcitable muscle membrane predicts intensive care unitacquired paresis in mechanically ventilated, sedated patients. Crit Care Med. 2009;37:2632-2637.

72. Dinglas VD, Aronson FL, Colantuoni E, et al. Muscle Weakness and 5- Year Survival in Acute Respiratory Distress Syndrome Survivors. Crit Care Med. 2017; 45(3): 446-453.

73. Vanhorebeek I, De Vos R, Mesotten D, Wouters PJ, Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. Lancet. 2005;365:53–9.

74. Van den Berghe G. How does blood glucose control with insulin save lives in intensive care? J Clin Invest. 2004;114:1187–95.

75. Cnop M, Welsh N, Jonas JC, et al. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes. 2005;54 Suppl 2:S97-107.

76. Callahan LA, Supinski GS. Hyperglycemia and acquired weakness in critically ill patients: potential mechanisms. Crit Care. 2009;13(2):125.

77. Nanas S, Kritikos K, Angelopoulos E, Siafaka A, Tsikriki S, Poriazi M, et al. Predisposing factors for critical illness polyneuromyopathy in a multidisciplinary intensive care unit. Acta Neurol Scand. 2008;118:175–81.

78. Witt NJ, Zochodne DW, Bolton CF, Grand'Maison F, Wells G, Young GB, et al. Peripheral nerve function in sepsis and multiple organ failure. Chest. 1991;99:176–84.

79. Patel BK, Pohlman AS, Hall JB, Kress JP. Impact of early mobilization on glycemic control and ICU-acquired weakness in critically ill patients who are mechanically ventilated. Chest. 2014;146:583–9.

80. Puthucheary ZA, Rawal J, Mchphail M, et al. Acute Skeletal Muscle Wasting in Critical Illness. JAMA. 2013;310:1591-1600.

81. Garnacho-Montero J, Madrazo-Osuna J, Garcia-Garmendia JL, Ortiz-Leyba C, Jimenez-Jimenez FJ, Barrero-Almodovar A, et al. Critical illness polyneuropathy: risk factors and clinical consequences. A cohort study in septic patients. Intensive Care Med. 2001;27:1288–96.

82. Paramore S. Effects of the use of neuromuscular blocking agents on acute respiratory distress syndrome outcomes: A systematic review. J Am Assoc Nurse Pract. 2018;30(6):327-332.

83. Slutsky AS. Neuromuscular blocking agents in ARDS. N Engl J Med. 2010;363(12):1176-80.

84. Warr J, Thiboutot Z, Rose L, Mehta S, Burry LD. Current therapeutic uses, pharmacology, and clinical considerations of neuromuscular blocking agents for critically ill adults. Ann Pharmacother. 2011;45(9):1116-26.

85. Leatherman JW, Fluegel WL, David WS, Davies SF, Iber C. Muscle weakness in mechanically ventilated patients with severe asthma. Am J Respir Crit Care Med. 1996;153(5):1686-90.

86. Adnet F, Dhissi G, Borron SW, Galinski M, Rayeh F, Cupa M, Pourriat JL, Lapostolle F. Complication profiles of adult asthmatics requiring paralysis during mechanical ventilation. Intensive Care Med. 2001;11:1729-36.

87. Behbehani NA, Al-Mane F, D'yachkova Y, Paré P, FitzGerald JM. Myopathy following mechanical ventilation for acute severe asthma: the role of muscle relaxants and corticosteroids. Chest. 1999;115(6):1627-31.

88. Kupfer Y, Namba T, Kaldawi E, Tessler S. Prolonged weakness after long-term infusion of vecuronium bromide. Ann Intern Med. 1992;15;117(6):484-6.

89. Price DR, Mikkelsen ME, Umscheid CA, Armstrong EJ. Neuromuscular Blocking Agents and Neuromuscular Dysfunction Acquired in Critical Illness: A Systematic Review and Meta-Analysis. Crit Care Med. 2016;44(11):2070-2078.

90. Papazian L, Forel JM, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, Jaber S, Arnal JM, Perez D, Seghboyan JM, Constantin JM, Courant P, Lefrant JY, Guérin C, Prat G, Morange S, Roch A; ACURASYS Study Investigators. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med. 2010;363(12):1107-16.

91. National Heart, Lung, and Blood Institute PETAL Clinical Trials Network, Moss M, Huang DT, Brower RG, Ferguson ND, Ginde AA, Gong MN, Grissom CK, Gundel S, Hayden D, Hite RD, Hou PC, Hough CL, Iwashyna TJ, Khan A, Liu KD, Talmor D, Thompson BT, Ulysse CA, Yealy DM, Angus DC. Early Neuromuscular Blockade in the Acute Respiratory Distress Syndrome. N Engl J Med. 2019;380(21):1997-2008.

92. Forel JM, Roch A, Marin V, Michelet P, Demory D, Blache JL, Perrin G, Gainnier M, Bongrand P, Papazian L. Neuromuscular blocking agents decrease inflammatory response in patients presenting with acute respiratory distress syndrome. Crit Care Med. 2006;34(11):2749-57.

93. Weber-Carstens S, Deja M, Koch S, Spranger J, Bubser F, Wernecke KD, Spies CD, Spuler S, Keh D. Risk factors in critical illness myopathy during the early course of critical illness: a prospective observational study. Crit Care. 2010;14(3):R119.

ARTIGO 1

Diabetes associates with mortality in critically ill patients with SARS-CoV-2 pneumonia: no diabetes paradox in COVID-19

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Diabetes associates with mortality in critically ill patients with SARS-CoV-2 pneumonia: no diabetes paradox in COVID-19

Running title: No diabetes paradox in critically ill patients with COVID-19

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PB participated in the study design, data interpretation, and draft of the manuscript. LS participated in data acquisition and draft of the manuscript. AFS participated in the study conception and design and data acquisition. LHR participated in data acquisition. JLC acquired and analyzed data from the Biobank. FG participated in data interpretation. CBL participated in the study conception and design, data interpretation, and statistical analysis. THR participated in the study conception and

design, data interpretation, statistical analysis, and draft of the manuscript. All authors revised the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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ABSTRACT

Background: Diabetes mellitus (DM) is not associated with increased mortality in critically ill patients, a phenomenon known as the "diabetes paradox". However, DM is a risk factor for increased mortality in patients with COVID-19. This study aims to investigate the association of DM and stress-induced hyperglycemia at intensive care unit (ICU) with mortality in this population.

Methods: This is a retrospective study. Electronic medical records from patients admitted from March 2020 to September 2020 were reviewed. Primary outcome was mortality. Secondary outcomes were ICU and hospital mortality and stay, and need for mechanical ventilation and renal replacement therapy.

Results: 187 patients were included. Overall mortality was 43.2%, higher in patients with DM (55.7% vs. 34%; p=0.007), even after adjustment for age, hypertension, and disease severity. When patients were separated into groups, named normoglycemia (without DM and glycemia ≤140 mg/dL), stress-induced hyperglycemia (without DM and glycemia >140mg/dL), and DM (previous diagnosis or HbA1c ≥6.5%), the mortality rate was 25.8%, 37.3%, and 55.7%, respectively (p=0.021). Mortality was higher in patients with higher glycemic variability. No statistical difference related to secondary outcomes was observed.

Conclusions: DM, hyperglycemia, and glycemic variability associated with increased mortality in critically ill patients with severe COVID-19, but did not increase the rates of other clinical outcomes. More than stress-induced hyperglycemia, DM was associated with mortality.

ABBREVIATION LIST

DM: diabetes mellitus

ICU: intensive care unit

HbA1c: glycates hemoglobin

MV: mechanical ventilation

ICU-AW: ICU-acquired muscular weakness

LOS: length of stay

SAPS III: Simplified Acute Physiology Score III

ADA: American Diabetes Association

RRT: renal replacement therapy

SD: standard deviation

HR: hazard ratio

BMI: body mass index

INTRODUCTION

During the COVID-19 pandemic, several studies have reported that patients with diabetes mellitus (DM) have a higher risk of severe SARS-CoV-2 infection [1]. These patients have a greater likelihood of hospitalization, intensive care unit (ICU) admission, and need for mechanical ventilation [1]. Interestingly, prior to the pandemic, DM was not associated with increased mortality in ICU patients [2], contrary to what might be anticipated based on the known complications and risks associated with DM in other settings. In general, DM is recognized as a chronic condition that predisposes individuals to various health complications, including cardiovascular disease, renal dysfunction, infections, retinopathy, and neuropathy [3]. These complications can contribute to higher morbidity and mortality rates in the general population [4]. However, within the ICU, the impact of DM on mortality appears to be different. This phenomenon is known as the "diabetes paradox". It suggests that in the ICU setting other factors such as the severity of the acute illness may overshadow the direct impact of DM on mortality.

While stress-induced hyperglycemia is linked to higher mortality, the literature does not clarify whether hyperglycemia is a marker of disease severity or a determinant of prognosis [5,6]. Furthermore, other altered glycemic parameters like hypoglycemia, glycemic variability, and glycemic gap [7,8] are related to worse outcomes [9]. In a general population of critically ill patients, Bellaver et al. [9] demonstrated that hypoglycemia and hyperglycemia on ICU admission were independently associated with increased mortality, whereas a higher glycemic gap and a higher glycemic variability were associated with other negative clinical outcomes, such as need for renal replacement therapy and shock incidence. In patients with COVID-19, Bhatti et al. [10] found that a more strict metabolic control, with random blood glucose levels lower than 160 mg/dL

and fasting blood glucose levels lower than 126 mg/dL, is associated with a considerably lower risk of mortality.

Chronic hyperglycemia may trigger protective mechanisms against cell damage, which may explain the diabetes paradox and why patients with poor chronic glycemic control have worse outcomes when treated with intensive glycemic control in ICU [11]. By measuring the glycemic gap preexisting chronic hyperglycemia can be distinguished from stress-induced hyperglycemia [12]. Although the unfavorable effects of dysglycemia on critically ill patients are well known, the impact of stress-induced hyperglycemia in ICU patients with COVID-19 and the diabetes paradox are still unclear. Therefore, this study aims to explore the association of acute and chronic glycemic parameters with clinical outcomes in critically ill patients with severe acute respiratory failure due to SARS-CoV-2 infection.

MATERIAL AND METHODS

Ethical Considerations

The study was approved by the Ethics Committee at the Hospital de Clínicas de Porto Alegre (project number 2020-0218) and adhered to the Helsinki Declaration.

Study population

This is a retrospective study. Informed consent was obtained. The study assessed critically ill adults (aged >18 years) admitted to the ICU from March 2020 to September 2020. The inclusion criterion was patients admitted to ICU with SARS-CoV-2 infection

with one HbA1c measurement available at ICU admission or with the possibility to measure it by using blood stored at the COVID-19 Collection in the Biobank. Exclusion criteria were diabetic ketoacidosis, hyperosmolar hyperglycemic state, hemoglobinopathies, and ICU length of stay (LOS) less than 24 hours. Clinical and laboratory data were recorded for all patients. The Simplified Acute Physiology Score 3 (SAPS 3) was used to score disease severity [13]. Data referring to demographic characteristics of the study population, as well as coexisting medical conditions, reasons for ICU admission, origin before ICU admission, nutrition and insulin therapy were extracted from the review of the electronic medical record. SAPS 3 and other variables related to disease severity, such as use of vasopressor, need for MV and RRT, were also extracted from medical records. Routine laboratory tests provided data related to biochemical measurements. Especially regarding HbA1c, it could be available at ICU admission or measured from blood sample stored ate the Biobank. All data extracted from electronic medical records were independently reviewed by two researchers. Outcomes were adjudicated by two researchers independently.

Diabetes was defined based on previous diagnosis or whenever HbA1c was ≥6.5%. Hyperglycemia was defined according to the American Diabetes Association (ADA) proposed threshold for in-hospital hyperglycemia, and severe hyperglycemia as any blood glucose measurement >200 mg/dL. Patients without previous history of DM, HbA1c <6.5%, and glycemia ≤140 mg/dL were classified as the normoglycemia group. Those without previous history of DM, HbA1c <6.5%, and glycemia >140 mg/dL were classified as stress-induced hyperglycemia group [14]. Hypoglycemia was defined as any glycemic level <70 mg/dL [11,14], and serious hypoglycemia was defined as <54 mg/dL during the first day in the ICU [14]. Glucose variability was calculated as the absolute difference in capillary blood glucose during the first ICU day [15,16]. The glycemic gap

was calculated as the difference between the ICU admission serum blood glucose and the estimated mean blood glucose, estimated by the HbA1c [12, 17]. Patients were separated by a cutoff value of 80 mg/dL for glycemic gap and 40 mg/dL for glycemic variability based on Bellaver et al. [9] and Liao et al. [18].

The outcomes of interest were mortality (primary endpoint) and ICU and hospital mortality, need for mechanical ventilation (VM), need for renal replacement therapy (RRT), length of stay at the ICU and at the hospital, and ICU readmission (secondary endpoints).

Statistical analysis

Statistical analysis included the use of the Student's t-test, Mann–Whitney U test, or chi-square test, as appropriate. Univariate linear regression or logistic regression models were constructed depending on the characteristics of the outcomes of interest. Kaplan-Meier method was used to perform time-to-event analyses. Time-to-event effect size (hazard ratios, HR) was estimated by Cox proportional hazard analyses with mortality as outcome and variables adjusted for age, presence of hypertension, and disease severity using SAPS 3 score and need of vasopressors. A sample size of 182 patients was calculated for this study. Values were considered statistically significant if p<0.05. Statistical analyses were conducted in the software program SPSS 20.0 (Chicago, IL, USA).

RESULTS

Figure 1 shows the summary of the study.

Patient characteristics

A total of 556 patients admitted to the ICU with SARS-CoV-2 infection were evaluated for eligibility. Then, 187 patients had HbA1c results available and were included in this study. Table 1 shows their main characteristics. Briefly, 60% were male, mean age was 60 ± 15 years, and SAPS 3 score was 62 ± 16. The most common primary coexisting condition was hypertension (61.6%), which was more frequent in non-survivors than in survivors (70.4% vs. 50.4%, p=0.029). Presence of DM was based on previous diagnosis in 62 patients (33.2%) and on HbA1c quantification in additional 17 patients (9%), totalizing 79 patients with DM (42,7%). Acute respiratory failure was the main reason for ICU admission (96%), with 74.1% of patients requiring invasive MV. Most patients received antibiotics for presumed sepsis from pulmonary origin (87%) and 71.2% received corticosteroids.

Metabolic parameters and mortality

Table 2 shows that the overall mortality rate was 43.2% (n=80), which was higher in critically ill patients with severe COVID-19 pneumonia with DM than in those without DM (55.7% vs. 34%; p=0.007). This association persists even after adjustment for age, hypertension, and disease severity estimated by the need for vasopressors (p=0.033). Figure 2 shows the probability of survival according to the presence or absence of DM, after adjusting for age, hypertension, SAPS 3 score, and vasopressors use. When patients were separated into the normoglycemia, stress-induced hyperglycemia, and DM groups mortality rate was 25.8%, 37.3%, and 55.7%, respectively, with significant difference of mortality between patients with DM and those with normoglycemia

(p=0.021). Figure 3 shows the individuals' time until death in the three groups. The mean HbA1c was 6.8 ± 2% and the mean blood glucose was 174 ± 106 mg/dL, different among patients with normoglycemia, stress-induced hyperglycemia, or DM, as expected (Table 1), but no difference was found between survivors and non-survivors (Supplementary Table 1). Hyperglycemia (glucose >140 mg/dL) was present in 80% (n=149) of patients and severe hyperglycemia (glucose >200 mg/dL) in 43% (n=81) within the first 24 hours of ICU admission. Patients who had hyperglycemia >140 mg/dL and hyperglycemia >200 mg/dL in the first 24h of ICU admission had higher mortality rates compared to those who presented lower glycemic parameters (47% vs. 26%, p=0.048) and (56% vs. 34%, p=0.003), respectively.

The median glycemic variability was 67 mg/dL (38 to 149 mg/dL) and the glycemic gap varied from −16 to 65 mg/dL, with a median of 14 mg/dL. In critically ill patients with severe COVID-19 pneumonia, mortality rate was higher in those with glycemic variability >40 mg/dL compared to those with <40 mg/dL (48.4% vs. 26.7%, p=0.011). No differences in mortality rates were detected between patients with a glycemic gap below or above >80 mg/dL (42.3% vs. 48.6%, p=0.484) (Table 3).

Hypoglycemia was a rare event. Glycemic values <70 mg/dL occurred in five patients (2.7%) and severe hypoglycemia <54 mg/dL occurred in one patient (0.5%), not affecting mortality (p=0.45 and p=0.38, respectively).

Mean body mass index (BMI) was 30.7 ± 7.2 kg/m^2, similar between patients with normoglycemia, stress-induced hyperglycemia, or DM (Table 1), but higher in survivors than in non-survivors (31.8 ± 7.6 vs. 29.2 ± 6.4, p=0.012).

Glycemic parameters and other outcomes

Table 2 shows that the need for MV, need for RRT, ICU and hospital length of stay, and ICU readmission rate were similar between patients with normoglycemia, stress-induced hyperglycemia, or DM. Table 3 shows the impact of glycemic gap, glycemic variability, and hypoglycemia <70 mg/dL on secondary outcomes. All these glycemic parameters were not different for secondary outcomes. Mean glycated hemoglobin was not different between survivors and non-survivors (6.2 ± 1.9 vs. 7 ± 2, p=0.131), between patients who need RRT or not (6.7 ± 1.5 vs. 6.8 ±2.1, p=0.736), and between patients who need MV or not (6.7 ± 1.8 vs. 7.1 ± 2.5, p=0.275).

DISCUSSION

In this retrospective cohort study of 187 critically ill patients with severe COVID-19 pneumonia, DM and hyperglycemia at ICU admission were independently associated with increased mortality, but not with increased need for supportive therapies, ICU and hospital length of stay, and ICU readmission. Most interestingly, more than stress-induced hyperglycemia, DM was associated with mortality, contrary to the "diabetes paradox" in ICU patients.

Throughout the SARS-CoV-2 pandemic, people with chronic diseases, including DM and hypertension, have been disproportionately affected, with an increased risk of hospitalization and mortality [19,20]. The prevalence of DM in our sample was 42.7%, 1.5-fold higher than the general population of critically ill patients [9] and the prevalence of hypertension was 61.6%. This high prevalence confirms DM and hypertension as risks factor for ICU admission during COVID-19 [21], in agreement with a systematic review of 18 studies that reported a high risk of severe COVID-19 in patients with DM compared with those without DM [22]. The mortality rate in our study was significantly higher in

patients with hyperglycemia >140 mg/dL and severe hyperglycemia (>200 mg/dL), as expected. However, and most importantly, the mortality was higher in patients with DM than in those without DM, even after adjustment for age, hypertension, and disease severity, a new aspect for ICU patients, specifically associated with COVID-19. Accordingly, a meta-analysis of 33 studies conducted by Kumar et al. demonstrated that patients with DM faced increased odds of developing a composite endpoint of severe COVID-19 or death (2.49 [95% CI: 1.98–3.14], $p < 0.01$) [23]. DM associates with increased risk of developing complications during critical illnesses, but the presence of DM was not independently associated with increased risk of death in critically ill patients before the COVID-19 pandemic [2]. The "diabetes paradox" [2] suggests that chronic hyperglycemia before the acute insult may be associated with favorable outcomes in critical illnesses [24,27]. Cellular adaptations to chronic hyperglycemia of DM would "prepare" the cellular antioxidant apparatus to deal with a subsequent hyperglycemia during an acute illness [28]. Thus, acute hyperglycemia would be more deleterious in critically ill patients without DM, who have no previous cellular conditioning against glucose toxicity. Additionally, patients with DM receive more insulin than those without DM, but with in-hospital hyperglycemia. Insulin has many beneficial non-glycemic effects, including modulation of inflammation, reduction of circulating free fatty acids, regulation of apoptosis, prevention of endothelial dysfunction and hypercoagulation, decrease in neutrophil chemotaxis and leukocyte adhesion, attenuation of the catabolic state of critical illness, and prevention of excessive nitric oxide generation, reducing oxidative stress [29]. In our sample, DM chronic hyperglycemia, rather than stress-induced hyperglycemia, was associated with higher mortality. We demonstrated this phenomenon by two separate analyses. First, by Cox-regression, in which we separated the sample into three groups (normoglycemia, stress-induced hyperglycemia, and DM),

and second by the glycemic gap analysis. Thus, our results were consistent, robust, and presented a new aspect of critically ill patients with COVID-19, in which the diabetes paradox cannot be identified.

This population of critically ill patients with SARS-CoV-2 infection clearly presents particularities compared to other ICU patients, especially regarding metabolic pathways of glycemic control and inflammatory mechanisms. In SARS-CoV-2 infection, excessive production of inflammatory mediators leads to a condition known as "cytokine storm [30]". DM is also a chronic pro-inflammatory state characterized by an exaggerated cytokine response, where patients with DM had significantly higher levels of interleukin-6 (IL-6), ferritin, and C-reactive protein compared to individuals without DM [31]. The "cytokine storm" condition results in an increase of oxidative stress and cellular damage, including muscle and liver tissue, which play a central role in the regulation of glucose metabolism, leading to insulin resistance [32]. This suggests that individuals with uncontrolled blood glucose levels may be more vulnerable to cytokine production outbursts, which consequently may lead to rapid exacerbation of COVID-19, with the development of ARDS and shock [33]. Furthermore, previous research found that COVID-19 subjects with DM had higher D-dimer levels than those without DM, probably indicating hemostatic system over-activation [31]. This preexisting pro-thrombotic hypercoagulable state of COVID-19 [34] exacerbated by the presence of DM may result in severe thromboembolic outcomes and eventually higher mortality.

Our sample of critically ill patients with severe COVID-19 pneumonia showed an extremely high rate of hyperglycemia (80%). Hyperglycemia is deleterious to the microvasculature, which may facilitate the mechanisms by which viral replication damages the cells, leading to a vicious circle of inflammation and hyperglycemia [35]. In addition to the severity of the pulmonary disease and inflammation, resulting in stress

hyperglycemia as an adaptive response, high doses of corticosteroids used in many patients at the time of ICU admission may have contributed to high glucose levels. In our sample, 28.8% of patients were using corticosteroids before hospital admission. Besides, the high incidence of sepsis (89.7% on admission) probably had a role in inducing hyperglycemia [6].

Glycemic parameters that distinguish the effect of stress-induced hyperglycemia from chronic hyperglycemia did not affect clinical outcomes of these patients. In our sample, glycemic gap and glycated hemoglobin levels were not associated with the outcomes, whereas high glycemic variability was associated with a higher mortality rate. Interestingly, a low rate of severe hypoglycemia was evidenced, despite the high use of short-acting insulin therapy to control acute hyperglycemia on admission. Two main interpretations can be explored: 1) the severity of the disease evolving with stress-induced hyperglycemia, and 2) the high number of patients already using corticosteroids at admission. We believe both explanations are complementary, although corticosteroids were used more frequently and at higher doses than they usually are in ICU patients.

DM is strongly associated with obesity and hypertension. According to the diabetes paradox, obesity is also protective during critical illnesses, with reports of better outcomes in patients with obesity with ARDS [36] and sepsis [37-40]. The chronic low-grade inflammation status observed in patients with obesity could modulate the sepsis host response, resulting in an attenuated cytokine response, thus leading to lower mortality rates compared to individuals with normal weight [41]. In our sample, the mean BMI was 30.7 ± 7.2 kg/m^2, which was higher in survivors than in non-survivors (p=0.012), corroborating the protective effect of moderate obesity in critically ill patients. Besides, hypertension has also been associated with poorer outcomes in patients with

COVID-19 [42]. Our results align with this observation, as we observed a higher prevalence of hypertension in in non-survivors.

The findings of our study provide new insights regarding the concept of the "diabetes paradox". Traditionally, DM is not associated with increased mortality rates in critically ill patients. However, our results reveal that in the context of COVID-19, DM emerges as a significant risk factor for mortality. This observation introduces a new hypothesis-generating concept, suggesting that the impact of DM on mortality may vary in different population of critically ill patients.

This study has limitations. First, due to the retrospective design, some information may have been overlooked. However, the outcomes of interest were decided by two researchers. Second, our analysis revealed no significant differences in the secondary outcomes among the different glycemic groups under investigation. These findings suggest that factors other than glycemic control might influence these outcomes, or that the sample size might have been insufficient to detect subtle differences, as the sample size calculations was estimated for the primary outcome (mortality). Third, glucose monitoring was not continuous, increasing the possibility that some extreme glucose values may have gone unrecorded. Fourth, the assessment of glycemic variability was performed based on capillary measurements, which is a less reliable method in hemodynamic unstable patients. However, this is the most common method for glucose measurement worldwide. Lastly, the glycemic control during all ICU length of stay was not evaluated.

In summary, this retrospective cohort study showed that DM – more than stress-induced hyperglycemia – was associated with higher mortality rates compared to normoglycemia. Although patients with DM have more comorbidities, they do not have higher mortality rates than patients without DM when admitted to the ICU before the

COVID-19 pandemic. However, our results suggest that the "diabetes paradox" is not present in critically ill patients with COVID-19. DM seems to be "unprotective" in this population, presenting a new aspect of COVID-19.

REFERENCES

[1] Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response!, Crit Care. 17 (2) (2013) 305. doi: 10.1186/cc12514.

[2] Krinsley JS, Fisher M. The diabetes paradox: diabetes is not independently associated with mortality in critically ill patients, Hosp Pract. 40 (2) (2012) 31-35. doi: 10.3810/hp.2012.04.967.

[3] Schiborn C, Schulze MB. Precision prognostics for the development of complications in diabetes, Diabetologia. 65 (11) (2022) 1867-1882. doi: 10.1007/s00125-022-05731-4.

[4] Li S, Wang J, Zhang B, Li X, Liu Y. Diabetes Mellitus and Cause-Specific Mortality: A Population-Based Study, Diabetes Metab J. 43 (3) (2019) 319-341. doi: 10.4093/dmj.2018.0060.

[5] Lepper PM et al. Serum glucose levels for predicting death in patients admitted to hospital for community acquired pneumonia: prospective cohort study, BMJ. 344 (2012) e3397. doi: 10.1136/bmj.e3397.

[6] Van Vught LA et al. Admission hyperglycemia in critically ill sepsis patients: association with outcome and host response, Crit Care Med. 44 (7) (2016) 1338-1346. doi: 10.1097/CCM.0000000000001650.

[7] Kompoti M et al. Glycated hemoglobin at admission in the intensive care unit: clinical implications and prognostic relevance, J Crit Care. 30 (1) (2015) 150–155. doi: 10.1016/j.jcrc.2014.08.014.

[8] Viana MV et al. Contrasting effects of preexisting hyperglycemia and higher body size on hospital mortality in critically ill patients: a prospective cohort study, BMC Endocr Disord. 14 (1) (2014) 50. doi: 10.1186/1472-6823-14-50.

[9] Bellaver P et al. Association of multiple glycemic parameters at intensive care unit admission with mortality and clinical outcomes in critically ill patients, Sci Rep. 9 (1) (2019) 18498. doi: 10.1038/s41598-019-55080-3.

[10] Bhatti JM, Raza SA, Shahid MO, Akhtar A, Ahmed T, Das B. Association between glycemic control and the outcome in hospitalized patients with COVID-19, Endocrine. 77 (2) (2022) 213-220. doi: 10.1007/s12020-022-03078-9.

[11] Study investigators N-S et al. Hypoglycemia and risk of death in critically ill patients, N Engl J Med. 367 (12) (2012) 1108–1118. doi: 10.1056/NEJMoa1204942.

[12] Liao WI et al. An elevated glycemic gap is associated with adverse outcomes in diabetic patients with acute myocardial infarction, Sci Rep. 6 (2016) 27770. doi: 10.1038/srep27770.

[13] Moreno RP et al. 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 (10) (2005) 1345–1355. doi: 10.1007/s00134-005-2763-5.

[14] American Diabetes A. 15. Diabetes care in the hospital: standards of medical care in diabetes-2019, Diabetes Care. 42 (Suppl 1) (2019) S173-S181. doi: 10.2337/dc19-S015.

[15] Egi M, Bellomo R, Reade MC. Is reducing variability of blood glucose the real but hidden target of intensive insulin therapy?, Crit Care. 13 (2) (2009) 302. doi: 10.1186/cc7755.

[16] Egi M et al. Variability of blood glucose concentration and short-term mortality in critically ill patients, Anesthesiology. 105 (2) (2006) 244–252. doi: 10.1097/00000542-200608000-00006.

[17] Liao WI et al. An elevated gap between admission and A1C-derived average glucose levels is associated with adverse outcomes in diabetic patients with pyogenic liver abscess, PLoS One. 8 (5) (2013) e64476. doi: 10.1371/journal.pone.0064476.

[18] Liao WI et al. Usefulness of glycemic gap to predict icu mortality in critically ill patients with diabetes, Medicine (Baltimore). 94 (36) (2015) e1525. doi: 10.1097/MD.0000000000001525.

[19] Khunti K, Valabhji J, Misra S. Diabetes and the COVID-19 pandemic, Diabetologia. 66 (2) (2023) 255-266. doi: 10.1007/s00125-022-05833-z.

[20] Hartmann-Boyce J, Rees K, Perring JC, et al. Risks of and from SARS-CoV-2 infection and COVID-19 in people with diabetes: a systematic review of reviews, Diabetes Care. 44 (12) (2021) 2790–2811. doi: 10.2337/dc21-0930.

[21] Gao YD et al. Risk factors for severe and critically ill COVID-19 patients: A review, Allergy. 76 (2) (2021) 428-455. doi: 10.1111/all.14657.

[22] Singh AK, Gillies CL, Singh R, et al. Prevalence of co-morbidities and their association with mortality in patients with COVID-19: a systematic review and meta-analysis, Diabetes Obes Metab. 22 (10) (2020)1915–1924. doi: 10.1111/dom.14124.

[23] Kumar A, Arora A, Sharma P, Anikhindi SA, Bansal N, Singla V, Khare S, Srivastava A. Is diabetes mellitus associated with mortality and severity of COVID-19? A meta-analysis, Diabetes Metab Syndr. 14 (4) (2020) 535-545. doi: 10.1016/j.dsx.2020.04.044.

[24] Siegelaar SE et al. The effect of diabetes on mortality in critically ill patients: a systematic review and meta-analysis, Crit Care. 15 (5) (2011) R205. doi: 10.1186/cc10440.

[25] Vincent JL et al. Insulin-treated diabetes is not associated with increased mortality in critically ill patients, Crit Care. 14 (1) (2010) R12. doi: 10.1186/cc8866.

[26] Graham BB et al. Diabetes mellitus does not adversely affect outcomes from a critical illness, Crit Care Med. 38 (1) (2010) 16–24. doi: 10.1097/CCM.0b013e3181b9eaa5.

[27] Krinsley JS et al. The impact of premorbid diabetic status on the relationship of the three domains of glycemic control and mortality in critically ill patients, Curr Opin Clin Nutr Metab Care. 15 (2) (2012) 151–160. doi: 10.1097/MCO.0b013e32834f0009.

[28] Honiden S, Gong MN. Diabetes, insulin, and development of acute lung injury, Crit Care Med. 37 (8) (2009) 2455–2464. doi: 10.1097/CCM.0b013e3181a0fea5.

[29] Vanhorebeek I et al. Glycemic and nonglycemic effects of insulin: how do they contribute to a better outcome of critical illness?, Curr Opin Crit Care. 11 (4) (2005) 304–311.

[30] Tang Y et al. Cytokine storm in covid-19: the current evidence and treatment strategies, Front Immunol. 11 (2020) 1708. doi: 10.3389/fimmu.2020.01708.

[31] Guo W, Li M, Dong Y et al. Diabetes is a risk factor for the progression and prognosis of COVID-19, Diabetes Metab Res Rev. 31 (2020) e3319. doi: 10.1002/dmrr.3319.

[32] Böni-Schnetzler M, Meier DT. Islet inflammation in type 2 diabetes, Semin Immunopathol. 41 (4) (2019) :501-513. doi: 10.1007/s00281-019-00745-4.

[33] Sharma P, Behl T, Sharma N, Singh S, et al. COVID-19 and diabetes: Association intensify risk factors for morbidity and mortality, Biomed Pharmacother. 151 (2022) 113089. doi: 10.1016/j.biopha.2022.113089.

[34] Pellegrini JAS, Rech TH, Schwarz P, et al. Incidence of venous thromboembolism among patients with severe COVID-19 requiring mechanical ventilation compared to other causes of respiratory failure: a prospective cohort study, J Thromb Thrombolysis. 52 (2) (2021) 482-492. doi: 10.1007/s11239-021-02395-6.

[35] Michalakis K, Ilias I. SARS-CoV-2 infection and obesity: Common inflammatory and metabolic aspects, Diabetes Metab Syndr. 14 (4) (2020) 469-471. doi: 10.1016/j.dsx.2020.04.033.

[36] Ni YN et al. Can body mass index predict clinical outcomes for patients with acute lung injury/acute respiratory distress syndrome? A meta-analysis, Critical Care. 21 (1) (2017) 36. doi: 10.1186/s13054-017-1615-3.

[37] Pepper DJ et al. Increased body mass index and adjusted mortality in ICU patients with sepsis or septic shock: a systematic review and meta-analysis, Critical Care. 20 (1) (2016) 181. doi: 10.1186/s13054-016-1360-z.

[38] Wang S et al. The role of increased body mass index in outcomes of sepsis: a systematic review and meta-analysis, BMC Anesthesiol. 17 (1) (2017) 118. doi: 10.1186/s12871-017-0405-4.

[39] Pepper DJ et al. Does Obesity Protect Against Death in Sepsis? A Retrospective Cohort Study of 55,038 Adult Patients, Crit Care Med. 47 (5) (2019) 643-650. doi: 10.1097/CCM.0000000000003692.

[40] Jagan N et al. Sepsis and the obesity paradox: size matters in more than one way, Crit Care Med. 48 (9) (2020) e776-e782. doi: 10.1097/CCM.0000000000004459.

[41] Robinson J et al. The obesity paradox in sepsis: a theoretical framework, Biol Res Nurs. 22 (2) (2020) 287-294.

[42] Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study, Lancet. 395 (2020) 1054–62. doi: 10.1016/S0140-6736(20)30566-3.

FIGURES

QUESTION: Are DM and stress-induced hyperglycemia at ICU associated with mortality in critical ill patients with COVID-19?

POPULATION

111 Men   76 Women

Inclusion criteria: adult ICU patients with COVID-19 and with a HbA1c measurement

Exclusion criteria: diabetic ketoacidosis, hyperosmolar hyperglycemic state, hemoglobinopathies, ICU LOS < 24h

Mean age: **60 years**

SAPS 3: 62

LOCATION

Brazilian tertiary ICU

DESIGN

Retrospective cohort study

FINDINGS

Primary outcome: overall mortality = **43.2%**

	Mortality
Normoglycemia	25.8%
Stress-induced hyperglycemia	37.3%
Diabetes mellitus	55.7%
	p=0.021

No statistical difference related to secondary outcomes (ICU and hospital mortality, need for MV, need for RRT, LOS at the ICU and at the hospital, and ICU readmission)

- Mortality was higher in patients with higher glycemic variability (> 40mg/dL)

Mortality (adjusted for age and SAPS 3)

Diabetes mellitus 55.7%   Non-diabetes mellitus 34%   p=0.024

CONCLUSION

DM, more than stress-induced hyperglycemia, is associated with higher mortality rates compared to normoglycemia.
It is suggested that the "diabetes paradox" does not occur in critically ill patients with COVID-19.

Figure 1. Flow chart of the study. DM: diabetes mellitus; ICU: intensive care unit; LOS: lenght of stay; MV: mechanical ventilation; RRT: renal replacement therapy.
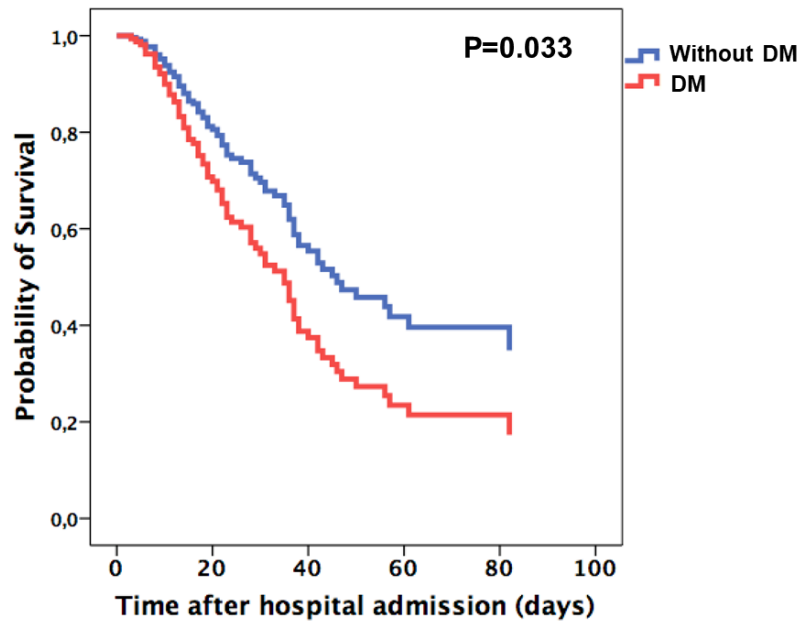
Figure 2. Probability of survival according to the presence or absence of DM after adjusting for age, presence of hypertension, and disease severity using SAPS 3 score and need of vasopressors. DM: diabetes mellitus.
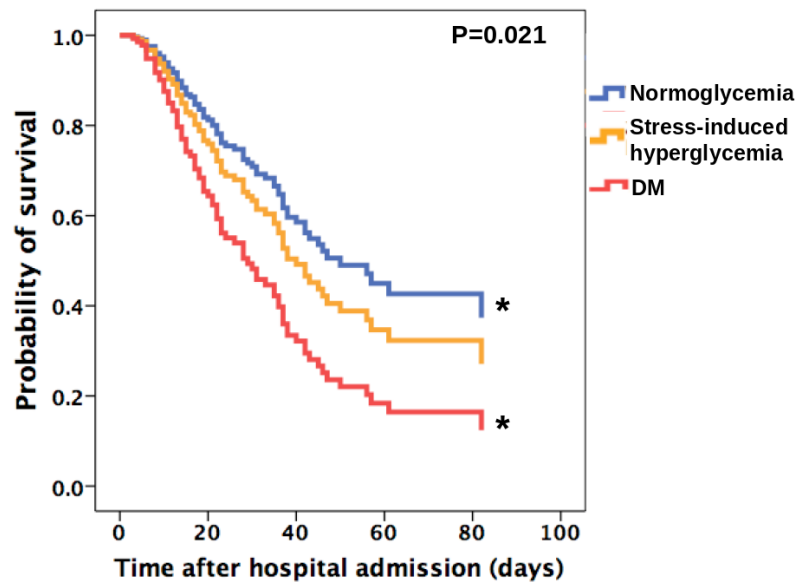
Figure 3. Probability of survival according to glycemic status. DM: diabetes mellitus. *: difference between groups.

Table 1. Baseline characteristics of patients.

Characteristics	All patients	Patients with normoglycemia n = 31	Patients with stress- induced hyperglycemia n = 75	Patients with DM n = 79	p
Demographycs					
Age (years)	60 ± 15	53 ± 16 [a]	59 ± 15	63 ± 12 [a]	0.03
Men (n, %)	111 (60)	17 (54.8)	48 (64)	46 (58.2)	0.623
BMI (kg/m^2)	30.7 ± 7.2	30.8 ± 7	29.7 ± 6.6	31.7 ± 7.8	0.226
Coexisting conditions					
Hypertension (n, %)	114 (61.6)	13 (41.9) [b, c]	41 (54.7) [b]	60 (76) [b, c]	0.001
Diabetes (n, %)	79 (42.7)	0	0	79 (100)	-
Cancer (n, %)	16 (8.6)	3 (9.7)	6 (8)	7 (8.9)	0.958
Chronic kidney disease (n, %)	16 (8.6)	2 (6.5)	6 (8)	8 (10.1)	0.799
Ischemic heart disease (n, %)	20 (10.8)	1 (3.2)	7 (9.3)	12 (15.2)	0.166
Heart failure (n %)	15 (8.1)	1 (3.2)	7 (9.3)	7 (8.9)	0.548
COPD (n, %)	18 (9.7)	2 (6.5)	6 (8)	10 (12.7)	0.495
Asthma (n, %)	21 (11.4)	4 (12.9)	7 (9.3)	10 (12.7)	0.774
Transplantation (n, %)	6 (3.2)	0	4 (5.3)	2 (2.5)	0.331
Immunosuppresion (n, %)	10 (5.4)	1 (3.2)	6 (8)	3 (3.8)	0.433
Previous use of corticosteroids	51 (28.8)	7 (23.3)	22 (31)	22 (28.9)	0.74
Reasons for ICU admission					0.814
Acute respiratory failure (n, %)	177 (96)	29 (93)	72 (96)	76 (96)	
Other (n, %)	8 (4)	2 (7)	3 (4)	3 (4)	
Place before ICU admission					0.228
Emergency room (n, %)	54 (29)	8 (26)	18 (24)	28 (35)	
Medical unit (n, %)	46 (25)	11 (35)	21 (28)	14 (18)	
External transfer (n, %)	85 (46)	12 (38)	36 (38)	37 (47)	
Disease severity					
SAPS 3 score	62 ± 16	57 ± 16	61 ± 15	64 ± 16	0.15
Presence of sepsis (n, %)	166 (89.7)	28 (90.3)	68 (90.7)	70 (88.6)	0.9
Pulmonary (n, %)	161 (87)	26 (83.4)	65 (86.7)	70 (88.6)	

Other (n, %)	5 (2.7)	2 (7.1)	3 (4.4)	0	
Need for vasopressors (n, %)	99 (53.5)	10 (32.3) [d]	43 (57.3)	46 (58.2)	0.034
Need for renal replacement therapy (n, %)	60 (32.4)	11 (35.5)	21 (28)	28 (35.4)	0.568
Need for invasive mechanic ventilation (n, %)	137 (74.1)	22 (71)	56 (74.7)	59 (74.7)	0.912
Prone position (n, %)	71 (38.4)	13 (41.9)	30 (40)	28 (35.4)	0.764
ECMO (n, %)	2 (1.1)	1 (3.2)	1 (1.3)	0	0.326
Use of corticosteroids (n, %)	126 (71.2)	19 (70.4)	54 (74)	53 (68.8)	0.781
Nutrition					0.876
None (n, %)	30 (16)	5 (16)	11 (37)	14 (47)	
Oral or enteral (n, %)	155 (84)	26 (84)	64 (85)	65 (42)	
Insulin therapy					
Long-acting insulin, first 24h from admission (n, %)	28 (15.3)	0	2 (2.7)	26 (33.3)	<0.0001
Short-acting insulin, first 24h from admission (n, %)	89 (51.4)	4 (13.8)	29 (41.4)	56 (75.7)	<0.0001
Biochemical measurements					
Hematocrit (%)	37.1 ± 5.9	36.6 ± 5.7	37.5 ± 6	36.9 ± 6	0.723
Hemoglobin (g/dL)	12.3 ± 2.1	12.2 ± 2	12.4 ± 2.2	12.2 ± 2.1	0.788
Leukocytes (10^3/mm³)	10.9 ± 5.8	9 ± 3.9	11.4 ± 6.7	11.1 ± 5.4	0.135
Lymphocytes (%)	9.4 ± 7	10.3 ± 6.5	7.9 ± 5.8	10.4 ± 8	0.071
Platelets (10^3/uL)	230 (164 - 284)	203 (148 - 250)	233 (167 – 278)	229 (159 - 306)	0.315
Creatinine (mg/dL)	1.1 (0.8 - 1.7)	0.9 (0.7 – 1.9)	1.1 (0.8 – 1.8)	1.1 (0.8 - 1.6)	0.715
Potassium (mEq/L)	4.4 ± 0.6	4.3 ± 0.5	4.4 ± 0.7	4.4 ± 0.6	0.668
CRP (mg/dL)	151 (86 - 220)	142 (77 - 216)	146 (86 - 219)	152 (95 – 225)	0.316
Lactate (mmol/L)	1.9 ± 0.4	1.2 ± 0.5	2.3 ± 0.7	1.9 ± 0.3	0.54
Blood glucose (mg/dL)	174 ± 106	104 ± 12 [a]	125 ± 44 [e]	245 ± 122 [a, e]	<0.0001
HbA1C (%)	6.8 ± 2	5.6 ±0.4 [a]	5.7 ± 0.4 [e]	8.3 ± 2.2 [a, e]	<0.0001

DM: diabetes mellitus; BMI: body mass index; SAPS 3 score: Simplified Acute Physiology 3 score; COPD: chronic obstructive pulmonary disease; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit; CRP: C-reactive protein; HbA1c: glycated hemoglobin. Values are mean ± SD or median and interquartile range.

[a, e] values are statistically different between groups, ANOVA with Bonferroni post-hoc test.

[b, c,] values are statistically different between groups, chi-square test.

[d] values are statistically different from other groups, chi-square test.

Table 2. Effects of hyperglycemia on clinical outcomes in critically ill patients with severe Covid-19.

Outcomes	All patients	Patients with normoglycemia	Patients with stress-induced hyperglycemia	Patients with DM	p
Mortality (n, %)	80 (43.2)	8 (25.8) [a, b]	28 (37.3) [b]	44 (55.7) [a, b]	**0.021**
Need for RRT (n, %)	60 (32.4)	11 (35.5)	21 (28)	28 (35.4)	0.568
Need for VM (n, %)	137 (74.1)	22 (71)	56 (74.7)	59 (74.7)	0.912
ICU readmission (n, %)	4 (2.2)	0	2 (2.7)	2 (2.5)	0.662
Time on VM (days)	16 (9-26)	17.5 (8.3-26.3)	15 (6.5-23.8)	17 (10.3-28)	0.685
LOS, hospital (days)	19 (10-36)	22 (10-35)	21 (9-37)	17 (10-29)	0.708
LOS, ICU (days)	14 (6-26)	19 (8-27)	14 (6-26)	14 (6-23)	0.839

RRT: renal replacement therapy; MV: mechanical ventilation; LOS: length of stay; ICU: intensive care unit. Values are mean ± SD or median and interquartile range.
[a, b]: values are statistically different between groups, chi-square test.

Table 3. Effects of glycemic gap and glycemic variability on clinical outcomes in critically ill patients with severe COVID-19 pneumonia.

Outcomes	Glycemic gap			Glycemic variability		
	<80 mg/dL n = 149	>80 mg/dL n = 37	p	<40 mg/dL n = 45	>40 mg/dL n = 128	p
Mortality (n, %)	63 (42.3)	18 (48.6)	0.484	12 (26.7)	62 (48.4)	**0.011**
Need for RRT (n, %)	47 (31.5)	13 (35.1)	0.676	13 (28.9)	43 (33.6)	0.562
Need for MV (n, %)	109 (73.2)	30 (81.1)	0.321	32 (71.1)	95 (74.2)	0.685
ICU readmission (n, %)	4 (2.7)	0	0.314	0	4 (3.1)	0.23
Time on MV (days)	16 (9-26)	17 (8-27.3)	0.969	16.5 (9.3-26.8)	15 (8-26)	0.594
LOS, hospital (days)	24 (14-39.5)	19.5 (12.5-37)	0.757	28.5 (16-39.8)	21 (13-37)	0.415
LOS, ICU (days)	20 (20-31.5)	18 (9-28)	0.613	21 (11.2-35)	17 (9-29)	0.406

RRT: renal replacement therapy; MV: mechanical ventilation; LOS: length of stay; ICU: intensive care unit. Hypoglycemia was defined as any blood or capillary glucose <70 mg/dL during the first ICU day. Hyperglycemia was defined as any blood glucose >140 mg/dL at ICU admission. Values are mean ± SD or median and interquartile range.

Material suplementar:

Supplementary Table 1. Glycated hemoglobin and clinical outcomes in patients with severe COVID-19 pneumonia.

	Mortality			Need for RRT			Need for MV			ICU readmission		
	Yes	No	p	Yes	No	p	Yes	No	p	Yes	No	p
HbA1c (%)	7 ± 2	6.2 ± 1.9	0.131	6.7 ± 1.5	6.8 ±2.1	0.736	6.7 ± 1.8	7.1 ± 2.5	0.275	5.7 ± 1	6.8 ± 2	0.255

HbA1c: glycated hemoglobin RRT: renal replacement therapy; MV: mechanical ventilation; ICU: intensive care unit. Values are mean ± SD.

ARTIGO 2

Association between diabetes and stress-induced hyperglycemia with skeletal muscle gene expression of *IRS1*, *IRS2*, *INSR*, *SLC2A1,* and *SLC2A4* in critically ill patients: a prospective cohort study

Artigo submetido na revista Diabetes & Metabolism (fator de impacto 7,2)

Dados ainda não publicados

ARTIGO 3

Association between neuromuscular blocking agents and the development of intensive care unit-acquired weakness (ICU-AW): A systematic review with meta-analysis and trial sequential analysis

Artigo publicado na revista Anaesthesia, Critical Care & Pain Medicine (fator de impacto 5,5)

Abstract

Background: To systematically review the literature and to synthesize evidence concerning the effects of the use of neuromuscular blocking agents (NMBA) regarding the development of intensive care unit-acquired weakness (ICU-AW).

Methods: This study was registered in the PROSPERO database CRD42020142916. We performed a systematic review in PubMed, Embase, and the Cochrane Central Register of Controlled Trials. Randomized clinical trials (RCTs), and cohort studies with adults that reported the use of NMBA and the development of ICU-AW were included. Two reviewers independently screened citations, conducted data extraction, and assessed risk of bias using the Newcastle-Ottawa scale for cohort studies and the Cochrane Collaboration tool for RCTs. Pre-specified subgroup analyses were performed for presence of sepsis and type of NMBA used. Exploratory subgroup analyses were performed for study design (RCTs vs cohort studies), and cause of respiratory failure (acute respiratory distress syndrome and severe asthma), and studies with concomitant use of NMBA and corticosteroids. The quality of evidence for intervention effects was summarized. Certainty of evidence was assessed using the GRADE approach.

Results: We included 30 studies, four RCTs, 21 prospective and five retrospective cohorts, enrolling a total of 3,839 patients. Most of the included studies were observational with high heterogeneity, whereas the RCTs had a high risk of bias. The use of NMBA was associated with risk for the developing ICU-AW (OR 2.77; 95% CI 1.98-3.88, I^2 =62%). An exploratory subgroup analysis of RCTs vs observational studies showed an effect size not significantly related to the incidence of ICU-AW (OR 1.44; 95% CI 0.61-3.4, I^2 =75%). A trial sequential analysis showed the need to include 14,023 patients in order to provide evidence for either beneficial or harmful intervention effects.

In subgroup analysis, the effect of NMBA was more pronounced in patients with sepsis, patients with asthma and with the use of corticosteroid drugs.

Conclusions: This meta-analysis suggests that the use of NMBA might be implicated in development of ICU-AW. However, there is not enough evidence to definitively conclude about the association between the use of NMBA and the development of ICU-AW, as these results are based mostly on observational studies with high heterogeneity.

Keywords: Neuromuscular blocking agents; intensive care unit-acquired weakness; meta-analysis; critical illness; trial sequential analysis.

Abbreviation list

ARDS: acute respiratory distress syndrome

aOR: adjusted odds ratio

CI: confidence intervals

ICU-AW: intensive care unit-acquired weakness

MD: mean differences

MV: mechanical ventilation

MRC: Medical Research Council

NMBA: neuromuscular blocking agents

OR: odds ratio

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RCT: randomized clinical trial

ROB: risk of bias

TSA: trial sequential analysis

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Authors' Contribution

PB participated in the study conception and design, data acquisition, analysis and interpretation of data, statistical analysis, and drafting and revising the manuscript. AFS participated in data acquisition, analysis, and drafting and manuscript revision. CBL participated in statistical analysis and manuscript revision. THR participated in the study conception and design, interpretation of data, and drafting and the revision of the manuscript. WN participated in the study conception and design, data acquisition, analysis and interpretation of data, statistical analysis, and revision.

PB is the guarantor of this work and has full access to all data and takes responsibility for the integrity of the data and the accuracy of data analysis.

Acknowledgements

1. Introduction

Intensive care unit-acquired weakness (ICU-AW) is a devastating neuromuscular condition that aggravates the course of critical illness (1), which, in the pathophysiology, includes both peripheral nerve alterations, called critical illness polyneuropathy (2, 3, 4), and myopathic injuries, named critical illness myopathy (2, 5, 6, 7). A combination of inflammatory and toxic factors, including nutritional deficits, and prolonged immobilization, leads to ICU-AW (1, 8). The incidence of ICU-AW is associated with difficulties in weaning from mechanical ventilation (MV) (9, 10, 11, 12, 13), increased mortality and hospital costs (9, 13, 14), as well as prolonged functional disabilities in survivors (1, 15).

Several risk factors are involved in the development of ICU-AW namely: sepsis, multiple organ dysfunction, severity of the disease, need for renal replacement therapy, and hyperglycemia (8, 16). Furthermore, medications are meticulously implicated, especially aminoglycoside antibiotics, corticosteroids, and neuromuscular blocking agents (NMBA) (9, 17, 18).

NMBA are commonly used to facilitate rapid sequence intubation and to maintain patients on prolonged MV, reducing patient-ventilator asynchrony, and decreasing the work of breathing, notably in patients with severe gas exchange impairments, as in critically ill patients with acute respiratory distress syndrome (ARDS) (19, 20, 21).

The use of NMBA has been suggested as an independent predictor of ICU-AW in several diseases (6, 9, 22, 23, 24, 25), leading to both myopathic (22, 23) and polyneuropathic (24) components of the syndrome. However, evidence does not corroborate the association between NMBA use and ICU-AW development (26, 27, 28,

29). Additionally, most studies were conducted in selected patient populations, such as patients with ARDS (26, 27, 28, 30), severe asthma (6, 22, 23, 24), septic shock (9), and Sars-CoV-2 infection (31, 32). The heterogeneity of the studies in terms of the type of NMBA used, total dose and duration of use, and the cause of respiratory failure, results in unclear conclusions.

ICU-AW is a very common condition with deleterious consequences for patients admitted to the ICU. Understanding its pathophysiological mechanisms can be useful to implement measures capable of reducing its incidence. Therefore, this study aims to systematically review the literature and to synthesize evidence concerning the use of NMBA compared to nonuse in the development of ICU-AW in critically ill adult patients.

2. Material and methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses: the PRISMA statement. This study was registered in the PROSPERO database CRD42020142916.

2.1 Search strategy

We aim to identify all studies evaluating the use of NMBA and its association with the development ICU-AW. We defined the comparison of the following pre-specified subgroups: sepsis and type of NMBA used (benzyl quinolinic NMBAs or others). Study design (RCT or cohort studies), cause of respiratory failure (ARDS or non-ARDS, and severe asthma or non-severe asthma) were analyzed a posteriori. A systematic review of the literature of studies restricted to English language was undertaken in PubMed, Embase, and Cochrane Central Register of Controlled Trials from inception to November 2022. Search terms are available in Supplementary Appendix 1. Moreover, a

manual search of references cited by the selected articles and relevant reviews was performed.

2.2 Eligibility criteria

We included randomized clinical trials (RCT) and prospective or retrospective cohort studies that evaluated the use of NMBA and the development of ICU-AW in adult critically ill patients. The studies selected describe the primary outcome of interest (ICU-AW) as follows: ICU-AW clinically diagnosed by the use of Medical Research Council (MRC) scale for muscle strength, electrophysiological results, or histopathology of muscle or nerve tissue analysis. We excluded studies involving patients with previous diagnosis of myopathy or polyneuropathy, reported insufficient data, exclusively enrolled pediatric patients, and experimental studies. The most complete report with the longest follow-up was included if duplicate papers were identified.

2.3 Study selection and data abstraction

Two reviewers (PB and AFS) independently screened titles and abstracts and selected studies for full-text analysis based on inclusion criteria. Data were extracted independently by each researcher using a standardized form. The following data were collected from each study: author, year of publication, country of origin, study design, the number of subjects who received or not received NMBA, and the number of outcomes of interest in each group. When available, the type of intensive care unit (ICU), the cause of respiratory failure, and data about dose, type, and the duration of use of NMBA were recorded. The outcome of interest was ICU-AW. Disagreements were resolved by consensus or by a third-party decision (WN).

2.4 Study Quality Assessment

Two reviewers (PB and AFS) independently assessed risk of bias (ROB) of each study using the Newcastle-Ottawa scale (33) for cohort studies and the Cochrane Collaboration tool for RCT (33). Level of certainty was assessed using the GRADE approach.

2.5 Data synthesis and analysis

This meta-analysis was in accordance with the Cochrane Collaboration guidelines (33). The outcome of interest evaluated was ICU-AW (primary outcome). No secondary outcomes were analyzed. All statistical analyses were performed in Review Manager version 5.4 (Nordic Cochrane Center, Copenhagen, Denmark). The number of events in each group was presented as absolute numbers. Characteristics of population in each study was tabulated in a Microsoft Excel spreadsheet in order to define which studies were eligible for each synthesis. This material can be provided to interested parties through direct contact with the main investigator (PB). We expressed the pooled effect estimates for binary and continuous variables using odds ratio (OR) and mean differences (MD) with 95% confidence intervals (CI). The Cochran Q and I^2 test were used to evaluate heterogeneity between studies. Substantial heterogeneity was defined as I^2 >50%. A random-effect model with Mantel-Haenszel weighting was used for all analyses. Heterogeneity was explored using subgroup analysis. Interaction between subgroups were evaluated based on Cochrane Handbook recommendations (33), and meta regressions using subgroups as dichotomous variables were performed. The goal of the interaction test is to understand if the magnitude of the effect of the intervention differs within categories of a subgroup. If the effect is different within subgroups we call this effect modification of the intervention on the outcome due to the additional presence of

the subgroup variable. Funnel plot asymmetry was used to assess potential publication bias. We performed two a priori subgroup analyses based on the type of NMBA used and the presence of sepsis. We also performed five exploratory analyses: study design (RCT or observational study), of studies with adjustment for potential confounders (by adjusted OR), studies with patients with severe asthma, studies with patients with ARDS, and studies with concomitant use of NMBA and corticosteroids.

We performed the trial sequential analysis (TSA) using TSA version 0.9.5.10 Beta (Centre for Clinical Intervention Research Department, Copenhagen, Denmark) to determine whether sufficient data from RCTs were available to draw definitive conclusions (34). We also performed the analysis with 80% power, 5% type I error, and the expected difference between groups as 20% and 40% to create a Z-curve and boundaries to identify benefit, harm or futility. If the curve crosses one of the boundaries or reaches the optimal sample size line, definitive conclusions can be assumed (35, 36). The proportion of ICU-AW in controls was set at 40%, as it varies widely in the literature. The choice of this cutoff was based on a systematic review including 31 studies that reported a median prevalence of 43% (interquartile range 25–75%) (37). The TSA was adjusted for diversity (model variance based). Inconsistency (I^2) and diversity (D^2) were calculated and results were adjusted for diversity (38). TSA analysis was not pre-specified in PROSPERO and, as a protocol deviation, should be interpreted as exploratory.

3. Results

3.1 Search and selection of studies

Search terms are available in Supplementary Appendix 1. The initial electronic search yielded a total of 23,315 potentially relevant citations. The manual search did not

result in any additional studies. After removing duplicates and screening titles and abstracts, 83 articles were selected for full-text review, out of them, 53 were excluded for the reasons presented in Figure 1, whereas 30 articles met the inclusion criteria and were included. Figure 1 shows the search strategy. Supplementary Appendix 2 shows the 53 articles excluded from meta-analysis.

3.2 Study characteristics and quality

The main characteristics of the studies included in this systematic review are presented in Supplementary Table 1. The search identified four RCT (26, 27, 28, 30) and 26 cohort studies.

Supplementary Table 2 presents the methodological quality assessment of the studies. According to the Cochrane Collaboration Tool, ROB was high in three RCT (27, 28, 30) due to lack of blinding. Newcastle-Ottawa scale evaluated the overall quality of prospective studies as acceptable.

3.3 Use of NMBA and the development of ICU-AW

The 30 studies included in data synthesis enrolled a total of 3,839 patients, 1,725 patients who received NMBA and 2,114 patients who did not receive. Ten studies (3, 12, 14, 17, 39, 40, 41, 42, 43, 44) did not provide information on the type of NMBA used. The most commonly used NMBA was vecuronium in 13 studies (4, 5, 6, 7, 9, 10, 22, 23, 24, 45, 46, 47, 48), followed by atracurium in seven studies (4, 6, 9, 10, 22, 23, 48), cisatracurium in seven studies (22, 26, 27, 28, 29, 30, 32), and pancuronium in four studies (6, 22, 23, 46). The use of more than one type of NMBA was described in eight studies (4, 6, 9, 10, 22, 23, 31,46, 48), whereas seventeen studies (3, 4, 6, 10, 14, 17, 22, 23, 31, 39, 40, 41, 42, ,43, 44, 45, 48) did not report the dose of NMBA and 14 studies

did not report the duration of use (5, 6, 9, 10, 17, 29, 39, 40, 41, 42, 43, 44, 45, 46). All RCT used cisatracurium with a bolus dose followed by a continuous infusion for approximately 48 hours. Supplementary Table 3 shows the type of NMBA used, dose, and duration of use in each study.

The incidence of ICU-AW ranged from 0 to 90% among patients who received NMBA and from 0 to 100% in the control group. The timing of evaluation of the development of ICU-AW varied widely between studies, whereas eight studies did not report this information (4, 7, 22, 23, 31, 39, 42, 48). Supplementary Table 4 shows the ICU-AW assessment time in each study, as well as the muscle strength assessment by the MRC scale.

Figure 2 shows the forest plot for the 30 studies pooled together. The size of the effect indicated that the use of NMBA was associated with risk of developing ICU-AW (OR 2.77; 95% CI 1.98-3.88). According to GRADE approach, there is a low level of certainty of evidence, as demonstrated in Supplementary Table 5. Data were pooled using a random effects model considering the observed heterogeneity ($\tau2$ =0.36; $\chi2$ =73.55, df =28 (P < 0.00001); I^2 =62%). Publication bias was not identified.

3.4 A priori subgroup analyses

Patients with sepsis

Three studies (9, 10, 41) included patients with sepsis (68 patients in the NMBA group and 169 in the control group). In this population, the use of NMBA increased the odds of developing ICU-AW (OR 14.79; 95% CI 1.19-183.84). Data were pooled using a random effects model considering the observed heterogeneity ($\tau2$ =4.03; $\chi2$ =10.96, df

=2 (P =0.004); I^2= 82%) (Supplementary Figure 1). Publication bias was not identified. The p-value for the interaction test for subgroups analysis (sepsis vs non-sepsis) was 0.16.

Type of NMBA

Taking into account the type of NMBA, there was no difference in relation to the development of ICU-AW among patients who received benzyl quinolinic NMBA (atracurium and cisatracurium) (26-30, 32) (OR 1.79; 95% CI 0.9 - 3.6) χ2 =11.36, df =4 (P =0.02); I^2 =65%) (Supplementary Figure 2). This analysis included 728 patients in the NMBA group and 653 in the control group. The p-value for the interaction test for subgroups analysis (cisatracurium use vs non-cisatracurium use) was 0.13.

However, risk to develop ICU-AW was detected among patients who received amino-steroid NMBA (vecuronium and pancuronium) (5, 7, 24, 45, 46, 47) (OR 3.68; 95% CI 1.51-9) (τ2 = 0.42; χ2 = 7.88, df = 5 (P = 0.16); I^2 = 37%) (Supplementary Figure 3). This analysis included 109 patients in the NMBA group and 143 in the control group. Publication bias was not identified. The p-value for the interaction test for subgroups analysis (aminosteroid use vs non-aminosteroid use) was 0.73.

3.5 Exploratory analysis

RCT versus cohort studies and ARDS versus non-ARDS studies

When we separate the studies according to the study design, cohort studies, that included a total of 1,082 patients in NMBA group and 1,486 in the control group, it demonstrated an effect size pointing to increased odds of developing ICU-AW (OR 3.27; 95% CI 2.19 - 4.88) (Supplementary Figure 4). However, when RCT (coincidentally all ARDS studies are RCTs), including a total of 643 patients in the NMBA group and 628

in the placebo group, were analyzed separately, the effect size was not significant (OR 1.44; 95% CI 0.61-3.4) (Figure 3). The data were pooled using a random effects model, considering the observed heterogeneity ($\tau 2$ =0.36; $\chi 2$ =7.93, df =2 (P <0.02); I^2 =75%). Publication bias was not identified. The p-value for the interaction test for subgroups analysis (RCT vs non-RCT / ARDs vs non-ARDS) was 0.08.

Patients with severe asthma

Five cohort studies (6, 7, 22, 23, 48) evaluated patients with severe asthma (233 patients in NMBA group and 160 in the control group), one prospective (7) and four retrospective (6, 22, 23, 48). Among them, the effect size analysis indicated that the use of NMBA was significantly associated with risk of developing ICU-AW (OR 7.91; 95% CI 1.76-35.66). Data were pooled using a random effects model considering the observed heterogeneity ($\tau 2$ =1.39; $\chi 2$ =7.77, df =4 (P <0.1); I^2 =48%) (Supplementary Figure 5). Publication bias was not identified. The p-value for the interaction test for subgroups analysis (asthma vs non-asthma) was 0.16.

Other exploratory analysis

We conducted other exploratory analyses of studies with specific populations of patients, presented in Supplementary Table 6.

In studies where all patients received corticosteroids concomitantly with NMBA (n =328) (5, 6, 7, 22, 43), NMBA use was associated with increased risk of ICU-AW (OR 4.14, 95% CI 1.29 – 13.30, p =0.02, I^2 =30%). The p-value for the interaction test for subgroups analysis (corticosteroids plus NMBA use vs non-corticosteroid plus NMBA use) was 0.6. Similarly, when only studies that included more than 50% of patients receiving corticosteroids (n = 999) (5, 6, 7,

14, 22, 44, 46, 48) were evaluated, there was an increased risk of ICU-AW with the use of NMBA compared to nonuse (OR 2.88, 95% CI 1.41 – 5.85, p =0.04, I^2 =53%).

When studies that included more than 50% of patients with sepsis were evaluated in separate (n = 1,986) (4, 9, 14, 27, 39, 41, 44), the use of NMBA was associated with increased risk of ICU-AW (OR 2.47, 95% CI 1.34 – 4.54, p =0.004, I^2 =78%), but when studies that included exclusively patients with sepsis (n =173) (9, 41) were evaluated, there was no association with increased risk of ICU-AW with NMBA use (OR 32.4, 95% CI 0.78 – 1343.61, p =0.07, I^2 =84%).

We also performed an exploratory analysis, evaluating only observational studies that performed analyses adjusted for potential confounders. In an analysis for adjusted OR (aOR) of three studies (9, 23, 43) (n =322 patients), the use of NMBA was associated with an increased ICU-AW risk: aOR 2.31 (95% CI 1.38 – 3.86, p =0.001, I^2 =20%).

In our study, we did not identify any significant interaction between subgroups, as evaluated by a random effects model.

3.6 Trial sequential analysis for RCT

Although not included in the initial protocol, exploratory analysis of specific patient populations and TSA were performed. TSA was carried out in order to determine whether the question under study was already definitively answered based on the evidence of RCTs available to date.

The TSA analysis calculated an optimal sample size of 14,023 patients considering an increase in risk of 20% and 3,310 patients for a risk of 40%. So, the optimal sample size, harm boundary, and futility boundaries were not reached for the association of the use of NMBA with the incidence of ICU-AW (Supplementary Figure 6). TSA

analysis indicated a high heterogeneity (I2 = 71% and D2 = 92%) and the pooled effect) was 1.25 (CI adjusted for diversity 0.13 – 12.41).

The study review protocol according to the PRISMA 2020 checklist can be found in Supplementary Appendix 3.

4. Discussion

The main safety concern of NMBA use is its association with the development of ICU-AW. The present meta-analysis, which includes 30 studies, shows that the use of NMBA is associated with an increased risk of developing ICU-AW. This finding is especially supported by cohort studies with high heterogeneity and provides a low level of certainty based on GRADE approach.

The four placebo-controlled RCT included in this meta-analysis enrolled exclusively patients with ARDS and used cisatracurium for myorelaxation lasting for 48 hours. When polled together, a high heterogeneity was observed and no statistical difference for the use of NMBAs and the development of ICU-AW was demonstrated. The Cochrane Risk of Bias Toll classified three of the RCT (27, 28, 30) as having high ROB due to the absence of blinding. Also, three RCTs were conducted by the same research group. Particularly, the RCTs did not use electromyography examination, and ICU-AW diagnosis was based on clinical evaluation, suggesting that cases of ICU-AW may have gone undetected, since Gainnier et al (30) did not report any cases of ICU-AW. Besides, the RCTs did not assess the effectiveness of the neuromuscular blockade with train-of-four stimulation, raising a question about adequate blockade. Moreover, the short duration of NMBA use (48 hours) may explain the low incidence of ICU-AW. Furthermore, the early assessment (5 days) of ICU-AW incidence of the studies by Forel et al. (28) and Gainnier et al. (30) might have been insufficient to recognize muscle

weakness in patients requiring prolonged mechanical ventilation, particularly when they are sedated.

Regarding the pre-specified subgroup analysis of studies enrolling patients with sepsis, we showed an association between the use of NMBA and the development of ICU-AW in this population, but the result has a very wide IC and high heterogeneity. Moreover, most studies that enrolled patients with sepsis evaluated the incidence of ICU-AW after 28 days, but did not specify the duration of NMBA use.

One interesting finding of our meta-analysis is the type of NMBA and the development of ICU-AW. Unlike the amino-steroid class of NMBA (vecuronium and pancuronium), the benzyl quinolinic class (cisatracurium and atracurium) was not associated with an increased risk of ICU-AW; however the high heterogeneity of the studies should be considered. Changes in body composition and hepatorenal function led to changes in NMBA pharmacokinetics (49). In this sense, cisatracurium and atracurium have a particular metabolization mechanism. Hofmann clearance is an elimination reaction that leads to spontaneous inactivation of the agent in the bloodstream, dependent on temperature and pH (50), accounting for 77% of total body clearance of cisatracurium and atracurium, whereas organ-dependent clearance accounts for only 23% (51). We hypothesized that this special feature of drug kinetics might explain the rapid metabolism of this type of NMBA, preventing its accumulation and potential side effects in critically ill patients with multiorgan dysfunction, as the varying degrees of renal and liver failure do not significantly affect its metabolism and elimination.

The exploratory analyses of studies that evaluated patients with severe asthma showed an association between NMBA use and ICU-AW. None of the studies with patients with severe asthma clearly stated the timing of the assessment of ICU-AW, despite these pitfalls, their results are in line with its pathophysiology. Patients with severe

asthma and sepsis are at high risk of developing ICU-AW, as many risk factors for the development of muscle weakness, such as hyperglycemia, systemic inflammation, and the use of corticosteroids and antibiotics, are concomitantly present in these diseases.

We used TSA to verify our results from RCT, as this is the appropriate study design to analyze a drug benefit or risk. Our TSA showed that there was insufficient data to reach numerical conclusions about the association between NMBA use and ICU-AW development.

This review has some strengths. Updated searching in several databases avoided the bias of excluding potential articles, while broad search terms led to a large initial screening. The TSA analysis, despite being an exploratory analysis, demonstrated that numerical conclusions cannot be reached at this time for RCT, and further studies are necessary.

However, there are also limitations. First, the search for articles was restricted to the English language. Second, the time for muscle weakness assessment, the diagnostic methods, and the choice and doses of NMBA used varied widely among studies; most of the included studies were observational with high heterogeneity, whereas the RCT had a high ROB. Third, the subgroup analysis of study design (RCT versus observational study) was not pre-specified, and as a protocol deviation, should be interpreted as exploratory. Finally, the substantial statistical heterogeneity remained, despite the outcome being analyzed in pre-specified subgroups, in an effort to reduce methodological and clinical heterogeneity.

5. Conclusion

The pathophysiology of ICU-AW is complex and poorly understood. Isolating the role of each risk factor that contributes to its development is difficult. This meta-analysis suggests that the use of NMBA might be implicated in development of ICU-AW. However, this conclusion is based on observational studies with high heterogeneity. The use of NMBA for short periods, especially the benzyl quinolinic class, seems to be safer, but the TSA analysis indicates the need for further RCT to better answer this question.

6. References

1- Deem S, Lee CM, Curtis JR. Acquired neuromuscular disorders in the intensive care unit. Am J Respir Crit Care Med. 2003 Oct 1;168(7):735-9.

2- Latronico N, Peli E, Botteri M. Critical illness myopathy and neuropathy. Curr Opin Crit Care. 2005 Apr;11(2):126-32.

3- Coakley JH, Nagendran K, Honavar M, Hinds CJ. Preliminary observations on the neuromuscular abnormalities in patients with organ failure and sepsis. Intensive Care Med. 1993;19(6):323-8.

4- Coakley JH, Nagendran K, Yarwood GD, Honavar M, Hinds CJ. Patterns of neurophysiological abnormality in prolonged critical illness. Intensive Care Med. 1998 Aug;24(8):801-7.

5- Amaya-Villar R, Garnacho-Montero J, García-Garmendía JL, Madrazo-Osuna J, Garnacho-Montero MC, Luque R, Ortiz-Leyba C. Steroid-induced myopathy in patients intubated due to exacerbation of chronic obstructive pulmonary disease. Intensive Care Med. 2005 Jan;31(1):157-61.

6- Leatherman JW, Fluegel WL, David WS, Davies SF, Iber C. Muscle weakness in mechanically ventilated patients with severe asthma. Am J Respir Crit Care Med. 1996 May;153(5):1686-90. doi: 10.1164/ajrccm.153.5.8630621. PMID: 8630621.

7- Douglass JA, Tuxen DV, Horne M, Scheinkestel CD, Weinmann M, Czarny D, Bowes G. Myopathy in severe asthma. Am Rev Respir Dis. 1992 Aug;146(2):517-9.

8- Hermans G, Van den Berghe G. Clinical review: intensive care unit acquired weakness. Crit Care. 2015 Aug 5;19(1):274.

9- Garnacho-Montero J, Madrazo-Osuna J, García-Garmendia JL, Ortiz-Leyba C, Jiménez-Jiménez FJ, Barrero-Almodóvar A, Garnacho-Montero MC, Moyano-Del-Estad MR. Critical illness polyneuropathy: risk factors and clinical consequences. A cohort study in septic patients. Intensive Care Med. 2001 Aug;27(8):1288-96.

10- Garnacho-Montero J, Amaya-Villar R, García-Garmendía JL, Madrazo-Osuna J, Ortiz-Leyba C. Effect of critical illness polyneuropathy on the withdrawal from mechanical ventilation and the length of stay in septic patients. Crit Care Med. 2005 Feb;33(2):349-54.

11- Spitzer AR, Giancarlo T, Maher L, Awerbuch G, Bowles A. Neuromuscular causes of prolonged ventilator dependency. Muscle Nerve. 1992 Jun;15(6):682-6.

12- 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 Dec 11;288(22):2859-67.

13- Vanhorebeek I, Latronico N, Van den Berghe G. ICU-acquired weakness. Intensive Care Med. 2020 Apr;46(4):637-653.

14- Hermans G, Van Mechelen H, Clerckx B, Vanhullebusch T, Mesotten D, Wilmer A, Casaer MP, Meersseman P, Debaveye Y, Van Cromphaut S, Wouters PJ, Gosselink R, Van den Berghe G. Acute outcomes and 1-year mortality of intensive care unit-acquired weakness. A cohort study and propensity-matched analysis. Am J Respir Crit Care Med. 2014 Aug 15;190(4):410-20.

15- Schweickert WD, Hall J. ICU-acquired weakness. Chest. 2007 May;131(5):1541-9.

16- Bednarík J, Vondracek P, Dusek L, Moravcova E, Cundrle I. Risk factors for critical illness polyneuromyopathy. J Neurol. 2005 Mar;252(3):343-51.

17- Hermans G, Wilmer A, Meersseman W, Milants I, Wouters PJ, Bobbaers H, Bruyninckx F, Van den Berghe G. Impact of intensive insulin therapy on neuromuscular complications and ventilator dependency in the medical intensive care unit. Am J Respir Crit Care Med. 2007 Mar 1;175(5):480-9.

18- Stevens RD, Dowdy DW, Michaels RK, Mendez-Tellez PA, Pronovost PJ, Needham DM. Neuromuscular dysfunction acquired in critical illness: a systematic review. Intensive Care Med. 2007 Nov;33(11):1876-91.

19- Paramore S. Effects of the use of neuromuscular blocking agents on acute respiratory distress syndrome outcomes: A systematic review. J Am Assoc Nurse Pract. 2018 Jun;30(6):327-332.

20- Slutsky AS. Neuromuscular blocking agents in ARDS. N Engl J Med. 2010 Sep 16;363(12):1176-80.

21- Warr J, Thiboutot Z, Rose L, Mehta S, Burry LD. Current therapeutic uses, pharmacology, and clinical considerations of neuromuscular blocking agents for critically ill adults. Ann Pharmacother. 2011 Sep;45(9):1116-26.

22- Adnet F, Dhissi G, Borron SW, Galinski M, Rayeh F, Cupa M, Pourriat JL, Lapostolle F. Complication profiles of adult asthmatics requiring paralysis during mechanical ventilation. Intensive Care Med. 2001 Nov;27(11):1729-36.

23- Behbehani NA, Al-Mane F, D'yachkova Y, Paré P, FitzGerald JM. Myopathy following mechanical ventilation for acute severe asthma: the role of muscle relaxants and corticosteroids. Chest. 1999 Jun;115(6):1627-31.

24- Kupfer Y, Namba T, Kaldawi E, Tessler S. Prolonged weakness after long-term infusion of vecuronium bromide. Ann Intern Med. 1992 Sep 15;117(6):484-6.

25- Price DR, Mikkelsen ME, Umscheid CA, Armstrong EJ. Neuromuscular Blocking Agents and Neuromuscular Dysfunction Acquired in Critical Illness: A Systematic Review and Meta-Analysis. Crit Care Med. 2016 Nov;44(11):2070-2078.

26- Papazian L, Forel JM, Gacouin A, Penot-Ragon C, Perrin G, Loundou A, Jaber S, Arnal JM, Perez D, Seghboyan JM, Constantin JM, Courant P, Lefrant JY, Guérin C, Prat G, Morange S, Roch A; ACURASYS Study Investigators. Neuromuscular blockers in early acute respiratory distress syndrome. N Engl J Med. 2010 Sep 16;363(12):1107-16.

27- National Heart, Lung, and Blood Institute PETAL Clinical Trials Network, Moss M, Huang DT, Brower RG, Ferguson ND, Ginde AA, Gong MN, Grissom CK, Gundel S, Hayden D, Hite RD, Hou PC, Hough CL, Iwashyna TJ, Khan A, Liu KD, Talmor D, Thompson BT, Ulysse CA, Yealy DM, Angus DC. Early Neuromuscular Blockade in the Acute Respiratory Distress Syndrome. N Engl J Med. 2019 May 23;380(21):1997-2008.

28- Forel JM, Roch A, Marin V, Michelet P, Demory D, Blache JL, Perrin G, Gainnier M, Bongrand P, Papazian L. Neuromuscular blocking agents decrease inflammatory response in patients presenting with acute respiratory distress syndrome. Crit Care Med. 2006 Nov;34(11):2749-57.

29 - Weber-Carstens S, Deja M, Koch S, Spranger J, Bubser F, Wernecke KD, Spies CD, Spuler S, Keh D. Risk factors in critical illness myopathy during the early course of critical illness: a prospective observational study. Crit Care. 2010;14(3):R119.

30- Marc Gainnier, MD; Antoine Roch, MD; Jean-Marie Forel, MD; Xavier Thirion, MD, PhD; Jean-Michel Arnal, MD; Stéphane Donati, MD; Laurent Papazian. Effect of

neuromuscular blocking agents on gas exchange in patients presenting with acute respiratory distress syndrome. Crit Care Med. 2004; 32:113-119.

31- Frithiof R, Rostami E et al. Critical illness polyneuropathy, myopathy and neuronal biomarkers in COVID-19 patients: A prospective study. Clinical Neurophysiology 132 (2021) 1733–1740.

32- Núñez-Seisdedos MN, Lázaro-Navas I et al. Intensive Care Unit- Acquired Weakness and Hospital Functional Mobility Outcomes Following Invasive Mechanical Ventilation in Patients with COVID-19: A Single-Centre Prospective Cohort Study. Journal of Intensive Care Medicine 2022, Vol. 37(8) 1005-1014.

33- Higgins JPT, Green S: Cochrane handbook for systematic reviews of interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011 London, UK. Available at: http://handbook.cochrane.org.

34- Thorlund K, Engstrom J, Wetterslev J, Brok J, Imberger G, Gluud C. User Manual for Trial Sequential Analysis (TSA). Copenhagen: Copenhagen Trial Unit, 2nd ed, 2017.

35- Wetterslev J, Thorlund K, Brok J, Gluud C (2008) Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. J Clin Epidemiol 61: 64±75.

36- Thorlund K, Devereaux PJ, Wetterslev J, Guyatt G, Ioannidis JP, Thabane L, et al. (2009) Can trial sequential monitoring boundaries reduce spurious inferences from meta-analyses? Int J Epidemiol 38:276±286.

37- Fan E, Cheek F, Chlan L et al. An official American Thoracic Society Clinical Practice guideline: the diagnosis of intensive care unit-acquired weakness in adults. Am J Respir Crit Care Med 190:1437–46 - 37

38- (Lan KKG. and DeMets G. Discrete sequential boundaries for clinical trials. Biometrika 1983; 70(3): 659-663- 38

39- Ali NA, O'Brien JM Jr, Hoffmann SP, et al (Ohio State Univ, Columbus, OH; et al). Acquired Weakness, Handgrip Strength, and Mortality in Critically III Patients. Am J Respir Crit Care Med 178:261-268, 2008.

40- Anna-Giulia Brunello, Matthias Haenggi, Oliver Wigger, Francesca Porta, Jukka Takala, Stephan M. Jakob. Usefulness of a clinical diagnosis of ICU-acquired paresis to predict outcome in patients with SIRS and acute respiratory failure. Intensive Care Med (2010) 36:66–74.

41- Gupta S, Mishra M. Acute Physiology and Chronic Health Evaluation II score of ≥ 15: A risk fator for sepsis-induced critical illness polyneuropathy. Neurol India 2016;64:640-5.

42- S. Nanas, K. Kritikos, E. Angelopoulos, A. Siafaka, S. Tsikriki, M. Poriazi, D. Kanaloupiti, M. Kontogeorgi, M. Pratikaki, D. Zervakis, C. Routsi, C. Roussos. Predisposing factors for critical illness polyneuromyopathy in a multidisciplinar intensive care unit. Acta Neurol Scand 2008: 118:175–181.

43- Luan Nguyen The, Cong Nguyen Huu. Critical illness polyneuropathy and myopathy in a rural area in Vietnam. Journal of the Neurological Sciences 357 (2015) 276–281.

44- Luuk Wieske, Esther Witteveen, Camiel Verhamme, Daniela S. Dettling-Ihnenfeldt, Marike vander Schaaf, Marcus J. Schultz, Ivo N. van Schaik, Janneke Horn. Early Prediction of Intensive Care Unit–Acquired Weakness Using Easily Available Parameters: A Prospective Observational Study. PLoS ONE 9(10): e111259.

45- Marie-An C. J. de Letter, MD; Paul I. M. Schmitz, PhD; Leo H. Visser, PhD, MD; Freek A. M. Verheul, MD; Ronald L. L. A. Schellens, MD; Dolf A. W. Op de Coul, PhD,

MD; Frans G. M. van der Meché, PhD, MD. Risk factors for the development of polyneuropathy and myopathy in critically ill patients. Crit Care Med 2001 Vol. 29, No. 12:2281-86.

46- Rudolf I. Thiele, Heinz Jakob, Ernst Hund, Harald Genzwuerker, Ulf Herold, Peter Schweiger, Siegfried Hagl. Critical illness polyneuropathy: a new iatrogenically induced syndrome after cardiac surgery?. European Journal of Cardio-thoracic Surgery 12 (1997) 826–835.

47- F. S. S. Leijten, A.W. De Weerd, D. C.J. Poortvliet, V.A. De Ridder, C. Ulrich, J.E. Harinek-De Weerd. Critical illness polyneuropathy in multiple organ dysfunction syndrome and weaning from the ventilator. Intensive Care Med (1996) 22:856-861.

48- Sarah M. Kesler, Mark D. Sprenkle, William S. David, James W. Leatherman. Severe weakness complicating status asthmaticus despite minimal duration of neuromuscular paralysis. Intensive Care Med (2009) 35:157–160.

49- McLeskey CH. Anesthesia for the geriatric patients, Annual Refresher Course Lectures-ASA, 1990; 165.

50- Stoelting RK. Pharmacology and physiology in anes - thetic practice, 2nd Ediction, J.B.Lippincott Company, 1991: 172-225.

51- Kisor DF, Schmith VD. Clinical pharmacokinetics of cisatracurium besilate. Clin Pharmacokinet, 1996;36:27-40.

TITLE AND LEGENDS

Title: Association between neuromuscular blocking agents and the development of intensive care unit-acquired weakness (ICU-AW): a systematic review with meta-analysis and trial sequential analysis

Identification of studies via databases and registers

Identification

Records identified from Databases (n = 25318):
Embase (n = 18716)
PubMed (n = 3504)
Cochrane (n = 3098)

Records removed *before screening*:
Duplicate records removed (n = 2003)

Screening

Records screened (n = 23315)

Records excluded based on titles and abstracts (n = 23232)

Reports assessed for eligibility (n = 83)

Reports excluded:
Insufficient data (n = 17)
Articles that did not fulfill the proposed design (n = 14)
Abscense of control group (n = 11)
No data on ICU-AW (n = 6)
No data on NMBA (n = 5)

Included

Studies included in review (n = 30)

Figure 1. Flow chart of the study selection process.

1. Randomized clinical trials

Study or Subgroup	NMB Events	NMB Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Forel 2006 *	1	18	1	18	7.8%	1.00 [0.06, 17.33]
Gainnier 2004 *	0	28	0	28		Not estimable
Moss 2019 *	234	501	139	505	51.1%	2.31 [1.77, 3.00]
Papazian 2010 *	28	96	25	77	41.1%	0.86 [0.45, 1.64]
Total (95% CI)		643		628	100.0%	1.44 [0.61, 3.40]
Total events	263		165			

Heterogeneity: Tau² = 0.36; Chi² = 7.93, df = 2 (P = 0.02); I² = 75%
Test for overall effect: Z = 0.83 (P = 0.41)

2. Observational studies

Study or Subgroup	NMB Events	NMB Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Adnet 2001	10	55	1	46	2.5%	10.00 [1.23, 81.40]
Ali 2008	8	28	27	108	5.8%	1.20 [0.47, 3.04]
Amaya-Villar 2005	4	7	5	14	3.0%	2.40 [0.38, 15.32]
Behbehani 1999	9	30	0	56	1.6%	49.93 [2.78, 895.59]
Brunello 2009	7	10	6	29	3.5%	8.94 [1.76, 45.37]
Coakley 1993	14	15	8	8	1.2%	0.57 [0.02, 15.58]
Coakley 1998	28	33	9	11	3.1%	1.24 [0.20, 7.56]
De Jonghe 2002	15	44	9	51	5.7%	2.41 [0.93, 6.26]
De Letter 2001	16	34	20	62	6.0%	1.87 [0.79, 4.40]
Douglass 1992	9	22	0	3	1.4%	4.93 [0.23, 106.88]
Frithiof 2021	11	56	0	55	1.6%	28.05 [1.61, 489.11]
Garnacho-Montero 2001	9	10	41	63	2.5%	4.83 [0.57, 40.63]
Garnacho-Montero 2005	10	13	24	51	4.1%	3.75 [0.92, 15.24]
Gupta 2016	36	45	1	55	2.5%	216.00 [26.22, 1779.10]
Hermans 2007	34	53	47	155	6.8%	4.11 [2.13, 7.94]
Hermans 2014	130	191	97	224	7.7%	2.79 [1.86, 4.17]
Hund 1997	6	17	1	27	2.3%	14.18 [1.52, 132.06]
Kesler 2008	8	57	2	17	3.4%	1.22 [0.23, 6.40]
Kupfer 1992	7	10	0	18	1.4%	79.29 [3.64, 1729.03]
Leatherman 1996	20	69	0	38	1.6%	31.89 [1.87, 544.08]
Leijten 1996	11	19	7	19	4.4%	2.36 [0.64, 8.68]
Nanas 2008	15	61	29	124	6.6%	1.07 [0.52, 2.19]
Nguyen 2015	53	83	20	50	6.6%	2.65 [1.29, 5.45]
Núñez-Seisdedos 2022	44	61	2	9	3.4%	9.06 [1.71, 48.03]
Weber-Carstens 2010	14	24	8	16	4.5%	1.40 [0.39, 5.00]
Wieske 2014	17	35	86	177	6.6%	1.00 [0.48, 2.06]
Total (95% CI)		1082		1486	100.0%	3.27 [2.19, 4.88]
Total events	545		450			

Heterogeneity: Tau² = 0.50; Chi² = 63.95, df = 25 (P < 0.0001); I² = 61%
Test for overall effect: Z = 5.79 (P < 0.00001)

3. All studies

	NMB Total	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Total (95% CI)	1725	2114	100.0%	2.77 [1.98, 3.88]
Total events	808	615		

Heterogeneity: Tau² = 0.36; Chi² = 73.55, df = 28 (P < 0.00001); I² = 62%
Test for overall effect: Z = 5.93 (P < 0.00001)

Figure 2. Forest plot for the 30 studies evaluating the effect of NMBA use and the development of ICU-AW. ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents; *: randomized clinical trial.

Table 1
Summary of studies evaluating the use of NMBA in the incidence of ICU-AW.

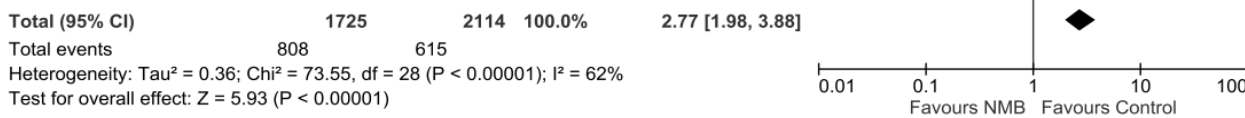| Study	Country of origin	Study design	Study setting	Population	ICU-AW diagnosis	NMBA (n)	Control (n)	ICU-AW incidence
Adnet, 2001 [22]	France	Retrospective cohort	MICU	Severe asthma + MV	Clinical + ENMG	55	45	18% vs. 2%
Ali, 2008 [37]	United States	Prospective cohort	MICU	MV > 5 days	Clinical	28	108	28% vs. 25%
Amaya-Villar, 2005 [5]	Spain	Retrospective cohort	MICU	COPD + MV > 48 h + high dose corticosteroids	Clinical + ENMG	7	19	57% vs. 36%
Behbehani, 1999 [23]	Canada	Retrospective cohort	?	Severe asthma + MV	Clinical	30	56	30% vs. 0%
Brunello, 2010 [38]	Switzerland	Prospective cohort	MSICU	SIRS + MV >48 h	Clinical	10	29	70% vs. 21%
Coakley, 1993 [3]	United Kingdom	Prospective cohort	MSICU	ICU LOS > 7 days + organ dysfunction	Biopsy	15	8	93% vs. 100%
Coakley, 1998 [4]	United Kingdom	Prospective cohort	MSICU	ICU LOS > 7 days + MV	ENMG	33	11	85% vs. 82%
De Jonghe, 2002 [10]	France	Prospective cohort	MICU + SICU	MV > 7 days	Clinical + ENMG + biopsy	44	51	34% vs. 18%
De Letter, 2001 [44]	Netherlands	Prospective cohort	?	MV > 4 days	Clinical + ENMG	31	66	43% vs. 30%
Douglass, 1992 [43]	Australia	Prospective cohort	MICU	Severe asthma + MV	Clinical + ENMG + biopsy	22	3	41% vs. 0%
Forel, 2006 [36]	France	RCT	MICU + MSICU	ARDS	Clinical	18	18	5% vs. 5%
Frithiof, 2021 [29]	Sweden	Prospective cohort	MSICU	SARS-CoV-2 infection	ENMG	56	55	19.6% vs. 0%
Gainnier, 2004 [28]	France	RCT	MICU + MSICU	ARDS	Clinical	28	28	0% vs. 0%
Gamacho-Montero, 2001 [12]	Spain	Prospective cohort	MSICU	Sepsis + multiorgan dysfunction + MV > 10 days	Clinical + ENMG	10	63	90% vs. 65%
Gamacho-Montero, 2005 [8]	Spain	Prospective cohort	MSICU	Severe sepsis/septic shock + VM > 7 days	ENMG	13	51	77% vs. 47%
Gupta, 2016 [39]	India	Prospective cohort	MSICU	Sepsis	ENMG	45	55	80% vs. 2%
Hermans, 2007 [16]	Belgium	Prospective cohort	MICU + SICU	ICU LOS > 7 days	ENMG	53	155	64% vs. 30%
Hermans, 2014 [11]	Belgium	Prospective cohort	MICU + SICU + CICU	ICU LOS > 8 days	Clinical	191	224	68% vs. 43%
Kesler, 2009 [47]	United States	Retrospective cohort	MICU	Severe asthma + MV	Clinical	57	17	14% vs. 12%
Kupfer, 1992 [24]	United States	Prospective cohort	?	Need for NMBA > 6 h	Clinical + ENMG	10	18	70% vs. 0%
Leatherman, 1996 [6]	United States	Retrospective cohort	MICU	Severe asthma + MV	Clinical + ENMG + biopsy	69	38	29% vs. 0%
Leijten, 1996 [46]	Netherlands	Prospective cohort	MSICU	MV > 7 days	ENMG	19	19	58% vs. 37%
Moss, 2019 [26]	United States	RCT	MICU	ARDS	Clinical	501	505	41% vs. 31% at D7 47% vs. 27% at D28
Nanas, 2008 [40]	Greece	Prospective cohort	MSICU	ICU LOS > 10 days	Clinical	61	124	25% vs. 23%
Nguyen, 2015 [41]	Vietnam	Prospective cohort	MSICU	ICU LOS > 10 days	Clinical + ENMG	83	50	63% vs. 40%
Núñez-Seisdedos, 2022 [30]	Spain	Prospective cohort	MSICU	SARS-CoV-2 infection	Clinical	61	9	72.1% vs. 22.2%
Papazian, 2010 [25]	France	RCT	MSICU	ARDS	Clinical	178	162	29% vs. 32% at D28 36% vs. 31% at ICU discharge
Weber-Carstens, 2010 [27]	Germany	Retrospective cohort	SICU	MV with SAPS > 20 for 3 consecutive days in the first week of ICU stay	ENMG	24	16	58% vs. 50%
Wieske, 2014 [42]	Netherlands	Prospective cohort	MSICU	MV > 48 h	Clinical	35	177	48% vs. 48%

NMBA: neuromuscular blocking agents; ICU-AW: intensive care unit-acquired weakness; ARDS: adult respiratory distress syndrome; CICU: cardiovascular intensive care unit; COPD: chronic obstructive pulmonary disease; ENMG: eletroneuromyography; ICU: intensive care unit; LOS: length of stay; MICU: medical intensive care unit; MSICU: medical-surgical intensive care unit; MV: mechanical ventilation; RCT: randomized controlled trial; SAPS: simplified acute physiology score; SICU: surgical intensive care unit; SIRS: systemic inflammatory response syndrome.

Material suplementar do Artigo 3:

Supplementary Table 1. Methodology and reporting assessment.

Chocrane Collaboration tool for assessing risk of bias

Study	Sequence generation	Allocation concealment	Blinding of participants	Blinding outcome assessment	Incomplete outcome data	Other potential threats to validity	Risk of bias
Forel, 2006	🟢	🟢	🔴	🟢	🟢	🟢	High
Gainnier, 2004	🟢	🟢	🔴	🟢	🟢	🟢	High
Moss, 2019	🟢	🟢	🔴	🔴	🟢	🟢	High
Papazian, 2010	🟢	🟢	🟢	🟢	🟢	🟢	Low

Newcastle-Ottawa quality assessment scale for cohort studies

Study	Exposed representative	Nonexposed representative	Ascertainment of exposure	Outcome of interest not present at start	Comparability	Assessment of oucome	Adequate duration of follow-up	Completeness of follow-up	Score
Adnet, 2001	🟢	🟢	🟢	🟢	🟢🟢	🟢	🔴	🟢	8
Ali, 2008	🟢	🟢	🔴	🟢	🟢🟢	🟢	🔴	🟢	7
Amaya-Villar, 2005	🟢	🟢	🔴	🟢	🟢🟢	🟡	🔴	🟢	6-7
Behbehani, 1999	🟢	🟢	🔴	🟢	🟢🟢	🟢	🟢	🟢	8
Brunello, 2009	🟢	🟢	🔴	🟢	🟢🟢	🟢	🟢	🔴	7
Coakley, 1993	🟢	🟢	🔴	🟢	🟢🟢	🟡	🟢	🟢	7-8
Coakley, 1998	🟢	🟢	🔴	🟢	🟢🟢	🟢	🔴	🟢	7
De Jonghe, 2002	🟢	🟢	🟢	🟢	🟢🟢	🟢	🟢	🟡	8-9
De Letter, 2001	🟢	🟢	🟢	🟢	🟢🟢	🟢	🟢	🟢	9
Douglass, 1992	🟢	🟢	🟢	🟢	🔴🟢	🟢	🔴	🟢	7
Frithiof, 2021	🟢	🟢	🟢	🟢	🟢🔴	🟢	🔴	🟢	6
Garnacho-Montero, 2001	🟢	🟢	🟢	🟢	🟢🟢	🟢	🟢	🟢	9
Garnacho-Montero, 2005	🟢	🟢	🔴	🟢	🟢🟢	🟢	🟢	🟢	8
Gupta, 2016	🟢	🟢	🔴	🟢	🟢🟢	🟢	🟢	🟢	8
Hermans, 2007	🟢	🟢	🔴	🟢	🟢🟢	🟢	🔴	🟢	7
Hermans, 2014	🟢	🟢	🔴	🟢	🟢🟢	🟢	🟢	🟢	8
Hund, 1997	🟢	🟢	🟢	🟢	🔴🟢	🟢	🟢	🟢	8
Kesler, 2008	🟢	🟢	🟢	🟢	🔴🟢	🟢	🔴	🟢	7
Kupfer, 1992	🟢	🟢	🟢	🟢	🔴🔴	🟢	🔴	🟢	6
Leatherman, 1996	🟢	🟢	🟢	🟢	🔴🟢	🟢	🟢	🔴	7
Leijten, 1996	🟢	🟢	🟢	🟢	🟢🟢	🟢	🟢	🟢	9
Nanas, 2008	🟢	🟢	🔴	🟢	🟢🟢	🟢	🔴	🟢	7
Nguyen, 2015	🟢	🟢	🔴	🟢	🔴🟢	🟢	🟢	🟢	7
Núñez-Seisdedos, 2022	🟢	🟢	🟢	🟢	🟢🔴	🟢	🟢	🟢	8
Weber-Carstens, 2010	🟢	🟢	🟢	🟢	🟢🟢	🟢	🟢	🟢	9
Wieske, 2014	🟢	🟢	🔴	🟢	🟢🟢	🟢	🔴	🟢	7

🟢 Low risk of bias 🟡 Incert risk of bias 🔴 High risk of bias

Supplementary Table 2. Details about NMBA use in each study.

Study	NMBA	NMBA dose	Duration of use
Adnet, 2001	Cisatracurim 5.4% Atracurium 3.6% Pancuronium 41.8% Vecuronium 49%	Not available	Overall median 46 h (20-91) ICU-AW group: 101 h (70-148) Without ICU-AW group: 22 h (46-70)
Ali, 2008	Not available	Not available	Not available
Amaya-Villar, 2005	Vecuronium	ICU-AW group: 13 Without ICU-AW group: 11 (it does not mention the units)	Not available
Behbehani, 1999	Atracurium Pancuronium Vecuronium	Not available	2-7 days
Brunello, 2009	Not available	Not available	Not available
Coakley, 1993	Not available	Not available	>48 h in continuous infusion
Coakley, 1998	Atracurium Vecuronium	Not available	ICU-AW group: use of NMBA for more than 24 h in 45.9% Without ICU-AW group: use of NMBA for more than 24 h in 71%
De Jonghe, 2002	Not available	ICU-AW group: 13.3 mg Without ICU-AW:16 mg (vecuronium-equivalent dosage)	ICU-AW: 3.3 days (4 patients received continuous infusion) Without ICU-AW group: 2.1 days (2 patients received continuous infusion)
De Letter, 2001	Vecuronium	Registered, but not available in the article	Registered, but not available in the article
Douglass, 1992	Vecuronium	Overall media 402 ± 674 mg ICU-AW group: 1065 ± 674 mg Without ICU-AW group: 231 ± 163 mg	Overall media: 2.5 ± 2.5 days ICU-AW group: 5.4 ± 2 days Without ICU-AW group: 1.3 ± 0.9 days
Forel, 2006	Cisatracurium	0.2 mcg/kg bolus + initial infusion 5 mcg/kg/min and titrated after	48 h
Frithiof, 2021	Atracurium Rocuronium	Continuous use, dose not available	MV free days ICU-AW group: 22 (0-23) Without ICU-AW group: 25 (21-27)
Gainnier, 2004	Cisatracurium	50 mg bolus + initial infusion 5 mcg/kg/min and titrated after	48 h
Garnacho-Montero, 2001	Atracurium Vecuronium	Vecuronium 95-708 mg Atracurium 1520-5040 mg	Not available
Garnacho-Montero, 2005	Atracurium Vecuronium	Not available	Not available
Gupta, 2016	Not available	Not available	Not available
Hermans, 2007	Not available	Not available	Not available, but it was extended (continuous infusion)
Hermans, 2014	Not available	Not available, but included single dose for intubation	Overall media: 0 days (0-1)
Hund, 1997	Pancuronium Vecuronium	ICU-AW group: 85.7 mg/kg (0.37 ± 0.3) Without ICU-AW group: 29.7 mg/kg (0.52 ± 0.6)	Not available, but they applied repeated cycles of 4-8 mg bolus
Kesler, 2008	Atracurium Vecuronium	Not available	ICU-AW group: 3.5 h ± 6.2 Without ICU-AW group: 1.5 h ± 3.5

Kupfer, 1992	Vecuronium	Overall media: 31-3799 mg ICU-AW group: 1352 mg Without ICU-AW group: 528 mg	7 h -13 days ICU-AW group media: 7.2 days Without ICU-AW group media: 3.8 days
Leatherman, 1996	Atracurium Pancuronium Vecuronium	Not available, but included single, intermittent, or continuous infusion	Not available
Leijten, 1996	Vecuronium	0.1 mg/kg for intubation + maintenance at 0.03 mg/kg/h or more (discontinued daily)	ICU-AW group media: 2 days (0-17) Without ICU-AW group media: 0.5 day (0-16)
Moss, 2019	Cisatracurium	15 mg bolus + 37.5 mg/h; cumulative: 1807 mg (1706-1815)	47.8 h (43.8-48 h)
Nanas, 2008	Not available	Not available	Not available, but it was for a short period
Nguyen, 2015	Not available	Not available	Not available
Núñez-Seisdedos, 2022	Cisatracurium	Overall media: 744mg (288.5-1501) ICU-AW group: 941mg (392-1977) Without ICU-AW group: 493mg (10-1089) (cumulative dose bolus and continuous infusion)	Overall media: 5 days (2-8) ICU-AW group: 6 days (3-9) Withou ICU-AW group: 3 days (0-6)
Papazian, 2010	Cisatracurium	15 mg bolus + 37.5 mg/h	48 h
Weber-Carstens, 2010	Cisatracurium	Cumulative dose in first 8 days: ICU-AW group: 27.5 mg Without ICU-AW group: 10 mg	Not available
Wieske, 2014	Not available	More than one administration of any NMBA in the first 2 days of ICU stay	Not available

ICU: intensive care unit; ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents.

Supplementary Table 3. Time and MRC scale assessment of ICU-AW development in each study included in the systematic review.

Study	ICU-AW assessment time	MRC in control group	MRC in NMBA group
Adnet, 2001	Not available	Not available	Not available
Ali, 2008	Not available (awake patient)	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
Amaya-Villar, 2005	Mean of 6 days (2-12) between MV start and evaluation	Not available	Not available
Behbehani, 1999	Not available	Not available	Not available
Brunello, 2009	Within 48 hours of ICU admission, at day 7, and at ICU discharge	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
Coakley, 1993	Biopsy performed around day 10 of ICU admission (3-37 days)	Not available	Not available
Coakley, 1998	Not available	Not available	Not available
De Jonghe, 2002	Day 7 after been awake, weekly for 1 month, and monthly for 9 months until muscle weakness regression or death	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
De Letter, 2001	Clinical criteria 2x/week in the ICU + 1x/week in the ward; ENMG days 4, 11, and 25 after starting MV	Not available	Not available
Douglass, 1992	Not available (clinical reassessments from admission throughout hospitalization)	Not available	Not available
Forel, 2006	120 hours	Not available	Not available
Frithiof, 2021	Not available	Not available	Not available
Gainnier, 2004	120 hours	Not available	Not available
Garnacho-Montero, 2001	Days 10 and 21 after starting MV	Not available	Not available
Garnacho-Montero, 2005	At the beginning of weaning: mean of 16.4 days after starting MV in patients with ICU-AW and 11.3 days in patients without ICU-AW	Not available	Not available
Gupta, 2016	Day 14, 21, or 28 after ICU admission	Not available	Not available
Hermans, 2007	Day 7 after ICU admission	Not available	Not available

Hermans, 2014	Time between waking up and first MRC: 12 days (9-20) in the group with ICU-AW and 9 days (8-12) in the group without ICU-AW	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
Hund, 1997	ENMG 5-7 days after starting MV, repeated weekly for one month, every 15 days for the next 2 months and every 21 days until discharge from ICU	Not available	Not available
Kesler, 2008	Not available	Not available	Not available
Kupfer, 1992	24 hours after stopping NMBA and, if abnormal, followed by reaching stability; control group were submitted to serial neurological assessment during and after MV (time not specified)	Not available	Not available
Leatherman, 1996	Not available	Not available	Not available
Leijten, 1996	7-9 days from starting MV, after 3 weeks, and 2 months (if still in ICU)	Not available	Not available
Moss, 2019	Days 7 and 28	49.5 +-12.3 at D7 | 49.8 +- 10.6 at D28	46.7 +- 14.4 at D7 | 45.7 +- 13.9 at D28
Nanas, 2008	Not available	Not available	Not available
Nguyen, 2015	Days 15, 30, 60, and 90 after ICU admission	Not available	Not available
Núñez-Seisdedos, 2022	ICU and hospital discharge	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
Papazian, 2010	Day 28 after ICU admission and on ICU discharge	55 (39-60) at D28 | 55 (44-60) at ICU discharge	55 (46-60) at D28 | 55 (43-60) at ICU discharge
Weber-Carstens, 2010	Day 8 from ICU admission	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA
Wieske, 2014	Mean of 9 days from ICU admission in group with ICU-AW and 7 days in group without ICU-AW	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA	MRC assessment in patients who developed ICU-AW or not and not in the groups that used or did not used NMBA

ENMG: electroneuromyography; ICU: intensive care unit; ICU-AW: intensive care unit-acquired weakness; MRC: Medical Research Council, MV: mechanical ventilation; NMBA: neuromuscular blocking agents.

Supplementary Table 4. Quality of evidence assessment based on GRADE approach.

N° of studies / N° of participants	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall quality of evidence
4 RCTs, n = 1271 26 observational studies, n = 2568	Serious[a]	Serious[b]	Unlikely	Serious[c]	Unlikely	++, low

[a] Risk of bias was rated serious because of no blinding in three out of four RCTs; because inadequate duration of follow-up in eleven out of 26 cohort studies; and because impaired comparability between groups in eight out of 26 cohort studies

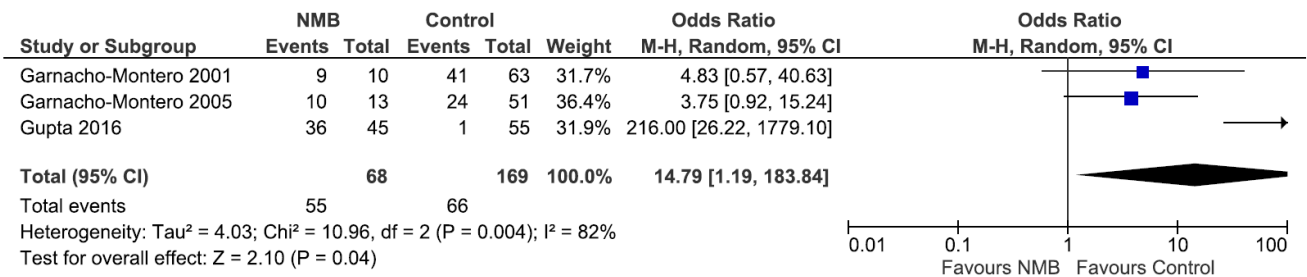[b] Inconsistency across studies based on I^2 was considered serious (high heterogeneity)

[c] Imprecision was considered serious, considering that studies have wide confidence intervals around the effect, which does not allow a clear estimate of its magnitude

GRADE: grading of recommendations assessment, development and evaluation

Supplementary Table 5. Exploratory analysis of studies with specific populations of patients.

Exploratory analysis	Number of studies included	n NMBA	n control	OR (95% CI)	p value	I^2	Results
Entire population of patients with sepsis	2	55	118	32.4 (0.78 – 1343.61)	0.07	84%	NMBA use was not associated with ICU-AW in this population
>50% of patients with sepsis in the population	7	843	1143	2.47 (1.34 – 4.54)	0.004	78%	In studies that included more than 50% of the patients with sepsis, NMBA use was associated with an increased incidence of ICU-AW
Entire population receiving corticosteroids	5	210	118	4.14 (1.29 – 13.3)	0.02	30%	In studies that analysed exclusively patients with concomitant use of corticosteroids, NMBA use was associated with an increased incidence of ICU-AW
>50% of patients receiving corticosteroids in the population	8	453	546	2.88 (1.41 – 5.85)	0.004	53%	In studies that analysed a population mostly composed of patients using corticosteroids, NMBA use was associated with an increased incidence of ICU-AW
Entire population receiving cisatracurium	5	667	644	1.45 (0.74 – 2.88)	0.28	63%	In studies that analysed a population composed exclusively of patients using cisatracurium, the use of this NMBA was not associated with ICU-AW

ICU: intensive care unit; ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents; OR: odds ratio; I^2 heterogeneidade.

Study or Subgroup	NMB Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Garnacho-Montero 2001	9	10	41	63	31.7%	4.83 [0.57, 40.63]
Garnacho-Montero 2005	10	13	24	51	36.4%	3.75 [0.92, 15.24]
Gupta 2016	36	45	1	55	31.9%	216.00 [26.22, 1779.10]
Total (95% CI)		68		169	100.0%	14.79 [1.19, 183.84]
Total events	55		66			

Heterogeneity: Tau² = 4.03; Chi² = 10.96, df = 2 (P = 0.004); I² = 82%
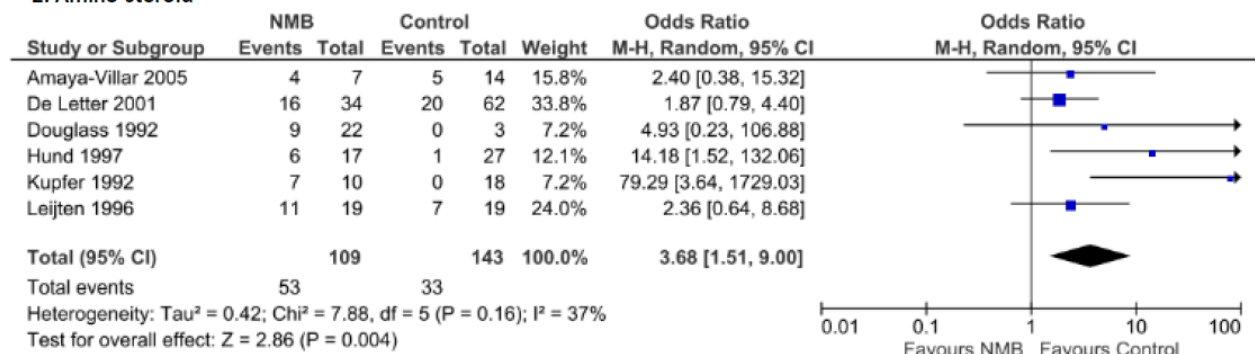Test for overall effect: Z = 2.10 (P = 0.04)

Supplementary Figure 1. Forest plot for the effect of NMBA use and the development of ICU-AW of studies enrolling patients with sepsis. ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents.
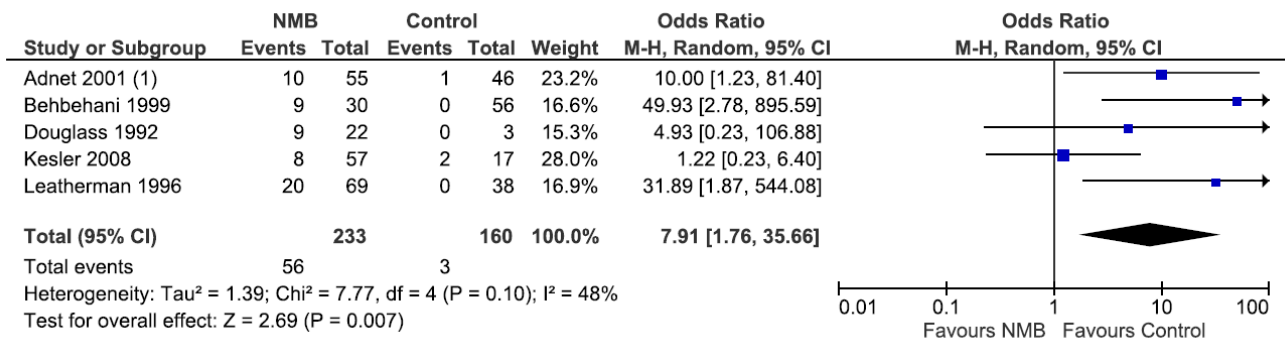
1. Benzyl quinolinic

Study or Subgroup	NMB Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Forel 2006 *	1	18	1	18	5.2%	1.00 [0.06, 17.33]
Gainnier 2004 *	0	28	0	28		Not estimable
Moss 2019 *	234	501	139	505	36.9%	2.31 [1.77, 3.00]
Núñez-Seisdedos 2022	44	61	2	9	12.0%	9.06 [1.71, 48.03]
Papazian 2010 *	28	96	25	77	29.1%	0.86 [0.45, 1.64]
Weber-Carstens 2010	14	24	8	16	16.9%	1.40 [0.39, 5.00]
Total (95% CI)		728		653	100.0%	1.79 [0.90, 3.60]
Total events	321		175			

Heterogeneity: Tau² = 0.32; Chi² = 11.36, df = 4 (P = 0.02); I² = 65%
Test for overall effect: Z = 1.65 (P = 0.10)

2. Amino-steroid

Study or Subgroup	NMB Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Amaya-Villar 2005	4	7	5	14	15.8%	2.40 [0.38, 15.32]
De Letter 2001	16	34	20	62	33.8%	1.87 [0.79, 4.40]
Douglass 1992	9	22	0	3	7.2%	4.93 [0.23, 106.88]
Hund 1997	6	17	1	27	12.1%	14.18 [1.52, 132.06]
Kupfer 1992	7	10	0	18	7.2%	79.29 [3.64, 1729.03]
Leijten 1996	11	19	7	19	24.0%	2.36 [0.64, 8.68]
Total (95% CI)		109		143	100.0%	3.68 [1.51, 9.00]
Total events	53		33			

Heterogeneity: Tau² = 0.42; Chi² = 7.88, df = 5 (P = 0.16); I² = 37%
Test for overall effect: Z = 2.86 (P = 0.004)

**Supplementary Figure 2.** Forest plot for the effect of benzyl quinolinic and amino-steroid NMBA use and the development of ICU-AW. ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents; *: randomized clinical trial.

Study or Subgroup	NMB Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Adnet 2001 (1)	10	55	1	46	23.2%	10.00 [1.23, 81.40]
Behbehani 1999	9	30	0	56	16.6%	49.93 [2.78, 895.59]
Douglass 1992	9	22	0	3	15.3%	4.93 [0.23, 106.88]
Kesler 2008	8	57	2	17	28.0%	1.22 [0.23, 6.40]
Leatherman 1996	20	69	0	38	16.9%	31.89 [1.87, 544.08]
Total (95% CI)		233		160	100.0%	7.91 [1.76, 35.66]
Total events	56		3			

Heterogeneity: Tau² = 1.39; Chi² = 7.77, df = 4 (P = 0.10); I² = 48%
Test for overall effect: Z = 2.69 (P = 0.007)

Supplementary Figure 3. Forest plot for the effect of NMBA use and the development of ICU-AW of studies enrolling patients with severe asthma. ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents.
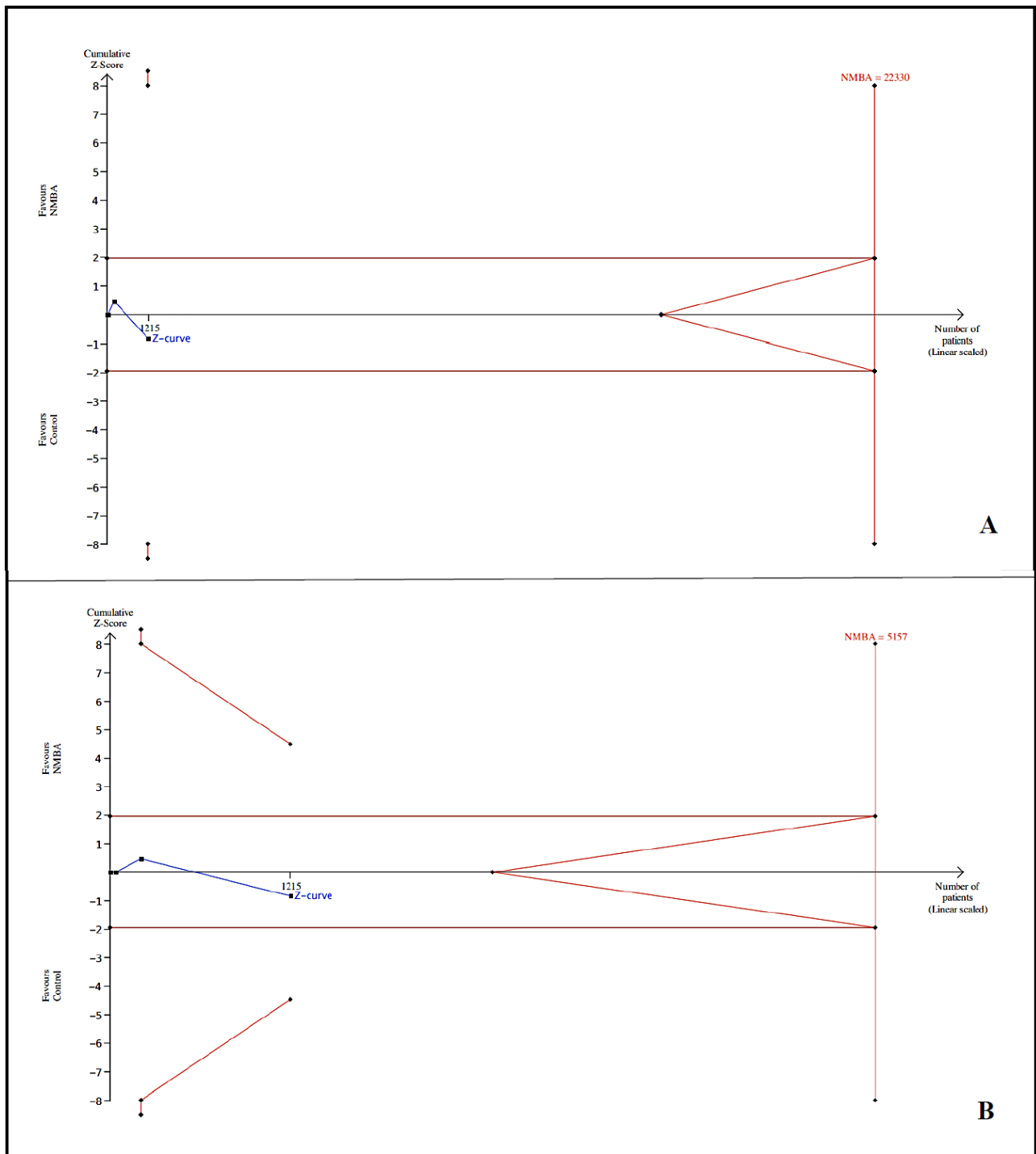
Supplementary Figure 4. Trial sequential analysis for the RCTs. (A) TSA considering an increase in the risk for the development of ICU-AW of 20% with NMBA use. (B) TSA considering an increase in risk for the development of ICU-AW of 40% with NMBA use. ICU-AW: intensive care unit-acquired weakness; NMBA: neuromuscular blocking agents; RCTs: randomized clinical trials; TSA: trial sequential analysis.

CONSIDERAÇÕES FINAIS

Com os dados apresentados nessa tese podemos afirmar que DM, hiperglicemia de estresse e variabilidade glicêmica associaram-se à mortalidade em pacientes criticamente doentes internados por COVID-19, mas não houve relação com demais desfechos clínicos. Mais do que a hiperglicemia de estresse, a presença de DM associou-se à mortalidade neste grupo de pacientes. Esses achados são contrários aos encontrados nos demais pacientes criticamente doentes, onde existe o "paradoxo do DM", ou seja, pacientes com DM internados na UTI não apresentam aumento de mortalidade, provavelmente por adaptação à hiperglicemia crônica, a qual seria capaz de gerar um acondicionamento celular protetor contra o dano mediado pela hiperglicemia aguda durante a doença crítica.

No que diz respeito ao estudo molecular realizado em biópsias musculares de pacientes críticos com e sem DM, não foram identificadas mudanças na expressão dos genes *IRS-1, IRS-2, SLC2A1* e *SLC2A4*. No entanto, o gene *INSR* apresentou sua expressão reduzida em pacientes com hiperglicemia de estresse, sugerindo uma provável ligação causal entre essa anormalidade e a patogênese da hiperglicemia em doentes criticamente doentes.

Por fim, a patogênese da FA-UTI é complexa e pouco compreendida. Da mesma forma, isolar o papel de cada fator de risco que contribui para o seu desenvolvimento é extremamente difícil. A metanálise parte dessa tese sugere que o uso de bloqueadores neuromusculares pode estar implicado no desenvolvimento de FA-UTI, porém essa conclusão deriva de estudos observacionais com alta heterogeneidade. Possivelmente, o uso de bloqueadores neuromusculares por curtos períodos, especialmente da classe dos benzis-quinolínicos, parece ser seguro, mas a *trial sequential analysis* sugere a

necessidade de mais ensaios clínicos randomizados para responder adequadamente essa questão.

Os três estudos apresentados nessa tese trazem contribuições importantes para o melhor entendimento do papel da hiperglicemia de estresse e do DM na população específica de pacientes com COVID-19 e seu comportamento diferente daquele apresentado pelos demais pacientes criticamente doentes, da relação entre o efeito da hiperglicemia de estresse sobre a expressão do gene *INSR*, sugerindo que a hiperglicemia aguda possa causar danos maiores que as alterações metabólicas crônicas em pacientes críticos, e da associação entre uso de bloqueadores neuromusculares e o desenvolvimento de FA-UTI, condição extremamente frequente, igualmente associada à presença de hiperglicemia, e com grande impacto deletério em pacientes criticamente doentes.

Embora estudos clínicos, genéticos e moleculares sejam difíceis de serem realizados em ambiente de terapia intensiva, eles são cada vez mais necessários, uma vez que podem contribuir para a compreensão dos mecanismos fisiopatológicos pelos quais a doença crítica aguda pode levar a danos em longo prazo, permitindo a correção de fatores de risco, a implementação de uma medicina de precisão e, consequentemente, a obtenção de melhores desfechos nessa população.

Com base na fundamentação teórica e nos resultados apresentados nesta tese, nossos objetivos seguem visando a uma melhor compreensão dos mecanismos subjacentes pelos quais a hiperglicemia causa danos ao organismo na fase de injúria aguda. Com esse intuito, está em desenvolvimento um novo estudo que se propõe a investigar a relação entre hiperglicemia de estresse, inflamação e alterações na expressão gênica e proteica da musculatura esquelética por meio de técnicas de análises histológicas e moleculares do tecido muscular esquelético. Portanto, mantendo a mesma linha de pesquisa desenvolvida nessa tese, seguiremos investigando de que forma a hiperglicemia,

as respostas inflamatórias e as alterações genéticas e moleculares no tecido muscular esquelético podem contribuir em conjunto para a ocorrência da FA-UTI. Esperamos que essa abordagem ampla em diferentes domínios (endócrino, metabólico, inflamatório, molecular e genético) aprofunde nosso entendimento sobre a fisiopatologia implicada no desenvolvimento dessa condição tão incidente e catastrófica e possa, eventualmente, levar a estratégias terapêuticas mais eficazes para prevenir ou tratar os danos musculares em pacientes criticamente doentes.

OUTRAS PRODUÇÕES BIBLIOGRÁFICAS NO PERÍODO DO DOUTORADO

Além dos artigos que fazem parte da presente tese, ao longo do período do doutorado foram publicados os seguintes manuscritos:

1. Piardi DS, Butzke M, Mazzuca ACM, Gomes BS, Alves SG, Kotzian BJ, Ghisleni EC, Giaretta V, Bellaver P, Varaschin GA, Garbin AP, Beck-da-Silva L. Effect of adding hydrochlorothiazide to usual treatment of patients with acute descompensated heart failure: a randomized clinical trial. Sci Rep, 2021;11(1):17370.

2. Henrique LR, Crispim D, Vieceli T, Schaeffer AF, Bellaver P, Leitão CB, Rech TH. Copeptin and stress-induced hyperglycemia in critically ill patients: A prospective study. PLoS One, 2021;16(4):e0250035.

3. Piltcher-da-Silva R, Chedid MF, Grezzana Filho TJM, Leipnitz I, de Araújo A, Gazzana MB, Saueressig MG, Lorenzi W, Cardoni MG, Bellaver P, Alvares-da-Silva MR, Feier FH, Chedid AD, Kruel CRP. Severe hepatopulmonary syndrome with hypoxemia refractory to liver ransplant: Recovery after 67 days of ECMO support. Int J Artif Organs, 2022;45(1):121-123.