UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL
PROGRAMA DE PÓS-GRADUAÇÃO EM DOENÇAS INFECCIOSAS E PARASITÁRIAS

PATRICIA VIEIRA DA SILVA

FATORES DE RISCO PARA ÓBITO PELA COVID-19 E EFETIVIDADE DAS VACINAS CORONAVAC E CHADOX1 FRENTE A VARIANTE GAMA NO BRASIL

CAMPO GRANDE-MS
2022

PATRICIA VIEIRA DA SILVA

FATORES DE RISCO PARA ÓBITO PELA COVID-19 E EFETIVIDADE DAS VACINAS CORONAVAC E CHADOX1 FRENTE A VARIANTE GAMA NO BRASIL

Tese apresentada ao Programa de Pós-graduação em Doenças Infecciosas e Parasitárias, da Universidade Federal de Mato Grosso do Sul, como requisito parcial para obtenção do título de Doutora.

Orientador: Prof. Dr. Julio Croda

CAMPO GRANDE-MS

2022

A tese intitulada FATORES DE RISCO PARA ÓBITO PELA COVID-19 E EFETIVIDADE DAS VACINAS CORONAVAC E CHADOX1 FRENTE A VARIANTE GAMA NO BRASIL, como exigência para obtenção do grau de Doutor em Doenças Infecciosas e Parasitárias, à banca examinadora de QUALIFICAÇÃO, na Faculdade de Medicina da Universidade Federal de Mato Grosso do Sul, MS, obteve conceito _____.

BANCA EXAMINADORA	NOTA/CONCEITO
_____	_____
Dr. Julio Croda - PPGDIP/UFMS; FIOCRUZ-MS	
_____	_____
Drª. Ana Lúcia Lyrio de Oliveira – PPGDIP/UFMS	
_____	_____
Dr. Marco Antônio Moreira Puga - PPGDIP/UFMS	
_____	_____
Dr. Rivaldo Venâncio da Cunha PPGDIP/UFMS; FIOCRUZ-MS	
_____	_____
Dr. Roberto Dias de Oliveira – UEMS	
_____	_____
Drª. Karla Regina Warszawski de Oliveira - UFMS (Suplente)	

Campo Grande, _____ de maio de 2022.

RESUMO

Em dezembro de 2019, um surto de pneumonia de origem desconhecida foi relatado na China. O agente etiológico foi identificado como coronavírus causador da síndrome respiratória aguda grave 2 (SARS-CoV-2) e a doença nomeada como doença do novo coronavírus 2019 (COVID-19). O vírus se espalhou por todo mundo e no Brasil foram registrados mais de 30 milhões de casos e 663 mil mortes. Números altamente desproporcionais de mortes foram identificados entre alguns grupos, levando a necessidade de elucidar quais fatores de risco estão associados a desfechos graves pela COVID-19. As vacinas contra o SARS-CoV-2 foram desenvolvidas, comprovadamente eficazes e implantadas em campanhas de vacinação em massa. Destacam-se entre elas, as duas primeiras vacinas distribuídas no Brasil, a CoronaVac e a ChAdOx1. O surgimento das variantes de preocupação (VOCs), como a variante Gama, criou a necessidade do monitoramento contínuo da efetividade das vacinas, uma vez que mutações observadas nessas variantes podem conferir escape a imunidade e neutralização dos imunizantes. Diante do exposto, este estudo teve como objetivo descrever os fatores de risco para óbito pela COVID-19 e estimar a efetividade das duas primeiras vacinas frente a variante Gama no Brasil. Diferentes metodologias foram utilizadas neste estudo. Para identificar os fatores de risco para óbito, foi realizada uma análise de sobrevida com todos os pacientes internados com COVID-19 confirmada por RT-PCR no estado de São Paulo cadastrados no Sistema de Vigilância Epidemiológica da Gripe (SIVEP-GRIPE) no período de 26 de fevereiro de 2020 a 10 de outubro de 2020. Para o estudo de efetividade foi realizado um caso-controle pareado com teste negativo, comparando os casos de COVID-19 confirmada por RT-PCR, hospitalização e óbito, em idosos durante a epidemia com alta prevalência da variante Gama no estado de São Paulo, com as doses aplicadas da CoronaVac e ChAdOx1. Observamos que homens, idosos, e pessoas com comorbidades tiveram maior risco de óbito pela COVID-19. A efetividade ajustada da CoronaVac contra COVID-19 sintomática foi de 24,7% em 0-13 dias e 46,8% em ≥14 dias após a segunda dose. Já a efetividade ajustada contra internações foi de 55,5% e contra óbitos foi de 61,2% ≥14 dias após a segunda dose. A efetividade da CoronaVac diminuiu com o aumento da idade. A partir de 28 dias após a primeira dose, a efetividade da ChAdOx1 foi de 33,4% contra COVID-19 sintomática, 55,1% contra hospitalização e 61,8% contra óbito. A partir de 14 dias após a segunda dose,

a efetividade do esquema de duas doses da ChAdOx1 foi 77,9% contra COVID-19, 87,6% contra hospitalização e 93,6% contra óbito. De acordo com os resultados obtidos, pessoas que apresentam fatores de risco para agravamento da infecção devem ser priorizadas na vacinação, afim de diminuir a mortalidade pela COVID-19. Além disso, a conclusão do cronograma vacinal de duas doses com CoronaVac e ChAdOx1 oferece proteção contra casos leves e graves da COVID-19 em idosos durante a circulação generalizada da variante Gama.

Palavras chave: COVID-19, Fatores de Risco, Variante Gama, Vacina

ABSTRACT

In December 2019, an outbreak of pneumonia of unknown origin was reported in China. The etiologic agent was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease named as novel coronavirus disease 2019 (COVID-19). The virus has spread all over the world and in Brazil more than 30 million cases and 663 thousand deaths have been recorded. Highly disproportionate numbers of deaths have been identified among some groups, prompting the need to elucidated which risk factors are associated with severe COVID-19 outcomes. Vaccines against SARS-CoV-2 have been developed, proven effective and deployed in mass vaccination campaigns. Among them, the first two vaccines distributed in Brazil, CoronaVac and ChAdOx1, stand out. The emergence of variants of concern (VOCs), such as the Gamma variant, created the need for continuous monitoring of the effectiveness of vaccines, since mutations observed in these variants can provide escape immunity and neutralization of immunizers. Given the above, this study aims to describe the risk factors for death from COVID-19 and estimate the effectiveness of the first two vaccines against the Gamma variant in Brazil. Different methodologies were used in this study. To identify risk factors for death, a survival analysis was performed with all hospitalized patients with COVID-19 confirmed by RT-PCR in the state of São Paulo registered in the Influenza Epidemiological Surveillance System (SIVEP-GRIPE) in the period from February 26, 2020 to October 10, 2020. For the effectiveness study, a matched, test-negative case-control study was carried out, comparing the cases of COVID-19 confirmed by RT-PCR, hospitalization and death, in the elderly during the epidemic with a high prevalence of the Gamma variant in the state of São Paulo, with administered doses of CoronaVac and ChAdOx1. We observed that men, the elderly, and people with comorbidities had a higher risk of death from COVID-19. The adjusted effectiveness of CoronaVac against symptomatic COVID-19 was 24.7% at 0-13 days and 46.8% at ≥14 days after the second dose. The adjusted effectiveness against hospitalizations was 55.5% and against deaths it was 61.2% ≥14 days after the second dose. The effectiveness of CoronaVac decreased with increasing age. From 28 days after the first dose, the effectiveness of ChAdOx1 was 33.4% against symptomatic COVID-19, 55.1% against hospitalization, and 61.8% against death. As of 14 days after the second dose, the effectiveness of the two-dose regimen was 77.9% against COVID-19, 87.6% against hospitalization, and 93.6%

against death. According to the results obtained, people who have risk factors for worsening the infection should be prioritized in vaccination, in order to reduce mortality from COVID-19. Additionally, completion of the two-dose vaccine schedule with CoronaVac and ChAdOx1 provides protection against both mild and severe COVID-19 outcomes in older adults during widespread Gamma variant circulation.

Key words: COVID-19, Risk factors, Gamma variant, Vaccine

SUMÁRIO

1 INTRODUÇÃO

Em dezembro de 2019, um surto de pneumonia de origem desconhecida foi relatado na cidade de Wuhan, província de Hubei, China (1). O agente etiológico foi posteriormente identificado como coronavírus causador da síndrome respiratória aguda grave 2 (SARS-CoV-2) e a infecção como doença do novo coronavírus 2019 (COVID-19) pela Organização Mundial da Saúde (2). No Brasil, o primeiro caso de COVID-19 foi relatado na cidade de São Paulo em um homem de 61 anos cujo os sintomas começaram em 23 de fevereiro de 2020 (3). Desde então, mais de 30 milhões de casos e 663 mil óbitos foram relatados no país (4).

Números altamente desproporcionais de óbitos pela COVID-19 foram identificados entre alguns grupos populacionais, indicando a necessidade de investigar quais fatores estão associados a desfechos graves pela doença, principalmente entre pessoas hospitalizadas (5). Estudos mostraram que homens, idosos e pessoas com comorbidades têm maior risco de hospitalização e óbito (6). Uma análise retrospectiva das 250 mil primeiras hospitalizações pela COVID-19 no Brasil observou que a idade média dos pacientes foi de 60 anos e que a mortalidade aumentou acentuadamente com a idade e a presença de comorbidades (7).

Cento e noventa e cinco vacinas foram desenvolvidas desde o início da pandemia, trinta e oito estão em fase 3 do ensaio clínico e dezenove foram autorizadas para uso (8). Dentre as vacinas autorizadas destacam-se, principalmente em países de baixa e média renda, a vacina de vírus inativado CoronaVac (Sinovac) e a vacina de vetor viral ChAdOx1 (AstraZeneca) (9,10). Ensaios controlados randomizados mostraram eficácia de 70,4% para ChAdOx1 (10) e 51% para CoronaVac (9) contra COVID-19 sintomática após administração de duas doses. No Brasil, a distribuição dessas vacinas iniciou em janeiro de 2021 e desde então aproximadamente 240 milhões de doses foram aplicadas (11).

O surgimento das variantes de preocupação (VOCs), associadas à diminuição da atividade de neutralização pelos anticorpos vacinais, criou a necessidade do monitoramento contínuo da eficácia das vacinas (12). A VOC Gama foi detectada pela primeira vez na cidade de Manaus (13) e acumula mutações que levam a maior transmissibilidade, gravidade e diminuição da soroneutralização (14,15). Desde o início da pandemia foi a cepa responsável pelo maior número de óbitos no Brasil (16).

Evidências sugerem que a eficácia seja reduzida para uma única dose de ChAdOx1 contra a variante Gama (17) e CoronaVac (18).

Diante deste cenário, além de conhecer os fatores de risco para desfechos graves, uma questão-chave é investigar se as vacinas ChAdOx1 e CoronaVac contra a COVID-19 são efetivas nas populações em maiores riscos desses desfechos graves, como os idosos, que podem ter respostas imunológicas prejudicadas e estão sub-representados em ensaios clínicos randomizados. Ademais, o surgimento de variantes emergentes é uma preocupação para o controle da pandemia, com isso conhecer a efetividade das vacinas frente a variante Gama é fundamental para o monitoramento e tomadas de decisão como a necessidade de doses de reforço.

2 REFERENCIAL TEÓRICO

2.1 Os coronavírus

Os coronavírus (CoV) são um grupo de vírus de RNA envelopados, fita simples de sentido positivo, pertencente à subfamília *Orthocoronavirinae*, da família *Coronaviridae*, da ordem *Nidovirales*. Os coronavírus da subfamília *Orthocoronaviridae* se dividem em quatro gêneros: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus* e *Deltacoronavirus*.(19) Dentre esses gêneros, sete coronavírus são conhecidos por infectar humanos, dois *Alphacoronavirus* (HCoV-229E e HCoV-NL63) e cinco *Betacoronavirus* (HCoV-OC43, HCoV-HKU1, SARS-CoV, SARS-CoV-2 e MERS-CoV) (20).

A caracterização genômica dos coronavírus mostrou que morcegos e roedores são as prováveis fontes gênicas dos *Alphacoronavirus* e *Betacoronavirus,* enquanto espécies aviárias parecem representar as fontes genéticas de *Gammacoronavirus* e *Deltacoronavirus*. Os coronavírus se tornaram os principais patógenos de surtos de doenças respiratórias emergentes, podendo causar também doenças entéricas, hepáticas e neurológicas em diferentes espécies animais, incluindo camelos, bovinos, gatos e morcegos. Por razões ainda desconhecidas, esses vírus podem atravessar as barreiras das espécies e causar, em humanos, doenças que variam do resfriado comum a doenças mais graves que levam a óbito (21).

Os primeiros relatos de coronavírus humano endêmico datam da década de 1960 (22), quando HCoV-OC43 (23) e HCoV-229E (24) foram reconhecidos como importantes causas de infecções do trato respiratório superior, ocasionalmente associados a doenças pulmonares mais graves em idosos, recém-nascidos e imunocomprometidos (25). Em 2004 e 2005, outros dois coronavírus foram isolados, HCoV-NL63 (26) de pacientes hospitalizados e crianças com doença respiratória grave e HCoV-HKU1 (27) de pacientes idosos com comorbidades, respectivamente. Além desses quatro, dois coronavírus epidêmicos surgiram em humanos nas últimas duas décadas e foram responsáveis por alta letalidade, a Síndrome Respiratória Aguda Grave (SARS-CoV) (28) em 2002 e a Síndrome Respiratória do Oriente Médio (MERS-CoV) (29) em 2012.

O coronavírus da Síndrome Respiratória Aguda Grave (SARS-CoV) foi identificado pela primeira vez no sul da China (província de Guangdong) em novembro

de 2002 (28,30). Ao final da epidemia, a China relatou mais de 8.000 casos da doença e 774 óbitos e letalidade de 7% (31). O surto global de SARS foi contido em julho de 2003 e desde 2004 não houve nenhum caso relatado (32). Após o surgimento do SARS-CoV, o MERS-CoV foi o segundo coronavírus que resultou em uma crise global de saúde pública (33). A MERS surgiu pela primeira vez em 2012 na Arábia Saudita, quando um homem de 60 anos apresentou pneumonia grave (29). Desde então, 27 países reportaram casos, sendo 2.494 confirmados laboratorialmente e 858 óbitos e letalidade de 35% (34,35).

No final de 2019, o sétimo coronavírus conhecido por infectar humanos foi identificado como causador de uma epidemia de pneumonia na China (1,36). Em 11 de fevereiro de 2020, a Organização Mundial da Saúde (OMS) nomeou a nova doença como “COVID-19”, abreviação de “coronavirus disease 2019” (2). Em março do mesmo ano, o Grupo de Estudo *Coronaviridae,* do Comitê Internacional de Taxonomia de Vírus, responsável pela classificação de vírus e nomenclatura de táxons da família *Coronaviridae*, reconheceu este vírus provisoriamente denominado 2019-nCoV como formando um clado irmão do coronavírus da síndrome respiratória aguda grave (SARS-CoV), e o nomeou como SARS-CoV-2 (36).

Embora o SARS-CoV-2 tenha sido identificado pela primeira vez em Wuhan, até o momento sua origem é desconhecida (36). A OMS divulgou um relatório no qual explorou várias hipóteses sobre a origem do vírus e apesar de não ter encontrado resultados conclusivos, mostrou a importante participação de animais no ciclo de transmissão (37). Análises genômicas sugerem que o SARS-CoV-2 provavelmente evoluiu de uma cepa encontrada em morcegos e semelhante ao SARS-CoV e MERS-CoV, avançou para hospedeiros intermediários como pangolins e martas e por último para humanos (21,38,39). A sequência genômica mais próxima do SARS-CoV-2 foi encontrada em um coronavírus de morcego (*Rhinolophus affinis*) RaTG13 com 96,2% de semelhança (36).

2.2 A COVID-19

2.2.1 Características e manifestações clínicas

A COVID-19, doença causada pelo SARS-CoV-2, pode afetar os seres humanos de diferentes maneiras, podendo variar de formas assintomática ou oligossintomática, à doença clínica caracterizada por insuficiência respiratória aguda com necessidade de ventilação mecânica, choque séptico, falência múltipla de órgãos e óbito (21). O período médio de incubação do SARS-CoV-2, assim como a variante Gama, é estimado em até 5 dias e a maioria dos pacientes desenvolverá sintomas dentro de até 11,5 dias após a infecção (40). Entretanto, esse período pode mudar de acordo com a cepa circulante, uma vez que a variante Delta apresentou período médio de incubação de 4 dias e a Ômicron 3 dias (41).

Os Institutos Nacionais de Saúde (NIH) emitiram diretrizes que classificam a COVID-19 em cinco tipos distintos: infecção assintomática ou pré-sintomática, doença leve, doença moderada, doença grave e doença crítica. A infecção assintomática ou pré-sintomática é caracterizada por indivíduos com teste positivo para SARS-CoV-2 sem quaisquer sintomas clínicos consistentes com a COVID-19 (42). Já a doença leve caracteriza-se por indivíduos que apresentam algum sintoma da COVID-19, como febre, tosse, dor de garganta, mal-estar, dor de cabeça, dor muscular, náusea, vômito, diarreia, anosmia ou disgeusia, mas sem falta de ar ou imagem torácica anormal. A doença moderada é definida por indivíduos que apresentam sintomas clínicos ou evidência radiológica de doença do trato respiratório inferior e que têm saturação de oxigênio (SpO_2) ≥ 94% em ar ambiente (42).

A doença grave é caracterizada por indivíduos que têm (SpO_2) ≤ 94% em ar ambiente; uma relação entre pressão parcial de oxigênio arterial e fração inspirada de oxigênio, (PaO_2/FiO_2) <300 com taquipnéia acentuada com frequência respiratória >30 respirações/min ou infiltrados pulmonares >50% (42). Por último, a doença crítica é definida por indivíduos com insuficiência respiratória aguda, choque séptico e/ou disfunção de múltiplos órgãos. Pacientes com doença grave de COVID-19 podem desenvolver a síndrome do desconforto respiratório agudo (SDRA), que tende a ocorrer aproximadamente uma semana após o início dos sintomas (42).

A síndrome do desconforto respiratório agudo (SDRA) é uma doença respiratória aguda caracterizada por opacidades radiográficas bilaterais do tórax com

hipoxemia grave devido a edema pulmonar não cardiogênico (43). A definição de Berlim classifica a SDRA em três tipos com base no grau de hipóxia: leve (200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg em pacientes que não recebem ventilação mecânica ou naqueles controlados por ventilação não invasiva (VNI) usando pressão expiratória final positiva (PEEP) ou pressão positiva contínua nas vias aéreas (CPAP) ≥ 5 cmH_2O); moderada (100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg) e grave (PaO_2/FiO_2 ≤ 100 mmHg) (44).

Embora a COVID-19 afete predominantemente o sistema respiratório, pode ser considerada uma doença viral sistêmica, que afeta diversos órgãos, principalmente em indivíduos hospitalizados, geralmente associada a maior gravidade (21). Entre as manifestações em múltiplos órgãos temos: manifestações renais (ex. lesão renal aguda) (45); manifestações cardíacas (ex. lesão miocárdica, arritmias, cardiomiopatia e choque cardiogênico); manifestações hematológicas (ex. linfopenia e trombocitopenia); manifestações gastrointestinais (ex. diarreia, náusea e/ou vômito); manifestações hepatobiliares (ex. disfunção hepática); manifestações endocrinológicas (ex. níveis anormais de glicose no sangue e cetose euglicêmica) (46); manifestações neurológicas (ex. anosmia e ageusia) (47) e manifestações cutâneas (ex. lesões acrais semelhantes a pseudo-frieiras) (48).

2.2.2 Transmissão e prevenção

A transmissão do SARS-CoV-2 se dá predominantemente de pessoa-a-pessoa, através de gotículas ou partículas respiratórias contaminadas com o vírus expelidas ao tossir, espirrar e falar. A exposição pode ocorrer quando alguém inala essas gotículas ou partículas ou caso o indivíduo toque uma superfície que contenha essas gotículas ou partículas contaminadas e, a seguir, leve suas mãos aos olhos, nariz ou boca (49). Sabe-se que as gotículas não alcançam mais de 1,8 metros de quem as expeliu, revelando que essa é uma distância segura que se deve manter de quem apresenta manifestações respiratórias (49).

A transmissão por superfícies inanimadas contaminadas com SARS-CoV-2 foi bem caracterizada com base em muitos estudos que relatam a viabilidade desse vírus em várias superfícies porosas e não porosas. Em condições experimentais, observou-se que o SARS-CoV-2 é mais estável em superfícies de aço inoxidável e plástico em comparação com superfícies de cobre e papelão, com o vírus viável sendo detectado

até 72 horas após a inoculação das superfícies (50). O SARS-CoV-2 pode se manter viável por períodos mais longos quando em baixas temperaturas, por até 28 dias a 20 °C em superfícies comuns, como vidro, aço inoxidável e cédulas de papel e polímero. Por outro lado, o agente infeccioso sobrevive menos de 24h a 40°C em algumas superfícies (51).

Menos comumente, pode ocorrer a transmissão aérea de pequenas gotículas e partículas de SARS-CoV-2 para pessoas a mais de 1,8 metros de distância; em casos raros, as pessoas que passam por uma sala anteriormente ocupada por uma pessoa infectada podem ser infectadas. A infecção por SARS-CoV-2 por transmissão aérea de pequenas partículas tende a ocorrer após exposição prolongada (ou seja, > 15 minutos) a uma pessoa infectada que está em um espaço fechado com pouca ventilação (49). A transmissão vertical é possível, mas ocorre em um pequeno percentual de casos de infecção materna pela COVID-19 (52). Por fim, a transmissão por via oral-fecal ainda é discutida, pois apesar RNA viral ter sido encontrado nas fezes, é necessário que o vírus tenha capacidade infecciosa, o que até o momento não foi comprovado (53).

O risco de transmissão do SARS-CoV-2 pode ser reduzido com o uso de máscaras (54), lavagem das mãos (55) e intervenções não farmacêuticas, como o distanciamento social (56). As máscaras destinam-se principalmente a reduzir a emissão de gotículas carregadas com o vírus pelo indivíduo infectado (fonte), especialmente relevante para infectados assintomáticos, que se sentem bem e podem não estar cientes de sua infecciosidade para os outros. As máscaras também ajudam a reduzir a inalação dessas gotículas pelo usuário. A relação entre o controle da fonte e a proteção do usuário é provavelmente complementar e possivelmente sinérgica, de modo que o benefício individual aumenta com o aumento do uso de máscaras na comunidade (57).

As intervenções não farmacêuticas (INF) como distanciamento social, quarentena, isolamento, bloqueios, toque de recolher, restrições de viagem, entre outros, foram adotadas por diferentes governos em todo mundo para proteger a população da COVID-19. Essas estratégias têm sido usadas para reduzir o número de casos para níveis aceitáveis ou eliminar a transmissão pessoa-pessoa do vírus e mostrou-se eficaz. Entretanto, as INFs são uma forma complexa de intervenção, pois dependem de fatores culturais, sociais e econômicos, para serem implementadas e seguidas de forma adequada em um sistema de saúde (58).

Além da importância de medidas de saúde pública para prevenir ou diminuir a transmissão do SARS-CoV-2, o passo crucial para conter a pandemia global é a vacinação. A vacinação é sem dúvidas a maneira mais eficaz de prevenir a COVID-19. Esforços extraordinários de instituições e pesquisadores em todo o mundo resultaram no desenvolvimento de vacinas contra o SARS-CoV-2 em uma velocidade sem precedentes (59). Vacinas altamente protetoras estão sendo distribuídas ao redor do mundo e estudos tem demonstrado que elas podem mitigar substancialmente as taxas de infecção, hospitalizações e óbitos pela doença, especialmente entre indivíduos vulneráveis com comorbidades e fatores de risco associados à COVID-19 grave (60).

2.2.3 Diagnóstico e tratamento

O teste padrão ouro para o diagnóstico da COVID-19 é baseado em um teste molecular de transcriptase reversa seguida pela reação em cadeia da polimerase em tempo real (qRT-PCR), visando detectar o cDNA do vírus em amostras respiratórias como swab nasofaríngeo e oral ou aspirado brônquico. O teste qRT-PCR fornece um teste sensível e específico para detectar SARS-COV-2, com diferentes protocolos de diagnóstico incluindo sequências de primers alvo disponíveis no banco de dados público da OMS (61). Entretanto, esse teste também pode resultar em falsos negativos se a quantidade de genoma viral for insuficiente ou se a janela de tempo correta de replicação viral for perdida. Além disso, o processo de RT-PCR necessita de equipe especializada, infraestrutura laboratorial complexa e alto custo, dificultando a ampla disponibilidade, principalmente em países de baixa e média renda (61).

Outro teste para diagnóstico da infecção pela COVID-19 é o teste de antígeno. O teste de antígeno é um exame imunocromatográfico rápido, que ao invés de detectar o material genético do vírus, como na qRT-PCR, identifica as proteínas do SARS-CoV-2 (ex. NCP). Os testes de antígeno são menos sensíveis, mas têm um tempo de resposta mais rápido em comparação com qRT-PCR e são uma alternativa em locais com escassez de recursos (62,63). Os testes sorológicos avaliam a presença de anticorpos IgM e IgG que aparecem em resposta a infecção. Como os anticorpos geralmente são detectados apenas 1-3 semanas após o início dos sintomas, esses testes são mais utilizados na vigilância epidemiológica, para avaliar a taxa geral de infecção pelo SARS-CoV-2 na comunidade (61).

Considerando que essa doença viral comumente se manifesta como pneumonia, a imagem radiológica tem papel fundamental no processo de diagnóstico, tratamento e seguimento. Os exames de imagem podem incluir radiografia de tórax, ultrassonografia pulmonar ou tomografia computadorizada (TC) de tórax (21). Não há diretrizes disponíveis sobre o momento e a escolha dos exames de imagem pulmonar em pacientes com COVID-19 e o tipo de imagem deve ser considerado com base na avaliação clínica. Outros exames laboratoriais complementares podem ser utilizados no manejo da doença como hemograma, testes bioquímicos de função renal, marcadores inflamatórios, entre outros (21).

A pandemia trouxe muitos desafios, entre eles o manejo terapêutico para tratar o aumento súbito de casos pela COVID-19 em todo mundo. No início, o conhecimento sobre a doença era limitado, o que criou a necessidade de mitigar essa nova infecção com terapias experimentais e reaproveitamento de medicamentos, com foco nos sinais e sintomas. A partir de então ocorreram avanços significativos, o que levou a uma melhor compreensão do manejo e desenvolvimento de novas terapêuticas (21).

Entre opções terapêuticas potenciais propostas, autorizadas ou aprovadas para uso clínico no manejo da COVID-19 estão: terapias antivirais (ex. Molnupiravir, Remdesivir, Paxlovid, ritonavir em combinação com nirmatrelvir e Lopinavir/ritonavir), produtos de anticorpos neutralizantes anti-SARS-CoV-2 (REGN-COV2, ex. Casirivimab, Imdevimab, Bamlanivimab/Etesevimab e Sotrovimabe) e agentes imunomoduladores (ex. corticosteroides, interferon-β-1a, tocilizumab, Sarilumab/Siltuximab, Baricitinibe e tofacitinibe) (21). No Brasil, cinco medicamentos estão aprovados pela ANVISA: Remdesivir, Sotrovimabe, Baricitinibe, Paxlovid e Evusheld® (cilgavimabe + tixagevimabe), sendo o último um anticorpo monoclonal, aprovado para uso emergencial em 24 de fevereiro de 2022 (64).

2.3 Epidemiologia

Os primeiros casos da COVID-19 foram relatados na cidade de Wuhan, província de Hubei (China) no início de dezembro de 2019 (65). No entanto, devido a doença ser assintomática ou causar apenas sintomas leves em muitos indivíduos (66,67), é provável que a transmissão possa ter ocorrido na comunidade antes desse período (37). Após a identificação do SARS-CoV-2 como causador de casos de pneumonia em Hubei, o vírus se espalhou para as demais províncias chinesas (68).

Não demorou até que outros países apresentassem circulação viral significativa, tendo como fonte de transmissão pessoas que retornaram de viagens da China. Inicialmente o vírus se dispersou pela Coréia do Sul, Itália, Irã e Japão e a seguir para os demais países da Europa, do Continente Americano e da África (69).

Em 30 de janeiro de 2020, a OMS declarou que o surto da COVID-19 constituía-se em uma Emergência de Saúde Pública de Importância Internacional (ESPII), o mais alto nível de alerta da Organização, conforme previsto no Regulamento Sanitário Internacional. Essa decisão buscou aprimorar a coordenação, a cooperação e a solidariedade global para interromper a propagação viral (70). Após o vírus se espalhar para vários países e regiões do mundo, a OMS declarou o surto da COVID-19 uma pandemia, em 11 de março de 2020 (71,72).

Desde então, o SARS-CoV-2 se espalhou para 223 países, com mais de 500 milhões de casos e aproximadamente 6 milhões de óbitos relatados globalmente. Os EUA experimentaram o maior número de infecções por SARS-CoV-2 (mais de 78 milhões) e óbitos (mais de 900 mil) relacionados a COVID-19, seguidos pelo Brasil e pela Índia no número de óbitos. A estimativa atual da OMS da taxa global de mortalidade pela COVID-19 é de 2,2% (73). Já a mortalidade intra-hospitalar variou em média de 10% a 30% em diferentes países, com taxas mais elevadas encontradas em pacientes que foram admitidos em Unidade de Terapia Intensiva (UTI), homens, idosos e pacientes com doenças preexistentes (6,7,74–77).

2.3.1 Brasil

O primeiro caso de COVID-19 no Brasil foi confirmado em 26 de fevereiro de 2020, na cidade de São Paulo, em um homem de 61 anos com histórico de viagem ao norte da Itália, local que já havia relatado grande número de infectados e óbitos (3). Um estudo estimou que 54,8% de todos os casos importados no Brasil no início da pandemia seriam provenientes de viajantes infectados na Itália, seguido de China (9,3%) e França (8,3%) (78). Apesar das medidas adotadas pelo governo brasileiro, o vírus se espalhou rapidamente por todo território nacional (79).

Transcorreram pouco menos de 5 meses desde a descoberta do primeiro infectado pelo SARS-CoV-2 (8ª semana epidemiológica - SE), até o pico da primeira onda (30ª SE) com mais de 65 mil novos casos e 1500 óbitos registrados em um único dia. Nesse momento o Brasil já apresentava aproximadamente 2,5 milhões de casos

confirmados, 213 mil hospitalizações e mais de 85 mil óbitos, sendo o segundo país com maior número de casos e óbitos pela COVID-19 no ranking mundial. A partir da 30ª até a 44ª SE ocorreu um declínio substancial de casos e óbitos (80).

A primeira onda foi caracterizada pela introdução e dispersão do vírus no país. O estado de São Paulo foi responsável por 35% das hospitalizações e 25% do total de óbitos nesse período (80). Com a implementação de medidas de isolamento e biossegurança o número de infectados e óbitos teve um declínio como pôde ser observado da 30ª a 44ª SE (81). Análises genômicas demonstraram que as variantes com maior prevalência durante o início e meio da primeira onda foram B.1.1.28 (20 a 30%) e B.1.1.33 (10 a 35%) e ao final a prevalência foi de aproximadamente 20% da variante P.2 (Zeta) (82).

Em meados de novembro de 2020 começou a segunda onde (80). Essa, por sua vez, com maior impacto do que a primeira, com novos recordes diários é impulsionada pela introdução de uma nova variante descoberta em Manaus, a P.1 (Gama) (83). Durante a segunda onda as variantes Zeta e Gama foram as mais prevalentes. A Zeta teve maior prevalência durante a 50ª/2020 a 7ª/2021 SE (~20%). A partir da 7ª SE a Gama dominou o cenário, variando de 60% a 90% entre as variantes circulantes no Brasil (81).

A segunda onda foi marcada pelo colapso no sistema de saúde de Manaus, noticiado mundialmente, com dezenas de pessoas mortas por asfixia devido a falta de oxigênio para suprir a demanda de casos graves (84). Entretanto, não foi apenas o estado do Amazonas que sofreu com a disseminação da variante Gama. À medida que ela foi se disseminando pelo território nacional, novos recordes de óbitos foram registrados em todos os estados (80). Na 11ª SE a taxa de ocupação de leitos de UTI COVID-19 para adultos encontrava-se em nível crítico (taxas superiores a 80% de ocupação) em 18 de 19 estados e Distrito federal (85). O estado de São Paulo se destacou novamente, com acúmulo de quase 20 mil internações em uma semana (11ª SE) (14) e mais de 1300 óbitos em um dia, correspondendo a 30% das óbitos no pico da segunda onda (80).

Pesquisadores compararam as internações pela COVID-19 durante a primeira e segunda onda no Brasil, com dados disponibilizados no Sistema de Informação de Vigilância Epidemiológica da Gripe - SIVEP-GRIPE. Os números médios de admissões por semana aumentaram em 59% da primeira para a segunda onda. Já o número de pacientes que necessitaram de suporte ventilatório não invasivo ou

invasivo aumentou 192% e os óbitos intra-hospitalares passaram de 33% para 40%. Outro dado importante é que a mediana da idade dos pacientes hospitalizados baixou de 63 para 59 anos (14). Análise realizada pela Fiocruz reforçou essa tendência de hospitalizações em menores de 60 anos, incluindo também pessoas que necessitaram de atendimento em Unidade de Terapia Intensiva (UTI) (86).

A segunda onda, com predominância da variante Gama, foi a responsável pelo maior número de internações e óbitos ocorridos no Brasil desde o início da pandemia, onde o número de óbitos chegou a mais de 4 mil em um único dia. A partir de então, as infecções, internações e óbitos têm uma queda, mas mantêm estabilidade em altos patamares até a 21ª SE. Entretanto, a estabilidade não dura muito tempo e o número de casos e óbitos voltou a subir. Na 25ª SE o Brasil atingiu um novo recorde diário de casos novos, aproximadamente 124 mil pessoas foram diagnosticadas com a doença (80,87).

A vacinação contra a COVID-19 foi iniciada no final de janeiro de 2021, ainda durante a segunda onda, com duas vacinas aprovadas pela ANVISA para uso emergencial: a CoronaVac (Sinovac/Butantan) e ChAdOx1 (AstraZeneca/Fiocruz) (88). A aplicação foi dividida à princípio por quatro grupos prioritários, um deles, os idosos, o que pode explicar a diminuição nas hospitalizações (14,86) e mortalidade(89) dos indivíduos desse grupo a partir da 6ª SE. Apesar de a vacinação ter começado na 3ª SE, a implementação foi muito lenta, devido principalmente a questões burocráticas e dificuldade de resolutividade dos governos estaduais e federal. A redução efetiva de óbitos pode ser observada apenas a partir da 24ª SE, em meados de junho (80,87).

A queda de casos e óbitos se mantiveram em queda até a 1ª SE de 2022, com um pequeno aumento de casos no meio desse período, na 38ª SE/2021, devido a disseminação da variante Delta (16,87). A rápida introdução e disseminação da variante Ômicron, e o relaxamento das medidas de biossegurança e a alta mobilidade devido as comemorações de final de ano, fizeram o número de casos da COVID-19 disparar, com novo recorde diário de 287 mil novos casos registrados em apenas um dia na 5ª SE (80). Apesar do aumento exponencial de infecções, as hospitalizações e óbitos não ultrapassaram os números observados durante o pico da variante Gama, compatível com estudos de outros países que demonstraram que pessoas infectadas pela variante Ômicron tem menor probabilidade de serem hospitalizadas e ter desfechos graves (90,91).

O Brasil atingiu mais de 30 milhões de casos confirmados da COVID-19. O número de óbitos chegou a mais de 660 mil, com uma taxa de letalidade de 2,2%. Destes, 5 milhões de casos (~17%) e 167 mil óbitos (~25%) estão concentrados no estado de São Paulo (4). Segundo o Registro Nacional de Terapia Intensiva, 19% das admissões em UTI foram devido a COVID-19, desses ~35% foram a óbito. Homens, idosos e pessoas com comorbidades evoluíram mais para desfechos graves no Brasil e no mundo (6,92).

2.4 Fisiopatologia do SARS-COV-2

2.4.1 Genótipo e fenótipo

O SARS-CoV-2 é um vírus envelopado esférico ou pleomórfico, com diâmetro de aproximadamente 60-140 nm (93). Seu genoma é composto por RNA de fita simples de sentido positivo associado a uma nucleoproteína dentro de um capsídeo composto por proteína da matriz (94). A característica mais proeminente dos coronavírus são as projeções de glicoproteínas em forma de “espinhos” que emergem da superfície do envelope. Esses “espinhos” trazem uma característica marcante ao vírion, formando um aspecto de coroa, que levou ao nome Coronavírus. Alguns coronavírus também contêm uma proteína hemaglutinina-esterase (HE) em sua superfície (95), entretanto, o sequenciamento do genoma completo e análises filogenéticas demonstraram que o SARS-CoV-2 não possui o gene HE e consequentemente a proteína HE (96).

Os coronavírus possuem os maiores genomas entre todos os vírus de RNA conhecidos (26,4 kb a 31,7 kb). Esse grande genoma conferiu a essa família de vírus uma habilidade extra para acomodar e modificar genes, com seu conteúdo guanidina + citosina variando de 32% a 43%. As extremidades 5' e 3' contêm regiões curtas não traduzidas. Para as regiões de codificação, as organizações genômicas de todos os coronavírus são semelhantes. Os genes para as principais proteínas estruturais ocorrem na ordem 5'-3': 5'-replicase ORF1ab, spike (S), envelope (E), membrana (M), nucleocapsídeo (N)-3', sendo as quatro últimas proteínas codificadas pelas ORFs 10 e 11 no terço do genoma próximo ao terminal 3' (97).

Além dessas quatro proteínas estruturais principais (S, E, M e N) diferentes CoVs codificam proteínas estruturais e acessórias especiais, como proteína HE,

proteína 3a/b e proteína 4a/b. Essas proteínas maduras são responsáveis por várias funções importantes na manutenção do genoma e na replicação viral (94).

A proteína spike (S) (~150 kDa) é um componente essencial para a infecção da célula hospedeira, responsável tanto pela ligação aos receptores celulares quanto pela subsequente fusão das membranas virais e celulares. Ela está presente na superfície do vírus e usa sinais N-terminais como porta de entrada para o retículo endoplasmático do hospedeiro (98). A glicoproteína S trimérica não requer nenhuma outra proteína de superfície para a fusão das membranas, classificada como proteína de fusão de classe 1 (99). Na maioria dos coronavírus, a proteína S é clivada por uma protease semelhante à furina da célula hospedeira em dois polipeptídeos separados, S1 e S2. A subunidade S1 atua como domínio de ligação ao receptor; e a S2 forma o pedúnculo que fornece a estrutura de espinho (100).

A proteína M é uma proteína pequena (~ 25-30 kDa) com três domínios transmembranares, sendo a proteína estrutural mais abundante no vírion (101). Acredita-se que dê ao vírion sua forma (100), possui um pequeno ectodomínio glicosilado N-terminal e um endodomínio C-terminal muito maior que se estende de 6 a 8 nm na partícula viral (102). Apesar de serem inseridas por co-tradução na membrana do retículo endoplasmático, a maioria das proteínas M não contém uma sequência sinal. Essa proteína pode permanecer em duas conformações que ajudam a desencadear a curvatura da membrana e a afinidade de ligação ao nucleocapsídeo. A proteína M do coronavírus desempenha um papel central na montagem do vírus, transformando as membranas celulares em oficinas onde os fatores do vírus e do hospedeiro se unem para produzir novas partículas virais (103).

A proteína E (~8–12 kDa) é encontrada em pequenas quantidades dentro do vírion. A característica de sua membrana não está completamente resolvida, mas a maioria dos dados sugerem que é uma proteína transmembrana (100). Essa proteína tem um papel muito significativo na patogênese e na manutenção da atividade do canal iônico influenciando respostas inflamatórias do hospedeiro, mas não é essencial no processo de replicação viral (104). Dados sugerem que a proteína E trabalha junto com a proteína M para produzir envelopes de coronavírus (105), entretanto não se sabe como a proteína E auxilia a proteína M na montagem do vírion (100).

A proteína N constitui a única proteína presente no nucleocapsídeo. Ela é composta por dois domínios separados, um domínio N-terminal (NTD) e um domínio C-terminal (CTD). Embora a ligação ideal requeira contribuição dos dois domínios,

cada um deles utiliza mecanismos diferentes para se ligarem ao RNA (106). A proteína N é fortemente fosforilada o que desencadeia uma mudança estrutural, aumentando a afinidade para RNA viral versus não viral. A proteína N liga-se ao genoma viral em uma conformação do tipo miçangas em um barbante (100). Dois substratos de RNA específicos foram identificados para a proteína N; os Sequência Reguladora Transcricional - TRSs e o sinal de empacotamento genômico. Verificou-se que o sinal de empacotamento genômico se liga especificamente ao CTD (107). A proteína N também se liga à nsp3, um componente chave do complexo da replicase, e à proteína M. Essas interações de proteínas provavelmente ajudam a ligar o genoma viral ao complexo replicase-transcriptase e, posteriormente, empacotam o genoma encapsulado em partículas virais (100).

2.4.2 Replicação e patogênese

A entrada celular dos coronavírus depende da ligação da proteína spike (S) aos receptores celulares, e da ativação dessa proteína pelas enzimas proteases da célula hospedeira. O SARS-CoV-2 se liga à enzima conversora de angiotensina 2 (ECA-2) por meio de sua proteína spike, o que permite que o vírus entre e infecte as células. Para que o vírus complete a entrada na célula, a proteína spike deve ser preparada pela protease. Semelhante ao SARS-CoV, o SARS-CoV-2 usa uma enzima chamada serino protease transmembranar II (TMPRSS2) para concluir esse processo. Para anexar o receptor do vírus (proteína spike) ao seu ligante celular (ECA-2), a ativação por TMPRSS2 é necessária (94,108).

Depois que o vírus entra nas células, o genoma do RNA viral é liberado no citoplasma e traduzido em duas poliproteínas e proteínas estruturais. Em seguida o genoma viral começa a se replicar. As glicoproteínas do envelope recém-formadas são inseridas na membrana do retículo endoplasmático ou Golgi, e o nucleocapsídeo é formado pela combinação do RNA genômico e da proteína do nucleocapsídeo. Em seguida, as partículas virais germinam no compartimento intermediário do retículo endoplasmático-Golgi. Por fim, as vesículas contendo as partículas do vírus se fundem com a membrana plasmática para liberar o vírus (109).

A partir do momento que o vírus entra na célula, seu antígeno é apresentado às células apresentadoras de antígeno (APC do inglês Antigen Presenting Cells). Peptídeos antigênicos são apresentados pelo complexo principal de

histocompatibilidade (MHC) ou antígeno leucocitário humano (HLA) e então reconhecidos por linfócitos T citotóxicos (109). A apresentação do antígeno estimula a imunidade humoral e celular do hospedeiro, que são mediadas por células B e T (110). A resposta humoral inclui anticorpos dirigidos contra proteínas S e N, incluindo IgM, IgG e IgA (111). As interações celulares produzem um conjunto diversificado de mediadores imunológicos contra o vírus invasor, produzindo importante resposta inflamatória (95).

As formas leves e graves da COVID-19 resultam em alterações nos subgrupos de leucócitos circulantes e na secreção de citocinas, particularmente IL-6, IL-1β, IL-10, TNF, fator estimulador de colônias de granulócitos e macrófagos (GM-CSF), proteína 10 induzida por IFN (IP-10), IL-17, proteína quimiotática de monócitos 3 (MCP-3) e IL-1ra. As manifestações clínicas de uma resposta leve, com baixa liberação de citocinas em resposta à infecção, incluem: febre, mialgia, artralgia, náusea, erupção cutânea, depressão e outros sintomas semelhantes aos da gripe (112,113).

Em contraste, a hiperprodução de citocinas, também conhecida como tempestade de citocinas, está relacionada a forma grave da doença. Ela é ocasionada por um aumento súbito nos níveis circulantes de diferentes citocinas pró-inflamatórias, incluindo IL-6, IL-1, TNF-α e interferon. Este aumento resulta no influxo de várias células imunes como macrófagos, neutrófilos e células T da circulação para o local da infecção, com efeitos destrutivos no tecido humano resultantes da desestabilização das interações entre as células endoteliais, danos na barreira vascular, lesão alveolar difusa, falência múltipla dos órgãos e óbito (114).

O SARS-CoV-2 atinge predominantemente os sistemas respiratório e vascular (21). Uma das principais causas de óbito entre os pacientes com COVID-19 é insuficiência respiratória pela síndrome do desconforto respiratório agudo (SDRA) (68,115). Embora o mecanismo exato da SDRA pela COVID-19 não seja totalmente compreendido, a produção excessiva de citocinas pró-inflamatórias é considerada um dos principais fatores contribuintes (113). Mecanicamente, as complicações pulmonares resultam da ruptura da barreira vascular levando a edema tecidual (causando acúmulo de líquido nos pulmões), endotelite, ativação das vias de coagulação com potencial desenvolvimento de coagulação intravascular disseminada (CIVD) e infiltração de células inflamatórias desreguladas (116).

O aumento da permeabilidade vascular e o subsequente desenvolvimento de edema pulmonar em pacientes com COVID-19 grave são explicados por múltiplos mecanismos, entre eles temos: endotelite como resultado de lesão viral direta e inflamação perivascular levando à deposição microvascular e microtrombos; desregulação da renina-angiotensina-aldosterona (SRAA) pelo aumento da ligação do vírus aos receptores ECA-2; ativação da via calicreína-bradicinina e contração aumentada das células epiteliais causando inchaço das células e desordem das junções intercelulares (21,116,117).

O sistema respiratório não é o único alvo do SARS-CoV-2, ele pode afetar outros sistemas vitais, como sistema nervoso central, hematológico, gastrointestinal, renal, hepático e cardiovascular (21). A ECA-2, receptor funcional do SARS-CoV-2, está envolvida na função cardíaca e no desenvolvimento de hipertensão e diabetes mellitus (118). Estudos tem demonstrado que pacientes com sintomas graves geralmente apresentam complicações envolvendo lesão miocárdica aguda (65,119). Embora o mecanismo exato do envolvimento cardíaco na COVID-19 seja desconhecido, é provável que seja multifatorial e inclui a liberação de citocinas pró-inflamatórias causando redução do fluxo sanguíneo coronariano, diminuição do suprimento de oxigênio, desestabilização da placa coronariana e microtrombogênese. A IL-6 pode levar à inflamação vascular, miocardite e arritmias cardíacas (120).

A leucopenia é uma das anormalidades laboratoriais mais comuns encontradas na COVID-19. Esse achado pode ser devido a destruição de linfócitos mediada por ECA-2 na invasão direta pelo vírus, apoptose de linfócitos devido a citocinas pró-inflamatórias e possível invasão do vírus aos órgãos linfáticos (121). A patogênese da hipercoagulação associada à COVID-19 provavelmente é induzida por dano viral direto ou lesão induzida por citocinas. A lesão no endotélio vascular leva a ativação de plaquetas, monócitos e macrófagos, aumento da expressão do fator tecidual, fator de von Willebrand e fator VIII que resulta na geração de trombina e formação de coágulos de fibrina (122).

Achados clínicos e laboratoriais demonstraram que o SARS-CoV-2 é um vírus neurotrópico e pode invadir tecidos nervosos (123). A forma de entrada do vírus no sistema nervoso central (SNC), particularmente no cérebro, e seus mecanismos fisiopatológicos ainda não são totalmente conhecidos. Teorias sugerem que a invasão e penetração viral ocorrem principalmente através da ECA-2 (124). A ECA-2 é expressa em diferentes estruturas cerebrais, particularmente em núcleos implicados

na regulação central da função cardiovascular, como o tronco encefálico, bem como em regiões não cardiovasculares, como o córtex motor e rafe (125). As possíveis rotas pelas quais o SARS-CoV-2 pode invadir o sistema nervoso central são a transferência transsináptica através de neurônios infectados por meio do nervo olfatório, infecção de células endoteliais vasculares ou migração de leucócitos através da barreira hematoencefálica (125).

As manifestações gastrointestinais da COVID-19 incluem diarreia, náusea, vômito e dor abdominal. RNA do SARS-CoV-2 foi detectado na matéria fecal em pacientes assintomáticos, após amostras respiratórias testarem negativas para o vírus. Entre os possíveis mecanismos da patogênese viral no trato gastrointestinal temos: 1. os receptores ECA-2 são expressos no epitélio do trato gastrointestinal, criando o potencial de replicação viral neste local; 2. pode haver uma lesão direta no sistema gastrointestinal devido a resposta inflamatória. Os enterócitos absortivos podem ser infectados e destruídos pelo SARS-CoV-2, potencialmente levando à má absorção, secreção intestinal desequilibrada e ativação do sistema nervoso entérico, resultando nos sintomas descritos (126,127).

A patogênese da lesão hepática em pacientes com COVID-19 é explicada por muitos mecanismos isolados ou em combinação que incluem replicação viral mediada pela ECA-2 no fígado, lesão mediada diretamente pelo vírus, lesão isquêmica, resposta inflamatória, lesão hepática induzida por drogas ou agravamento de doença hepática preexistente (126). Assim como em outros sistemas, o mecanismo para no comprometimento renal encontramos lesão direta do vírus, desequilíbrio no SRAA, estado hiperinflamatório induzido por citocinas, lesão microvascular e hipercoagulação associada a COVID-19. Além disso, hipovolemia, agentes nefrotóxicos e sepse também podem contribuir potencialmente para a lesão renal (21,128).

2.5 Fatores de risco para desfechos graves

O conhecimento dos fatores de risco que levam a desfechos graves como hospitalização, admissão em terapia intensiva (UTI) e óbito, causados pela COVID-19, é de extrema importância. Ele auxilia nas tomadas de decisão no manejo clínico, principalmente quando há escassez iminente de recursos de saúde, como leitos de UTI; nas medidas preventivas específicas com priorização de intervenções e triagem

não farmacêuticas das pessoas em risco; e pode ser importante para o desenho e interpretação de ensaios clínicos sobre a eficácia de tratamentos. Intervenções urgentes de saúde pública devem ser cuidadosamente adaptadas e implementadas para os grupos em risco, afim de reduzir a mortalidade pela COVID-19. Estudos tem demonstrado que os principais fatores de risco incluem características demográficas e condições clínicas como comorbidades subjacentes (6,129).

Muitos relatórios verificaram o impacto das comorbidades na gravidade da COVID-19. A porcentagem de pacientes com condições médicas preexistentes infectados pelo SARS-CoV-2 que necessitaram de hospitalização foi seis vezes maior do que pacientes sem comorbidades (21). Diversas doenças estão relacionadas a maior risco de desfechos graves e óbito pela COVID-19, incluindo câncer (130), doença hepáticas crônica (126), HIV (131), obesidade (132), tuberculose (133), condições de saúde mental (134), fibrose cística (135), condições cardíacas (136), doença pulmonar obstrutiva crônica (DPOC) (137), diabetes mellitus (138), doença renal crônica (139) e condições neurológicas (140).

Os homens apresentaram maior risco de infecção, doença grave, admissão em UTI e óbito pela COVID-19 (6,129,141). Além de diferenças socioeconômicas, comportamentais e de estilo de vida, entre homens e mulheres, acredita-se que a distinção do sistema imunológico de homens e mulheres pode explicar parcialmente a discrepância na incidência e gravidade pela COVID-19 (129). Dentro do sistema imunológico adaptativo, os homens têm números mais baixos de células T CD8+, células T CD4+(142) e diminuição da produção de células B em comparação com as mulheres (143). Além disso, uma vez que alguns importantes genes reguladores imunológicos estão localizados no cromossomo X, as pacientes mulheres podem ser beneficiadas devido a uma expressão mais alta de TLR7 (Toll Like Receptor 7), sensor imune inato responsável pela indução de interferon tipo I e outras citocinas inflamatórias importantes na resposta imune antiviral (6).

Outro risco demográfico relacionado a gravidade pela COVID-19 é a idade. A relação entre idade avançada e aumento do risco de infecção, hospitalização, admissão em UTI e óbito é amplamente relatada (6,144,145). Estes achados podem ser explicados pela imunossenescência, definida pela reestruturação do sistema imune durante o envelhecimento, com alguns parâmetros diminuídos, inalterados ou mesmo aumentados (146). O envelhecimento é caracterizado por um estado pró-inflamatório crônico com ativação imune inata persistente de baixo grau que pode

aumentar o dano tecidual causado por infecções em idosos. Em adição, com a idade as células do sistema imune inato e adaptativo exibem redução de performance (129).

O envelhecimento também está associado a uma alta prevalência de comorbidades, que são fatores de risco para COVID-19. Além disso, idosos apresentam diminuição da capacidade de reserva fisiológica dos órgãos vitais, o que pode levar ao aumento da fragilidade e, juntamente com um sistema imunológico envelhecido, pode colocar os idosos em risco de pior prognóstico e maior risco de mortalidade quando infectados pelo SARS-CoV-2 (129). É importante destacar que alguns idosos, pessoas com doenças subjacentes ou os dois, continuam desenvolvendo doença grave e óbito pela COVID-19, com o passar do tempo após a vacinação, mostrando a necessidade de monitoramento periódico dessas populações e implementação de doses de reforço da vacina (147,148).

A gravidade da infecção e a mortalidade relacionadas a COVID-19 também variam entre os diferentes grupos étnico-raciais. Segundo o Centers for Disease Control and Prevention (CDC), minorias étnico-raciais nos EUA como pretos, hispânicos e asiáticos tiveram uma porcentagem maior de hospitalizações relacionadas a COVID-19 do que pacientes brancos (149). Dados semelhantes foram encontrados no Brasil, em que a mortalidade na população preta/parda foi notavelmente maior em comparação com a população branca (150). Essa alta porcentagem de hospitalizações e óbitos pela COVID-19 entre esses grupos foi impulsionada pela maior exposição ao SARS-CoV-2, relacionada a desigualdade socioeconômica e maior incidência de doenças associadas (151).

Além dos indivíduos com risco para agravamento e óbito devido às condições clínicas e demográficas, existem grupos com elevado grau de vulnerabilidade social e, portanto suscetíveis a um maior impacto ocasionado pela COVID-19 (152). A identificação das populações vulneráveis é de vital importância para que as intervenções possam reduzir a propagação da doença, controlando a disseminação do vírus, afim de proteger a vida, diminuir o risco de casos graves de COVID-19 e surgimento de novas variantes (153). Entre os grupos vulneráveis temos indígenas, ribeirinhos, quilombolas, pessoas em situação de rua, privados de liberdade, refugiados, pessoas com deficiência permanente, entre outros (88).

Os trabalhadores de saúde constituem outro grupo que precisa ser destacado. Apesar de relatórios não demonstrarem maiores taxas de hospitalização e óbitos nesse grupo em relação a população geral, eles estão na linha de frente do combate

a COVID-19, correndo maior risco de adquirir a doença e, posteriormente, contaminar pacientes e outros que se encontram em maior perigo de desfechos graves (154,155).

Pesquisadores do Reino Unido realizaram uma revisão sistemática e meta-análise e observaram que, mulheres gestantes e puérperas são mais propensas a serem internadas em UTI ou necessitarem de ventilação invasiva quando comparada as mulheres não grávidas em idade reprodutiva. Comorbidades pré-existentes, etnia não branca, hipertensão crônica, diabetes pré-existente, idade materna elevada e índice de massa corporal elevado são fatores de risco para COVID-19 grave na gravidez. Gestantes com COVID-19 versus sem COVID-19 têm maior probabilidade de parto prematuro e podem ter um risco aumentado de óbito materno e de serem internadas na UTI. Outro dado importante é que os bebês nascidos de gestantes infectadas pelo SARS-CoV-2 são mais propensos a serem admitidos na unidade neonatal (156).

2.6 Variantes do SARS-COV-2

Assim como outros vírus, o SARS-CoV-2 é propenso a constantes mudanças genéticas. Essas mudanças resultam em múltiplas variantes que podem ter características diferentes em comparação as suas cepas ancestrais (21). A maioria das mudanças tem pouco ou nenhum impacto nas propriedades do vírus, entretanto, algumas delas podem modificar a forma com que ele se dissemina, a gravidade da doença associada e o desempenho de vacinas, medicamentos, ferramentas de diagnóstico ou outras medidas sociais e de saúde pública. Devido ao risco aumentado à saúde global provocada pelas variantes e a necessidade de um monitoramento contínuo, o CDC e a OMS estabeleceram um sistema de classificação para distinguir as variantes emergentes do SARS-CoV-2 em: variantes de interesse (em inglês VOI – Variants of interest), variantes de preocupação (VOC - Variants of concern) e variantes sob monitoramento (VUM - Variants under monitoring) (157).

Para uma variante ser classificada como VOC ela deve estar associada a uma ou mais das seguintes alterações em um grau de significância para a saúde pública global: aumento da transmissibilidade ou alteração prejudicial na epidemiologia da COVID-19; aumento da virulência ou mudança na apresentação clínica da doença; ou diminuição da eficácia das medidas sociais e de saúde pública ou diagnósticos, vacinas e terapias disponíveis. Já para uma variante ser considerada como uma VOI,

seu genoma deve conter mutações que mudem o fenótipo do vírus e: ter sido identificada como causadora de transmissão comunitária, de múltiplos casos ou de clusters (agrupamentos de casos) de COVID-19 ou ter sido detectada em vários países; ou ser de outra forma avaliada como uma VOI pela OMS em consulta com o Grupo de Trabalho de Evolução do Vírus SARS-CoV-2 (158).

Uma variante do SARS-CoV-2 com alterações genéticas suspeitas de afetar as características do vírus com alguma indicação de que pode representar um risco futuro, mas a evidência de impacto fenotípico ou epidemiológico não é claro, exigindo monitoramento aprimorado e avaliação repetida até novas evidências, é classificada como variantes sob monitoramento (VUM). Até o momento, cinco linhagens foram consideradas VOC: Alfa (B.1.1.7), Beta (B.1.351), Gama (P.1), Delta (B.1.617.2) e Ômicron (B.1.1.529); oito VOIs: Epsilon (B.1.427 e B.1.429); Zeta (P.2); Eta (B.1.525); Teta (P.3); Iota (B.1.526); Capa (B.1.617.1); Lambda (C.37) e Mu (B.1.621); e duas estão em monitoramento: B.1.640 e XD (157).

2.6.1 Variantes de preocupação (VOCs)

Alfa

A variante Alfa, também conhecida como linhagem B.1.1.7 ou GRY (anteriormente GR/501Y.V1), foi descrita pela primeira vez no Reino Unido, após relato do aumento na incidência de infecções por SARS-CoV-2 no leste e sudeste da Inglaterra e na área metropolitana de Londres, associado a uma nova variante. A primeira amostra sequenciada identificada data de setembro de 2020 (159). A variante Alfa é caracterizada por ser mais transmissível e patogênica do que linhagens preexistentes, causando maior número de hospitalizações e óbitos comparada com cepas circulantes anteriormente (160). Em 18 de dezembro de 2020 ela foi classificada como uma VOC (202012/01) (157). No Brasil, a variante Alfa foi descrita pela primeira vez em dezembro de 2020(161) e circulou até meados de agosto de 2021, porém com menor impacto do que outras VOCs (16). Até o momento essa variante já foi confirmada em 181 países (162).

A linhagem B.1.1.7 apresenta 17 mutações no genoma viral. Destas, oito estão na proteína S (21). Uma delas é a mutação N501Y caracterizada pela substituição do aminoácido asparagina pela tirosina na posição 501, no domínio de ligação ao

receptor (RDB) da proteína S (163). Essa mutação aumenta a afinidade do vírus pelo receptor ECA-2 (164), o que pode justificar sua rápida disseminação e maior resistência a neutralização por anticorpos (165–167). Além da mutação N501Y, essa variante carrega deleções que resultaram na perda de aminoácidos no domínio N-terminal (NTD) da proteína S. Essas deleções também podem contribuir para o escape da resposta imune, considerando que o NTD constitui um sítio de ligação de anticorpos neutralizantes. A perda desses aminoácidos pode influenciar também na sensibilidade de kits diagnósticos que empregam sondas voltadas à detecção desta sequência, gerando resultado falso negativo (168,169).

Beta

A variante Beta, também conhecida como B.1.351 ou 20H/501Y.V2, foi identificada na África do Sul em outubro de 2020 (170), e em poucas semanas se tornou dominante na região. Em 18 de dezembro do mesmo ano foi classificada pela OMS como uma VOC (157). Desde então, esta variante já foi detectada em 121 países (171). No Brasil, ela foi identificada em abril de 2021 (172), sem impacto importante na epidemiologia da doença. Apesar de ter se espalhado rapidamente nas províncias de Eastern Cape e Western Cape e na Europa Ocidental, não existem, até o momento, evidências robustas que comprovem maior grau de virulência ou maior gravidade da doença relacionada à variante Beta (173).

Comparada com a cepa de referência (Wuhan), a variante B.1.351 possui 12 mutações não sinônimas e uma deleção. Dentre essas, nove mutações e a delação estão localizadas na proteína spike (L18F, D80A, D215G, LAL 242–244 del, R246I, K417N, E484K, N501Y, D614G e A701V), enquanto as demais estão localizadas em ORF1a (K1655N), envelope (P71L) e proteína N (T205I) (174). Assim como a variante Alfa, a variante Beta possui a mutação N501Y no RBD da proteína spike. No entanto, além da mutação N501Y, essa variante acumula duas mutações adicionais no mesmo domínio RBD (K417N e E484K) que podem desempenhar um papel fundamental tanto na interação com o receptor quanto na evasão imune (175). Esses dados apontam para uma possível diminuição na eficácia dos tratamentos baseados em anticorpos monoclonais ou policlonais, bem como um aumento na taxa de reinfecção em regiões onde essa variante se espalha predominantemente (173).

Gama

A terceira variante de preocupação do SARS-CoV-2 é a Gama, também conhecida como P.1 ou B.1.1.28.1. Ela foi detectada pelo Instituto Nacional de Doenças Infecciosas do Japão, em 6 de janeiro de 2021, isolada de quatro viajantes que chegaram a Tóquio vindos do Amazonas, Brasil, em 2 de janeiro de 2021 (159). Através do relógio molecular foi demonstrado que a linhagem P.1 surgiu em torno de 15 de novembro de 2020, apenas 3 a 4 semanas antes do novo aumento de casos confirmados de SARS-CoV-2 em Manaus (176). Em dezembro essa variante já estava presente em 42% das amostras de Manaus, chegando a 91% em janeiro de 2021 e rapidamente se espalhando por outras regiões. No dia 11 de janeiro de 2021 a OMS a designou como VOC (157) e, desde então, 79 países identificaram essa variante em seus territórios (177).

A variante Gama é responsável pelo maior número de óbitos pela COVID-19 durante a pandemia no Brasil (178). Por meio de um modelo dinâmico de duas categorias que integra dados genômicos e de mortalidade, pesquisadores estimaram que essa variante pode ser 1,7 a 2,4 vezes mais transmissível do que o vírus anterior (não-P.1) (176). A P.1 apresenta valores mais baixos de *Cycle Threshold* (Ct), ou seja, apresenta alta carga viral. Cargas virais altas persistentes após 1 semana do início dos sintomas foram associadas a piores desfechos pela COVID-19 e pode estar relacionada a alta letalidade observada durante a onda da variante Gama (13,179).

Análises filogenéticas indicaram que a P.1 é descendente da linhagem B.1.1.28 que foi detectada pela primeira vez no Brasil no início de março de 2020 (180). A linhagem P.1 apresenta uma constelação de mutações, aproximadamente 35 substituições de aminoácidos, sendo 10 delas de aminoácidos que definem a linhagem na proteína spike do vírus (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y e T1027I) em comparação com seu ancestral imediato (B.1.1.28) (176). Algumas das mutações na P.1 são comuns a outras variantes de preocupação, em linhagens geograficamente distintas, indicando um processo de adaptação molecular convergente. Dentre as mutações na proteína spike, três mutações chave estão localizadas no RBD: K417T, N501Y e E484K (180).

A mutação N501Y é compartilhada com as variantes Alfa e Beta, e como descrito anteriormente, aumenta a afinidade do vírus pela ECA2, elevando a taxa de transmissão e potencialmente resultando em cargas virais mais altas (181). A mutação

E484K também aumenta a afinidade da proteína S para ECA2, em particular, alternando a carga na região de *loop* flexível de RBD, o que leva à formação de novos contatos favoráveis. Além disso, a presença de E484K, pode induzir escape da neutralização de anticorpos anti-SARS-CoV-2 e consequente reinfecção de indivíduos convalescentes da COVID-19 (175). A K417T encontra-se no mesmo local da mutação K417N na variante Beta, com a mudança da asparagina (N) para treonina (T). Assim como a K417N, tem sido associada à diminuição da atividade de neutralização de alguns anticorpos monoclonais (mAb) e aumento da afinidade com ECA2 (182,183).

Além das mutações descritas acima, há evidências de diversificação contínua na linhagem P.1, com mutações raras e inéditas localizadas em NTD da proteína spike. A substituição P209H pode ter um efeito na estrutura em NTD que pode afetar a atividade de anticorpos neutralizantes. Vários sítios da variante Gama demonstraram estar sob seleção positiva e negativa. A seleção negativa tem um papel importante para os mecanismos de infecção viral, consequentemente sendo conservados entre os genomas do SARS-CoV-2. Já a seleção positiva está levando a um aumento na variabilidade de aminoácidos em alguns sítios virais, o que resulta em um potencial amplificado para adaptação viral e sucesso evolutivo. A falta de evidências claras de recombinação e a seleção positiva sugerem que a emergência desta linhagem resultou principalmente em fortes forças evolutivas e acúmulo progressivo de um conjunto de assinaturas favoráveis à mutações (183).

Delta

A variante Delta ou B.1.617.2 foi relatada pela primeira vez na Índia em dezembro de 2020 (184). Nova Délhi, a capital nacional da Índia, vivenciou vários surtos de SARS-CoV-2 em 2020 e atingiu soropositividade populacional de mais de 50% em 2021. Com alta soropositividade na população, esperava-se alguma proteção contra futuros surtos pelo SARS-CoV-2 entretanto, em abril de 2021 a cidade ficou sobrecarregada devido ao exponencial aumento no número de casos e óbitos pela COVID-19, causada por uma nova variante, a B.1.617.2 (185). Desde então, a variante Delta se espalhou por 185 países em todo o mundo, ultrapassando rapidamente as variantes existentes para se tornar a variante dominante em muitos

países. Ela chegou a ser designada como uma VOI, mas devido sua alta transmissibilidade, foi classificada como VOC em 11 de maio de 2021 (186).

No Brasil, a variante Delta foi identificada em maio de 2021, substituindo o predomínio da variante Gama em agosto deste mesmo ano, mas causando menor número de óbitos do que sua antecessora (16). Segundo a OMS, a linhagem B.1.617 foi classificada em três sublinhagens, denominadas B.1.617.1, B.1.617.2 e B.1.617.3, com pequenas diferenças e distinta distribuição geográfica (69). Entre as mutações observadas na linhagem B.1.617, 15 estão localizadas na proteína spike: T19R, (V70F*), T95I, G142D, E156-, F157-, R158G, (A222V*), (W258L*), (K417N*), L452R, T478K, D614G, P681R, D950N (49).

Dentre essas mutações, a mutação P681R, no local de clivagem da furina polibásica no limite S1/S2, se mostrou altamente conservada, se tornando uma marca registrada do fenótipo virológico da variante Delta. Estudos tem demonstrado que essa mutação facilita a clivagem da proteína spike e aumenta a fusogenicidade viral, além disso, o vírus portador de P681R apresenta maior patogenicidade em comparação com seu vírus parental (187). Além da mutação P681R, outras não foram observadas em linhagens virais anteriores como a L452R que se acredita aumentar a infectividade e pode ajudar o vírus a evitar a destruição pelas células imunes e T478K ligada ao escape imunológico (188,189).

A variante Delta mostra características distintas que não são encontradas em outras variantes, que podem explicar sua alta transmissibilidade. Quando a proteína Delta S é expressa na superfície da célula em um nível de saturação, essas células se fundem de forma mais eficiente com as células-alvo que produzem níveis mais baixos de ECA2 do que as células de variantes antecessoras. Em adição, os pseudovírus contendo a construção Delta S entram nas células que expressam ECA2 mais rapidamente do que outras variantes. Esses dados sugerem que a proteína Delta S evoluiu para otimizar a etapa de fusão para entrar nas células que expressam baixos níveis do receptor. Essa otimização pode explicar por que a variante Delta é transmitida mesmo com uma breve exposição e infecta rapidamente muitas células hospedeiras, resultando em curto período de incubação e altas cargas virais durante a infecção (187,190–192).

Ômicron

A Ômicron, linhagem B.1.1.529, foi a quinta variante de preocupação designada pela OMS. Foi identificada pela primeira vez em 23 de novembro de 2021, a partir de uma amostra coletada no dia 09 deste mês, por pesquisadores usando o sequenciamento do genoma para investigar um aumento intrigante no número de casos na África do Sul, onde os casos diários passaram de 274 em 11 de novembro para 1.000 quinze dias depois (193). Em 24 de novembro a Ômicron passou a ser uma VUM e apenas dois dias depois foi designada como uma VOC (157). Desde então, foi identificada em 185 países (194). No Brasil, o primeiro caso da variante foi registrado em 12 de dezembro de 2021. A partir desse momento, ela se espalhou rapidamente por todo país e foi responsável pelo recorde no número de casos na pandemia, com mais de 3 mil infectados registrados em um único dia (80).

A variante Ômicron é caracterizada por apresentar um número elevado de mutações em relação a cepa inicial Wuhan-Hu-1 e outras VOCs, incluindo T91 no envelope, P13L, E31del, R32del, S33del, R203K, G204R na proteína do nucleocapsídeo, D3G, Q19E, A63T na matriz, N211del/L212I, Y145del, Y144del, Y143del, G142D, T95I , V70del, H69del, A67V em NTD, Y505H, N501Y, Q498R, G496S, Q493R, E484A, T478K, S477N, G446S, N440K, K417N, S375F, S373P, S371L, G339D em RBD, D796Y no peptídeo de fusão do pico, L981F, N969K, Q954H na repetição heptada 1 da proteína spike, bem como várias outras mutações nas proteínas não estruturais (49). Análises da sequência do genoma da Ômicron sugerem que ela não é derivada de nenhuma das variantes que circulam atualmente e pode ter uma origem diferente (195).

A variante Ômicron compartilha mutações RBD com outras VOCs, como as mutações N501Y e K417N que conferem afinidades a ligação a ECA2 aumentadas e diminuídas, respectivamente (196). No entanto, a Ômicron possui mutações adicionais em RBD, a maioria das quais demonstrou diminuir a ligação a ECA2 (164). Apesar disso, essa variante mostrou afinidade a ECA2 semelhante a Delta, revelando que ela evoluiu para manter sua capacidade de se ligar ao receptor de forma eficiente. As interações envolvendo as novas mutações nos resíduos 493, 496, 498 e 501 mostraram restaurar a eficiência da ligação a ECA2, que seria perdida como resultado de outras mutações, como K417N. Além disso, o número incomumente alto de mutações na proteína spike parece conferir amplo escape de anticorpos em relação às variantes emergidas anteriormente do SARS-CoV-2 (196).

Inicialmente, a subvariante conhecida como BA.1 era a versão circulante mais comum da Ômicron, mas uma subvariante geneticamente distinta conhecida como BA.2 tem se espalhado rapidamente por todo mundo. BA.2 difere de BA.1 em sua sequência genética, incluindo algumas diferenças de aminoácidos na proteína spike e outras proteínas. Dados iniciais sugerem que BA.2 parece mais transmissível do que BA.1, entretanto esta diferença parece ser muito menor do que a diferença de transmissibilidade entre BA.1 e Delta. Além disso, estudos de reinfecção em nível populacional sugerem que a infecção com BA.1 fornece forte proteção contra a reinfecção com BA.2, mas a BA.2 pode causar doença mais grave. Em países nos quais a imunidade vacinal ou infecção natural é alta, não houve diferença na gravidade entre BA.2 e BA.1 (197).

2.6.2 Variantes de interesse (VOIs)

Até o momento, a OMS designou oito variantes de interesse (VOIs) (157). A variante Épsilon (B.1.427 e B.1.429), também chamada de CAL.20C/L452R, surgiu nos EUA por volta de junho de 2020 e foi responsável por um aumento de até 24% na transmissibilidade em relação a cepa de origem. Essa variante abriga mutações específicas, L452R, D614G na B.1.427 e S13I, W152C, L452R, D614G na B.1.429 (198). Devido à sua alta transmissibilidade, o CDC a classificou como uma variante de preocupação nos EUA (49). Já a OMS a classificou como VOI em 06 de julho de 2021 (157).

A variante Zeta ou P.2, foi detectada pela primeira vez no Brasil em abril de 2020. Apresenta mutações na proteína S (L18F; T20N; P26S; F157L; E484K; D614G; S929I; e V1176F) e foi classificada como VOI pela OMS em 06 de julho de 2021 devido à potencial redução na neutralização por tratamentos com anticorpos e soros vacinais (21,157). A variante Eta (B.1.525) foi detectada em vários países em dezembro de 2020 e designada como uma VOI em 20 de setembro de 2021 (157). Ela difere de todas as outras variantes por ter a mutação E484K e uma nova mutação F888L no domínio S2 da proteína spike (199). A variante Iota (B.1.526) foi identificada pela primeira vez nos EUA em novembro de 2020 e foi designada pela OMS como uma VOI em 20 de setembro de 2021 (157). Essa variante apresentou duas mutações notáveis na proteína S: E484K e S477N, ligadas a neutralização de anticorpos e maior afinidade pelo receptor (200).

A variante Teta ou P.3, também chamada de GR/1092K.V1, carrega mutações chave na proteína spike (deleção 141-143 E484K; N501Y; e P681H). Ela foi detectada pela primeira vez nas Filipinas em janeiro de 2021 e foi classificada como uma variante de interesse em 06 de julho de 2021 pela OMS. A variante Kappa (B.1.617.1) foi primeiramente identificada na Índia em outubro de 2020 e designada como VOI em 20 de setembro de 2021. Abriga mutações chave como: T95I, G142D, E154K, L452R, E484Q, D614G, P681R e Q1071H (157,200).

A variante Lambda ou C.37, foi detectada pela primeira vez no Peru em dezembro de 2020 e foi designada como VOI pela OMS em 14 de junho de 2021. Entre suas mutações, encontramos as seguintes na proteína spike: G75V, T76I, Δ246-252, L452Q, F490S, D614G e T859N. A variante Mu (B.1.621) foi identificada na Colômbia em janeiro de 2021 e designada como VOI pela OMS em agosto de 2021. O genoma da Mu tem um número total de 21 mutações, incluindo 9 mutações na proteína spike, entre elas temos: T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H e D950N (157,200).

2.7 Vacinas

Atualmente, 153 vacinas em desenvolvimento estão em fase clínica e 196 em fase pré-clínica. Das vacinas em fase clínica, a maioria utiliza proteínas recombinantes como plataforma de produção (51/153 – 34%); duas doses (89/152 – 58%) e por via intramuscular (118/153 – 77%). Das vacinas em estágio clínico, 39 encontram-se em fase 3, 19 foram autorizadas apenas para uso emergencial e 12 foram completamente aprovadas (8).

Entre as completamente aprovadas, temos vacinas de RNA (Comirnaty ou BNT162b2 produzida pela Pfizer e BioNTech; e Spikevax ou mRNA-1273 da Moderna e NIH); vacinas de vetor viral (Sputnik V ou Gam-Covid-Vac da Gamaleya Research Institute; Vaxzevria/ AZD1222/ ou ChAdOx1 nCoV-19 da AstraZeneca e Universidade de Oxford; Convidecia ou Ad5-nCoV da CanSino Biologics; Ad26.COV2.S da Janssen e Covaxin ou BBV152 da Bharat Biotech); vacinas de subunidade de proteína (NVX-CoV2373 ou Covovax da Novavax e EpiVacCorona ou Aurora-CoV do Vector Institute); vacinas de vírus inativado (BBIBP-CorV da Sinopharm; Inactivated SARS-CoV-2 vaccine (Vero cell) da Sinopharm e Instituto de Produtos Biológicos de Wuhan

e CoronaVac da Sinovac) e uma vacina de partícula semelhante ao vírus (CoVLP ou Covifenz da Medicago e GSK) (201).

Aproximadamente 12 bilhões de doses de vacinas foram administradas ao redor do mundo, somando 58,6% da população global vacinada com pelo menos 1 dose. Cinco países já vacinaram mais de 90% da sua população com o esquema completo, são eles: Emirados Árabes, Gibraltar, Brunei, Cingapura, Chile e Malta. O Brasil ocupa o 46° país no ranking mundial com 75,53% da população vacinada com o esquema completo. Quando considerada as doses de reforço, 22,05% da população global receberam pelo menos uma dose e o Brasil cai para 60° no ranking mundial, com 38,33% de vacinados (202). Em contrataste a esses números, um terço da população mundial ainda não recebeu uma única dose, incluindo 83% da população da África. Existem países com baixíssima cobertura vacinal, 21 não vacinaram nem 10% de sua população e 3 aparecem com 0% de cobertura (Coreia do Norte, Burundi e Eritreia). Dos 21 abaixo de 10%, 16 estão na África (203).

No dia 18 de janeiro de 2021, iniciou-se a Campanha Nacional de Vacinação contra a COVID-19 no Brasil. Desde então, mais de 470 milhões de doses foram distribuídas em todo país. No ranking dos cinco estados brasileiros com maior cobertura vacinal, temos em primeiro lugar o estado de São Paulo com 85% da população completamente imunizada (2 doses ou dose única) e 53% com a dose reforço. Em segundo lugar vem o estado do Piauí com 83,3%, seguido de Ceará (79,5%), Paraná (77,8%) e Rio Grande do Sul (77,5%) (204).

Devido a disponibilidade limitada de doses em um primeiro momento, as campanhas vacinais foram direcionadas a reduzir a morbimortalidade causada pela COVID-19, bem como proteger a força de trabalho para manutenção do funcionamento dos serviços de saúde e dos serviços essenciais. Assim, o cronograma de vacinação no Brasil foi organizado para atender primeiramente os grupos prioritários definidos pelo Ministério da saúde e seguindo as recomendações do SAGE - Grupo Consultivo Estratégico de Especialistas em Imunização (em inglês, Strategic Advisor Group of Experts on Immunization) da OMS, começando pelos idosos e deficientes institucionalizados, indígenas, trabalhadores de saúde, idosos, ribeirinhos e quilombolas, pessoas com comorbidades, pessoas em situação de rua, privados de liberdade e demais grupos (88).

Inicialmente duas vacinas foram aprovadas para uso emergencial no Brasil, a Coronavac - Sinovac/Butantan e a ChAdOx1 - Oxford AstraZeneca/Fiocruz. Desde

então, mais dois imunizantes foram autorizados e utilizados nas campanhas nacionais, a Comirnaty ou BNT162b2 produzida pela Pfizer e BioNTech (23 de fevereiro de 2021) e Ad26.COV2.S da Janssen (31 de março de 2021). No dia 12 de março de 2021 a Anvisa concedeu registro definitivo a vacina ChAdOx1 e em 20 de janeiro de 2022 aprovou a ampliação para o uso emergencial da vacina Coronavac para crianças e adolescentes com idade entre 6 e 17 anos, exceto imunocomprometidas (88).

2.7.1 Coronavac - Sinovac / Instituto Butantan

A Coronavac é uma vacina produzida com antígeno do vírus inativado SARS-CoV-2. É apresentada em suspensão injetável no frasco-ampola com 10 doses, de aplicação intramuscular (IM). O esquema vacinal é composto por duas doses de 0,5 mL, com intervalo de 4 semanas entre elas. Cada dose contém 600SU de antígeno do vírus inativado e excipiente de hidróxido de alumínio, hidrogenofosfato dissódico, di-hidrogenofosfato de sódio, cloreto de sódio, água para injetáveis e hidróxido de sódio para ajuste de pH. É armazenada em temperatura de 2°C a 8°C e depois de aberto pode ser utilizado em até 8 horas (88).

Essa vacina foi desenvolvida pela empresa privada chinesa Sinovac, surgiu como uma das principais vacinas da China. No início de 2020, a empresa observou em experimentos com macacos, que a vacina reduziu significativamente a concentração de coronavírus nos animais infectados com o vírus e que os animais vacinados se recuperaram mais rápido do que os macacos não vacinados. Após a descoberta, a vacina foi submetida aos testes de fase 1/2 e subsequentemente ao de fase 3 no Brasil e Turquia (205).

Os testes de fase 3 mostraram que a vacina protegia contra a COVID-19, entretanto, os resultados de eficácia foram consideravelmente diferentes entre os dois países. Na Turquia, a eficácia contra COVID-19 sintomática confirmada por RT-PCR foi de 83,5%, com um bom perfil de segurança e tolerabilidade (206). Já no Brasil, a eficácia contra COVID-19 sintomática foi de 50,7%, 83,7% contra casos que requerem assistência e 100% para casos moderados e graves (9). Após a Coronavac ser aprovada em outros países, alguns deles realizaram estudos para medir sua eficácia. Um exemplo foi o Chile, em que pesquisadores analisaram uma coorte com aproximadamente 10,2 milhões de pessoas e encontraram uma eficácia de 65,9%

para a prevenção da COVID-19, 87,5% para hospitalização, 90,3% para prevenção de internação em UTI e 86,3% para prevenção de óbito relacionado à Covid-19 (207).

Em 20 de janeiro de 2022 a Coronavac recebeu aprovação para ser aplicada em crianças e adolescentes no Brasil (88), após a fase 1/2 de testes e ter se mostrado segura e eficaz (208). Outro estudo realizado no Chile observou pessoas na faixa etária de 6 a 16 anos e encontrou eficácia de 74,5% para a prevenção da infecção e mais de 90% para hospitalização e admissão em UTI (209). A Coronavac também mostrou ser eficaz na prevenção da COVID-19 sintomática e grave em gestantes (210). Apesar da eficácia comprovada, principalmente após o esquema completo (18,211), diversos estudos tem demonstrado que ela diminui significantemente com o tempo (212–214). Essa é uma preocupação especialmente com os grupos em maior risco como os idosos (212), o que levou a OMS recomendar que pessoas que receberam a vacina da Sinovac recebam uma dose de reforço com a mesma (esquema homólogo) ou outra vacina (esquema heterólogo) (215). O esquema heterólogo resultou em respostas imunes robustas e pode aumentar a proteção (216).

Considerando as variantes emergentes há uma preocupação importante com a persistência da eficácia das vacinas frente as diversas mutações desenvolvidas pelo vírus. A Coronavac é uma vacina de vírus inativado e espera-se que essa plataforma tenha uma vantagem sobre outras plataformas de vacinas, uma vez que elas tendem a produzir respostas imunes amplas, pois como todo o vírus é apresentado ao sistema imunológico as respostas imunes não atingem apenas a proteína S, mas também a M e N. De fato, a Coronavac apresentou capacidade de neutralização, mesmo que reduzida, contra as variantes Alfa, Épsilon e Gama (214) após o esquema de duas doses. Já contra as variantes Beta, Delta e Ômicron ainda n ao se tem muitas evidências (217,218). Quando considerada a dose de reforço, seja homóloga ou heteróloga, a Coronavac proporcionou proteção sustentada contra doença grave causada pela variante Ômicron (219).

A Sinovac anunciou um acordo em 12 de julho de 2021 para fornecer até 550 milhões de doses à COVAX, iniciativa de distribuição de vacinas para países de baixa e média renda e hoje é a vacina mais usada em todo mundo. Atualmente, essa vacina está sendo distribuída em 54 países com mais de 2 bilhões de doses. O Brasil é o segundo maior consumidor da vacina chinesa, com mais de 117 milhões de doses distribuídas, entre elas mais de 28 milhões no estado de São Paulo (215).

2.7.2 ChAdOx1 - AstraZeneca/ Universidade de Oxford

A ChAdOx1 é apresentada em suspensão injetável no frasco-ampola com 10 doses, de aplicação intramuscular (IM). O esquema vacinal é composto por duas doses de 0,5 mL, com intervalo de 4 a 8 semanas entre elas. Cada dose contém 5×10^{10} partículas virais (pv) do vetor adenovírus recombinante de chimpanzé, deficiente para replicação (ChAdOx1), que expressa a glicoproteína SARS-CoV-2 Spike (S). É armazenada em temperatura de 2°C a 8°C e depois de aberto pode ser utilizado em até 6 horas (88). A ChAdOx1 foi implementada em mais de 180 países, com mais de 2,5 bilhões de doses liberadas para fornecimento em todo o mundo, destas, mais de 128 milhões foram distribuídas no Brasil e 28 milhões no estado de São Paulo (204,220).

Uma vacina projetada pela Universidade de Oxford e produzida pela empresa anglo-sueca AstraZeneca, a ChAdOx1 foi baseada em uma plataforma de vacinas que pesquisadores de Oxford vinham desenvolvendo há anos para outras doenças. Eles começaram com um adenovírus que normalmente infecta chimpanzés e o modificaram geneticamente para transportar genes virais. No início de 2020, os cientistas desenvolveram uma vacina baseada no vetor ChAdOx1 que codifica a proteína spike. Em macacos rhesus, apemas uma dose de ChAdOx1 induziu respostas imunes humorais e celulares e proteção contra a infecção do trato respiratório inferior após o teste com altas doses de SARS-CoV-2 (221,222).

Em dezembro de 2020 a AstraZeneca e Oxford publicaram o primeiro artigo científico com os resultados preliminares da Fase 3. A vacina foi avaliada em quatro ensaios em três continentes, mostrando eficácia de 70,4% após duas doses e proteção de 64,1% após pelo menos uma dose padrão, contra doença sintomática. Apesar de ser eficaz, muitas questões ficaram abertas, como a proteção em pessoas idosas e o grande número de eventos adversos durante o estudo (10). Entretanto, devido a urgência de disponibilidade de imunizantes, o baixo custo e a facilidade de armazenamento, a vacina foi adquirida por diversos países e em março de 2021 anexada ao consórcio COVAX (223).

Em março de 2021, O comitê de segurança da Agência Europeia de Medicamentos – EMA, alertou o mundo sobre a possível ligação da vacina ChAdOx1 a casos muito raros de coágulos sanguíneos incomuns com plaquetas baixas (224).

Estudos concluíram que a vacinação com ChAdOx1 pode resultar no raro desenvolvimento de trombocitopenia trombótica imune mediada por anticorpos ativadores de plaquetas contra PF4, que mimetiza clinicamente a trombocitopenia autoimune induzida por heparina (225,226). Mesmo após a descoberta, pesquisadores e agências reguladoras endossaram o uso da vacina e enfatizaram que o evento é extremamente raro e que os benefícios do imunizante superam os riscos (224). Apesar disso, vários países restringiram o uso da vacina da AstraZeneca a certas faixas etárias, e a Dinamarca optou por não a usar completamente (227).

A ChAdOx1 demonstrou proteção para internação, admissão em UTI ou óbito relacionado a COVID-19 de cerca de 90%, entretanto, assim como a Coronavac e outras vacinas, a eficácia diminuiu com o passar do tempo após a segunda dose, principalmente nos idosos (228,229). Estudos tem demonstrado aumento na imunogenicidade com reforço homólogo ou heterólogo da terceira dose após duas doses de ChAdOx1, com maior eficácia para os esquemas heterólogos, porém, o risco de reatogenicidade na aplicação de imunizantes diferentes é maior e deve ser tratado com cautela. Pesquisadores demonstraram que a aplicação da vacina Ad26.COV2.S da Janssen após ChAdOx1/ChAdOx1 causou aumento de eventos adversos sistêmicos e locais, mas que a vacina da Pfizer aplicada após duas doses da AstraZeneca aumentou significantemente a proteção (230,231).

Além da ChAdOx1 ser usada como imunizante primário, ela pode ser utilizada como terceira dose ou dose de reforço. Pesquisadores relataram o aumento da proteção em esquemas vacinais utilizando a vacina da AstraZeneca como reforço, mas também um possível aumento dos efeitos colaterais em alguns casos, como exemplo Pfizer/Pfizer seguido de AstraZeneca (231,232). Ademais, a utilização de dose fracionada (meia-dose) como reforço após 2 doses de Coronavac mostrou imunogenicidade não inferior, mas menor reatogenicidade sistêmica, sendo uma alternativa a ser considerada, especialmente em ambientes com recursos limitados (233).

Frente as variantes emergentes, a ChAdOx1 mostrou neutralização geral reduzida, mas retenção de eficácia contra as variantes Alfa (234), Gama (17) e Delta (235) do SARS-CoV-2. Um estudo demonstrou atividade mínima de neutralização contra a variante Ômicron após duas doses (236). No entanto, a vacina falhou em proteger contra infecção leve a moderada pela COVID-19 devido à variante Beta (234). Pensando nas mutações e eficácia contra as variantes, a AstraZeneca produziu

uma vacina de segunda geração, a AZD2816, com a mesma tecnologia usada na AZD1222, mas com pequenas alterações genéticas para a proteína spike com base na variante Beta. Foi observado aumento na imunogenicidade frente as variantes Beta, Gama e Delta em animais, entretanto, novos estudos são necessários para verificar a capacidade neutralizante no mundo real e a outras variantes, como a Ômicron (237).

3 OBJETIVOS

3.1 Geral

- Descrever os fatores de risco para óbito pela COVID-19 e estimar a efetividade das vacinas CoronaVac e ChAdOx1 frente à variante Gama no Brasil

3.2 Específicos

- Descrever os fatores de risco para óbito nos pacientes hospitalizados com COVID-19 no estado de São Paulo, de 26 de fevereiro de 2020 a 10 de outubro de 2020;
- Estimar a efetividade da vacina CoronaVac (Sinovac/Biotech) na população idosa no estado de São Paulo, de 17 de janeiro de 2021 a 29 de abril de 2021;
- Estimar a efetividade da vacina ChAdOx1 (Oxford/AstraZeneca), na população idosa no estado de São Paulo, de 17 de janeiro de 2021 a 09 de julho de 2021.

4 METODOLOGIA

Para atingir os objetivos propostos, metodologias distintas foram utilizadas e estão detalhadas nos artigos publicados apresentados na seção resultados e discussão e Anexo A.

5 RESULTADOS E DISCUSSÃO

Os resultados e discussão são apresentados na forma dos artigos:

Risk Factors for Death Among 120,804 Hospitalized Patients with Confirmed COVID-19 in São Paulo, Brazil. Publicado pela *The American Journal of Tropical Medicine and Hygiene*, fator de impacto 2.453, Qualis Capes A3.

Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. Publicado na *British Medical Journal*, fator de impacto 39.890, Qualis Capes A1.

Effectiveness of ChAdOx1 vaccine in older adults during SARS-CoV-2 Gamma variant circulation in São Paulo. Publicado na *Nature Communications*, fator de impacto 14.919, Qualis Capes A1.

Am. J. Trop. Med. Hyg., 105(1), 2021, pp. 88–92
doi:10.4269/ajtmh.20-1598

Risk Factors for Death Among 120,804 Hospitalized Patients with Confirmed COVID-19 in São Paulo, Brazil

Patricia Vieira da Silva,[1]† Silvano Barbosa de Oliveira,[2,3,4]† Juan José Cortez Escalante,[2,3] Maria Almiron,[2,3] Daniel Henrique Tsuha,[1] Helena Keico Sato,[5] Paulo Rossi Menezes,[6] Regiane Cardoso de Paula,[6] Tatiana Lang D'Agostini,[6] and Julio Croda[1,7]*†

[1]*Federal University of Mato Grosso do Sul, Campo Grande, Brazil;* [2]*Pan American Health Organization, Brasília, Brazil;* [3]*World Health Organization, Brasília, Brazil;* [4]*University of Brasilia, Brasília, Brazil;* [5]*Epidemiological Surveillance Center "Prof. Alexandre Vranjac" of the São Paulo State Department of Health, São Paulo, Brazil;* [6]*Disease Control Coordination of the São Paulo State Department of Health, São Paulo, Brazil;* [7]*Oswaldo Cruz Foundation, Campo Grande, Brazil*

Abstract. São Paulo is a state in Brazil with one of the highest numbers of confirmed and severe cases of coronavirus disease (COVID-19), with an incidence of 294 hospitalizations per 100,000 inhabitants. We report the clinical characteristics and outcomes of 120,804 hospitalized patients with confirmed COVID-19 from February 26 to October 10, 2020, in São Paulo. Characteristics of patients who died and survived were compared using a survival analysis. The median age was 60 years (interquartile range [IQR], 47–72), 67,821 (56.1%) were men, and 61,659 (51.0%) were white. Most hospitalized patients (79,812; 66.1%) reported one or more comorbidities, 41,708 (34.5%) hospitalized patients were admitted to intensive care units, and 33,079 (27.4%) died. Men (hazard ratio [HR], 1.22; 95% confidence interval [CI], 1.18–1.25), elderly individuals (HR, 3.85; 95% CI, 3.68–4.02), and patients with chronic cardiovascular disease including hypertension (HR, 1.05; 95% CI, 1.02–1.08), chronic lung disease (HR, 1.38; 95% CI, 1.31–1.45), diabetes mellitus (HR, 1.14; 95% CI, 1.11–1.18), and chronic neurological disease (HR, 1.48; 95% CI, 1.41–1.55) were at higher risk for death from COVID-19.

BACKGROUND

In December 2019, an outbreak of pneumonia of unknown origin was reported in Wuhan city, Hubei province, China. The etiological agent was subsequently identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the illness was identified as novel coronavirus disease (COVID-19) by the World Health Organization.[1] In Brazil, the first COVID-19 case was reported in Sao Paulo City in a 61-year-old man whose onset of symptoms began on February 23, 2020.[2] Since the writing of this report, more than 7 million confirmed cases and 180,000 deaths have been reported in Brazil, with the highest number of hospitalized patients and deaths occurring in the state of São Paulo.[3]

Because COVID-19 is an emerging disease, there is limited knowledge about its clinical characteristics and outcomes among hospitalized patients. Studies have shown that among individuals with confirmed cases of COVID-19, older people and those with underlying clinical conditions are at higher risk for hospitalization and death.[4] A systematic review conducted in China found that of 2,087 hospitalized patients with cases considered critical, 49% (1,023) died. Of these 1,023 patients, 81% (829 of 1,023) were 60 years or older. When considering the existence of underlying clinical conditions, mortality among patients without comorbidities was much lower (0.9%; 133 of 15,536) than that of patients with one or more comorbidities.[5] A similar pattern was observed in the United States[4] and Italy.[6]

Because Latin-America has a different demographic population than China and many countries in Europe, a notable concern is whether different age groups and severity might be observed as the virus spreads among the Brazilian population. During this study, we describe the risk factors for death among 120,804 hospitalized patients with confirmed COVID-19 cases in São Paulo, Brazil, from February 26 to October 10, 2020.

MATERIALS AND METHODS

This case series included all hospitalized patients with confirmed COVID-19 in the state of São Paulo registered in the Influenza Epidemiological Surveillance System (SIVEP-GRIPE) from February 26, 2020 to October 10, 2020.[7] The SIVEP-GRIPE is a system used by health professionals and institutions from the public or private sector throughout the national territory to report hospitalized patients with severe acute respiratory illness (SARI) and deaths attributable to SARI regardless of hospitalization. For this analysis, epidemiological, clinical, and outcome data of the hospitalized patients with COVID-19 (confirmed by real-time RT-PCR)[8] from SIVEP-GRIPE were used.

The criteria for hospitalization and case management according to the guidelines of the World Health Organization (WHO)[8] and Brazilian Ministry of Health[9] were followed during the observation period. According to these guidelines, cases of COVID-19 that progressed to SARI were observed in patients who presented with clinical criteria for flu and at least one of the following symptoms: shortness of breath (dyspnea/respiratory distress); sensation of persistent pressure in the chest; O_2 saturation less than 95% in ambient air; or bluish coloring of the lips or face.

Critical cases were defined as hospitalization with SARI, the need to be admitted to the intensive care unit (ICU) because of the presence of organic disorders or hemodynamic instability, and requiring mechanical ventilation or other intensive care procedures. To qualify for admission to the ICU, it was necessary to present at least one of the following criteria: acute respiratory failure requiring invasive mechanical ventilation; acute respiratory failure with the need for noninvasive ventilation (especially when

* Address correspondence to Julio Croda, Universidade Federal de Mato Grosso do Sul, Faculdade de Medicina – Prédio antigo, Av., Costa e Silva s/n, Universitário II, 79070-900, Campo Grande-MS, Brazil. E-mail: juliocroda@gmail.com
† These authors contributed equally to this work.

there is a need for $FiO_2 > 50\%$, or inspiratory positive airway pressure > 10 cm H_2O or expiratory positive airway pressure > 10 cm H_2O to maintain $SpO_2 > 94\%$ and/or respiratory frequency ≤ 24 rpm); $PaCO_2 \geq 50$ mm Hg, and pH ≤ 7.35; or hemodynamic instability or shock defined as arterial hypotension (systolic blood pressure < 90 mmHg or mean arterial pressure < 65 mmHg). The care of these patients with SARI followed the protocols nationally standardized by the Ministry of Health.

The χ^2 test was used to compare the differences among fatal cases. The Kaplan-Meier survival analysis was performed to analyze the probability of death during the hospitalization of severely ill patients with COVID-19. For the occurrence of death, the observation period was considered from the date of symptom onset until the occurrence of the fatal outcome. When the outcome of death did not occur, October 10 was selected as the final date of observation. Furthermore, the four more prevalent comorbidities among the patients included in the study were analyzed (chronic cardiovascular disease including hypertension, chronic lung disease, diabetes mellitus, and chronic neurological disease). The outcome was adjusted for age, sex, and all comorbidities, as well as their interactions using Cox regression. The survival analysis was performed using three age categories; younger 60 years, 60 to 79 years, and older than 80 years. Statistical analyses were conducted using R software with Survival package.

Although the research did not require approval, the researchers express their ethical commitment to the management, analysis, and publication of data in accordance with Resolution 466/12 and 510/16 of the National Health Council.

RESULTS

In São Paulo state, from February 26 to October 10, 2020, a total of 120,804 hospitalized patients with laboratory-confirmed COVID-19 were reported in 623 of 645 municipalities, comprising an incidence of 294 hospitalizations per 100,000 inhabitants. The characteristics of patients with COVID-19 during hospitalization and outcomes are summarized in Table 1. The median age of hospitalized patients was 60 years (interquartile range [IQR], 47–72 years), 67,821 (56.1%) were men, and 61,659 (51.0%) were white. Most hospitalized patients (79,812; 66.1%) reported one or more comorbidities; among them, 27,000 (33.8%) died. More than 41,000 hospitalized patients (34.5%) were admitted to ICUs and 33,079 (27.4%) died.

Associations among sex, age group, four other prevalent comorbidities (chronic cardiovascular disease plus hypertension, chronic lung disease, diabetes mellitus, and chronic neurological disease) and the risk of COVID-19-related death are shown in Table 2. The corresponding Kaplan-Meier graph is presented in Figure 1. Men were at higher risk for death than women (HR, 1.22; 95% CI, 1.18–1.25), and the number of deaths was slightly higher for men than for women (28.1% versus 26.4%).

The rates of death among races (white, black, yellow, mixed, and indigenous) were similar, ranging from 21.8% to 33.5%. When the groups were separated into white and non-white (blacks and other minority ethnicities), the death rates were similar (white: 29.0% [17.902 of 61.659]; non-white: 29.0% [9.102 of 31.355]).

Increasing age was strongly associated with the risk of death, with patients 80 years or older having a more than three-fold increased risk compared with patients younger than 60 years (hazard ratio [HR], 3.85; 95% confidence interval [CI]], 3.68–4.02). The death rates among the younger than 30 years (6.1%; 358 of 5,879) and 30 to 59 years (13.6%; 7,278 of 53,368) age groups were considerably lower than that of the 60 years or older (41.3%; 25,415 of 61,475) age group, again highlighting the possible relationship of old age and death (Table 1).

The four most prevalent comorbidities, chronic cardiovascular disease plus hypertension (HR, 1.05; 95% CI, 1.02–1.08), chronic lung disease (HR, 1.38; 95% CI, 1.31–1.45), diabetes mellitus (HR, 1.14; 95% CI, 1.11–1.18), and chronic neurological disease (HR, 1.48; 95% CI, 1.41–1.55), were associated with an increased risk of death. As can be observed in Table 1, almost half of the patients who had chronic lung disease and chronic neurological disease died. Approximately half of the patients who needed care in the ICU died (48.5%; 20,220).

DISCUSSION

The state of São Paulo is the most populous state in Brazil, with approximately 46 million inhabitants, and it is one of the main protagonists of the COVID-19 pandemic in Brazil. This study showed that a large number of laboratories confirmed that COVID-19 cases led to hospitalization in the state (120,804; incidence of 294 hospitalizations per 100,000 inhabitants), and that approximately one-third of cases of those hospitalized evolved into serious illness with the need for ICU care (34.5%) and death (27.4%). These results serve as an alert to the government and the population to recognize the need for urgent measures to control the pandemic.

Our findings revealed that men are at higher risk for death than women. These data are similar to those of recent studies that reported that SARS-CoV-2 infection was more likely to affect males.[4,5,10] Therefore, male sex is a well-established risk factor for serious COVID-19 outcomes.

The results also showed that the majority of hospitalized patients were white, consistent with the first report of cases among people who had traveled internationally[11]; however, the numbers of deaths among races were similar when analyzed individually and equal when compared between whites and non-whites (blacks and other minority ethnicities). Non-white race was previously found to be associated with a higher risk of COVID-19 infection, risk of ICU admission, and death.[12,13] This topic needs to be studied further because it is related to other characteristics, such as cultural and socioeconomic factors. Although there was no difference in the number of deaths among the non-white population in this study, there is an information gap for the race variable because this information only started to be collected more than 1 month after the beginning of the pandemic in Brazil, thus showing the disregard for racial vulnerability in the country during the pandemic.

Increasing risks of death were observed with increasing age, and advanced age is also a well-established risk factor for serious COVID-19 outcomes; these results were widely observed during other studies performed in several countries worldwides.[4,5,14,15] Although the death rates among young people are considerably lower than those among elderly

TABLE 1
Characteristics of patients with COVID-19 during hospitalization and outcomes

Characteristics	Total n (%)	Dead n (%)	Alive n (%)	*P* value
Total	120,804 (100.0)	33,079 (27.4)	87,725 (72.6)	
Sex				
Male	67,821 (100.0)	19,087 (28.1)	48,734 (71.9)	< 0.001
Female	52,974 (100.0)	13,992 (26.4)	38,982 (73.6)	
Missing	9 (100.0)	0 (0.0)	9 (100.0)	
Age group, y				
< 30	5879 (100.0)	358 (6.1)	5,521 (93.9)	< 0.001
30–59	53,368 (100.0)	7,278 (13.6)	46,090 (86.4)	
≥ 60	61,475 (100.0)	25,415 (41.3)	36,060 (58.7)	
Missing	82 (100.0)	28 (34.1)	54 (65.9)	
Race*				
White	61,659 (100.0)	17,902 (29.0)	43,757 (71.0)	< 0.001
Black	6,382 (100.0)	2,013 (31.5)	4,369 (68.5)	
Yellow	1,500 (100.0)	503 (33.5)	997 (66.5)	
Mixed	23,386 (100.0)	6,567 (28.1)	16,819 (71.9)	
Indigenous	87 (100.0)	19 (21.8)	68 (78.2)	
Missing	27,790 (100.0)	6,075 (21.9)	21,715 (78.1)	
Comorbidity (none, 1, or > 1†)				
Yes	79,812 (100.0)	27,000 (33.8)	52,812 (66.2)	< 0.001
No	224 (100.0)	46 (20.5)	178 (79.5)	
Missing	40,768 (100.0)	6,033 (14.8)	34,735 (85.2)	
Chronic cardiovascular disease + hypertension				
Yes	44,041 (100.0)	16,213 (36.8)	27,828 (63.2)	< 0.001
No	22,187 (100.0)	6,832 (30.8)	15,355 (69.2)	
Missing	54,576 (100.0)	10,034 (18.4)	44,542 (81.6)	
Chronic lung disease				
Yes	4,723 (100.0)	2,252 (47.7)	2,471 (52.3)	< 0.001
No	47,025 (100.0)	15,693 (33.4)	31,332 (66.6)	
Missing	69,056 (100.0)	15,134 (21.9)	53,922 (78.1)	
Diabetes mellitus				
Yes	31,903 (100.0)	11,702 (36.7)	20,201 (63.3)	< 0.001
No	29,847 (100.0)	9,806 (32.9)	20,041 (67.1)	
Missing	59,054 (100.0)	11,571 (19.6)	47,483 (80.4)	
Chronic neurological disease				
Yes	5,580 (100.0)	2,954 (52.9)	2,626 (47.1)	< 0.001
No	46,515 (100.0)	15,261 (32.8)	31,254 (67.2)	
Missing	68,709 (100.0)	14,864 (21.6)	53,845 (78.4)	
ICU				
Yes	41,708 (100.0)	20,220 (48.5)	21,488 (51.5)	< 0.001
No	79,096 (100.0)	12,859 (16.3)	66,237 (83.7)	

Severe cases: patients had been hospitalized in the general ward only. Critical cases: patients have been hospitalized in the ICU. Source: REDCap and Influenza Epidemiological Surveillance System (SIVEP-GRIPE) Epidemiological Surveillance Center of the State Health Secretary of São Paulo (CVE/SES-SP).

* Brazilian Institute of Geography and Statistics (IBGE) classification

† Chronic cardiovascular disease plus hypertension, diabetes mellitus, chronic lung disease, chronic liver disease, chronic hematological disease, Down syndrome, chronic neurological disease, immunodeficiency or immunodepression/HIV, chronic kidney disease, obesity, neoplasm (solid or hematological tumor).

individuals, it is very important to discuss the roles of these people in maintaining the disease. In general, young people are more resistant to maintaining social isolation and safety measures to contain the spread of the virus, and they can also be a vital source of transmission for individuals at increased risk for serious outcomes of COVID-19.

Several studies have shown that individuals with chronic diseases are at higher risk for serious outcomes of COVID-19.[15,16] Our findings support this information. Chronic cardiovascular disease plus hypertension, diabetes mellitus, chronic lung disease, and chronic neurological disease had a greater relationship with death, suggesting that these comorbidities may be associated with an increased risk of more serious outcomes among patients hospitalized with COVID-19.

The high death rate for patients admitted to the ICU observed during in this study was expected because of the critical condition of patients who are referred to this treatment unit because they require respiratory support and intensive care. However, other associated factors need to be studied further to determine if this high death rate is related to the severity of the illness also or also to the treatment methods used in the ICU.

There is a need for immediate measures to prevent and control the pandemic, especially for groups at higher risk for serious outcomes, so there will be fewer hospitalizations and deaths. Decreased mobility, social distancing, and the implementation of biosafety measures, such as wearing a mask, are essential for reducing the spread of the virus until vaccination coverage is sufficient to prevent high transmission. Another important factor is the government's attention to the socioeconomically vulnerable population, which is predominantly black. This population has greater difficulty maintaining social isolation and has less access to the health system.

Our investigation of risk factors for deaths in São Paulo state had several limitations. Data extraction from hospitalized SARS-COV-2-positive patients in São Paulo occurred on

TABLE 2

Cox multivariate regression analysis of epidemiological characteristics and comorbidities associated with death for COVID-19 patients

	Univariate analysis		Multivariate analysis	
Variable	HR	95% CI	HR	95% CI
Sex				
Female	1.00		1.00	
Male	1.07	1.04–1.09	1.22	1.18–1.25
Age group, y				
< 60	1.00		1.00	
60–79	3.22	3.13–3.31	2.20	2.11–2.28
≥ 80	6.04	5.86–6.22	3.85	3.68–4.02
CCD + H				
No	1.00		1.00	
Yes	1.23	1.20–1.23	1.05	1.02–1.08
CLD				
No	1.00		1.00	
Yes	1.58	1.51–1.65	1.38	1.31–1.45
DM				
No	1.00		1.00	
Yes	1.14	1.11–1.17	1.14	1.11–1.18
CND				
No	1.00		1.00	
Yes	1.94	1.86–2.02	1.48	1.41–1.55

CCD + H = chronic cardiovascular disease plus hypertension; CLD = chronic lung disease; DM = diabetes mellitus; CND = chronic neurological disease. Source: Influenza Epidemiological Surveillance System (SIVEP-GRIPE) Epidemiological Surveillance Center of the State Health Secretary of São Paulo (CVE/SES-SP).

October 10, 2020. The frequency and distribution of underlying conditions after that period may have changed as additional data became available. Many patients were still hospitalized at the time of data extraction. The notifications in the SIVEP-GRIPE system are detailed and longitudinal, but a considerably large amount of data were missing data, which may have impacted these results and introduced important information bias. Furthermore, some deaths related to COVID-19 may have been mistakenly classified as non-COVID-19, particularly during the beginning of the pandemic. However, it is possible that this inaccuracy has decreased rapidly as the number of deaths has increased.

CONCLUSIONS

In São Paulo state, men, elderly individuals, and patients with chronic cardiovascular disease, including hypertension, chronic lung disease, diabetes mellitus, and chronic neurological disease, are at higher risk for death from COVID-19.

Received December 17, 2020. Accepted for publication April 17, 2021.

Published online May 31, 2021.

Acknowledgment: The American Society of Tropical Medicine and Hygiene has waived the Open Access fee for this article due to the ongoing COVID-19 pandemic.

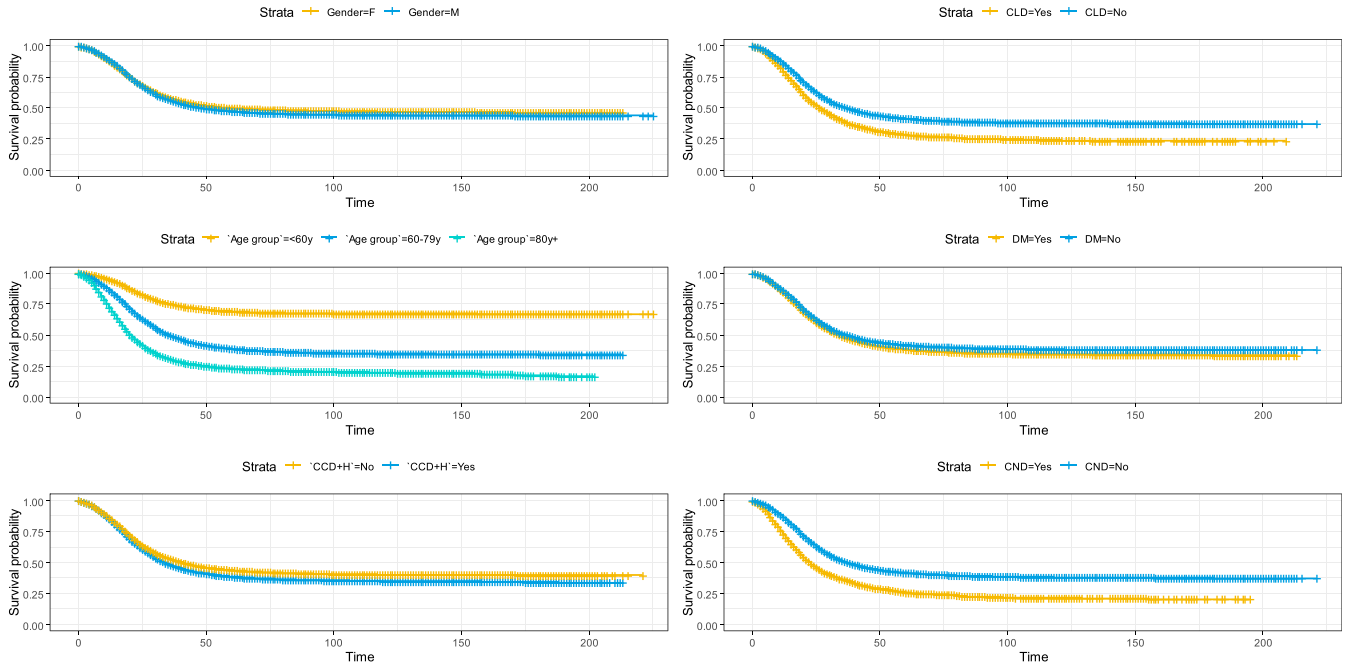


FIGURE 1. Kaplan-Meir plots for risk factors associated with death for coronavirus disease (COVID-19) patients. This figure appears in color at www.ajtmh.org.

Authors' addresses: Patricia Vieira da Silva, Daniel Henrique Tsuha, and Julio Croda, Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil, E-mails: patriciavieira.s@hotmail.com, daniel.tsuha@saude.ms.gov.br, and juliocroda@gmail.com. Silvano Barbosa de Oliveira, Juan José Cortez Escalante, and Maria Almiron, Pan American Health Organization, World Health Organization, University of Brasilia, Brasília, DF, Brazil, E-mails: silbaroli@gmail.com, cortezj@paho.org, and almironm@paho.org. Helena Keico Sato, Epidemiological Surveillance Center "Prof. Alexandre Vranjac" of the São Paulo State Department of Health, São Paulo, SP, Brazil, E-mail: helenakeicosato@gmail.com. Paulo Rossi Menezes, Regiane Cardoso de Paula, and Tatiana Lang D'Agostini, Disease Control Coordination of the São Paulo State Department of Health, São Paulo, SP, Brazil, E-mails: paulo.menezes@saude.sp.gov.br, depaula.regiane7@gmail.com, and tlang@saude.sp.gov.br.

REFERENCES

1. WHO, 2020. *Novel Coronavirus – China.* Geneva, Switzerland: World Health Organization. Available at: http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/. Accessed November 5, 2020.
2. WHO, 2020. *Coronavirus Disease (COVID-19) Situation Reports.* Geneva, Switzerland: World Health Organization. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports. Accessed November 5, 2020.
3. Coronavirus Brasil, 2020. Brazilian Ministry of Health. Available at: https://covid.saude.gov.br/. Accessed November 5, 2020.
4. CDC, 2020. COVID-19 response team. severe outcomes among patients with coronavirus disease 2019 (COVID-19)—United States, February 12–March 16, 2020. *MMWR Morb Mortal Wkly Rep 69:* 343–346.
5. Epidemiology Working Group for NCIP Epidemic Response, Chinese Center for Disease Control and Prevention, 2020. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *Zhonghua Liu Xing Bing Xue Za Zhi Zhonghua Liuxingbingxue Zazhi 41:* 145–151 (in Chinese).
6. EpiCentro, 2020. *Sorveglianza Integrata COVID-19: i Principali dati Nazionali.* Available at: https://www.epicentro.iss.it/coronavirus/sars-cov-2-sorveglianza-dati. Accessed November 5, 2020.
7. Dados Abertos, 2020. *Governo do Estado de São Paulo.* Available at: https://www.saopaulo.sp.gov.br/planosp/simi/dados-abertos/. Accessed November 17, 2020.
8. World Health Organization, 2020. *Clinical Management of Severe Acute Respiratory Infection when Novel Coronavirus 2019 Infection is Suspected: Interim Guidance, 28 January 2020.* Geneva, Switzerland: WHO. Available at: https://apps.who.int/iris/handle/10665/330893. Accessed April 2, 2021.
9. Correia de Medeiros A, Elcio Franco Filho A. *Orientações para manejo de Pacientes con COVID-19.*
10. Wang D et al., 2020. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA 323:* 1061–1069.
11. Candido DDS et al., 2020. Routes for COVID-19 importation in Brazil. *J Travel Med 27*. doi: 10.1093/jtm/taaa042.
12. Thebault R, Tran AB, Williams V, 2020. The coronavirus is infecting and killing black Americans at an alarmingly high rate. *Washington Post*. Available at: https://www.washingtonpost.com/nation/2020/04/07/coronavirus-is-infecting-killing-black-americans-an-alarmingly-high-rate-post-analysis-shows/. Published April 7, 2020. Accessed April 1, 2021.
13. Sze S et al., 2020. Ethnicity and clinical outcomes in COVID-19: a systematic review and meta-analysis. *EClinicalMedicine 29–30:* 100630.
14. Chen N et al., 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet 395:* 507–513.
15. CDC, 2020. *Hospitalization Rates and Characteristics of Patients Hospitalized with Laboratory-confirmed Coronavirus Disease 2019 — COVID-NET, 14 States, March 1–30,* 2020 | *MMWR.* Available at: https://www.cdc.gov/mmwr/volumes/69/wr/mm6915e3.htm. Accessed November 5, 2020.
16. Yang J et al., 2020. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. *Int J Infect Dis IJID Off Publ Int Soc Infect Dis 94:* 91–95.

FAST TRACK

Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study

Otavio T Ranzani,[1,2] Matt D T Hitchings,[3] Murilo Dorion,[4] Tatiana Lang D'Agostini,[5] Regiane Cardoso de Paula,[5] Olivia Ferreira Pereira de Paula,[5] Edlaine Faria de Moura Villela,[6] Mario Sergio Scaramuzzini Torres,[6] Silvano Barbosa de Oliveira,[7,8] Wade Schulz,[9] Maria Almiron,[7] Rodrigo Said,[7] Roberto Dias de Oliveira,[10] Patricia Vieira da Silva,[11] Wildo Navegantes de Araújo,[7,8,12] Jean Carlo Gorinchteyn,[13] Jason R Andrews,[14] Derek A T Cummings,[15,16] Albert I Ko,[4,17] Julio Croda[4,11,18]

For numbered affiliations see end of the article

Correspondence to: J Croda julio.croda@fiocruz.br (or @juliocroda on Twitter; ORCID 0000-0002-6665-6825)

Additional material is published online only. To view please visit the journal online.

Cite this as: *BMJ* 2021;374:n2015 http://dx.doi.org/10.1136/bmj.n2015

Accepted: 12 August 2021

ABSTRACT

OBJECTIVE

To estimate the effectiveness of the inactivated whole virus vaccine, CoronaVac (Sinovac Biotech), against symptomatic covid-19 in the elderly population of São Paulo state, Brazil during widespread circulation of the gamma variant.

DESIGN

Test negative case-control study.

SETTING

Community testing for covid-19 in São Paulo state, Brazil.

PARTICIPANTS

43 774 adults aged ≥70 years who were residents of São Paulo state and underwent reverse transcription polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 from 17 January to 29 April 2021. 26 433 cases with symptomatic covid-19 and 17 622 test negative controls with covid-19 symptoms were formed into 13 283 matched sets, one case with to up to five controls, according to age, sex, self-reported race, municipality of residence, previous covid-19 status, and date of RT-PCR test (±3 days).

INTERVENTION

Vaccination with a two dose regimen of CoronaVac.

MAIN OUTCOME MEASURES

RT-PCR confirmed symptomatic covid-19 and associated hospital admissions and deaths.

RESULTS

Adjusted vaccine effectiveness against symptomatic covid-19 was 24.7% (95% confidence interval 14.7% to 33.4%) at 0-13 days and 46.8% (38.7% to 53.8%) at ≥14 days after the second dose. Adjusted vaccine effectiveness against hospital admissions was 55.5% (46.5% to 62.9%) and against deaths was 61.2% (48.9% to 70.5%) at ≥14 days after the second dose. Vaccine effectiveness ≥14 days after the second dose was highest for the youngest age group (70-74 years)—59.0% (43.7% to 70.2%) against symptomatic disease, 77.6% (62.5% to 86.7%) against hospital admissions, and 83.9% (59.2% to 93.7%) against deaths—and declined with increasing age.

CONCLUSIONS

Vaccination with CoronaVac was associated with a reduction in symptomatic covid-19, hospital admissions, and deaths in adults aged ≥70 years in a setting with extensive transmission of the gamma variant. Vaccine protection was, however, low until completion of the two dose regimen, and vaccine effectiveness was observe to decline with increasing age among this elderly population.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Estimates of effectiveness of the inactivated whole virus vaccine, CoronaVac (Sinovac Biotech), against symptomatic covid-19 in randomised controlled trials have varied (51% to 84%)

Current evidence is limited on whether CoronaVac is effective against covid-19 associated severe disease or death, or in the setting of extensive circulation of the gamma variant

More evidence is needed for the real world effectiveness of CoronaVac and other inactivated vaccines among elderly people, a population that has been underrepresented in trials of these vaccines

WHAT THIS STUDY ADDS

A two dose regimen of CoronaVac was associated with 47% protection against symptomatic covid-19, 56% against hospital admissions, and 61% against deaths among adults aged ≥70 years in the setting of widespread transmission of the gamma variant

Protection is low until ≥14 days after the second dose of CoronaVac

The effectiveness of CoronaVac was observed to decline with increasing age in the elderly population

Introduction

As of early July 2021 the covid-19 pandemic has been responsible for 3.9 million deaths worldwide,[1] with a disproportionately high mortality and morbidity among elderly people.[2] A key question is whether the authorised covid-19 vaccines are effective in elderly people, who might have impaired immune responses[3 4] and are underrepresented in randomised controlled trials.[5-7] mRNA and adenovirus vector based vaccines have been shown to be effective against covid-19 in elderly people,[8 9] but evidence on the effectiveness of inactivated vaccines in this population is limited.[7 10-12]

CoronaVac (Sinovac Biotech), an inactivated whole virus vaccine, has been approved by 32 countries and jurisdictions[10] and been implemented as part of mass vaccination campaigns in low and middle income countries, many of which are experiencing covid-19 epidemics as a result of the emergence of SARS-CoV-2 variants of concern. Estimates from randomised controlled trials of vaccine efficacy

against symptomatic covid-19 of a two dose CoronaVac regimen in healthcare workers and the general population have varied (51% to 84%).[5 7 10] The World Health Organization's Emergency Use Listing (EUL) procedure approved the use of CoronaVac in early June 2021 but identified an evidence gap for the effectiveness of this vaccine in adults aged ≥60 years.[11] The WHO EUL cited an observational study in Chile,[10 12] which found that the adjusted effectiveness of CoronaVac among adults aged ≥60 years at 14 days or more after the second dose was 66.6%. During the study period, the gamma variant of concern was detected in 28.6% of SARS-CoV-2 genomes in Chile.[12] Furthermore, randomised controlled trials and observational studies have not investigated whether CoronaVac provides important protection after the first dose or in the setting of widespread transmission of variants of concern.[5 10 11]

Brazil has experienced one of the world's highest covid-19 burdens during the pandemic, with more than 18 million people affected and 526 000 deaths reported as of early July 2021.[1 13] Variants of concern, and in particular the gamma variant, have played an important role in the recent epidemic wave in Brazil, which began in early 2021.[14-16] The gamma variant, which was first detected in Manaus, shows increased transmissibility,[16] has accrued mutations associated with decreased in vitro seroneutralisation,[17-19] has a possible association with increased disease severity,[20 21] and, at present, accounts for most of the SARS-CoV-2 isolates genotyped in Brazil from 1 January 2021.[14 22] In the setting of a large epidemic associated with the gamma variant in São Paulo, the most populous state in Brazil, we conducted a matched, test negative[23] case-control study to evaluate the real world effectiveness of CoronaVac against symptomatic covid-19 and severe clinical outcomes in people aged ≥70 years.

Methods

Study setting

The State of São Paulo (23°3′S, 46°4′W) has 645 municipalities and 46 million inhabitants, 3.23 million of whom are aged ≥70 years.[24] The state experienced three successive waves of covid-19, during which 2 997 282 cases (cumulative incidence rate: 6475 per 100 000 population) and 100 649 deaths (cumulative mortality: 217 per 100 000 population) have been reported as of 9 May 2021 (fig 1, supplementary figure 1).[25] The state secretary of health of Sao Paulo initiated a covid-19 vaccination campaign for the general population on 17 January 2021 according to an age based prioritisation strategy (fig 1) and is administering a two dose regimen of CoronaVac with a two to four week interval between doses, and a two dose regimen of ChAdOx1 nCoV-19 (Oxford-AstraZeneca), with a 12 week interval.[26] As of 29 April 2021, 8.63 million doses (5.16 million first doses and 3.47 million second doses) of CoronaVac had been administered and 2.06 million doses (1.99 million first doses and 0.07 million second doses) of ChAdOx1 nCoV-19.

Study design

We conducted a matched test negative case-control study to estimate the effectiveness of CoronaVac in reducing the odds of reverse transcription polymerase chain reaction (RT-PCR) confirmed symptomatic covid-19 in adults aged ≥70 years from São Paulo state from 17 January 2021 (the start of covid-19 vaccination) to 29 April 2021. Test negative design studies have provided estimates of vaccine effectiveness in concordance with those obtained from randomised controlled trials,[27 28] and such studies have been used extensively to evaluate vaccines against respiratory infections,[29] including covid-19.[8 23 30] We chose the test negative design because of the feasibility of accessing information on people who were tested for SARS-CoV-2 through the São Paulo state surveillance systems and because of the opportunity to control for potential biases, such as healthcare seeking behaviour and access to testing.[23] The study population was adults aged ≥70 years who had a residential address in São Paulo state, underwent SARS-CoV-2 RT-PCR testing during the study period, and had complete and consistent information between data sources on age, sex, residence, and on vaccination and testing status and dates. We matched test negative controls with covid-19 symptoms to covid-19 cases by date of testing (±3 days) to address potential sources of bias that might vary during the course of an epidemic, as well as by participant characteristics of age, sex, self-reported race, municipality of residence, and previous covid-19 status.

In the protocol, we prespecified power thresholds for conducting analyses on the effectiveness of CoronaVac and ChAdOx1 nCoV-19. These thresholds were achieved for CoronaVac but not for ChAdOx1 nCoV-19 because of lower rates of ChAdOx1 nCov-19 administered in the population during the study period. We therefore restricted the evaluation of vaccine effectiveness to CoronaVac.

Amendment of protocol

The study design and statistical analysis plan were specified in advance of extracting information from data sources and are described in a publicly available protocol (https://github.com/juliocroda/VebraCOVID-19) and the supplementary file. We made two major changes to the original protocol: we added the analysis for hospital admissions and deaths inside the framework of a test negative design before submission to peer review (post hoc analysis), and after submission, as suggested by peer reviewers, we changed the matching procedure of the main analysis from one case matched to one control without replacement, to one case matched with up to five controls with replacement of controls between cases (unbalanced design); and we added two other sensitivity analyses for the matching procedure.

Data sources

We obtained individual level information on personal characteristics, comorbidities, SARS-CoV-2 testing,

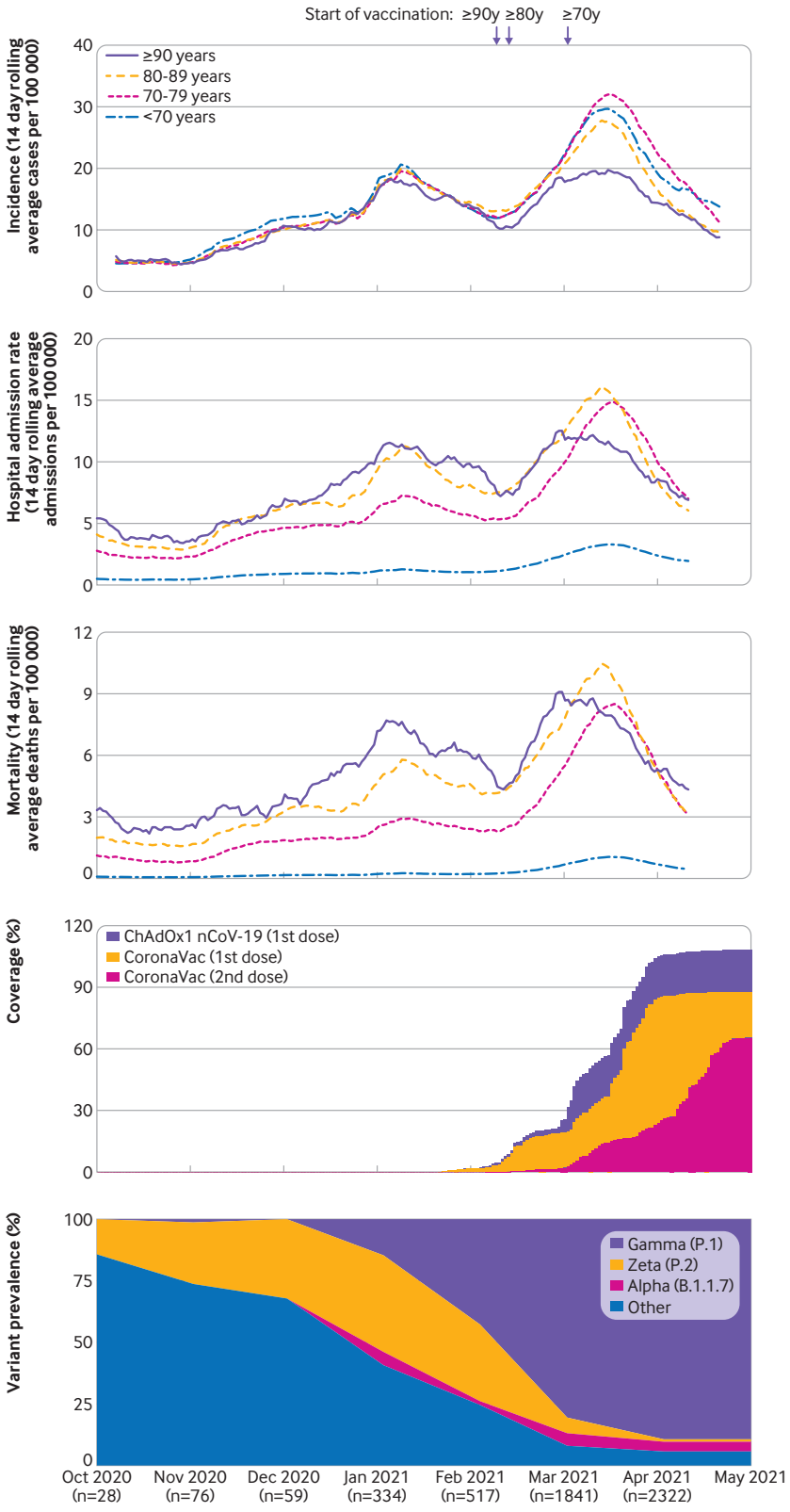


Fig 1 | Incidence of reported covid-19, vaccination coverage, and prevalence of SARS-CoV-2 variants of concern from 1 October 2020 to 29 April 2021 in São Paulo state, Brazil. Panels A-C show the 14 day rolling average of daily age group specific incidence of reported covid-19 cases, hospital admission rate, and mortality (events per 100 000 population). Panel D shows daily cumulative vaccination coverage in people aged ≥70 years. Population estimates for age groups were obtained from national projections for 2020.[24] Panel E shows the monthly prevalence of SARS-CoV-2 variants among genotyped isolates in the GISAID (global initiative on sharing avian influenza data) database (extraction on 20 June 2021).[22] Vertical bars show dates that adults aged ≥90, 80-89, and 70-79 years in the general population became eligible for vaccination

and covid-19 vaccination during the study period by extracting information on 6 May 2021 from the state secretary of health of Sao Paulo laboratory testing registry (GAL), the national surveillance databases for covid-19-like illnesses (e-SUS) and severe acute respiratory illness (SIVEP-Gripe), and the state secretary of health of Sao Paulo vaccination registry (Vacina Já). All people living in Brazil are eligible for testing and have access to the public health system. RT-PCR tests are performed by trained healthcare professionals following standard protocols. Notification of people with suspected covid-19, SARS-CoV-2 test results, and suspected deaths with covid-19 is compulsory in Brazil. Supplementary table 1 provides additional information on data sources. The information technology bureau of the São Paulo state government linked individual level records from the four databases using CPF (Cadastro de Pessoas Físicas) numbers (Brazilian citizens' unique identifier code) and provided anonymised datasets. The genotyping of all isolated SARS-CoV-2 in São Paulo state was not possible and these data are not available in the surveillance systems used in this study. We retrieved information on SARS-CoV-2 variants from genotyped isolates from São Paulo state deposited in the global initiative on sharing avian influenza data (GISAID) database.[22]

Selection of cases and matched controls

We selected cases from the study population who had covid-19 symptoms, defined as a covid-19-like illness, a positive SARS-CoV-2 RT-PCR test result from a respiratory sample that was collected within 10 days after the onset of symptoms, and did not have a positive RT-PCR test result in the preceding 90 days. We selected controls from the study population who had a covid-19-like illness, a negative SARS-CoV-2 RT-PCR test result from a respiratory sample that was collected within 10 days after the onset of symptoms,[23] and no positive RT-PCR test result in the previous 90 days during the study period, or in the subsequent 14 days. Cases and controls were excluded if they received the ChAdOx1 nCoV-19 vaccine before sample collection for RT-PCR testing. We defined covid-19-like illness as the presence of one or more reported covid-19 related symptoms.[31]

One case was matched with to up to five test negative controls according to RT-PCR sample collection date (±3 days), age category (five year age bands, eg, 70-74, 75-79 years), sex, municipality of residence, self-reported race (defined as brown, black, yellow, white, or indigenous),[32] and previous symptomatic events that were reported to the surveillance systems[31] between 1 February 2020 and 16 January 2021, as a proxy for previous SARS-CoV-2 infection. Matching factors were chosen from variables that were associated with vaccination coverage or timing, and with risk of SARS-CoV-2 infection or healthcare access (see protocol in supplementary file).[23] After identification of each case, we randomly chose up to five controls in an unbalanced design from the set of all eligible matching controls, allowing for replacement of controls between cases (main analysis). We conducted three sensitivity analyses, varying two features of the matching while keeping the same matching factors. In the first analysis we matched one case to one random control without replacement of controls (original analysis in the protocol); in the second analysis we matched one case to one random control, allowing for replacement of controls between cases; and in the third analysis we matched one case to two random controls, allowing for replacement of controls between cases.

Statistical analysis

We estimated the effectiveness of CoronaVac against symptomatic covid-19 in the 0-13 days and ≥14 days after the second dose and ≥14 days after the first dose. Furthermore, we estimated the effectiveness of a single dose 0-13 days after the first dose, when the vaccine has shown no or limited effectiveness.[5 33 34] An association during this period might serve as an indicator of unmeasured confounding in the effectiveness estimate.[35 36] We also expanded our bias indicator by evaluating the 0-13 days after the first dose as 0-6 days and 7-13 days.[36] The reference group for vaccination status was those who had not received a first vaccine dose before the date of sample collection.

Conditional logistic regression was used to estimate the odds ratio of vaccination among cases and controls: 1−odds ratio provided an estimate of vaccine effectiveness under the assumptions of a test negative design.[37] We included age and covid-19 associated comorbidities (cardiovascular, renal, neurological, haematological, and hepatic, diabetes, chronic respiratory disorder, obesity, or immunosuppression) as covariates in the model. Because age is a strong determinant of covid-19 outcomes, we adjusted for age after matching by age bands to control for potential residual confounding.[38] Non-linearity for age was evaluated using restricted cubic splines and we chose the most parsimonious model comparing nested models with a likelihood ratio test. To evaluate potential residual confounding by time varying factors that might not be dealt with by the matching criteria, we also conducted a post hoc sensitivity analysis that incorporated the calendar date of RT-PCR sample collection in the model.

In a post hoc analysis we estimated vaccine effectiveness against covid-19 associated hospital admission and death. To account for the competing event of dying before being admitted to hospital, we estimated vaccine effectiveness for the composite outcome of hospital admission or death, or both in a sensitivity analysis. In these separate analyses, we selected matched pairs in which the case had the secondary outcome of interest.[39 40] We fit the same conditional logistic regression model as for the primary outcome.

We conducted a prespecified analysis of vaccine effectiveness among age subgroups for the primary and secondary outcomes but could not perform analyses stratified by previous covid-19 documented infection

because of small numbers. The age subgroups were prespecified and followed the five year age categories used for matching, with age groups older than 80 collapsed into a single group. Additional post hoc analyses were performed of vaccine effectiveness for the primary outcome for subgroups stratified by sex, number of chronic comorbidities (none versus at least one), the two most common chronic comorbidities (cardiovascular disease and diabetes), and region of residence (Grande São Paulo health region versus others). Interaction terms were incorporated into the model to evaluate the association of each subgroup of interest with vaccine effectiveness ≥14 days after the second dose.

Power calculation

Our protocol specified that we would conduct proposed analyses after achieving ≥80% power to identify a vaccine effectiveness of 40% against symptomatic covid-19 for ≥14 days after the second dose of CoronaVac compared with not receiving a vaccine dose. The power was simulated fitting conditional logistic regressions on 1000 simulated datasets. After extracting information from the surveillance databases on 6 May 2021 and generating matched case-control pairs, we determined that the power of the study was 99.9% and proceeded to conduct the prespecified analyses. We did not perform an analysis for ChAdOx1 nCoV-19 because the simulated power was 31% to identify a vaccine effectiveness of 40% for ≥28 days after the first dose of ChAdOx1 nCoV-19 compared with not receiving a vaccine dose.

All analyses were done in R, version 4.0.2.

Patient and public involvement

Because this study used routine surveillance data sources and there was no direct funding, no members of the public or patients were directly involved. Nevertheless, we did speak to patients about the study and the outcomes to be evaluated, and we asked a member of the public to read our manuscript and provide inputs for its interpretation. No members of the public or patients were involved in writing up the results.

Results

São Paulo state experienced three covid-19 epidemic waves, with a peak incidence in July 2020 for the first wave and in January 2021 for the second wave (supplementary figure 1) and March 2021 for the third wave (fig 1). The second wave was preceded in November 2020 by an increase in the prevalence of the zeta variant among genotyped isolates from São Paulo state deposited into the GISAID database (fig 1). The third wave was preceded in January 2021 by an increase in the prevalence of the gamma variant among genotyped isolates (fig 1). The gamma variant replaced other SARS-CoV-2 variants[22] and accounted for 78.4% (3834/4887) of the genotyped isolates that were reported in GISAID during the study period and 85.5% (3584/4192) of genotyped isolates that were reported between 1 March and 29 April 2021, when the majority of discordant case-control sets were identified (supplementary figure 2). The vaccination campaign, initiated on 17 January 2021, achieved an estimated coverage of roughly 85% for the first CoronaVac dose (2.82 million) and 65% for the second dose (2.10 million) among adults aged ≥70 years by 29 April 2021 (fig 1). After initiation of the vaccination campaign and during the third epidemic wave, the incidence of covid-19 increased and peaked in late March in all age groups except for those aged ≥90 years (fig 1).

Study population

From 17 January 2021 to 29 April 2021, the rate of RT-PCR testing for SARS-CoV-2 in those age ≥70 years in São Paulo state was 25 per 1000 persons. Among 43 774 adults eligible for study inclusion (fig 2), 22 177 (50.7%) who provided 55 519 RT-PCR test results were included in matched case and control sets as follows: 3881 pairs matched 1:1, 1963 pairs matched 1:2, 1044 pairs matched 1:3, 678 pairs matched 1:4, and 5717 pairs matched 1:5. Overall, 6223 participants contributed more than one time as controls and 18 participants contributed as both control and case. Table 1 shows the characteristics of eligible participants with positive and negative RT-PCR test results, and selected cases and matched controls. Matched characteristics appear unbalanced because of the variable matching procedure. A higher proportion of cases than controls had reported comorbidities. Most of the discordant sets, based on vaccination status, were selected after 14 March 2021 (supplementary figure 2). For cases and controls who completed the two dose vaccine regimen, the intervals between doses were similar (mean 30 *v* 25 days). Likewise, the intervals between vaccine doses and RT-PCR testing were similarly distributed for cases and controls (table 1 and supplementary figure 3). Supplementary table 2 shows the distribution of matched sets according to the vaccination status of cases and controls at the time of RT-PCR testing. Supplementary tables 3 and 4 show the characteristics of the matched case and control sets selected for the analysis of secondary outcomes of hospital admission (n=30 308) and death (n=14 624).

Vaccine effectiveness against symptomatic covid-19

The adjusted effectiveness against symptomatic covid-19 was 24.7% (95% confidence interval 14.7% to 33.4%) at 0-13 days and 46.8% (38.7% to 53.8%) at ≥14 days after the second dose (table 2). No statistically significant change was identified in the odds of covid-19 in the 0-13 days after the first dose, which serves as a potential bias indicator. The bias indicator was similar 0-6 days and 7-13 days after the first dose (supplementary table 5). In the sensitivity analysis including calendar date of testing as a covariate, vaccine effectiveness after the second dose was 25.1% (15.2% to 33.8%) at 0-13 days and 47.1% (39.1% to 54.1%) at ≥14 days.

Vaccine effectiveness against symptomatic covid-19 was observed to decline with increasing age ≥14

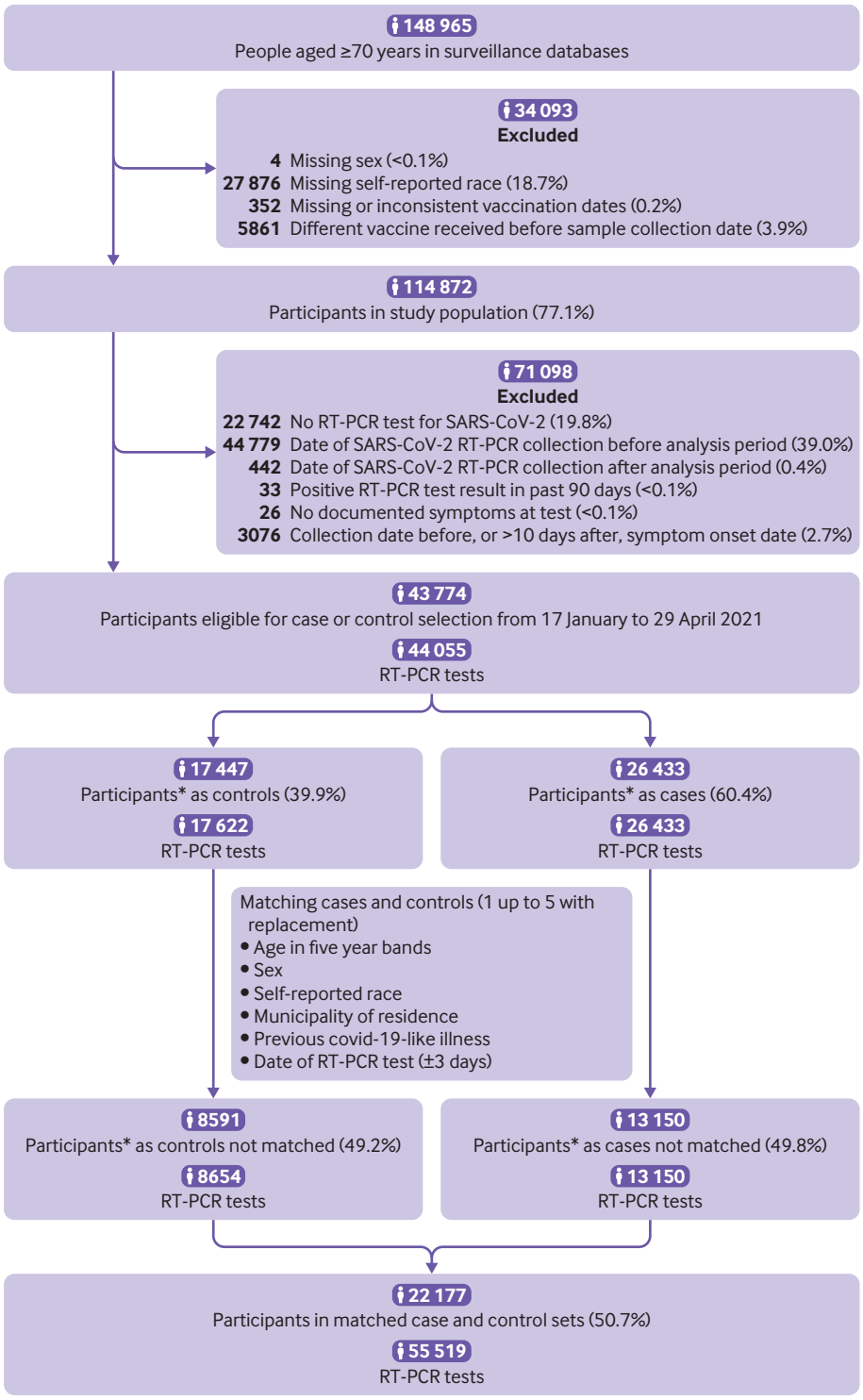


Fig 2 | Flowchart of study population from surveillance databases, and selection of matched cases and controls. *Some participants contributed as controls and cases, and matching allowed for replacement of controls between cases. RT-PCR=reverse transcription polymerase chain reaction

days after the second dose and was 59.0% (43.7% to 70.2%) among those aged 70-74 years, 56.2% (43.0% to 66.3%) among those aged 75-79 years, and 32.7% (17.0% to 45.5%) among those aged ≥80 years (P=0.007 for interaction; figure 3, supplementary table 6). Vaccine effectiveness against symptomatic covid-19 did not differ among subgroups defined by sex, presence of comorbidities, reported cardiovascular disease, or regions of residence. Participants with reported diabetes, however, had lower protection than those without reported diabetes (vaccine effectiveness 32.6% *v* 50.5%, P=0.008 for interaction) ≥14 days after the second dose (supplementary table 7 and supplementary figure 4)

Table 1 | Characteristics of adults aged ≥70 years in São Paulo state, Brazil, who were eligible for matching and selected into case test negative pairs. Values are numbers (percentages) unless stated otherwise

Characteristics	Eligible cases and controls		Matched sets	
	Test negative (n=17 622)*	Test positive (n=26 433)*	Controls (n=42 236)*	Cases (n=13 283)*
Mean (SD) age (years)	77.53 (6.8)	76.71 (6.2)	75.69 (5.44)	75.90 (5.64)
Age groups (years):				
70-79	12 123 (68.8)	19 673 (74.4)	34 134 (80.8)	10 543 (79.4)
80-89	4301 (24.4)	5437 (20.6)	7045 (16.7)	2311 (17.4)
≥90	1198 (6.8)	1323 (5.0)	1057 (2.5)	429 (3.2)
Men	7689 (43.6)	12 431 (47.0)	18 610 (44.1)	5919 (44.6)
Self-reported race†:				
White/branca	13 415 (76.1)	19 796 (74.9)	34 603 (81.9)	10 803 (81.3)
Brown/pardo	3192 (18.1)	4983 (18.9)	6797 (16.1)	2115 (15.9)
Black/preta	785 (4.5)	1258 (4.8)	727 (1.7)	287 (2.2)
Yellow/amarela	226 (1.3)	390 (1.5)	109 (0.3)	78 (0.6)
Indigenous/Indígena	4 (0.0)	6 (0.0)	-	-
Residence in Grande São Paul health region	12 381 (70.3)	16 538 (62.6)	14 368 (34.0)	6113 (46.0)
Reported No of comorbidities‡:				
0	10 027 (56.9)	12 668 (47.9)	23 961 (56.7)	5886 (44.3)
1 or 2	6984 (39.6)	12 548 (47.5)	16 626 (39.4)	6713 (50.5)
≥3	611 (3.5)	1217 (4.6)	1649 (3.9)	684 (5.1)
Cardiovascular disease	5293 (30.0)	10 079 (38.1)	12 563 (29.7)	5482 (41.3)
Diabetes	3233 (18.3)	6533 (24.7)	8269 (19.6)	3578 (26.9)
Past exposure to SARS-CoV-2§				
Previous symptomatic events notified to surveillance systems¶	685 (3.9)	354 (1.3)	47 (0.1)	37 (0.3)
Positive SARS-CoV-2 test result**	66 (0.4)	13 (0.0)	1 (0.0)	4 (0.0)
Median (interquartile range) interval between symptoms onset and RT-PCR testing (days)	3 (2-5)	4 (2-6)	3 (1-5)	4 (2-6)
Hospital admissions	4524/17 484 (25.9)	12 987/26 221 (49.5)	11 020/41 980 (26.3)	7043/13 175 (53.5)
Deaths	1594/16 710 (9.5)	7054/24 508 (28.8)	4072/40 134 (10.1)	3549/12 251 (29.0)
Median (interquartile range) interval between symptoms onset and hospital admission (days)	3 (2-6)	7 (4-10)	4 (2-7)	7 (4-10)
Median (interquartile range) interval between symptoms onset and deaths (days)	8 (4-13)	14 (9-21)	8 (4-16)	15 (10-22)
Vaccination status:				
Not vaccinated	11 986 (68.0)	17 233 (65.2)	27 994 (66.3)	8989 (67.7)
Single dose, within 0-13 days	1446 (8.2)	2976 (11.3)	4873 (11.5)	1565 (11.8)
Single dose, ≥14 days	1797 (10.2)	3312 (12.5)	4631 (11.0)	1489 (11.2)
Two doses, within 0-13 days	1041 (5.9)	1533 (5.8)	2445 (5.8)	700 (5.3)
Two doses, ≥14 days	1352 (7.7)	1379 (5.2)	2293 (5.4)	540 (4.1)
Mean (SD) interval between 1st and 2nd dose (days)	25 (6)	30 (12)	25 (6)	30 (12)
Mean (SD) interval between 1st dose and RT-PCR testing (days)	28 (19)	23 (16)	22 (17)	21 (16)
Mean (SD) interval between second dose and RT-PCR testing (days)	20 (15)	17 (14)	17 (14)	16 (14)

RT-PCR=reverse transcription polymerase chain reaction.
*Numbers refer to RT-PCR tests and represent 43 774 people for the eligible cases and controls and 22 177 people in matched cases and controls.
†Race/skin colour as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas).[32]
‡Comorbidities included cardiovascular, renal, neurological, haematological, or hepatic conditions, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
§Before start of study on 17 January 2021 and after systematic surveillance was implemented on 1 February 2020.
¶Reported illness with covid-19 associated symptoms in eSUS and SIVEP-Gripe databases.
**Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Vaccine effectiveness against covid-19 associated hospital admissions

The adjusted effectiveness against hospital admission was 39.1% (28.0% to 48.5%) at 0-13 days and 55.5% (46.5% to 62.9%) at ≥14 days after the second dose (table 2). No statistically significant reduction was observed in the odds of covid-19 in the periods after one dose, and the bias indicator effectiveness was close to zero (supplementary table 5).

Vaccine effectiveness against hospital admission was observed to decline with increasing age ≥14 days after the second dose and was 77.6% (62.5% to 86.7%) among those aged 70-74 years, 66.6% (51.8% to 76.9%) among those aged 75-79 years, and 38.9% (21.4% to 52.5%) among those aged ≥80 years (P<0.001 for interaction; fig 3, supplementary table 6).

Vaccine effectiveness against deaths with covid-19

The adjusted effectiveness against deaths with covid-19 was 31.2% (17.6% to 42.5%) ≥14 days after the first dose, 48.9% (34.4% to 60.1%) 0-13 days after the second dose, and 61.2% (48.9% to 70.5%) ≥14 days after the second dose (table 2). The bias indicator was close to zero 0-13 days after the first dose, and 0-6 days and 7-13 days after the first dose (supplementary table 5).

Vaccine effectiveness against deaths was observed to decline with increasing age ≥14 days after the second dose and was 83.9% (59.2% to 93.7%) among those aged 70-74 years, 78.0% (58.8% to 88.3%) among those aged 75-79 years, and 44.0% (20.3% to 60.6%) among those aged ≥80 years (P=0.001 for interaction; fig 3, supplementary table 6).

Table 2 | Effectiveness of CoronaVac (Sinovac Biotech) against symptomatic covid-19, hospital admissions, and deaths in adults aged ≥70 years in São Paulo state, Brazil

	Unadjusted analysis			Adjusted analysis*		
	Odds ratio (95% CI)	Vaccine effectiveness, % (95% CI)	P value	Odds ratio (95% CI)	Vaccine effectiveness, % (95% CI)	P value
Symptomatic covid-19 (n=55 519)						
One dose:						
0-13 days *v* unvaccinated†	1.02 (0.94 to 1.10)	−1.7 (−10.4 to 6.2)	0.68	1.01 (0.93 to 1.09)	−0.8 (−9.4 to 7.2)	0.86
≥14 days *v* unvaccinated†	0.88 (0.80 to 0.97)	11.9 (3.1 to 19.9)	0.01	0.88 (0.79 to 0.96)	12.5 (3.7 to 20.6)	0.01
Two doses:						
0-13 days *v* unvaccinated†	0.77 (0.68 to 0.87)	23.5 (13.5 to 32.3)	<0.001	0.75 (0.67 to 0.85)	24.7 (14.7 to 33.4)	<0.001
≥14 days *v* unvaccinated†	0.54 (0.47 to 0.62)	45.8 (37.7 to 52.9)	<0.001	0.53 (0.46 to 0.61)	46.8 (38.7 to 53.8)	<0.001
Hospital admissions associated with covid-19 (n=30 308)						
One dose:						
0-13 days *v* unvaccinated†	0.98 (0.89 to 1.09)	1.6 (−9.3 to 11.5)	0.76	0.93 (0.84 to 1.04)	6.6 (−4.3 to 16.3)	0.23
≥14 days *v* unvaccinated†	0.87 (0.77 to 0.99)	12.6 (1.3 to 22.6)	0.03	0.83 (0.73 to 0.94)	16.9 (5.7 to 26.8)	0.004
Two doses:						
0-13 days *v* unvaccinated†	0.66 (0.56 to 0.77)	34.4 (23.1 to 44.1)	<0.001	0.61 (0.52 to 0.72)	39.1 (28.0 to 48.5)	<0.001
≥14 days *v* unvaccinated†	0.48 (0.40 to 0.57)	51.9 (42.6 to 59.7)	<0.001	0.45 (0.37 to 0.54)	55.5 (46.5 to 62.9)	<0.001
Deaths associated with covid-19 (n=14 624)						
One dose:						
0-13 days *v* unvaccinated†	0.90 (0.78 to 1.04)	10 (−4.2 to 22.2)	0.16	0.87 (0.74 to 1.02)	13.1 (−1.5 to 25.6)	0.08
≥14 days *v* unvaccinated†	0.75 (0.63 to 0.89)	25.1 (11.2 to 36.9)	0.001	0.69 (0.58 to 0.82)	31.2 (17.6 to 42.5)	<0.001
Two doses:						
0-13 days *v* unvaccinated†	0.56 (0.44 to 0.70)	44.3 (29.6 to 55.9)	<0.001	0.51 (0.40 to 0.66)	48.9 (34.4 to 60.1)	<0.001
≥14 days *v* unvaccinated†	0.43 (0.33 to 0.56)	57.1 (44.3 to 67)	<0.001	0.39 (0.30 to 0.51)	61.2 (48.9 to 70.5)	<0.001

*Adjusted for age (linear term for symptomatic covid-19 and restricted cubic spline for hospital admissions and deaths) and number of comorbidities (0, 1 or 2, ≥3).
†At date of index sample collection for cases and controls.

The adjusted effectiveness for the composite outcome hospital admissions or deaths, or both, was 39.2% (28.3% to 48.4%) 0-13 days after the second dose, and 55.4% (46.5% to 62.8%) ≥14 days after the second dose (supplementary table 8).

Sensitivity analyses for the matching procedure

Overall, 13 150 cases (49.7%) could not be matched with a potential control. Thus, 30.1% of cases (7950 case-control pairs) could be matched in the first sensitivity analysis (1:1 without replacement), 50.3% of cases (13 283 case-control pairs) in the second sensitivity analysis (1:1, allowing for replacement of controls), and 35.6% of cases (9402 case-control pairs) in the third sensitivity analysis (1:2, allowing for replacement of controls). Supplementary table 9 shows the characteristics of the population in these three matching analyses. Overall, vaccine effectiveness was comparable to the findings of the main analysis, with varying precision. Vaccine effectiveness against symptomatic covid-19 was 41.6% (26.9% to 53.3%) in the first sensitivity analysis (n=15 900), 48.6% (38.9% to 56.8%) in the second sensitivity analysis (n=26 566), and 47.8% (38.2% to 56.0%) in the third

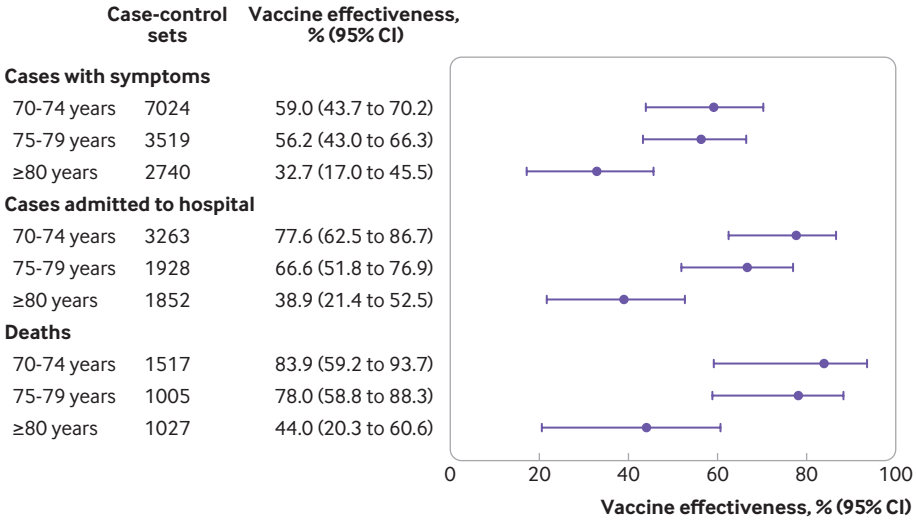


Fig 3 | Adjusted vaccine effectiveness ≥14 days after the second dose of CoronaVac (Sinovac Biotech) for subgroups of adults aged ≥70 years. Estimates of vaccine effectiveness were obtained from a conditional logistic regression model that included covariates of age and number of comorbidities and incorporated an interaction term between the category of interest and the period ≥14 days after the second dose

sensitivity analysis (n=28 206; supplementary tables 10-13). The same pattern of vaccine effectiveness observed in the main analysis when stratified by age and for severe outcomes was observed in the three sensitivity analyses (supplementary tables 10 and 14-16).

Discussion

In this test negative case-control study we found that the effectiveness of a two dose schedule of CoronaVac in the real world was 47% against symptomatic covid-19, 56% against covid-19 associated hospital admissions, and 61% against covid-19 associated deaths among those aged ≥70 years during a gamma variant associated epidemic in Brazil. Furthermore, we have addressed several evidence gaps for the use of CoronaVac: vaccination showed an effectiveness against covid-19, including associated severe outcomes, in the setting of widespread transmission of the gamma variant, which was similar to that found in the Brazilian randomised controlled trial conducted before the emergence of the gamma variant[5]; a single dose of CoronaVac was associated with low protection against symptomatic covid-19 or hospital admission; and vaccine effectiveness was observed to decline with increasing age among adults aged ≥70 years.

Research in context

A key evidence gap, as raised in the WHO EUL for CoronaVac,[11] has been the effectiveness of this vaccine in the elderly population, because this age group was not well represented in Brazilian and Turkish randomised controlled trials.[5 7 10 11] We found that two doses of CoronaVac administered at an average interval of four weeks had an overall effectiveness against symptomatic covid-19 of 47% (39% to 54%) in a population with a mean age of 76 years. This estimate is lower than the efficacy of 84% (95% confidence interval 65% to 92%) reported in the Turkish trial, with a participant median age of 45 years and two week dosing interval[7]; and comparable to the efficacy of 51% (95% confidence interval 36% to 62%) from the Brazilian trial in healthcare workers, with a participant mean age of 39 years and two week dosing interval.[5] Additionally, a cohort study in Chile reported an effectiveness for CoronaVac of 66.6% (95% confidence interval 65.4% to 67.8%) in those aged ≥60 years. It is not clear whether the observed differences are related to the age distribution, dosing interval, risk of infection in the community, or the presence of the gamma variant of concern, which was not prevalent during the trials' follow-up periods and was responsible for only 28.6% of genotyped infections in Chile during the study period.[10 12]

Among elderly people in our study, we observed a statistically significant decline in vaccine effectiveness against symptomatic covid-19 with increasing age, from 59.0% (43.7% to 70.2%) in those aged 70-74 year to 32.7% (17.0% to 45.5%) in those aged ≥80 years. These findings parallel real world evidence for the BNT162b2 mRNA vaccine, which showed reduced effectiveness in residents of long term care facilities in Denmark,[41] skilled nursing facilities in the USA,[42] and the general population aged ≥70 years in Finland[43] and ≥80 years in Israel.[44] As well as having a slower immune response and lower peak of neutralising antibodies than younger populations, elderly people seem to have faster decay of antibody titres.[4] Together, these findings suggest that specific vaccines or vaccination schedules might be required to effectively vaccinate the very elderly (≥80 years) population against covid-19.

Vaccine effectiveness was greater against severe outcomes than against symptomatic covid-19 in all age subgroups among elderly people. This finding, consistent with the findings from randomised controlled trials and observational studies for multiple covid-19 vaccines and across settings,[5 6 9 10 12] suggests that vaccination will reduce morbidity and mortality among elderly people even if effectiveness at preventing infections is reduced. The direct comparison of the effectiveness against hospital admission with other vaccines and between countries is not straightforward, because hospital admission depends on admission triage policies, which change according to age and hospital bed availability. Therefore, someone older than 80 years with symptomatic covid-19 has a higher likelihood of being admitted compared with younger patients even if the disease is not severe, and this likelihood varies between public and private facilities and whether the health system is overwhelmed.[13] Thus, we cannot generalise our findings for protection against hospital admission without considering this context. We evaluated vaccine effectiveness at the individual level, not accounting for the indirect effect and the total effect from the vaccination campaign. A preliminary aggregated analysis using weekly times series of covid-19 deaths in Brazil found a relative decrease in mortality among those aged ≥70 years compared with all ages after vaccination with CoronaVac and ChAdOx1 nCov-19,[45] suggesting a discernible impact of vaccination on mortality at the population level. Additional investigation is required to determine the duration of protection conferred by CoronaVac.[7 19 23]

The absence of demonstrable effectiveness of CoronaVac until completion of the two dose regimen has profound implications for use of this vaccine in response to an epidemic. In contrast with covid-19 vaccines that confer protection after the first dose,[9 46] CoronaVac showed low effectiveness until after the second dose (more than four weeks after the first dose).[19] Our findings suggest that in countries where CoronaVac supplies are constrained and there is high SARS-CoV-2 transmission, vaccination should prioritise completion of the two dose regimen among the highest risk populations and avoid being expanded to broader segments of the population for whom provisions for a second dose have not been secured.

Our study did not directly address the question of whether vaccination with CoronaVac is effective against gamma variant associated covid-19 because we had

no data on whether the analysed cases were related to the gamma variant. However, 91.0% (5054/5551) of the discordant sets in this matched case-control study were selected from 1 March to 29 April 2021, when the gamma variant accounted for 85% of the genotyped isolates during surveillance in São Paulo state. A test negative study in Canada evaluated adults aged ≥70 years and estimated an adjusted vaccine effectiveness of single dose mRNA vaccines of 61% (95% confidence interval 45% to 72%) against the gamma variant of concern compared with 72% (58% to 81%) for non-variants of concern.[47] Although further studies are required to determine the effectiveness of CoronaVac against the gamma variant and additional variants of concern, our findings provide supportive evidence for the use of CoronaVac in countries in South America that are experiencing epidemics due to extensive spread of the gamma variant[22] and are using CoronaVac as part of a mass vaccination campaign in response to the epidemic.

Strengths and limitations of this study

This study has several strengths, which include the large sample size and geospatial coverage, comprising the State of São Paulo with 46 million inhabitants distributed across 645 municipalities. We implemented a prespecified publicly available protocol, which is in accordance with the recent WHO guideline for evaluation of covid-19 vaccine effectiveness.[23] Using a test negative design, we have dealt with biases that affect observational studies on vaccine effectiveness, such as health seeking behaviour and access. Additionally, after matching and adjustment, the bias indicator association between recent vaccination with a single dose 0-13 days before sample collection was close to null, suggesting that the underlying risk of testing positive for SARS-CoV-2 did not differ between vaccinated and unvaccinated people.[8 35 36 48] Finally, we performed three sensitivity analyses for the matching procedure, which yielded comparable estimates to those of the main analysis, resulting in increased precision and showing the robustness of our vaccine effectiveness estimation.

Our study had limitations. We could not assess the influence of a previous SARS-CoV-2 infection on vaccine effectiveness because passive surveillance identified too few people with a positive RT-PCR or rapid antigen test result before the study period. Before the start of the vaccination campaign, the estimated seroprevalence of covid-19 in inhabitants aged ≥60 years in the capital of São Paulo state was 19.9% (14.9% to 29.9%) in January 2021.[49] Our estimates of vaccine effectiveness might therefore be subject to downward bias, as unvaccinated people were at lower risk of reinfection. We attempted to exclude false negative RT-PCR test results by excluding as controls those with a subsequent positive test result within 14 days after the initial test and including only tests performed within 10 days of symptom onset.[23] However, we cannot rule out some level of misclassification, although it is likely to be non-differential and thus would bias the estimate towards the null. In addition, we restricted our study population to elderly people because they were a priority group for vaccination and received the majority of CoronaVac doses during the initial stages of the vaccination campaign in Brazil; as a result, it was not possible to compare the effectiveness of CoronaVac between older and younger populations directly. Our analyses were also limited by the lack of more refined covariates, such as frailty, chronic illness status, and nursing home residence status, which could influence vaccine effectiveness in very elderly people and in itself would not be addressed by age and reported comorbidities. Finally, we cannot exclude the possibility of time varying changes in behaviour, non-drug interventions, or testing practices among participants. We tried to control for these by matching on time of RT-PCR testing (±3 days),[21] geography (ie, municipality of residence), and self-reported race, which is strongly associated with socioeconomic position in Brazil. When we tried to further adjust for unmeasured confounding by adjusting for the day of year, estimates of vaccine effectiveness remained similar.

Conclusion

This study found that a two dose schedule of CoronaVac was 47% effective in preventing symptomatic covid-19, with higher effectiveness against severe clinical outcomes, among elderly people aged ≥70 years in a setting with extensive transmission of the gamma variant. The delayed onset of vaccine mediated protection, however, underscores the need to prioritise vaccine supplies and maximise the number of people who complete the two dose schedule, when CoronaVac is used as part of a mass vaccination campaign that is implemented in response to a covid-19 epidemic.

AUTHOR AFFILIATIONS

[1]Barcelona Institute for Global Health, ISGlobal, Barcelona, Spain
[2]Pulmonary Division, Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil
[3]Department of Biostatistics, College of Public Health & Health Professions, University of Florida, Gainesville, FL, USA
[4]Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA
[5]Disease Control Coordination of the São Paulo State Department of Health, São Paulo, Brazil
[6]Municipal Health Secretary of Manaus, Brazil, AM, Brazil
[7]Pan American Health Organization, Brasília, DF, Brazil
[8]Universidade de Brasília, Brasília, DF, Brazil
[9]Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA
[10]State University of Mato Grosso do Sul - UEMS, Dourados, MS, Brazil
[11]Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande, MS, Brazil
[12]National Institute for Science and Technology for Health Technology Assessment, Porto Alegre, RS, Brazil
[13]Health Secretariat of the State of São Paulo, São Paulo, Brazil
[14]Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA
[15]Department of Biology, University of Florida, Gainesville, FL, USA
[16]Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA

[17]Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil
[18]Fiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, MS, Brazil

We thank the Pan American Health Organization for its support and the São Paulo state for making the databases available for analysis.

Contributors: All authors conceived the study. OTR and MDTH contributed equally as first authors and JRA, DATC, AIK, and JC contributed equally as senior authors. OTR, MDTH, and MD completed analyses with guidance from JRA, DATC, AIK, and JC. MSST, OFPP, OTR, and MDTH curated and validated the data. OTR and MDTH wrote the first draft of the manuscript. TLD, RCP, OFPP, EFMV, MA, RS, JCG, and WNA provided supervision. All authors contributed to, and approved, the final manuscript. JC is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding: No external funding was provided for this study. JC is supported by the Oswaldo Cruz Foundation (Edital Covid-19 – resposta rápida: 48111668950485). OTR is funded by a Sara Borrell fellowship (CD19/00110) from the Instituto de Salud Carlos III. OTR acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019-2023 programme and from the Generalitat de Catalunya through the Centres de Recerca de Catalunya (CERCA) programme. These institutions had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The health secretary of State of São Paulo and information technology bureau of the São Paulo state government reviewed the data and findings of the study, but the academic authors retained editorial control.

Competing interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval: This study was approved by the ethical committee for research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021).

Data sharing: Deidentified databases as well as the R codes will be deposited in the repository https://github.com/juliocroda/VebraCOVID-19 on publication of this article.

The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Dissemination to participants and related patient and public communities: The findings of this study will be disseminated through presentations to the São Paulo state health department and the national immunisation programme, as well as the World Health Organization. Blogs, press releases, and social media dissemination will also be prepared for public dissemination.

Provenance and peer review: Not commissioned; externally peer reviewed.

1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* 2020;20:533-4. doi:10.1016/S1473-3099(20)30120-1
2 O'Driscoll M, Ribeiro Dos Santos G, Wang L, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. *Nature* 2021;590:140-5. doi:10.1038/s41586-020-2918-0
3 Bajaj V, Gadi N, Spihlman AP, Wu SC, Choi CH, Moulton VR. Aging, Immunity, and COVID-19: How Age Influences the Host Immune Response to Coronavirus Infections? *Front Physiol* 2021;11:571416. doi:10.3389/fphys.2020.571416
4 Collier DA, Ferreira IATM, Kotagiri P, *et al.* Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* 2021; published online June 30. doi:10.1038/s41586-021-03739-1
5 Palacios R, Batista AP, Albuquerque CSN et al. Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study.*SSRN Journal*2021. doi:10.2139/ssrn.3822780
6 Voysey M, Clemens SAC, Madhi SA, et al, Oxford COVID Vaccine Trial Group. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021;397:99-111. doi:10.1016/S0140-6736(20)32661-1
7 Tanriover MD, Doğanay HL, Akova M, et al, CoronaVac Study Group. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey. *Lancet* 2021;398:213-22. doi:10.1016/S0140-6736(21)01429-X
8 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ* 2021;373:n1088. doi:10.1136/bmj.n1088
9 Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021;384:1412-23. doi:10.1056/NEJMoa2101765
10 Strategic Advisory Group of Experts on Immunization - SAGE (WHO). Evidence Assessment: Sinovac/CoronaVac COVID-19 vaccine. Report from 29/04/2021. https://cdn.who.int/media/docs/default-source/immunization/sage/2021/april/5_sage29apr2021_critical-evidence_sinovac.pdf (accessed May 26, 2021).
11 Strategic Advisory Group of Experts on Immunization - SAGE (WHO). Interim recommendations for use of the inactivated COVID-19 vaccine, CoronaVac, developed by Sinovac. 2021; published online May 24. https://apps.who.int/iris/bitstream/handle/10665/341454/WHO-2019-nCoV-vaccines-SAGE-recommendation-Sinovac-CoronaVac-2021.1-eng.pdf.
12 Jara A, Undurraga EA, González C, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile. *N Engl J Med* 2021. doi:10.1056/NEJMoa2107715
13 Ranzani OT, Bastos LSL, Gelli JGM, et al. Characterisation of the first 250,000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *Lancet Respir Med* 2021;9:407-18. doi:10.1016/S2213-2600(20)30560-9
14 Lamarca AP, de Almeida LGP, Francisco R da S, et al. Genomic surveillance of SARS-CoV-2 tracks early interstate transmission of P.1 lineage and diversification within P.2 clade in Brazil.*medRxiv* , 2021. doi:10.1101/2021.03.21.21253418
15 Naveca FG, Nascimento V, de Souza VC, et al. COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat Med* 2021;27:1230-8. doi:10.1038/s41591-021-01378-7.
16 Faria NR, Mellan TA, Whittaker C, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* 2021;372:815-21. doi:10.1126/science.abh2644
17 Jangra S, Ye C, Rathnasinghe R, et al, Personalized Virology Initiative study group. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *Lancet Microbe* 2021;2:e283-4. doi:10.1016/S2666-5247(21)00068-9
18 Hoffmann M, Arora P, Groß R, et al. SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies. *Cell* 2021;184:2384-2393.e12. doi:10.1016/j.cell.2021.03.036
19 Souza WM, Amorim MR, Sesti-Costa R, et al. Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. *Lancet Microbe* 2021;•••. doi:10.1016/S2666-5247(21)00129-4
20 Freitas ARR, Beckedorff OA, Cavalcanti LP de G, et al. The emergence of novel SARS-CoV-2 variant P.1 in Amazonas (Brazil) was temporally associated with a change in the age and sex profile of COVID-19 mortality: A population based ecological study. *The Lancet Regional Health - Americas* 2021. doi:10.1016/j.lana.2021.100021
21 Bastos LS, Ranzani OT, Souza TML, Hamacher S, Bozza FA. COVID-19 hospital admissions: Brazil's first and second waves compared. *Lancet Respir Med* 2021;9:e82-3. doi:10.1016/S2213-2600(21)00287-3
22 GISAID - hCov19 Variants. https://www.gisaid.org/hcov19-variants/ (accessed June 20, 2021).
23 Patel MK, Bergeri I, Bresee JS, et al. Evaluation of post-introduction COVID-19 vaccine effectiveness: Summary of interim guidance of the World Health Organization. *Vaccine* 2021;39:4013-24. doi:10.1016/j.vaccine.2021.05.099
24 Freire FHM de A, Gonzaga MR. Gomes MMF. Projeções populacionais por sexo e idade para pequenas áreas no Brasil. *RELAP* 2019;14:124-49. doi:10.31406/relap2020.v14.i1.n26.6
25 SEADE Foundation - São Paulo State. CORONAVIRUS - CASOS EM SP. Fundação SEADE. https://www.seade.gov.br/coronavirus/ (accessed May 9, 2021).
26 Brazilian Ministry of Health. Campanha Nacional de Vacinação contra a Covid-19. Décimo oitavo informe técnico. https://www.conasems.org.br/wp-content/uploads/2021/04/20a-distribuic%CC%A7a%CC%83o_Campanha_Nacional_de_Vacinacao_contra_a_Covid_19.pdf (accessed May 25, 2021).

27 De Serres G, Skowronski DM, Wu XW, Ambrose CS. The test-negative design: validity, accuracy and precision of vaccine efficacy estimates compared to the gold standard of randomised placebo-controlled clinical trials. *Euro Surveill* 2013;18:20585. doi:10.2807/1560-7917.ES2013.18.37.20585
28 Schwartz LM, Halloran ME, Rowhani-Rahbar A, Neuzil KM, Victor JC. Rotavirus vaccine effectiveness in low-income settings: An evaluation of the test-negative design. *Vaccine* 2017;35:184-90. doi:10.1016/j.vaccine.2016.10.077
29 Jackson ML, Chung JR, Jackson LA, et al. Influenza Vaccine Effectiveness in the United States during the 2015-2016 Season. *N Engl J Med* 2017;377:534-43. doi:10.1056/NEJMoa1700153
30 Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *N Engl J Med* 2021;385:585-94. doi:10.1056/NEJMoa2108891
31 Brazilian Ministry of Health. Epidemiologic Surveillance Guide. National Emergency of Public Health Concern due to the COVID-19 disease. https://www.conasems.org.br/wp-content/uploads/2021/03/Guia-de-vigila%CC%82ncia-epidemiolo%CC%81gica-da-covid_19_15.03_2021.pdf (accessed May 25, 2021).
32 Brazilian Institute of Geography and Statistics - IBGE. Ethnic and race characteristics of the population. Classifications and identities. 2013. https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf (accessed May 24, 2021).
33 Bueno SM, Abarca K, González PA, et al. Interim report: Safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy chilean adults in a phase 3 clinical trial.*medRxiv* , 2021. doi:10.1101/2021.03.31.21254494
34 Wu Z, Hu Y, Xu M, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021;21:803-12. doi:10.1016/S1473-3099(20)30987-7
35 Hitchings MDT, Lewnard JA, Dean NE, et al. Use of recently vaccinated individuals to detect bias in test-negative case-control studies of COVID-19 vaccine effectiveness.*medRxiv* , 2021. doi:10.1101/2021.06.23.21259415
36 Lewnard JA, Patel MM, Jewell NP, et al. Theoretical Framework for Retrospective Studies of the Effectiveness of SARS-CoV-2 Vaccines. *Epidemiology* 2021;32:508-17. doi:10.1097/EDE.0000000000001366
37 Sullivan SG, Tchetgen Tchetgen EJ, Cowling BJ. Theoretical Basis of the Test-Negative Study Design for Assessment of Influenza Vaccine Effectiveness. *Am J Epidemiol* 2016;184:345-53. doi:10.1093/aje/kww064
38 Pearce N. Analysis of matched case-control studies. *BMJ* 2016;352:i969. doi:10.1136/bmj.i969
39 Andrejko KL, Pry J, Myers JF, et al, California COVID-19 Case-Control Study Team. Prevention of COVID-19 by mRNA-based vaccines within the general population of California. *Clin Infect Dis* 2021. doi:10.1093/cid/ciab640
40 Crowe E, Pandeya N, Brotherton JML, et al. Effectiveness of quadrivalent human papillomavirus vaccine for the prevention of cervical abnormalities: case-control study nested within a population based screening programme in Australia. *BMJ* 2014;348:g1458. doi:10.1136/bmj.g1458
41 Moustsen-Helms IR, Emborg H-D, Nielsen J, et al. Vaccine effectiveness after 1st and 2nd dose of the BNT162b2 mRNA Covid-19 Vaccine in long-term care facility residents and healthcare workers – a Danish cohort study.*medRxiv* , 2021. doi.10.1101/2021.03.08.21252200
42 Britton A, Jacobs Slifka KM, Edens C, et al. Effectiveness of the Pfizer-BioNTech COVID-19 Vaccine Among Residents of Two Skilled Nursing Facilities Experiencing COVID-19 Outbreaks - Connecticut, December 2020-February 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:396-401. doi:10.15585/mmwr.mm7011e3
43 Baum U, Poukka E, Palmu AA, Salo H, Lehtonen TO, Leino T. Effectiveness of vaccination against SARS-CoV-2 infection and Covid-19 hospitalization among Finnish elderly and chronically ill – An interim analysis of a nationwide cohort study.*medRxiv* , 2021. doi:10.1101/2021.06.21.21258686
44 Yelin I, Katz R, Herzel E, et al. Associations of the BNT162b2 COVID-19 vaccine effectiveness with patient age and comorbidities. *medRxiv* , 2021 doi:10.1101/2021.03.16.21253686
45 Victora PC, Castro PMC, Gurzenda S, Medeiros AC, França GVA, Barros PAJD. Estimating the early impact of vaccination against COVID-19 on deaths among elderly people in Brazil: Analyses of routinely-collected data on vaccine coverage and mortality. *EClinicalMedicine* 2021;36:101036. doi:10.1016/j.eclinm.2021.101036
46 Voysey M, Costa Clemens SA, Madhi SA, et al, Oxford COVID Vaccine Trial Group. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* 2021;397:881-91. doi:10.1016/S0140-6736(21)00432-3
47 Skowronski DM, Setayeshgar S, Zou M, et al. Single-dose mRNA vaccine effectiveness against SARS-CoV-2, including Alpha and Gamma variants: a test-negative design in adults 70 years and older in British Columbia, Canada. *Clin Infect Dis* 2021;ciab616. doi:10.1093/cid/ciab616
48 Hitchings MDT, Ranzani OT, Torres MSS, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: A test-negative case-control study. *The Lancet Regional Health - Americas* 2021;100025. doi:10.1016/j.lana.2021.100025
49 SoroEpi Group. SoroEpi MSP study in São Paulo Capital - Phase 5 results. https://www.monitoramentocovid19.org (accessed May 17, 2021).

Supplementary information: additional tables 1-16 and figures 1-4

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-26459-6 OPEN

Effectiveness of ChAdOx1 vaccine in older adults during SARS-CoV-2 Gamma variant circulation in São Paulo

Matt D. T. Hitchings[1,19], Otavio T. Ranzani[2,3,19], Murilo Dorion[4], Tatiana Lang D'Agostini[5], Regiane Cardoso de Paula[5], Olivia Ferreira Pereira de Paula[5], Edlaine Faria de Moura Villela[5], Mario Sergio Scaramuzzini Torres[6], Silvano Barbosa de Oliveira[7,8], Wade Schulz[9], Maria Almiron[7], Rodrigo Said[7], Roberto Dias de Oliveira[10], Patricia Vieira Silva[11], Wildo Navegantes de Araújo[7,8,12], Jean Carlo Gorinchteyn[13], Jason R. Andrews[14], Derek A. T. Cummings[15,16], Albert I. Ko[4,17] & Julio Croda[4,11,18]✉

A two-dose regimen of the Oxford-AstraZeneca (ChAdOx1) Covid-19 vaccine with an inter-dose interval of three months has been implemented in many countries with restricted vaccine supply. However, there is limited evidence for the effectiveness of ChAdOx1 by dose in elderly populations in countries with high prevalence of the Gamma variant of SARS-CoV-2. Here, we estimate ChAdOx1 effectiveness by dose against the primary endpoint of RT-PCR-confirmed Covid-19, and secondary endpoints of Covid-19 hospitalization and Covid-19-related death, in adults aged ≥60 years during an epidemic with high Gamma variant prevalence in São Paulo state, Brazil using a matched, test-negative case-control study. Starting 28 days after the first dose, effectiveness of a single dose of ChAdOx1 is 33.4% (95% CI, 26.4–39.7) against Covid-19, 55.1% (95% CI, 46.6–62.2) against hospitalization, and 61.8% (95% CI, 48.9–71.4) against death. Starting 14 days after the second dose, effectiveness of the two-dose schedule is 77.9% (95% CI, 69.2–84.2) against Covid-19, 87.6% (95% CI, 78.2–92.9) against hospitalization, and 93.6% (95% CI, 81.9–97.7) against death. Completion of the ChAdOx1 vaccine schedule affords significantly increased protection over a single dose against mild and severe Covid-19 outcomes in elderly individuals during widespread Gamma variant circulation.

[1] Department of Biostatistics, College of Public Health & Health Professions, University of Florida, Gainesville, FL, USA. [2] Barcelona Institute for Global Health, ISGlobal, Barcelona, Spain. [3] Pulmonary Division, Heart Institute (InCor), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, São Paulo, São Paulo, Brazil. [4] Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT, USA. [5] Disease Control Coordination of the São Paulo State Department of Health, São Paulo, São Paulo, Brazil. [6] Municipal Health Secretary of Manaus, Manaus, Amazonas, Brazil. [7] Pan American Health Organization, Brasília, Distrito Federal, Brazil. [8] Universidade de Brasília, Brasília, Distrito Federal, Brazil. [9] Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT, USA. [10] State University of Mato Grosso do Sul - UEMS, Dourados, Mato Grosso do Sul, Brazil. [11] Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande, Mato Grosso do Sul, Brazil. [12] National Institute for Science and Technology for Health Technology Assessment, Porto Alegre, Rio Grande do Sul, Brazil. [13] Health Secretariat of the State of São Paulo, São Paulo, São Paulo, Brazil. [14] Division of Infectious Diseases and Geographic Medicine, Stanford University, Stanford, CA, USA. [15] Department of Biology, University of Florida, Gainesville, FL, USA. [16] Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA. [17] Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, Bahia, Brazil. [18] Fiocruz Mato Grosso do Sul, Fundação Oswaldo Cruz, Campo Grande, Mato Grosso do Sul, Brazil. [19] These authors contributed equally: Matt D. T. Hitchings, Otavio T. Ranzani. ✉email: julio.croda@fiocruz.br

Multiple vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent that causes coronavirus disease 19 (Covid-19), have been developed, proven efficacious, and deployed in mass vaccination campaigns[1–3]. Prominent among these vaccines, particularly in lower-income and middle-income countries, is the viral vector vaccine, ChAdOx1[4]. Randomized controlled trials (RCT) of ChAdOx1 delivered with a four-week inter-dose interval demonstrated 70.4% (95% CI: 54.8–80.6) efficacy against symptomatic Covid-19 in the period starting 14 days after the second vaccine dose[4], and 64.1% 95% CI: (50.5–73.9) starting at 21 days following the first dose[5]. Based on measured immunogenicity and efficacy following a single dose, many countries have implemented a dose-spacing strategy that uses an inter-dose interval of up to 12 weeks to maximize vaccine coverage[6] and has been endorsed by the World Health Organization (WHO)[7].

The emergence of variants of concern (VOC) associated with decreased neutralization activity has created an urgent need to continuously monitor vaccine effectiveness[8]. Recent evidence has suggested reduced effectiveness of a single dose of ChAdOx1 against the Gamma and Delta VOCs[9,10]. Local Gamma VOC circulation has been observed in countries in Latin America which are using ChAdOx1 in mass vaccination[11]. A key question for these countries is the effectiveness of ChAdOx1 by dose against mild and severe Covid-19 outcomes, particularly in priority populations for vaccination such as the elderly.

The Gamma VOC was first detected in the city of Manaus[12] and has been a driver of Covid-19 resurgence in Brazil and across South America[13]. The Brazilian national immunization program initiated a mass vaccination campaign in January 2021, which administered ChAdOx1 with three-month dose-spacing. In this work, we evaluate vaccine effectiveness following one and two doses during a epidemic with high Gamma variant prevalence in São Paulo, the most populous state in Brazil. We show that the effectiveness of the completed two-dose schedule is higher than effectiveness of a single dose, and demonstrate robust ChAdOx1 vaccine effectiveness against moderate and severe Covid-19 outcomes in this elderly population.

Results

Study setting. São Paulo State has experienced three Covid-19 epidemic waves, the latest peaking in March 2021, with cumulatively over 3.89 million reported cases, 430,000 hospitalizations, and 130,000 deaths due to Covid-19 as of 9 July 2021[14] (Fig. 1A). During the second and third waves, the Gamma variant increased in prevalence, reaching 80.2% from March to May 2021 among sequenced isolates, to become the predominant circulating variant in the state (Fig. 1B). The State Secretary of Health of São Paulo (SES-SP) initiated a mass vaccination campaign on 17 January 2021, prioritizing healthcare workers and elderly populations. Two primary vaccines are being distributed: a two-dose regimen of ChAdOx1, separated by a 12-week interval, and a two-dose regimen of CoronaVac, separated by a two- to four-week interval[15]. As of 9 July 2021, 1.61 million doses of ChAdOx1 (1.11 million first doses and 0.51 million second doses) and 9.07 million doses of CoronaVac (5.62 million first doses and 3.45 million second doses) (Fig. 1C) have been administered[16].

Study population. Among 137,744 individuals eligible for selection as a case or control (Fig. 2), 61,164 (44.4%) who provided 61,360 RT-PCR test results were selected into 30,680 matched case and control pairs. Table 1 and Supplementary Table 1 show the characteristics of eligible individuals and matched cases and controls. Supplementary Tables 2–4 show the distribution of matched pairs according to vaccination status of cases and controls at the time of RT-PCR testing for the analysis of symptomatic Covid-19, hospitalization, and death. Supplementary Fig. 1 shows the timing of discordant pair enrollment, while Supplementary Fig. 2 shows the distribution of intervals between administration of vaccine doses and RT-PCR testing. Among individuals testing positive for SARS-CoV-2 by RT-PCR or rapid antigen tests, 82,061 were eligible for analysis of effectiveness against progression to severe outcomes. Characteristics of this cohort are shown in Supplementary Table 5.

Vaccine effectiveness against symptomatic Covid-19. The adjusted effectiveness of a single dose of ChAdOx1 against symptomatic Covid-19 was 33.4% (95% CI: 26.4–39.7) for the period ≥28 days after administration of the first dose (Table 2). The effectiveness of a single dose reached a plateau after 28 days (Fig. 3), with no increase observed in later time periods. The adjusted effectiveness of the full two-dose schedule against symptomatic Covid-19 was 38.1% (95% CI: 11.9–56.5) in the period 0–13 days after administration of the second dose, and 77.9% (95% CI: 69.2–84.2) in the period ≥14 days after administration of the second dose. The estimated effectiveness in the period 0–13 days following the first dose, which serves as a negative control period to indicate bias, was −7.1% (95% CI: −19.6 to 4.1). Increasing number of comorbidities were significantly associated with increased odds of Covid-19 in the adjusted analyses (aOR 1.54, 95% CI: 1.49–1.60, for one-two comorbidities, and aOR 2.20, 95% CI: 1.98–2.45, for three or more comorbidities compared to no comorbidities). A previous positive SARS-CoV-2 viral test was associated with lower odds of Covid-19 (aOR 0.65, 95% CI: 0.37–1.17). Unadjusted, but matched, analyses provided similar effectiveness estimates (Supplementary Table 6).

Vaccine effectiveness against severe Covid-19 outcomes. In the period starting 28 days after the first dose, the adjusted effectiveness of a single dose was 55.1% (95% CI: 46.6–62.2) against hospitalization, and 61.8% (95% CI: 48.9–71.4) against death (Table 2). The adjusted effectiveness of the two-dose schedule starting 14 days after the second dose was higher: 87.6% (95% CI: 78.2–92.9) against hospitalization, and 93.6% (95% CI: 81.9–97.7) against death (Table 2). Effectiveness against ICU admission and mechanical ventilation was similar to effectiveness against hospitalization (Table 2). In general, vaccine effectiveness in the "bias-indicator" period 0–13 days after the first dose was low. Results were similar when performing different matching schemes (Supplementary Tables 7 and 8), with some small improvements in precision. Analysis of hospitalization, ICU admission, and death among those testing positive for SARS-CoV-2 estimated low effectiveness against progression before 28 days after the first dose, followed by increased effectiveness but with low precision for effectiveness starting 14 days after the second dose (Supplementary Table 9).

Subgroup analyses. The effectiveness of a single dose against symptomatic Covid-19 was lower among those with reported diabetes (24.2%, 95% CI: 11.0–35.4) than in those without reported diabetes (35.3%, 95% CI: 28.3–41.6) ($p_{interaction} = 0.03$) (Supplementary Table 10). Similarly, effectiveness was lower among those with at least one reported comorbidity compared to those without a reported comorbidity (Supplementary Table 10). Finally, single-dose effectiveness against hospitalization and death was lower among older individuals, but these analyses lacked sufficient power (Supplementary Table 11).

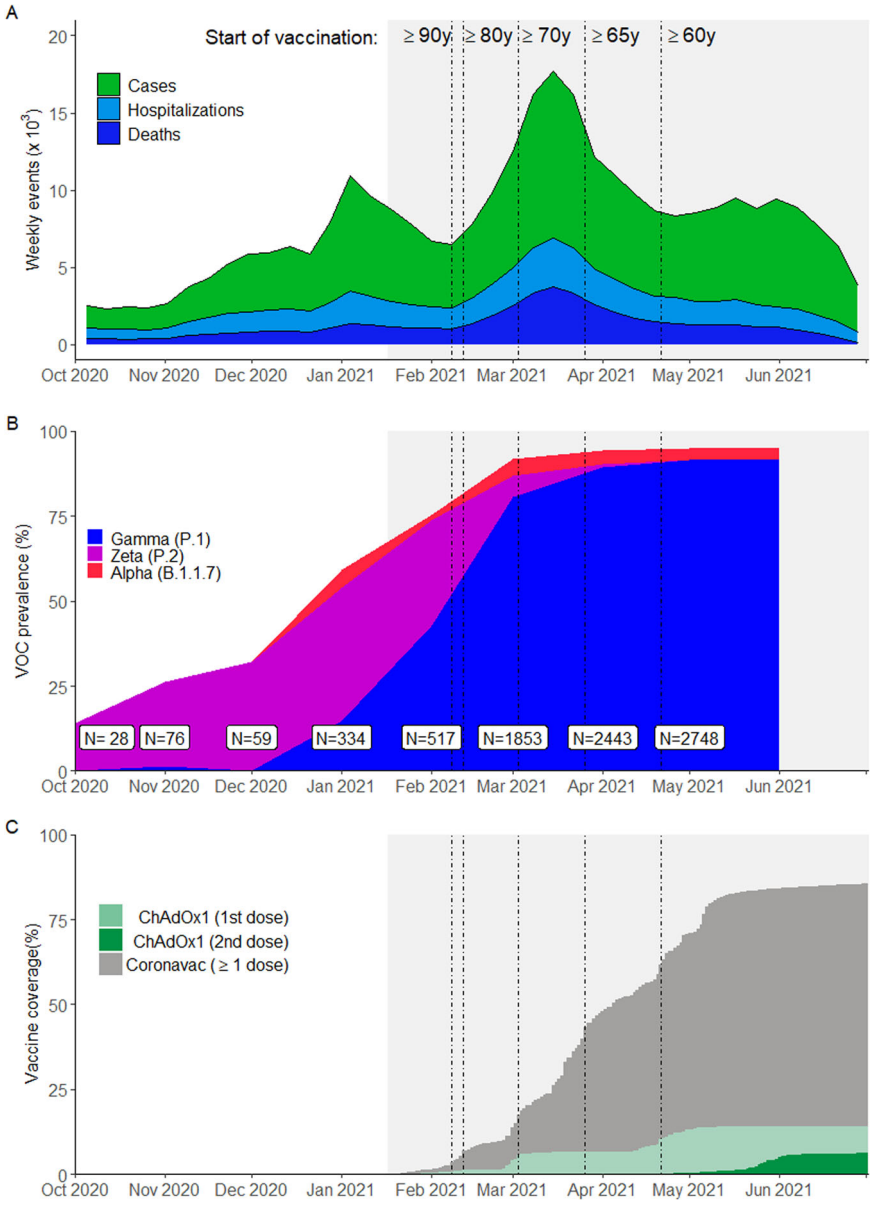


Fig. 1 Incidence of reported Covid-19, vaccination coverage, and prevalence of SARS-CoV-2 variants of concern from Oct 1, 2020 to July 2, 2021 in São Paulo State, Brazil. A The weekly case count of cases, hospitalizations, and deaths based on positive RT-PCR/Antigen tests for the age group ≥60 years. **B** The monthly prevalence of main SARS-CoV-2 variants of concern among genotyped isolates in the GISAID database[11] (extraction on July 7 2021). Prevalence was omitted for June and July due to low sample count. **C** Daily cumulative vaccination coverage for age group ≥60 years. Population estimates were obtained from national projections for 2020[35]. Vertical lines, from left to right in each panel, show the dates that adults ≥90, 80–89, 70–79, 65–69, and 60–64 years of age in the general population became eligible for vaccination. The gray shaded area represents the study period. Source data are provided as a Source Data file.

Discussion

A key priority for mass vaccination campaigns is to reduce morbidity and mortality in the elderly and other vulnerable populations, especially in the context of limited vaccine supply and VOC emergence. Our test-negative case–control study found that the two-dose schedule of ChAdOx1 in the elderly had robust effectiveness against Covid-19 and severe outcomes during a Covid-19 epidemic with high Gamma variant prevalence in the period starting 14 days after administration of the second dose: 77.9% (95% CI: 69.2–84.2) against symptomatic Covid-19, 87.6% (95% CI: 78.2–92.9) against Covid-19 hospitalization, and 93.6% (95% CI: 81.9–97.7) against Covid-19-related death. However, a single dose of ChAdOx1 in adults 60 years of age had effectiveness of 33.4% (95% CI: 26.4–39.7) against symptomatic Covid-19, 55.1% (95% CI: 46.6–62.2) against hospitalization, and 61.8% (95% CI: 48.9–71.4) against death. Additionally, no clinically significant effectiveness was detected within 28 days of administration of the first dose.

Randomized controlled trials of ChAdOx1 conducted in multiple countries reported pooled vaccine efficacy of 70.4% (95% CI: 54.8–80.6) against symptomatic Covid-19 in the period starting 14 days after the second vaccine dose, and 100% (95% CI, not calculated) against hospitalization for Covid-19[4]. A secondary analysis estimated efficacy of 64.1% (95% CI: 50.5–73.9) against symptomatic Covid-19 starting at 21 days following the first dose[5]. Subsequent observational studies have largely supported the effectiveness of ChAdOx1 against symptomatic Covid-19 and hospitalization in elderly populations[17–20]. In addition, these

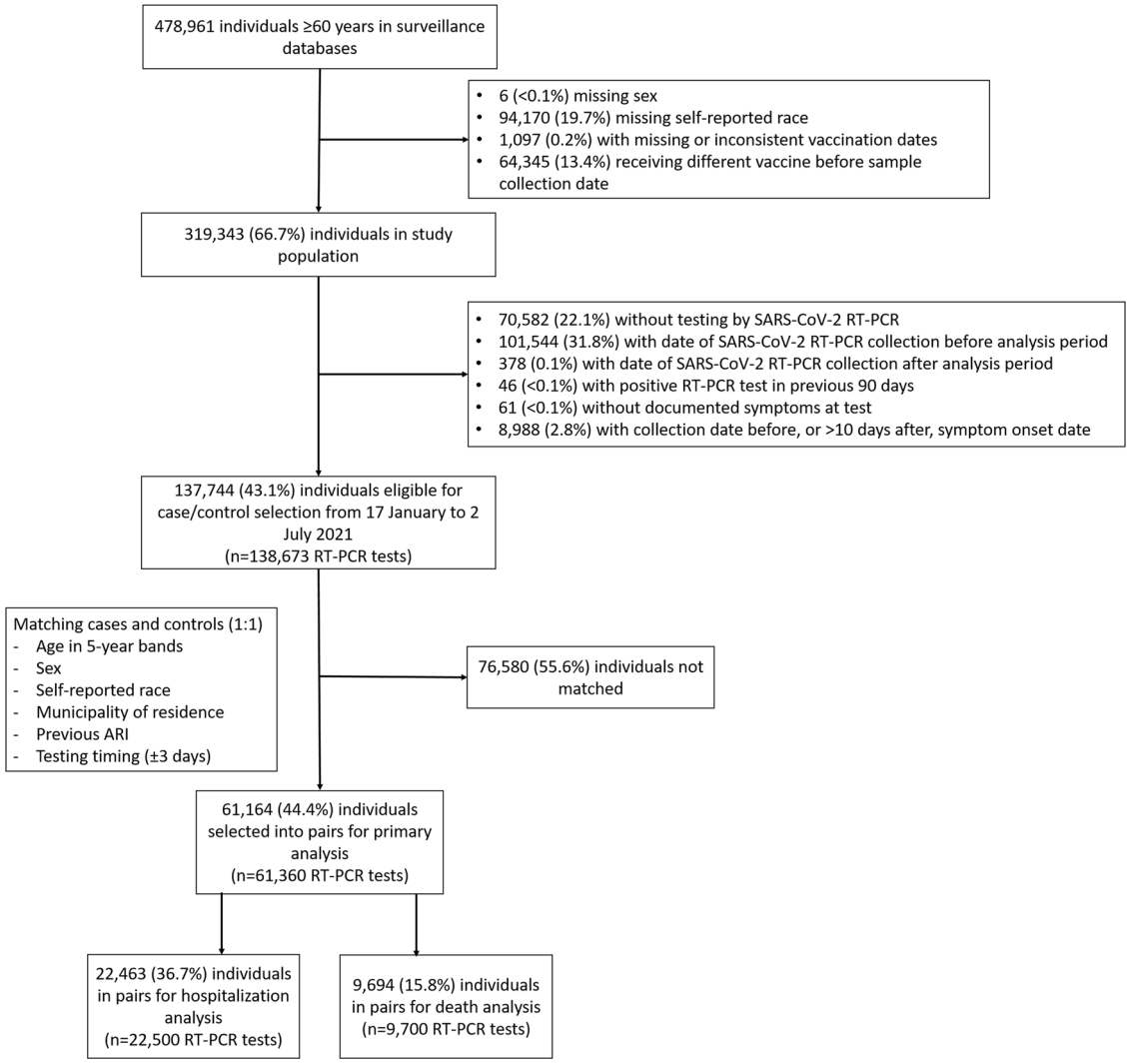


Fig. 2 Study flowchart. Flowchart of the identification of the study population from surveillance databases and selection of matched cases and controls.

studies provided further evidence for the effectiveness of a single dose of ChAdOx1 against infection with SARS-CoV-2, symptomatic Covid-19[17], and hospitalization[18,19], with onset of clinical effectiveness occurring between 21 and 28 days.

Emerging VOCs have been associated with reduced neutralization by serum from individuals who have been infected with non-VOC strains, and vaccinated[20,21], including those who are elderly[22], raising the possibility of decreased effectiveness. An RCT of ChAdOx1 conducted in South Africa found no effectiveness, albeit with low precision, of the two-dose vaccine schedule against mild-to-moderate Covid-19 caused by the Beta VOC[23]. Further evidence from observational studies has suggested reduced vaccine effectiveness against symptomatic disease for a single dose of vaccine against Gamma: 48% (95% CI: 28–63) after 14 days for ChAdOx1[10], 61% (95% CI: 45–72) after 21 days for mRNA vaccines[24], and 11% (95% CI: −4 to 23) after 14 days for CoronaVac[25]. However, the complete BNT162b2 schedule has shown robust effectiveness against the Gamma VOC[10], and a complete schedule of CoronaVac was effective against mild and severe outcomes in settings of high Gamma VOC prevalence[25]. These findings are consistent with reduced effectiveness of a single dose of BNT162b2 and ChAdOx1 against the Delta VOC observed in the UK[9]. Our study adds to this evidence base by estimating single-dose effectiveness of ChAdOx1 over the duration of the inter-dose interval, and demonstrating a substantial increase in effectiveness against Covid-19 and severe outcomes after the second dose in elderly individuals in a setting of high Gamma VOC prevalence.

Our findings have implications for vaccination policy in countries experiencing Covid-19 epidemics with high Gamma variant prevalence. Several countries, including Brazil, are administering the two-dose schedule of ChAdOx1 with a 12-week gap between doses to increase coverage, as WHO currently recommends[7]. The public health benefits of dose-spacing strategies were predicated on robust effectiveness following a single dose[26–28]. In the specific context of VOC emergence and spread, national programs should consider the reduced vaccine effectiveness of a single dose against the Gamma and Delta VOCs in the elderly, together with vaccine supply limitations, speed of vaccination, and logistics, when quantifying the benefits of dose-spacing strategies.

The design of this study lends strength to our findings. The six-month period during which the Gamma variant-associated epidemic and vaccination campaign occurred provided the opportunity to obtain robust estimates of single-dose effectiveness beyond 28 days, and effectiveness of the completed schedule in the same population for direct comparison. The test-negative design reduces bias caused by healthcare-seeking behavior[29], and we have controlled for additional sources of bias by matching on several predictors of healthcare access and utilization and

Table 1 Characteristics of adults ≥60 years of age who were eligible for matching and selected into case-test negative pairs.

Characteristics[a]	Eligible cases and controls		Matched pairs	
	Test-negative ($n = 56,676$)[b]	Test-positive ($n = 81,997$)[b]	Controls ($n = 30,680$)[b]	Cases ($n = 30,680$)[b]
Demographics				
Age, mean (SD), years	67.90 (7.8)	67.59 (7.2)	66.54 (6.5)	66.55 (6.5)
Male sex, *n* (%)	24,313 (42.9)	39,180 (47.8)	12,976 (42.3)	12,976 (42.3)
Self-reported race, *n* (%)[c]				
White/Branca	40,860 (72.1)	58,565 (71.4)	23,046 (75.1)	23,046 (75.1)
Brown/Pardo	12,484 (22.0)	18,463 (22.5)	6,572 (21.4)	6,572 (21.4)
Black/Preta	2,720 (4.8)	4,063 (5.0)	943 (3.1)	943 (3.1)
Yellow/Amarela	605 (1.1)	890 (1.1)	119 (0.4)	119 (0.4)
Indigenous/Indigena	7 (0.0)	16 (0.0)	-	-
Residence in "Grande São Paulo" Health Region, *n* (%)	39,767 (70.2)	53,540 (65.3)	17,771 (57.9)	17,771 (57.9)
Reported number of comorbidities, *n* (%)[d]				
None	37,434 (66.0)	47,262 (57.6)	20,604 (67.2)	17,520 (57.1)
One or two	18,121 (32.0)	32,093 (39.1)	9507 (31.0)	12,136 (39.6)
Three or more	1121 (2.0)	2642 (3.2)	569 (1.9)	1024 (3.3)
At least one previous ARI event, *n* (%)[e]	2722 (4.8)	1381 (1.7)	299 (1.0)	299 (1.0)
Positive SARS-CoV-2 test result, *n* (%)[f]	310 (0.5)	72 (0.1)	31 (0.1)	19 (0.1)
Vaccination status				
Not vaccinated, *n* (%)	44,285 (78.1)	65,582 (80.0)	24,868 (81.1)	25,215 (82.2)
Single dose, within 0–13 days, *n* (%)	1877 (3.3)	3535 (4.3)	1042 (3.4)	1141 (3.7)
Single dose, 14–27 days, *n* (%)	2543 (4.5)	4406 (5.4)	1427 (4.7)	1380 (4.5)
Single dose, ≥28 days, *n* (%)	6918 (12.2)	7704 (9.4)	3009 (9.8)	2731 (8.9)
2nd dose, within 0–13 days, *n* (%)	303 (0.5)	388 (0.5)	114 (0.4)	107 (0.3)
2nd dose, ≥14 days, *n* (%)	750 (1.3)	382 (0.5)	220 (0.7)	106 (0.3)

[a]Continuous variables are displayed as mean (SD); categorical variables are displayed as *n* (%).
[b]These numbers refer to RT-PCR tests and represent 120,483 individuals for the eligible cases and controls and 53,495 individuals in the matched cases and controls.
[c]Race/skin color as defined by the Brazilian national census bureau (Instituto Nacional de Geografia e Estatísticas)[35].
[d]Comorbidities included: cardiovascular, renal, neurological, hematological, or hepatic comorbidities, diabetes, chronic respiratory disorder, obesity, or immunosuppression.
[e]Prior to the start of the study on 17 January, 2021 and after systematic surveillance was implemented on 1 February, 2020. Reported illness with Covid-19 associated symptoms in the eSUS and SIVEP-Gripe databases.
[f]Defined as a positive SARS-CoV-2 RT-PCR or antigen detection test result.

Covid-19 risk[30]. We used a negative control period of 0–13 days within receiving the first dose to detect bias in our estimates, and found limited measured effectiveness in this period. This null association suggests that matched cases and controls were similar in their propensity to be vaccinated; differences observed for subsequent time periods were likely associated with vaccination rather than underlying characteristics of those who did and did not receive it[31]. The large sample size allowed us to produce robust estimates even against rare outcomes such as death and to perform subgroup analyses.

Our study has several limitations. We could not estimate the effectiveness against Gamma and non-Gamma Covid-19 cases within this study population, as we did not have access to individual-level genetic data on the virus. However, the majority of discordant case-control pairs selected (3728/3834, 97%) received RT-PCR tests after 1 March 2021, after which the prevalence of the Gamma variant among sequenced isolates was 80.2%. In addition, there was likely a proportion of the population that was seropositive without having received a previous positive RT-PCR or rapid antigen test before the study period. These individuals, even if unvaccinated, would be protected from reinfection by natural immunity, thus causing downward bias in our vaccine effectiveness estimates. Controls for the analysis of severe outcomes included controls with mild ARI, who may have had better access to healthcare, leading to bias in our estimates of effectiveness against severe outcomes. Exclusions due to missing data or lack of a matching control affect the generalizability of our results, as individuals who had complete data and were matched might not be representative of the general population. However, different matching schemes including a higher proportion of eligible cases returned similar results. Finally, our results cannot be extrapolated to younger populations.

In a setting of widespread circulation of the SARS-CoV-2 Gamma variant, in the general population of elderly individuals, completion of the two-dose schedule of ChAdOx1 was associated with a significant increase in protection against mild and severe Covid-19 outcomes compared to a single dose.

Methods

The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). We obtained local IRB approval to waive the Free and Informed Consent form. This was possible because the anonymized data bases from the surveillance system were sent to us only after the linkage and did not allow identification of the study participants. The second cohort was selected from within the surveillance data bases described, and did not involve any further prospective follow-up or data collection.

Study setting. The study setting and design have been described in detail elsewhere[25,32]. We obtained individual-level information on demographic characteristics, comorbidities, SARS-CoV-2 testing, and Covid-19 vaccination by extracting information on 9 July 2021 from the SES-SP laboratory testing registry (GAL), the national surveillance databases for acute respiratory illness (ARI) (e-SUS) and severe ARI (SIVEP-Gripe), and the SES-SP vaccination registry (Vacina Já), containing Covid-19 vaccine information for all individuals vaccinated in São Paulo State (Supplementary Table 12). The surveillance databases cover hospitalizations, as well as primary care, inpatient and specialty outpatient health visits conducted through public and private health systems. Notification of SARS-CoV-2 test results and suspected Covid-19 cases, hospitalizations, and deaths to these systems is compulsory. We retrieved information on SARS-CoV-2 variants from

Table 2 Adjusted effectiveness of a ChAdOx1 against clinical Covid-19 outcomes in adults ≥60 years of age.

	Symptomatic Covid-19 (*n* pairs = 30,680)	Covid-19 hospitalization (*n* pairs = 11,250)	ICU admission (*n* pairs = 4445)	Invasive mechanical ventilation (*n* pairs = 2672)	Covid-19-related death (*n* pairs = 4850)
Vaccine doses and timing	aVE (95% CI)	aVE (95% CI)	aVE (95% CI)	aVE (95% CI)	aVE (95% CI)
Single dose, within 0–13 days vs. unvaccinated*	−7.1% (−19.6–4.1)	13.1% (−4.4–27.7)	−9.1% (−46.6–18.8)	12.7% (−29.2–41)	16.1% (−11.9–37.2)
Single dose, 14–27 days vs. unvaccinated*	17.8% (8.0–26.5)	33.6% (19.9–45.0)	39.6% (15.4–56.8)	51.8% (26.3–68.4)	37.5% (15.2–54.0)
Single dose, ≥28 days vs. unvaccinated*	33.4% (26.4–39.7)	55.1% (46.6–62.2)	50.9% (33.6–63.8)	70.5% (54.9–80.8)	61.8% (48.9–71.4)
Two doses, within 0–13 days vs. unvaccinated*	38.1% (11.9–56.5)	59.2% (32.4–75.4)	50.9% (−41.8–83)	75.2% (−18.7–94.8)	77.8% (49.1–90.3)
Two doses, ≥14 days vs. unvaccinated*	77.9% (69.2–84.2)	87.6% (78.2–92.9)	89.9% (70.9–96.5)	96.5% (81.7–99.3)	93.6% (81.9–97.7)

aVE adjusted vaccine effectiveness. *At date of index sample collection for cases and controls.

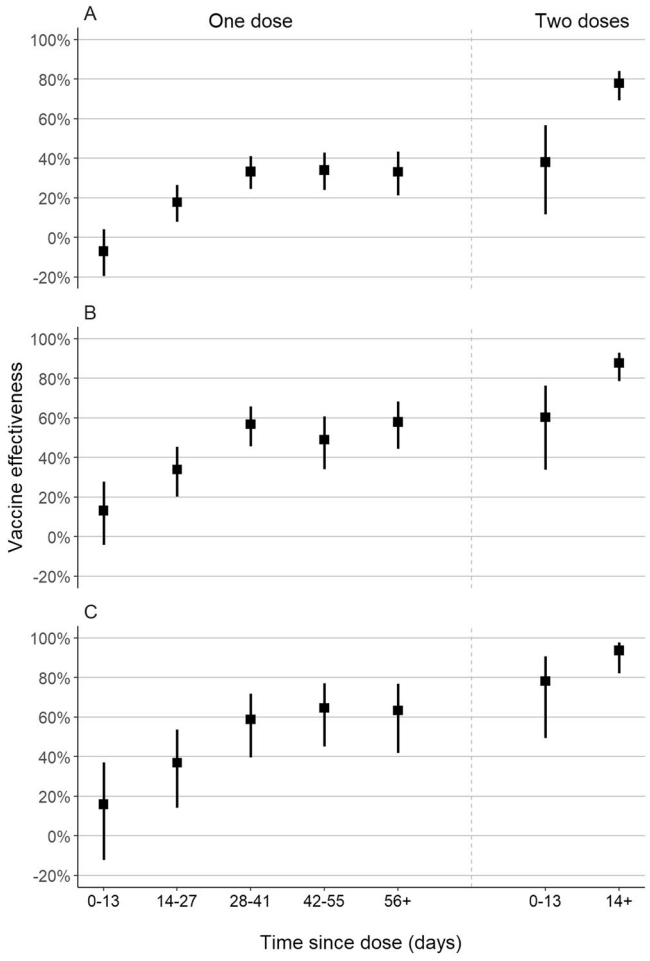


Fig. 3 ChAdOx1 vaccine effectiveness by dose. Adjusted vaccine effectiveness (squares) and 95% confidence intervals (lines) of one and two doses of ChAdOx1, by time since vaccination, against symptomatic Covid-19 (**A**), Covid-19 hospitalization (**B**), and Covid-19-related death (**C**) among matched case-control pairs ($n = 61{,}360$ RT-PCR tests). Source data are provided as a Source Data file.

genotyped isolates deposited in the GISAID database[11]. The STROBE checklist is shown in Supplementary Table 13. The protocol, including statistical analysis plan, further details of study design, and power calculations, is publicly available[33].

Study population and design. The study population was adults ≥60 years of age who had a residential address in São Paulo State and complete and consistent information between data sources on age, sex, residence, and vaccination and testing status and dates. We selected cases who had an ARI, received a positive SARS-CoV-2 RT-PCR test during the study period of 17 January 2021 to 2 July 2021 with sample collection date within 10 days after symptom onset, and without a positive SARS-CoV-2 RT-PCR test in the previous 90 days. We selected test-negative controls who had an ARI, received a negative SARS-CoV-2 RT-PCR test during the study period with sample collection date within 10 days after symptom onset, and without a positive SARS-CoV-2 RT-PCR test in the previous 90 days or following 14 days. Cases and controls who had received a dose of another Covid-19 vaccine before their RT-PCR test were excluded. We matched one control to each case by date of RT-PCR testing (±3 days), age (in 5-year bands), sex, self-reported race (brown, black, yellow, white, or indigenous)[34], municipality of residence, and prior ARI (defined as at least one previous symptomatic event that was reported to surveillance systems between 1 February 2020 and 16 January 2021). Each control RT-PCR test could serve as a control for only a single case. To assess the effect of different matching schemes, we performed two sensitivity analyses on the primary results: we matched controls with replacement (so that each control RT-PCR test could be matched to multiple cases), and we matched two controls to each case, with replacement.

To support the analysis of effectiveness against severe outcomes, we additionally estimated effectiveness of the vaccine against progression to severe outcomes among individuals with Covid-19. From the same surveillance database, we

selected individuals into a cohort who had an ARI with symptom onset date and sample collection date between 17 January 2021 and 4 June 2021 (28 days before the end of the study period, to allow for reporting of severe outcomes in recently infected individuals), received a positive SARS-CoV-2 RT-PCR or rapid antigen test within 14 days of symptom onset, and were otherwise eligible members of the study population.

Outcomes and covariates. We estimated the effectiveness of ChAdOx1 against the primary outcome of symptomatic Covid-19 during the period ≥28 days after a single vaccine dose, and 0–13 and ≥14 days after two vaccine doses. Furthermore, we estimated the effectiveness of a single dose during the period 14–27 days after the first dose to understand the onset of protection, and in the period 0–13 days, when the vaccine has no or limited effectiveness[17]. An association during this period may serve as a negative control period to detect unmeasured confounding in the effectiveness estimate in later time periods[31]. In a secondary analysis, we estimated vaccine effectiveness following the first dose in the time windows 28–41 days, 42–55 days, and ≥56 days separately. The reference group for vaccination status was individuals who had not received a first vaccine dose before the date of sample collection.

In addition, we estimated vaccine effectiveness against secondary outcomes of Covid-19 hospitalization, ICU admission with Covid-19, mechanical ventilation for Covid-19, and Covid-19-related death. We estimated single-dose effectiveness during the period ≥28 days after the first dose for all outcomes within subgroups defined by age (60–69 years vs. ≥70 years), sex, number of chronic comorbidities (none vs. at least one), reported cardiovascular disease, reported diabetes (the two most common reported comorbidities), and region of residence ("Grande São Paulo" health region vs. others). For the analysis among test-positive individuals, we analyzed the effectiveness of the vaccine against progression to hospitalization within 21 days of symptom onset, ICU admission within 21 days of symptom onset, and against death within 28 days of symptom onset.

Statistics. We performed conditional logistic regression to estimate vaccine effectiveness for each time window following vaccination, accounting for the matched design. Multivariable models were adjusted for the number of reported comorbidities (categorized as none, one-two, and at least three), previous positive SARS-CoV-2 RT-PCR or antigen test, and age as a continuous variable because we used 5-year age bands as a matching factor. For each outcome, we selected matched pairs in which cases had the outcome of interest, and fit the model described above to each subset. For severe outcomes, controls therefore represented test-negative patients from ambulatory and hospital settings who received RT-PCR testing. Finally, we conducted a Cox proportional hazards model to estimate effectiveness against progression within test-positive individuals. To account for variation in incidence and hospitalization practices by time and across the state, we stratified the baseline hazard by week of symptom onset and municipality of residence, and additionally adjusted for the matching factors and additional covariates listed above.

Our protocol specified that we would conduct proposed analyses after achieving ≥80% power to identify a vaccine effectiveness of 40% against symptomatic Covid-19 ≥28 days after a single dose of ChAdOx1, and 80% power to identify 50% effectiveness of two doses ≥14 days after the second dose. The power was estimated by fitting conditional logistic regressions on 1000 simulated datasets. After extracting the surveillance databases on July 9 2021 and generating matched case–control pairs, we determined that the power of the study was >99.8% for each analysis and performed the pre-specified analyses. All data processing and analyses were performed in R, version 4.0.2.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Deidentified analysis data sets generated from the surveillance and vaccine registry databases are available in the Github repository https://github.com/juliocroda/VebraCOVID-19[33]. Source data are provided as a Source Data file. For Fig. 1, vaccine data was obtained from OpenDataSUS (https://opendatasus.saude.gov.br/, access date 2021-07-09) and variant data from GISAID (https://www.gisaid.org/hcov19-variants/, access date 2021-07-07). Source data are provided with this paper.

Code availability

Code used to perform power calculations, conduct statistical analysis, and produce figures is available in the repository https://github.com/juliocroda/VebraCOVID-19[33].

Received: 10 September 2021; Accepted: 8 October 2021;
Published online: 28 October 2021

References

1. Polack, F. P. et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2021).
2. Baden, L. R. et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N. Engl. J. Med.* **384**, 403–416 (2021).
3. Dagan, N. et al. BNT162b2 mRNA covid-19 vaccine in a Nationwide mass vaccination setting. *N. Engl. J. Med.* **384**, 1–12 (2021).
4. Voysey, M. et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* **397**, 99–111 (2021).
5. Voysey, M. et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* **397**, 881–891 (2021).
6. Iacobucci, G. & Mahase, E. Covid-19 vaccination: What's the evidence for extending the dosing interval? *BMJ* **372**, n18 (2021).
7. World Health Organization. *COVID-19 Vaccine ChAdOx1-S [recombinant]* (WHO, 2021).
8. Krause, P. R. et al. SARS-CoV-2 variants and vaccines. *N. Engl. J. Med.* **385**, 179–186 (2021).
9. Bernal, J. L. et al. Effectiveness of covid-19 vaccines against the B.1.617.2 (Delta) variant. *N. Engl. J. Med.* **385**, 585–594 (2021).
10. Chung, H. et al. Effectiveness of BNT162b2 and mRNA-1273 covid-19 vaccines against symptomatic SARS-CoV-2 infection and severe covid-19 outcomes in Ontario, Canada: test negative design study. *BMJ* **374**, n1943 (2021).
11. GISAID. hCoV-19 Variants [Internet]. https://www.gisaid.org/hcov19-variants/ (2021).
12. Faria, N. R. et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science* **372**, 815–821 (2021).
13. Naveca, F. G. et al. COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nat. Med.* **27**, 1230–1238 (2021).
14. SEADE Foundation—São Paulo State. CORONAVIRUS—CASOS EM SP. Fundação SEADE. [Internet]. https://www.seade.gov.br/coronavirus/ (2021).
15. Palacios, R., Batista, A. P. & Albuquerque, C. S. N. Efficacy and safety of a COVID-19 inactivated vaccine in healthcare professionals in Brazil: the PROFISCOV study. *SSRN Electron J.* 3822780, https://doi.org/10.2139/ssrn.3822780 (2021).
16. Brazilian Ministry of Health. Campanha Nacional de Vacinação contra a Covid-19. Décimo oitavo informe técnico. [Internet]. https://www.conasems.org.br/wp-content/uploads/2021/04/20a-distribuição_Campanha_Nacional_de_Vacinacao_contra_a_Cov-id_19.pdf (2021).
17. Bernal, J. L. et al. Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. *BMJ* **373**, n1088 (2021).
18. Vasileiou, E. et al. Interim findings from first-dose mass COVID-19 vaccination roll-out and COVID-19 hospital admissions in Scotland: a national prospective cohort study. *Lancet* **397**, 1646–1657 (2021).
19. Hyams, C. et al. Effectiveness of BNT162b2 and ChAdOx1 nCoV-19 COVID-19 vaccination at preventing hospitalisations in people aged at least 80 years: a test-negative, case-control study. *Lancet Infect. Dis.* **63**, 1–9 (2021).
20. Jangra, S. et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. *Lancet Microbe.* **2**, e283–e284 (2021).
21. Souza, W. M. et al. Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. *Lancet Microbe* https://doi.org/10.1016/S2666-5247(21)00129-4 (2021).
22. Collier, D. A. et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature* **596**, 1–6 (2021).
23. Madhi, S. A. et al. Efficacy of the ChAdOx1 nCoV-19 Covid-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* **384**, 1885–1898 (2021).
24. Skowronski, D. M. et al. Single-dose mRNA vaccine effectiveness against SARS-CoV-2, including Alpha and Gamma variants: a test-negative design in adults 70 years and older in British Columbia, Canada. *Clin. Infect. Dis.* https://doi.org/10.1093/cid/ciab616 (2021).
25. Ranzani, O. T. et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. *BMJ* **374**, n2015 (2021).
26. Matrajt, L., Eaton, J., Swan, D. A. & Janes, H. Optimizing vaccine allocation for COVID-19 vaccines shows the potential role of single-dose vaccination. *Nat. Commun.* **12**, 1–18 (2021).
27. Romero-Brufau, S. et al. Public health impact of delaying second dose of BNT162b2 or mRNA-1273 covid-19 vaccine: simulation agent based modeling study. *BMJ* **373**, 1–9 (2021).

28. Moghadas, S. M. et al. Evaluation of COVID-19 vaccination strategies with a delayed second dose. *PLoS Biol.* **19**, 1–13 (2021).
29. Sullivan, S. G., Tchetgen, E. J. & Cowling, B. J. Theoretical basis of the test-negative study design for assessment of influenza vaccine effectiveness. *Am. J. Epidemiol.* **184**, 345–353 (2016).
30. Lewnard, J. A. et al. Theoretical framework for retrospective studies of the effectiveness of SARS-CoV-2 vaccines. *Epidemiology* **32**, 508–517 (2021).
31. Hitchings, M. D. T. et al. Use of recently vaccinated individuals to detect bias in test-negative case–control studies of COVID-19 vaccine effectiveness. Preprint at *medRxiv* (2021).
32. Hitchings, M. D. T. et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: a test-negative case–control study. *Lancet Reg. Health* **1**, 1–9 (2021).
33. Hitchings, M. D. T., Ranzani, O. T. & Croda, J. Study code [Internet]. https://doi.org/10.5281/zenodo.5542320 (2021).
34. Brazilian Insitute of Geography and Statistics—IBGE. Ethnic and racial characteristics of the population. Classifications and identities [Internet]. https://biblioteca.ibge.gov.br/visualizacao/livros/liv63405.pdf (2013).
35. de Araujo Freire, F. H., Gonzaga, M. R. & Gomes, M. M. F. Projeções populacionais por sexo e idade para pequenas áreas no Brasil. *RELAP* **14**, 124–149 (2019).

Acknowledgements

We are grateful for the Pan American Health Organization's support and the São Paulo State in making the databases available for analysis. J.C. is supported by the Oswaldo Cruz Foundation (Edital Covid-19—resposta rápida: 48111668950485). O.T.R. is funded by a Sara Borrell fellowship (CD19/00110) from the Instituto de Salud Carlos III. OTR acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019–2023 Program and from the Generalitat de Catalunya through the CERCA Program.

Author contributions

All authors conceived the study. J.R.A., D.A.T.C., A.I.K., and J.C. contributed equally. O.T.R., M.D.T.H., and M.D. completed analyses with guidance from J.R.A., D.A.T.C., A.I.K., and J.C. M.S.S.T., S.B.O., O.F.P.P., O.T.R., and M.D.T.H. curated and validated the data. OTR and MDTH wrote the first draft of the manuscript. T.L.D., R.C.P., O.F.P.P., E.F.M.V., M.A., R.S., J.C.G., and W.N.A. provided supervision. All authors, including W.S., R.D.O., and P.V.S., contributed to, and approved, the final manuscript. J.C. is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Ethical approval

The study was approved by the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). We obtained local IRB approval to waive consent. This was possible because the anonymized data bases from the surveillance system were sent to us only after the linkage and did not allow identification of the study participants. The second cohort was selected from within the surveillance data bases described, and did not involve any further prospective follow-up or data collection.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work. All funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The Health Secretary of State of São Paulo and PRODESP reviewed the data and findings of the study, but the academic authors retained editorial control. O.T.R., M.D.T.H., M.S.S.T., O.F.F.P., and J.C. had full access to de-identified data in the study and OTR and M.D.T.H. verified the data, and all authors approved the final version of the manuscript for publication.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-021-26459-6.

Correspondence and requests for materials should be addressed to Julio Croda.

Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

6 CONCLUSÕES

Entre os pacientes hospitalizados com COVID-19 confirmada no estado de São Paulo até outubro de 2020, foi observado que homens, idosos e aqueles com doenças cardiovasculares crônicas, incluindo hipertensão, doença pulmonar crônica, diabetes mellitus e doença neurológica crônica, apresentaram maior risco de óbito pela doença. Além disso, aproximadamente um terço dos pacientes evoluíram para doença grave ou óbito e a proporção de óbitos aumentou proporcionalmente com o aumento da idade.

A efetividade das vacinas CoronaVac (Sinovac) e ChAdOx1 (AstraZeneca) foram analisadas na população idosa do estado de São Paulo durante ampla circulação da variante Gama. A CoronaVac foi efetiva após a duas doses contra a COVID-19 sintomática, hospitalização e óbito e a efetividade aumentou progressivamente com o passar dos dias após a aplicação das doses. Entretanto, a CoronaVac não apresentou efetividade significativa após uma dose contra nenhum dos desfechos, foi menor em pessoas que relataram ter diabetes e diminuiu com o aumento da idade.

Diferente da CoronaVac, a ChAdOx1 foi efetiva após a aplicação de uma dose contra os desfechos graves como hospitalização, admissão em UTI, utilização de ventilação mecânica invasiva (VMI) e óbito, mas não apresentou efetividade significativa contra a COVID-19 sintomática. Após a aplicação de duas doses de ChAdOx1 foi observado aumento significativo da proteção contra todos os desfechos. Menor efetividade foi observada com o avanço da idade e em pessoas que relataram comorbidades.

Não foi possível analisar a efetividade das vacinas contra casos de COVID-19 causados pela variante Gama e não-Gama, uma vez que dados do sequenciamento individual não estavam disponíveis. A proporção da população soropositiva antes do período do estudo pode ter ocasionado viés e diminuição da estimativa de efetividade. Os achados de efetividade das vacinas na população idosa não podem ser extrapolados para a população mais jovem.

Os resultados dos estudos mostraram que as populações em maiores riscos de desfechos graves necessitam de intervenções prioritárias para a redução da morbidade e mortalidade causadas pela COVID-19. Ademais, mostraram a importância do esquema completo de duas doses na proteção contra a doença e

forneceu dados aos gestores para conduzirem a distribuição das vacinas, especialmente no contexto de oferta limitada dos imunizantes e emergência de transmissão de novas variantes. Por fim, evidenciou que a vacinação é fundamental para o controle e fim da pandemia.

REFERÊNCIAS

1. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. abril de 2020;5(4):536–44.
2. World Health Organization. Novel Coronavirus (2019-nCoV): situation report, 22 [Internet]. World Health Organization; 2020 fev [citado 21 de abril de 2022]. Disponível em: https://apps.who.int/iris/handle/10665/330991
3. First cases of coronavirus disease (COVID-19) in Brazil, South America (2 genomes, 3rd March 2020) - SARS-CoV-2 coronavirus / Genome Reports [Internet]. Virological. 2020 [citado 21 de abril de 2022]. Disponível em: https://virological.org/t/first-cases-of-coronavirus-disease-covid-19-in-brazil-south-america-2-genomes-3rd-march-2020/409
4. Coronavírus Brasil [Internet]. [citado 5 de novembro de 2020]. Disponível em: https://covid.saude.gov.br/
5. O'Driscoll M, Ribeiro Dos Santos G, Wang L, Cummings DAT, Azman AS, Paireau J, et al. Age-specific mortality and immunity patterns of SARS-CoV-2. Nature. fevereiro de 2021;590(7844):140–5.
6. Dessie ZG, Zewotir T. Mortality-related risk factors of COVID-19: a systematic review and meta-analysis of 42 studies and 423,117 patients. BMC Infectious Diseases. 21 de agosto de 2021;21(1):855.
7. Ranzani OT, Bastos LSL, Gelli JGM, Marchesi JF, Baião F, Hamacher S, et al. Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. The Lancet Respiratory Medicine. 1º de abril de 2021;9(4):407–18.
8. COVID-19 vaccine tracker and landscape [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines
9. Palacios R, Batista AP, Albuquerque CSN, Patiño EG, Santos J do P, Tilli Reis Pessoa Conde M, et al. Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study [Internet]. Rochester, NY: Social Science Research Network; 2021 abr [citado 21 de abril de 2022]. Report No.: 3822780. Disponível em: https://papers.ssrn.com/abstract=3822780
10. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. The Lancet. 9 de janeiro de 2021;397(10269):99–111.
11. Ministério da Saúde distribui mais de 5,3 milhões de vacinas Covid-19 para todo o Brasil [Internet]. Ministério da Saúde. [citado 21 de abril de 2022]. Disponível em: https://www.gov.br/saude/pt-br/assuntos/noticias/2022/marco/ministerio-da-saude-distribui-mais-de-5-3-milhoes-de-vacinas-covid-19-para-todo-o-brasil-1
12. Krause PR, Fleming TR, Longini IM, Peto R, Briand S, Heymann DL, et al. SARS-CoV-2 Variants and Vaccines. N Engl J Med. 8 de julho de 2021;385(2):179–86.
13. Naveca FG, Nascimento V, de Souza VC, Corado A de L, Nascimento F, Silva G, et al. COVID-19 in Amazonas, Brazil, was driven by the persistence of

endemic lineages and P.1 emergence. Nat Med. julho de 2021;27(7):1230–8.
14. Bastos LS, Ranzani OT, Souza TML, Hamacher S, Bozza FA. COVID-19 hospital admissions: Brazil's first and second waves compared. The Lancet Respiratory Medicine. 1º de agosto de 2021;9(8):e82–3.
15. Jangra S, Ye C, Rathnasinghe R, Stadlbauer D, Krammer F, Simon V, et al. SARS-CoV-2 spike E484K mutation reduces antibody neutralisation. Lancet Microbe. julho de 2021;2(7):e283–4.
16. Dashboard [Internet]. Genomahcov - Fiocruz. [citado 21 de abril de 2022]. Disponível em: http://www.genomahcov.fiocruz.br/dashboard/
17. Kunal S, Aditi, Gupta K, Ish P. COVID-19 variants in India: Potential role in second wave and impact on vaccination. Heart & Lung: The Journal of Cardiopulmonary and Acute Care. 1º de novembro de 2021;50(6):784–7.
18. Hitchings MDT, Ranzani OT, Torres MSS, de Oliveira SB, Almiron M, Said R, et al. Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: A test-negative case-control study. Lancet Reg Health Am. setembro de 2021;1:100025.
19. King AM, Lefkowitz E, Adams MJ, Carstens EB. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier; 2011. 1462 p.
20. Taxonomy [Internet]. [citado 22 de abril de 2022]. Disponível em: https://talk.ictvonline.org/taxonomy/
21. Cascella M, Rajnik M, Aleem A, Dulebohn SC, Di Napoli R. Features, Evaluation, and Treatment of Coronavirus (COVID-19). Em: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 [citado 22 de abril de 2022]. Disponível em: http://www.ncbi.nlm.nih.gov/books/NBK554776/
22. Corman VM, Muth D, Niemeyer D, Drosten C. Chapter Eight - Hosts and Sources of Endemic Human Coronaviruses. Em: Kielian M, Mettenleiter TC, Roossinck MJ, organizadores. Advances in Virus Research [Internet]. Academic Press; 2018 [citado 22 de abril de 2022]. p. 163–88. Disponível em: https://www.sciencedirect.com/science/article/pii/S0065352718300010
23. Hamre D, Procknow JJ. A New Virus Isolated from the Human Respiratory Tract. Experimental Biology and Medicine. 1º de janeiro de 1966;121(1):190–3.
24. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. Proc Natl Acad Sci USA. abril de 1967;57(4):933–40.
25. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol. junho de 2009;7(6):439–50.
26. van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJM, Wolthers KC, et al. Identification of a new human coronavirus. Nat Med. abril de 2004;10(4):368–73.
27. Characterization and Complete Genome Sequence of a Novel Coronavirus, Coronavirus HKU1, from Patients with Pneumonia | Journal of Virology [Internet]. [citado 22 de abril de 2022]. Disponível em: https://journals.asm.org/doi/10.1128/JVI.79.2.884-895.2005
28. Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome | NEJM [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nejm.org/doi/full/10.1056/nejmoa030747
29. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus ADME, Fouchier RAM. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia.

New England Journal of Medicine. 8 de novembro de 2012;367(19):1814–20.
30. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, et al. A Novel Coronavirus Associated with Severe Acute Respiratory Syndrome. N Engl J Med. 15 de maio de 2003;348(20):1953–66.
31. May RM, McLean AR, Pattison J, Weiss RA, Anderson RM, Fraser C, et al. Epidemiology, transmission dynamics and control of SARS: the 2002–2003 epidemic. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences. 29 de julho de 2004;359(1447):1091–105.
32. Peeri NC, Shrestha N, Rahman MS, Zaki R, Tan Z, Bibi S, et al. The SARS, MERS and novel coronavirus (COVID-19) epidemics, the newest and biggest global health threats: what lessons have we learned? International Journal of Epidemiology. 1º de junho de 2020;49(3):717–26.
33. Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. Virology Journal. 22 de dezembro de 2015;12(1):222.
34. Al-Omari A, Rabaan AA, Salih S, Al-Tawfiq JA, Memish ZA. MERS coronavirus outbreak: Implications for emerging viral infections. Diagnostic Microbiology and Infectious Disease. 1º de março de 2019;93(3):265–85.
35. Middle East respiratory syndrome coronavirus (MERS-CoV) [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/health-topics/middle-east-respiratory-syndrome-coronavirus-mers#tab=tab_1
36. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. abril de 2020;26(4):450–2.
37. WHO-convened global study of origins of SARS-CoV-2: China Part [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/publications-detail-redirect/who-convened-global-study-of-origins-of-sars-cov-2-china-part
38. Zhang T, Wu Q, Zhang Z. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak. Curr Biol. 6 de abril de 2020;30(7):1346-1351.e2.
39. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW, et al. SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020. Euro Surveill. 11 de junho de 2020;25(23):2001005.
40. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application | Annals of Internal Medicine [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.acpjournals.org/doi/10.7326/M20-0504
41. Jansen L. Investigation of a SARS-CoV-2 B.1.1.529 (Omicron) Variant Cluster — Nebraska, November–December 2021. MMWR Morb Mortal Wkly Rep [Internet]. 2021 [citado 22 de abril de 2022];70. Disponível em: https://www.cdc.gov/mmwr/volumes/70/wr/mm705152e3.htm
42. Clinical Spectrum [Internet]. COVID-19 Treatment Guidelines. [citado 22 de abril de 2022]. Disponível em: https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/
43. Meyer NJ, Gattinoni L, Calfee CS. Acute respiratory distress syndrome. The Lancet. 14 de agosto de 2021;398(10300):622–37.
44. The ARDS Definition Task Force*. Acute Respiratory Distress Syndrome: The Berlin Definition. JAMA. 20 de junho de 2012;307(23):2526–33.
45. Martinez-Rojas MA, Vega-Vega O, Bobadilla NA. Is the kidney a target of SARS-CoV-2? Am J Physiol Renal Physiol. 1º de junho de 2020;318(6):F1454–62.
46. Gupta A, Madhavan MV, Sehgal K, Nair N, Mahajan S, Sehrawat TS, et al.

Extrapulmonary manifestations of COVID-19. Nat Med. julho de 2020;26(7):1017–32.
47. Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, et al. Guillain–Barré Syndrome Associated with SARS-CoV-2. N Engl J Med. 25 de junho de 2020;382(26):2574–6.
48. Daneshgaran G, Dubin DP, Gould DJ. Cutaneous Manifestations of COVID-19: An Evidence-Based Review. Am J Clin Dermatol. 31 de agosto de 2020;1–13.
49. CDC. Coronavirus Disease 2019 (COVID-19) [Internet]. Centers for Disease Control and Prevention. 2020 [citado 22 de abril de 2022]. Disponível em: https://www.cdc.gov/coronavirus/2019-ncov/science/science-briefs/sars-cov-2-transmission.html
50. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. N Engl J Med. 17 de março de 2020;NEJMc2004973.
51. Riddell S, Goldie S, Hill A, Eagles D, Drew TW. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J. 7 de outubro de 2020;17:145.
52. Kotlyar AM, Grechukhina O, Chen A, Popkhadze S, Grimshaw A, Tal O, et al. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. Am J Obstet Gynecol. janeiro de 2021;224(1):35-53.e3.
53. Pedersen RM, Tornby DS, Bang LL, Madsen LW, Skov MN, Jensen TG, et al. Rectally shed SARS-CoV-2 lacks infectivity: time to rethink faecal–oral transmission? Nat Rev Gastroenterol Hepatol. setembro de 2021;18(9):669–669.
54. An evidence review of face masks against COVID-19 | PNAS [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.pnas.org/doi/10.1073/pnas.2014564118
55. Handwashing an effective tool to prevent COVID-19, other diseases [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/southeastasia/news/detail/15-10-2020-handwashing-an-effective-tool-to-prevent-covid-19-other-diseases
56. Changes in contact patterns shape the dynamics of the COVID-19 outbreak in China [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.science.org/doi/10.1126/science.abb8001
57. CDC. Masks and Respirators [Internet]. Centers for Disease Control and Prevention. 2020 [citado 22 de abril de 2022]. Disponível em: https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/about-face-coverings.html
58. Regmi K, Lwin CM. Factors Associated with the Implementation of Non-Pharmaceutical Interventions for Reducing Coronavirus Disease 2019 (COVID-19): A Systematic Review. International Journal of Environmental Research and Public Health. janeiro de 2021;18(8):4274.
59. Thanh Le T, Andreadakis Z, Kumar A, Gómez Román R, Tollefsen S, Saville M, et al. The COVID-19 vaccine development landscape. Nature Reviews Drug Discovery. 9 de abril de 2020;19(5):305–6.
60. Moghadas SM, Vilches TN, Zhang K, Wells CR, Shoukat A, Singer BH, et al. The Impact of Vaccination on Coronavirus Disease 2019 (COVID-19) Outbreaks in the United States. Clinical Infectious Diseases. 15 de dezembro de 2021;73(12):2257–64.

61. Böger B, Fachi MM, Vilhena RO, Cobre AF, Tonin FS, Pontarolo R. Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19. American Journal of Infection Control. 1º de janeiro de 2021;49(1):21–9.
62. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection - Dinnes, J - 2021 | Cochrane Library [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub2/full
63. Antigen-detection in the diagnosis of SARS-CoV-2 infection [Internet]. [citado 16 de maio de 2022]. Disponível em: https://www.who.int/publications-detail-redirect/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays
64. Medicamentos aprovados [Internet]. Agência Nacional de Vigilância Sanitária - Anvisa. [citado 22 de abril de 2022]. Disponível em: https://www.gov.br/anvisa/pt-br/assuntos/paf/coronavirus/medicamentos/medicamentos-aprovados
65. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.
66. Day M. Covid-19: four fifths of cases are asymptomatic, China figures indicate. BMJ. 2 de abril de 2020;369:m1375.
67. Kronbichler A, Kresse D, Yoon S, Lee KH, Effenberger M, Shin JI. Asymptomatic patients as a source of COVID-19 infections: A systematic review and meta-analysis. International Journal of Infectious Diseases. 1º de setembro de 2020;98:180–6.
68. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 7 de abril de 2020;323(13):1239–42.
69. Coronavirus Disease (COVID-19) Situation Reports [Internet]. [citado 5 de novembro de 2020]. Disponível em: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
70. IHR Emergency Committee on Novel Coronavirus (2019-nCoV) [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/director-general/speeches/detail/who-director-general-s-statement-on-ihr-emergency-committee-on-novel-coronavirus-(2019-ncov)
71. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. Acta Biomed. 2020;91(1):157–60.
72. Histórico da pandemia de COVID-19 - OPAS/OMS | Organização Pan-Americana da Saúde [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.paho.org/pt/covid19/historico-da-pandemia-covid-19
73. WHO Coronavirus (COVID-19) Dashboard [Internet]. [citado 22 de abril de 2022]. Disponível em: https://covid19.who.int
74. COVID-19 hospitalization and emergency department statistics | CIHI [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.cihi.ca/en/covid-19-hospitalization-and-emergency-department-statistics
75. Mortality Among US Patients Hospitalized With SARS-CoV-2 Infection in 2020 | Infectious Diseases | JAMA Network Open | JAMA Network [Internet]. [citado 22 de abril de 2022]. Disponível em:

https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2778237
76. Zimmermann IR, Sanchez MN, Frio GS, Alves LC, Pereira CC de A, Lima RT de S, et al. Trends in COVID-19 case-fatality rates in Brazilian public hospitals: A longitudinal cohort of 398,063 hospital admissions from 1st March to 3rd October 2020. PLOS ONE. 16 de julho de 2021;16(7):e0254633.
77. Auld SC, Harrington KRV, Adelman MW, Robichaux CJ, Overton EC, Caridi-Scheible M, et al. Trends in ICU Mortality From Coronavirus Disease 2019: A Tale of Three Surges. Crit Care Med. 1º de fevereiro de 2022;50(2):245–55.
78. Candido DDS, Watts A, Abade L, Kraemer MUG, Pybus OG, Croda J, et al. Routes for COVID-19 importation in Brazil. J Travel Med [Internet]. 18 de maio de 2020 [citado 5 de novembro de 2020];27(3). Disponível em: https://academic.oup.com/jtm/article/27/3/taaa042/5809508
79. SciELO - Brazil - COVID-19 in Brazil: advantages of a socialized unified health system and preparation to contain cases COVID-19 in Brazil: advantages of a socialized unified health system and preparation to contain cases [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.scielo.br/j/rsbmt/a/bwLKCs6ZfGhyFn3mp4RDhdQ
80. Boletins Epidemiológicos COVID-19 [Internet]. Ministério da Saúde. [citado 22 de abril de 2022]. Disponível em: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/boletins-epidemiologicos/covid-19/boletins-epidemiologicos-covid-19
81. Zeiser FA, Donida B, da Costa CA, Ramos G de O, Scherer JN, Barcellos NT, et al. First and second COVID-19 waves in Brazil: A cross-sectional study of patients' characteristics related to hospitalization and in-hospital mortality. The Lancet Regional Health - Americas. 1º de fevereiro de 2022;6:100107.
82. Candido DS, Claro IM, de Jesus JG, Souza WM, Moreira FRR, Dellicour S, et al. Evolution and epidemic spread of SARS-CoV-2 in Brazil. Science. 23 de julho de 2020;eabd2161.
83. SARS-CoV-2 reinfection by the new Variant of Concern (VOC) P.1 in Amazonas, Brazil - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology [Internet]. Virological. 2021 [citado 22 de abril de 2022]. Disponível em: https://virological.org/t/sars-cov-2-reinfection-by-the-new-variant-of-concern-voc-p-1-in-amazonas-brazil/596/2
84. Barreto IC de HC, Costa Filho RV, Ramos RF, Oliveira LG de, Martins NRAV, Cavalcante FV, et al. Health collapse in Manaus: the burden of not adhering to non-pharmacological measures to reduce the transmission of Covid-19. Saúde debate. 8 de dezembro de 2021;45:1126–39.
85. MonitoraCovid-19 [Internet]. [citado 22 de abril de 2022]. Disponível em: https://bigdata-covid19.icict.fiocruz.br/
86. Mediana de idade de internações por Covid-19 fica abaixo de 60 anos pela primeira vez [Internet]. Fiocruz. [citado 22 de abril de 2022]. Disponível em: https://portal.fiocruz.br/noticia/mediana-de-idade-de-internacoes-por-covid-19-fica-abaixo-de-60-anos-pela-primeira-vez
87. Brazil - COVID-19 Overview - Johns Hopkins [Internet]. Johns Hopkins Coronavirus Resource Center. [citado 22 de abril de 2022]. Disponível em: https://coronavirus.jhu.edu/region/brazil
88. Plano Nacional de Operacionalização da Vacinação contra Covid-19 - PNO [Internet]. Ministério da Saúde. [citado 22 de abril de 2022]. Disponível em: https://www.gov.br/saude/pt-br/coronavirus/vacinas/plano-nacional-de-operacionalizacao-da-vacina-contra-a-covid-19/plano-nacional-de-

operacionalizacao-da-vacinacao-contra-covid-19-pno
89. Victora CG, Castro MC, Gurzenda S, Medeiros AC, França GVA, Barros AJD. Estimating the early impact of vaccination against COVID-19 on deaths among elderly people in Brazil: Analyses of routinely-collected data on vaccine coverage and mortality. EClinicalMedicine. 1º de agosto de 2021;38:101036.
90. Early assessment of the clinical severity of the SARS-CoV-2 Omicron variant in South Africa | medRxiv [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.medrxiv.org/content/10.1101/2021.12.21.21268116v1
91. Lewnard JA, Hong VX, Patel MM, Kahn R, Lipsitch M, Tartof SY. Clinical outcomes among patients infected with Omicron (B.1.1.529) SARS-CoV-2 variant in southern California [Internet]. medRxiv; 2022 [citado 22 de abril de 2022]. p. 2022.01.11.22269045. Disponível em: https://www.medrxiv.org/content/10.1101/2022.01.11.22269045v2
92. Benchmarking - COVID-19 - UTIs Brasileiras [Internet]. [citado 22 de abril de 2022]. Disponível em: http://www.utisbrasileiras.com.br
93. Coronavirus Particle Assembly: Primary Structure Requirements of the Membrane Protein | Journal of Virology [Internet]. [citado 22 de abril de 2022]. Disponível em: https://journals.asm.org/doi/10.1128/JVI.72.8.6838-6850.1998
94. Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology, Immunology and Infection. 1º de abril de 2021;54(2):159–63.
95. Agrahari R, Mohanty S, Vishwakarma K, Nayak SK, Samantaray D, Mohapatra S. “Update vision on COVID-19: Structure, immune pathogenesis, treatment and safety assessment”. Sensors International. 1º de janeiro de 2021;2:100073.
96. Chan JFW, Kok KH, Zhu Z, Chu H, To KKW, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9(1):221–36.
97. Woo PCY, Huang Y, Lau SKP, Yuen KY. Coronavirus Genomics and Bioinformatics Analysis. Viruses. 24 de agosto de 2010;2(8):1804–20.
98. Beniac DR, Andonov A, Grudeski E, Booth TF. Architecture of the SARS coronavirus prefusion spike. Nat Struct Mol Biol. agosto de 2006;13(8):751–2.
99. Bosch BJ, van der Zee R, de Haan CAM, Rottier PJM. The Coronavirus Spike Protein Is a Class I Virus Fusion Protein: Structural and Functional Characterization of the Fusion Core Complex. J Virol. agosto de 2003;77(16):8801–11.
100. Fehr AR, Perlman S. Coronaviruses: An Overview of Their Replication and Pathogenesis. Em: Maier HJ, Bickerton E, Britton P, organizadores. Coronaviruses: Methods and Protocols [Internet]. New York, NY: Springer; 2015 [citado 22 de abril de 2022]. p. 1–23. (Methods in Molecular Biology). Disponível em: https://doi.org/10.1007/978-1-4939-2438-7_1
101. Armstrong J, Niemann H, Smeekens S, Rottier P, Warren G. Sequence and topology of a model intracellular membrane protein, E1 glycoprotein, from a coronavirus. Nature. 19 de abril de 1984;308(5961):751–2.
102. Nal B, Chan C, Kien F, Siu L, Tse J, Chu K, et al. Differential maturation and subcellular localization of severe acute respiratory syndrome coronavirus surface proteins S, M and E. J Gen Virol. maio de 2005;86(Pt 5):1423–34.
103. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. abril de 2011;174(1):11–22.

104. Nieto-Torres JL, DeDiego ML, Verdiá-Báguena C, Jimenez-Guardeño JM, Regla-Nava JA, Fernandez-Delgado R, et al. Severe Acute Respiratory Syndrome Coronavirus Envelope Protein Ion Channel Activity Promotes Virus Fitness and Pathogenesis. PLoS Pathog. 1º de maio de 2014;10(5):e1004077.
105. BOS ECW, LUYTJES W, MEULEN HVD, KOERTEN HK, SPAAN WJM. The Production of Recombinant Infectious DI-Particles of a Murine Coronavirus in the Absence of Helper Virus. Virology. 1º de abril de 1996;218(1):52–60.
106. Chang C ke, Sue SC, Yu T hung, Hsieh CM, Tsai CK, Chiang YC, et al. Modular organization of SARS coronavirus nucleocapsid protein. J Biomed Sci. 14 de outubro de 2005;13(1):59–72.
107. Kuo L, Masters PS. Functional Analysis of the Murine Coronavirus Genomic RNA Packaging Signal. J Virol. maio de 2013;87(9):5182–92.
108. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 16 de abril de 2020;181(2):271-280.e8.
109. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. Journal of Pharmaceutical Analysis. 1º de abril de 2020;10(2):102–8.
110. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell. 25 de junho de 2020;181(7):1489-1501.e15.
111. CDC. Labs [Internet]. Centers for Disease Control and Prevention. 2020 [citado 22 de abril de 2022]. Disponível em: https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html
112. Wang J, Jiang M, Chen X, Montaner LJ. Cytokine storm and leukocyte changes in mild versus severe SARS-CoV-2 infection: Review of 3939 COVID-19 patients in China and emerging pathogenesis and therapy concepts. J Leukoc Biol. 13 de junho de 2020;10.1002/JLB.3COVR0520-272R.
113. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Frontiers in Immunology [Internet]. 2020 [citado 22 de abril de 2022];11. Disponível em: https://www.frontiersin.org/article/10.3389/fimmu.2020.01446
114. Shimizu M. Clinical Features of Cytokine Storm Syndrome. Em: Cron RQ, Behrens EM, organizadores. Cytokine Storm Syndrome [Internet]. Cham: Springer International Publishing; 2019 [citado 22 de abril de 2022]. p. 31–41. Disponível em: http://link.springer.com/10.1007/978-3-030-22094-5_3
115. Contou D, Cally R, Sarfati F, Desaint P, Fraissé M, Plantefève G. Causes and timing of death in critically ill COVID-19 patients. Critical Care. 23 de fevereiro de 2021;25(1):79.
116. Teuwen LA, Geldhof V, Pasut A, Carmeliet P. COVID-19: the vasculature unleashed. Nat Rev Immunol. 2020;20(7):389–91.
117. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 9 de julho de 2020;383(2):120–8.
118. COVID-19 and the cardiovascular system | Nature Reviews Cardiology [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41569-020-0360-5
119. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of

138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA. 17 de março de 2020;323(11):1061–9.
120. Lippi G, Lavie CJ, Sanchis-Gomar F. Cardiac troponin I in patients with coronavirus disease 2019 (COVID-19): Evidence from a meta-analysis. Progress in Cardiovascular Diseases. 1º de maio de 2020;63(3):390–1.
121. Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study | Signal Transduction and Targeted Therapy [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41392-020-0148-4
122. Abou-Ismail MY, Diamond A, Kapoor S, Arafah Y, Nayak L. The hypercoagulable state in COVID-19: Incidence, pathophysiology, and management. Thromb Res. outubro de 2020;194:101–15.
123. Frontiers | Neuromechanisms of SARS-CoV-2: A Review | Frontiers in Neuroanatomy [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.frontiersin.org/articles/10.3389/fnana.2020.00037/full
124. Soltani Zangbar H, Gorji A, Ghadiri T. A Review on the Neurological Manifestations of COVID-19 Infection: a Mechanistic View. Mol Neurobiol. 1º de fevereiro de 2021;58(2):536–49.
125. Alenina N, Bader M. ACE2 in Brain Physiology and Pathophysiology: Evidence from Transgenic Animal Models. Neurochem Res. 1º de junho de 2019;44(6):1323–9.
126. Patel KP, Patel PA, Vunnam RR, Hewlett AT, Jain R, Jing R, et al. Gastrointestinal, hepatobiliary, and pancreatic manifestations of COVID-19. J Clin Virol. julho de 2020;128:104386.
127. Digestive system is a potential route of COVID-19: an analysis of single-cell coexpression pattern of key proteins in viral entry process | Gut [Internet]. [citado 22 de abril de 2022]. Disponível em: https://gut.bmj.com/content/69/6/1010
128. Ziemba R. Excess Death Estimates in Patients with End-Stage Renal Disease — United States, February–August 2020. MMWR Morb Mortal Wkly Rep [Internet]. 2021 [citado 22 de abril de 2022];70. Disponível em: https://www.cdc.gov/mmwr/volumes/70/wr/End-stage renal disease (ESRD) is a condition in which kidney function has permanently declined such that renal replacement therapy is required to sustain life.
129. Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies | BMJ Open [Internet]. [citado 22 de abril de 2022]. Disponível em: https://bmjopen.bmj.com/content/11/1/e044640.long
130. Saini KS, Tagliamento M, Lambertini M, McNally R, Romano M, Leone M, et al. Mortality in patients with cancer and coronavirus disease 2019: A systematic review and pooled analysis of 52 studies. Eur J Cancer. novembro de 2020;139:43–50.
131. Ssentongo P, Ssentongo AE, Heilbrunn ES, Ba DM, Chinchilli VM. Association of cardiovascular disease and 10 other pre-existing comorbidities with COVID-19 mortality: A systematic review and meta-analysis. PLoS One. 2020;15(8):e0238215.
132. Yang J, Hu J, Zhu C. Obesity aggravates COVID-19: A systematic review and meta-analysis. Journal of Medical Virology. 2021;93(1):257–61.
133. D-Interstitial-Lung-Disease-Review_Final.pdf.
134. Fond G, Nemani K, Etchecopar-Etchart D, Loundou A, Goff DC, Lee SW, et al. Association Between Mental Health Disorders and Mortality Among Patients

With COVID-19 in 7 Countries: A Systematic Review and Meta-analysis. JAMA Psychiatry. 1º de novembro de 2021;78(11):1208–17.
135. McClenaghan E, Cosgriff R, Brownlee K, Ahern S, Burgel PR, Byrnes CA, et al. The global impact of SARS-CoV-2 in 181 people with cystic fibrosis. Journal of Cystic Fibrosis. 1º de novembro de 2020;19(6):868–71.
136. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis. Int J Infect Dis. maio de 2020;94:91–5.
137. Lippi G, Henry BM. Chronic obstructive pulmonary disease is associated with severe coronavirus disease 2019 (COVID-19). Respiratory Medicine [Internet]. 1º de junho de 2020 [citado 22 de abril de 2022];167. Disponível em: https://www.resmedjournal.com/article/S0954-6111(20)30081-0/fulltext
138. Barron E, Bakhai C, Kar P, Weaver A, Bradley D, Ismail H, et al. Associations of type 1 and type 2 diabetes with COVID-19-related mortality in England: a whole-population study. The Lancet Diabetes & Endocrinology. 1º de outubro de 2020;8(10):813–22.
139. Khan MMA, Khan MN, Mustagir MG, Rana J, Islam MS, Kabir MI. Effects of underlying morbidities on the occurrence of deaths in COVID-19 patients: A systematic review and meta-analysis. J Glob Health. dezembro de 2020;10(2):020503.
140. Herman C, Mayer K, Sarwal A. Scoping review of prevalence of neurologic comorbidities in patients hospitalized for COVID-19. Neurology. 14 de julho de 2020;95(2):77–84.
141. Booth A, Reed AB, Ponzo S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: A global systematic review and meta-analysis. PLOS ONE. 4 de março de 2021;16(3):e0247461.
142. Stronger inflammatory/cytotoxic T-cell response in women identified by microarray analysis | Genes & Immunity [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/gene200912
143. Abdullah M, Chai PS, Chong MY, Tohit ERM, Ramasamy R, Pei CP, et al. Gender effect on in vitro lymphocyte subset levels of healthy individuals. Cellular Immunology. 1º de janeiro de 2012;272(2):214–9.
144. Palaiodimos L, Kokkinidis DG, Li W, Karamanis D, Ognibene J, Arora S, et al. Severe obesity, increasing age and male sex are independently associated with worse in-hospital outcomes, and higher in-hospital mortality, in a cohort of patients with COVID-19 in the Bronx, New York. Metabolism. julho de 2020;108:154262.
145. Iaccarino G, Grassi G, Borghi C, Ferri C, Salvetti M, Volpe M, et al. Age and Multimorbidity Predict Death Among COVID-19 Patients. Hypertension. agosto de 2020;76(2):366–72.
146. Cecí K, Torres lia de L, Patrí, Araú CD, Pereira J, Franç GS, et al. Immunosenescence. Geriatrics, Gerontology and Aging. 2011;5(3):163–9.
147. Yek C. Risk Factors for Severe COVID-19 Outcomes Among Persons Aged ≥18 Years Who Completed a Primary COVID-19 Vaccination Series — 465 Health Care Facilities, United States, December 2020–October 2021. MMWR Morb Mortal Wkly Rep [Internet]. 2022 [citado 22 de abril de 2022];71. Disponível em: https://www.cdc.gov/mmwr/volumes/71/wr/mm7101a4.htm
148. Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression - The Lancet [Internet]. [citado 22 de abril de 2022]. Disponível em:

https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)00152-0/fulltext
149. Romano SD. Trends in Racial and Ethnic Disparities in COVID-19 Hospitalizations, by Region — United States, March–December 2020. MMWR Morb Mortal Wkly Rep [Internet]. 2021 [citado 22 de abril de 2022];70. Disponível em: https://www.cdc.gov/mmwr/volumes/70/wr/mm7015e2.htm
150. Marinho MF, Torrens A, Teixeira R, Brant LCC, Malta DC, Nascimento BR, et al. Racial disparity in excess mortality in Brazil during COVID-19 times. European Journal of Public Health. 1º de fevereiro de 2022;32(1):24–6.
151. Disparities in COVID-19 Outcomes by Race, Ethnicity, and Socioeconomic Status: A Systematic Review and Meta-analysis | Health Disparities | JAMA Network Open | JAMA Network [Internet]. [citado 22 de abril de 2022]. Disponível em: https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2785980
152. Mah JC, Andrew MK. Social vulnerability indices: A pragmatic tool for COVID-19 policy and beyond. The Lancet Regional Health – Europe [Internet]. 1º de março de 2022 [citado 22 de abril de 2022];14. Disponível em: https://www.thelancet.com/journals/lanepe/article/PIIS2666-7762(22)00026-6/fulltext
153. Welsh CE, Sinclair DR, Matthews FE. Static Socio-Ecological COVID-19 Vulnerability Index and Vaccine Hesitancy Index for England. The Lancet Regional Health - Europe. 1º de março de 2022;14:100296.
154. COVID-19 in Health-Care Workers: A Living Systematic Review and Meta-Analysis of Prevalence, Risk Factors, Clinical Characteristics, and Outcomes | American Journal of Epidemiology | Oxford Academic [Internet]. [citado 22 de abril de 2022]. Disponível em: https://academic.oup.com/aje/article/190/1/161/5900120?login=false
155. Duprat IP, Melo GC de. Análise de casos e óbitos pela COVID-19 em profissionais de enfermagem no Brasil. Rev bras saúde ocup [Internet]. 9 de outubro de 2020 [citado 22 de abril de 2022];45. Disponível em: http://www.scielo.br/j/rbso/a/zvGPynQFqrnHkFW5VrqWYCt/?lang=pt
156. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis | The BMJ [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.bmj.com/content/370/bmj.m3320
157. Tracking SARS-CoV-2 variants [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
158. OMS anuncia nomenclaturas simples e fáceis de pronunciar para variantes de interesse e de preocupação do SARS-CoV-2 - OPAS/OMS | Organização Pan-Americana da Saúde [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.paho.org/pt/noticias/1-6-2021-oms-anuncia-nomenclaturas-simples-e-faceis-pronunciar-para-variantes-interesse-e
159. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology [Internet]. Virological. 2020 [citado 22 de abril de 2022]. Disponível em: https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563
160. Davies NG, Jarvis CI, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH.

Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature. maio de 2021;593(7858):270–4.
161. Claro IM, da Silva Sales FC, Ramundo MS, Candido DS, Silva CAM, de Jesus JG, et al. Local Transmission of SARS-CoV-2 Lineage B.1.1.7, Brazil, December 2020. Emerg Infect Dis. março de 2021;27(3):970–2.
162. Chen H, Huang X, Zhao X, Song Y, Hao P, Jiang H, et al. The First Case of New Variant COVID-19 Originating in the United Kingdom Detected in a Returning Student — Shanghai Municipality, China, December 14, 2020. China CDC Weekly. 2021;3(1):1–3.
163. Leung K, Shum MH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. Euro Surveill. janeiro de 2021;26(1).
164. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, et al. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell. 3 de setembro de 2020;182(5):1295-1310.e20.
165. Eurosurveillance | Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020 [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.26.1.2002106
166. Santos JC, Passos GA. The high infectivity of SARS-CoV-2 B.1.1.7 is associated with increased interaction force between Spike-ACE2 caused by the viral N501Y mutation [Internet]. bioRxiv; 2021 [citado 22 de abril de 2022]. p. 2020.12.29.424708. Disponível em: https://www.biorxiv.org/content/10.1101/2020.12.29.424708v1
167. Supasa P, Zhou D, Dejnirattisai W, Liu C, Mentzer AJ, Ginn HM, et al. Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. Cell. 15 de abril de 2021;184(8):2201-2211.e7.
168. McCarthy KR, Rennick LJ, Nambulli S, Robinson-McCarthy LR, Bain WG, Haidar G, et al. Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. Science. 12 de março de 2021;371(6534):1139–42.
169. Principais variantes do SARS-CoV-2 notificadas no Brasil [Internet]. Revista RBAC. [citado 22 de abril de 2022]. Disponível em: http://www.rbac.org.br/artigos/principais-variantes-do-sars-cov-2-notificadas-no-brasil/
170. Detection of a SARS-CoV-2 variant of concern in South Africa | Nature [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41586-021-03402-9
171. Cov-Lineages [Internet]. [citado 22 de abril de 2022]. Disponível em: https://cov-lineages.org/lineage.html?lineage=B.1.351
172. Slavov SN, Patané JSL, Bezerra R dos S, Giovanetti M, Fonseca V, Martins AJ, et al. Genomic monitoring unveil the early detection of the SARS-CoV-2 B.1.351 lineage (20H/501Y.V2) in Brazil [Internet]. medRxiv; 2021 [citado 22 de abril de 2022]. p. 2021.03.30.21254591. Disponível em: https://www.medrxiv.org/content/10.1101/2021.03.30.21254591v1
173. Gómez CE, Perdiguero B, Esteban M. Emerging SARS-CoV-2 Variants and Impact in Global Vaccination Programs against SARS-CoV-2/COVID-19. Vaccines (Basel). 11 de março de 2021;9(3):243.
174. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et

al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa [Internet]. medRxiv; 2020 [citado 22 de abril de 2022]. p. 2020.12.21.20248640. Disponível em: https://www.medrxiv.org/content/10.1101/2020.12.21.20248640v1

175. Nelson G, Buzko O, Spilman P, Niazi K, Rabizadeh S, Soon-Shiong P. Molecular dynamic simulation reveals E484K mutation enhances spike RBD-ACE2 affinity and the combination of E484K, K417N and N501Y mutations (501Y.V2 variant) induces conformational change greater than N501Y mutant alone, potentially resulting in an escape mutant [Internet]. bioRxiv; 2021 [citado 22 de abril de 2022]. p. 2021.01.13.426558. Disponível em: https://www.biorxiv.org/content/10.1101/2021.01.13.426558v1
176. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido D da S, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. Science. 21 de maio de 2021;372(6544):815–21.
177. Cov-Lineages [Internet]. [citado 22 de abril de 2022]. Disponível em: https://cov-lineages.org/lineage.html?lineage=P.1
178. Funk T, Pharris A, Spiteri G, Bundle N, Melidou A, Carr M, et al. Characteristics of SARS-CoV-2 variants of concern B.1.1.7, B.1.351 or P.1: data from seven EU/EEA countries, weeks 38/2020 to 10/2021. Euro Surveill. abril de 2021;26(16).
179. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19 | NEJM [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nejm.org/doi/full/10.1056/nejmoa2029849
180. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology [Internet]. Virological. 2021 [citado 22 de abril de 2022]. Disponível em: https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586
181. Golubchik T, Lythgoe KA, Hall M, Ferretti L, Fryer HR, MacIntyre-Cockett G, et al. Early analysis of a potential link between viral load and the N501Y mutation in the SARS-COV-2 spike protein [Internet]. medRxiv; 2021 [citado 22 de abril de 2022]. p. 2021.01.12.20249080. Disponível em: https://www.medrxiv.org/content/10.1101/2021.01.12.20249080v1
182. SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma | Nature Medicine [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41591-021-01285-x
183. Frontiers | Comparative Genomics and Characterization of SARS-CoV-2 P.1 (Gamma) Variant of Concern From Amazonas, Brazil | Medicine [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.frontiersin.org/articles/10.3389/fmed.2022.806611/full#B41
184. SARS-CoV-2 variants of concern are emerging in India | Nature Medicine [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41591-021-01397-4
185. Dhar MS, Marwal R, Radhakrishnan V, Ponnusamy K, Jolly B, Bhoyar RC, et al. Genomic characterization and Epidemiology of an emerging SARS-CoV-2 variant in Delhi, India. Science. 19 de novembro de 2021;374(6570):995–9.
186. Cov-Lineages [Internet]. [citado 22 de abril de 2022]. Disponível em: https://cov-lineages.org/lineage.html?lineage=B.1.617
187. Enhanced fusogenicity and pathogenicity of SARS-CoV-2 Delta P681R

mutation | Nature [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41586-021-04266-9
188. Di Giacomo S, Mercatelli D, Rakhimov A, Giorgi FM. Preliminary report on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Spike mutation T478K. Journal of Medical Virology. 2021;93(9):5638–43.
189. Emerging SARS-CoV-2 mutations L452R & Y453F evade cellular immunity [Internet]. News-Medical.net. 2021 [citado 22 de abril de 2022]. Disponível em: https://www.news-medical.net/news/20210722/Emerging-SARS-CoV-2-mutations-L452R-Y453F-evade-cellular-immunity.aspx
190. Membrane fusion and immune evasion by the spike protein of SARS-CoV-2 Delta variant [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.science.org/doi/10.1126/science.abl9463
191. Kim S, Liu Y, Lei Z, Dicker J, Cao Y, Zhang XF, et al. Differential Interactions Between Human ACE2 and Spike RBD of SARS-CoV-2 Variants of Concern. bioRxiv. 26 de julho de 2021;2021.07.23.453598.
192. Kannan SR, Spratt AN, Cohen AR, Naqvi SH, Chand HS, Quinn TP, et al. Evolutionary analysis of the Delta and Delta Plus variants of the SARS-CoV-2 viruses. Journal of Autoimmunity. 1º de novembro de 2021;124:102715.
193. Vaughan A. Omicron emerges. New Sci. 4 de dezembro de 2021;252(3363):7.
194. Cov-Lineages [Internet]. [citado 22 de abril de 2022]. Disponível em: https://cov-lineages.org/lineage.html?lineage=B.1.1.529
195. Wei C, Shan KJ, Wang W, Zhang S, Huan Q, Qian W. Evidence for a mouse origin of the SARS-CoV-2 Omicron variant. J Genet Genomics. dezembro de 2021;48(12):1111–21.
196. SARS-CoV-2 Omicron variant: Antibody evasion and cryo-EM structure of spike protein–ACE2 complex [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.science.org/doi/full/10.1126/science.abn7760
197. Statement on Omicron sublineage BA.2 [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/news/item/22-02-2022-statement-on-omicron-sublineage-ba.2
198. Zhang W, Davis BD, Chen SS, Sincuir Martinez JM, Plummer JT, Vail E. Emergence of a Novel SARS-CoV-2 Variant in Southern California. JAMA. 6 de abril de 2021;325(13):1324–6.
199. GISAID - hCov19 Variants [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.gisaid.org/hcov19-variants/
200. GISAID - hCov19 Variants [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.gisaid.org/hcov19-variants/
201. Covid-19 Vaccine Tracker: Latest Updates - The New York Times [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html#astrazeneca
202. Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, et al. Coronavirus Pandemic (COVID-19). Our World in Data [Internet]. 5 de março de 2020 [citado 22 de abril de 2022]; Disponível em: https://ourworldindata.org/covid-vaccinations
203. Weekly epidemiological update on COVID-19 - 12 April 2022 [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---12-april-2022
204. Painel de Distribuição de Vacinas [Internet]. [citado 22 de abril de 2022].

Disponível em: https://infoms.saude.gov.br/extensions/DEMAS_C19VAC_Distr/DEMAS_C19VAC_Distr.html

205. Zimmer C, Corum J, Wee SL, Kristoffersen M. Coronavirus Vaccine Tracker. The New York Times [Internet]. 10 de junho de 2020 [citado 22 de abril de 2022]; Disponível em: https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html
206. Efficacy and safety of an inactivated whole-virion SARS-CoV-2 vaccine (CoronaVac): interim results of a double-blind, randomised, placebo-controlled, phase 3 trial in Turkey - The Lancet [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)01429-X/fulltext
207. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Chile | NEJM [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nejm.org/doi/full/10.1056/NEJMoa2107715?query=recirc_curatedRelated_article
208. Han B, Song Y, Li C, Yang W, Ma Q, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. Lancet Infect Dis. dezembro de 2021;21(12):1645–53.
209. Jara A, Undurraga EA, Flores JC, Zubizarreta JR, Gonzalez C, Pizarro A, et al. Effectiveness of an Inactivated SARS-CoV-2 Vaccine in Children and Adolescents: A Large-Scale Observational Study [Internet]. Rochester, NY: Social Science Research Network; 2022 fev [citado 22 de abril de 2022]. Report No.: 4035405. Disponível em: https://papers.ssrn.com/abstract=4035405
210. Paixao ES, Wong KLM, Alves FJO, de Araújo Oliveira V, Cerqueira-Silva T, Júnior JB, et al. CoronaVac vaccine is effective in preventing symptomatic and severe COVID-19 in pregnant women in Brazil: a test-negative case-control study. BMC Medicine. 5 de abril de 2022;20(1):146.
211. Cerqueira-Silva T, Andrews JR, Boaventura VS, Ranzani OT, de Araújo Oliveira V, Paixão ES, et al. Effectiveness of CoronaVac, ChAdOx1 nCoV-19, BNT162b2, and Ad26.COV2.S among individuals with previous SARS-CoV-2 infection in Brazil: a test-negative, case-control study. Lancet Infect Dis. 31 de março de 2022;S1473-3099(22)00140-2.
212. Hitchings MDT, Ranzani OT, Lind ML, Dorion M, D'Agostini TL, de Paula RC, et al. Change in COVID-19 risk over time following vaccination with CoronaVac: A testnegative case-control study. medRxiv. 24 de dezembro de 2021;2021.12.23.21268335.
213. Cerqueira-Silva T, Katikireddi SV, de Araujo Oliveira V, Flores-Ortiz R, Júnior JB, Paixão ES, et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. Nat Med. abril de 2022;28(4):838–43.
214. Souza WM, Amorim MR, Sesti-Costa R, Coimbra LD, Brunetti NS, Toledo-Teixeira DA, et al. Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study. Lancet Microbe. outubro de 2021;2(10):e527–35.
215. Mallapaty S. China's COVID vaccines have been crucial — now immunity is waning. Nature. 14 de outubro de 2021;598(7881):398–9.
216. Clemens SAC, Weckx L, Clemens R, Mendes AVA, Souza AR, Silveira MBV, et al. Heterologous versus homologous COVID-19 booster vaccination in

previous recipients of two doses of CoronaVac COVID-19 vaccine in Brazil (RHH-001): a phase 4, non-inferiority, single blind, randomised study. The Lancet. 5 de fevereiro de 2022;399(10324):521–9.
217. Chen Y, Shen H, Huang R, Tong X, Wu C. Serum neutralising activity against SARS-CoV-2 variants elicited by CoronaVac. The Lancet Infectious Diseases. 1º de agosto de 2021;21(8):1071–2.
218. Thakur S, Sasi S, Pillai SG, Nag A, Shukla D, Singhal R, et al. SARS-CoV-2 Mutations and Their Impact on Diagnostics, Therapeutics and Vaccines. Front Med (Lausanne). 22 de fevereiro de 2022;9:815389.
219. Ranzani OT, Hitchings MDT, Melo RL de, França GVA de, Fernandes C de FR, Lind ML, et al. Effectiveness of an Inactivated Covid-19 Vaccine with Homologous and Heterologous Boosters against the Omicron (B.1.1.529) Variant [Internet]. medRxiv; 2022 [citado 22 de abril de 2022]. p. 2022.03.30.22273193. Disponível em: https://www.medrxiv.org/content/10.1101/2022.03.30.22273193v1
220. Full-year and Q4 2021 results [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.astrazeneca.com/media-centre/press-releases/2022/full-year-2021-and-q4-results.html
221. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial - The Lancet [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)31604-4/fulltext
222. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques | Nature [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41586-020-2608-y
223. AstraZeneca advances mass global rollout of COVID-19 vaccine through COVAX [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.astrazeneca.com/media-centre/press-releases/2021/astrazeneca-advances-mass-global-rollout-of-covid-19-vaccine-through-covax.html
224. EMA. AstraZeneca's COVID-19 vaccine: EMA finds possible link to very rare cases of unusual blood clots with low platelets [Internet]. European Medicines Agency. 2021 [citado 22 de abril de 2022]. Disponível em: https://www.ema.europa.eu/en/news/astrazenecas-covid-19-vaccine-ema-finds-possible-link-very-rare-cases-unusual-blood-clots-low-blood
225. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination | NEJM [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nejm.org/doi/full/10.1056/NEJMoa2104840
226. Schultz NH, Sørvoll IH, Michelsen AE, Munthe LA, Lund-Johansen F, Ahlen MT, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. New England Journal of Medicine. 3 de junho de 2021;384(22):2124–30.
227. Vacinas COVID e coágulos sanguíneos: cinco questões-chave [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/d41586-021-00998-w
228. Cerqueira-Silva T, Oliveira V de A, Boaventura VS, Pescarini JM, Júnior JB, Machado TM, et al. Influence of age on the effectiveness and duration of protection of Vaxzevria and CoronaVac vaccines: A population-based study. The Lancet Regional Health - Americas. 1º de fevereiro de 2022;6:100154.
229. Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of Protection against Mild and Severe Disease by Covid-19 Vaccines.

New England Journal of Medicine. 27 de janeiro de 2022;386(4):340–50.
230. Liu X, Munro APS, Feng S, Janani L, Aley PK, Babbage G, et al. Persistence of immunogenicity after seven COVID-19 vaccines given as third dose boosters following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK: three month analyses of the COV-BOOST trial. J Infect. 8 de abril de 2022;S0163-4453(22)00200-6.
231. Munro APS, Janani L, Cornelius V, Aley PK, Babbage G, Baxter D, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. The Lancet. 18 de dezembro de 2021;398(10318):2258–76.
232. Liu X, Shaw RH, Stuart ASV, Greenland M, Aley PK, Andrews NJ, et al. Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. The Lancet. 4 de setembro de 2021;398(10303):856–69.
233. Nanthapisal S, Puthanakit T, Jaru-Ampornpan P, Nantanee R, Sodsai P, Himananto O, et al. A randomized clinical trial of a booster dose with low versus standard dose of AZD1222 in adult after 2 doses of inactivated vaccines. Vaccine. 20 de abril de 2022;40(18):2551–60.
234. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. Lancet. 10 de abril de 2021;397(10282):1351–62.
235. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant | NEJM [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nejm.org/doi/full/10.1056/nejmoa2108891
236. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization | Nature [Internet]. [citado 22 de abril de 2022]. Disponível em: https://www.nature.com/articles/s41586-021-04389-z
237. Spencer AJ, Morris S, Ulaszewska M, Powers C, Kailath R, Bissett C, et al. The ChAdOx1 vectored vaccine, AZD2816, induces strong immunogenicity against SARS-CoV-2 beta (B.1.351) and other variants of concern in preclinical studies. eBioMedicine [Internet]. 1º de março de 2022 [citado 22 de abril de 2022];77. Disponível em: https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(22)00086-X/fulltext

ANEXO A

PROTOCOL - Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID)

Sub-Study: A Test-Negative Case-Control Study on the Effectiveness of COVID-19 Vaccines amongst the General Population of São Paulo State in Brazil

Version: 01.4 / July 12th 2021

PROTOCOL

Evaluation of Vaccine Effectiveness in Brazil against COVID-19 (VEBRA-COVID)
Sub-Study: A Test-Negative Case-Control Study on the Effectiveness of COVID-19 Vaccines amongst the General Population of São Paulo State in Brazil

Version: 01.4 / July 12th 2021

Table 1. Protocol Revisions

Changes in Version 1.4	Justification
Test-negative study for severe clinical outcomes	We specified an analysis method for severe clinical outcomes (hospitalization, ICU admission, respiratory support, and death) associated with the positive RT-PCR SARS-CoV-2 test, restricting the analysis data set to pairs in which the case experiences the outcome of interest. This analysis is in addition to the analysis of severe outcomes among individuals who test positive.
Power calculation for analysis of two-dose schedule of ChAdOx1	We added specification of the power required to trigger an analysis of effectiveness of the two-dose schedule of ChAdOx1 ≥14 days following administration of the second dose.
Specification of subgroup analyses	Examination of effect modification of vaccine effectiveness by demographic and other factors of interest
Secondary definition of exposure including longer time intervals following a single dose	We are interested in whether higher levels of immunity build up over time with a single dose
Timing of two-dose analysis for ChAdOx1	Based on published results of randomized trials, as well as new evidence from real-world effectiveness studies, we increased the threshold to trigger the two-dose analysis for ChAdOx1 to 50% effectiveness.

I. Background

Since the emergence of severe acute respiratory virus coronavirus 2 (SARS-CoV-2), Brazil has experienced one of the world's highest incidence and mortality rates in the world, with over 13 million reported infections as of the middle of April 2021.[1–3] São Paulo, the most populous state in Brazil (~ 46 million inhabitants), is the state with highest number of cases and deaths: 2,827,833 cases and 92,548 deaths as by April 24th 2021.[4] Variants of Concern (VOC) also had a key role on the recent several surges in Brazil and São Paulo State. The P.1 VOC, which was first detected in Manaus on Jan 12, 2021, [5–7] and now consists the majority of new infections, being dominant in several states in Brazil. P1. has accrued mutations associated with decreased neutralization,[8,9] and has since spread throughout Brazil, synchronizing the epidemic in country in a scenario of relaxed non-pharmacological interventions.

The rapid development of novel vaccines against COVID-19 allowed countries to start vaccine distribution programs within a year of the identification of the novel virus. Among the first vaccines to be developed was Sinovac's CoronaVac vaccine.[10–12] Phase III trials were conducted in Turkey, Chile, Singapore and Brazil. The Brazilian trial was conducted among a study population of healthcare professionals, and reported that the effectiveness of CoronaVac after 14 days following completion of a two dose schedule was 50.7% (95% CI 36.0-62.0) for all symptomatic cases of COVID-19, 83.7% (95% CI 58.0-93.7) for cases requiring medical attention, and 100% (95% CI 56.4-100) for hospitalized, severe, and fatal cases.[12] CoronaVac was approved for emergency use on 17 January in Brazil, and used to vaccinate healthcare workers and the general population. AstraZeneca-Oxford's ChAdOx1 vaccine[13,14] was approved on the same day and was administered beginning on 23 January 2021. In Brazil, ChAdOx1 schedule is for 12 weeks between first and second dose.

As vaccine programs continue, there has been much interest in estimation of vaccine effectiveness through observational studies, and specifically in settings where VOC are circulating. Such studies have advantages over clinical trials, including increased size and follow-up time, and reduced cost. However, as vaccinated and unvaccinated individuals are likely different in their SARS-CoV-2 risk and healthcare access, these studies must address bias through design and analysis. Several studies have demonstrated the effectiveness of COVID-19 vaccines against infection caused by the B.1.1.7 variant.[15] However, large-scale real-world investigations on vaccine effectiveness have not been conducted in regions where the P.1 variant is prevalent.

We propose a test-negative case-control study[16,17] of the general population from the São Paulo State to evaluate the effectiveness of COVID-19 vaccines in preventing symptomatic disease in a setting of widespread P.1 VOC transmission.[6] The study will initially evaluate the effectiveness of COVID-19 vaccines, CoronaVac and ChAdOx1 amongst the population with age ≥70 years, since the vaccination campaign prioritized this age group in its first months. We will expand the study population as additional age groups become eligible for vaccination. Furthermore, we expect that additional vaccines will be approved and will evaluate their effectiveness. We will therefore continue to amend the protocol and its objectives accordingly to address these new questions.

II. Objectives

To estimate the effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection amongst the general population from the São Paulo State. Our initial analyses will focus on estimating vaccine effectiveness in the age group of ≥70 years.

III. Methods

1. Study Design: We will conduct a retrospective matched case-control study, enrolling cases who test positive for SARS-CoV-2 and controls who test negative for SARS-CoV-2 amongst the general population (Section 3) as of the day that the COVID-19 vaccination campaign was initiated at the study sites. The study will evaluate vaccine effectiveness on the primary outcome of symptomatic SARS-CoV-2 infection. We will identify cases and matched controls by extracting information from health surveillance records and ascertain the type and data of vaccination by reviewing the state COVID-19 vaccination registry. In this design, one minus the odds ratio (1-OR) of vaccination comparing cases and controls estimates the direct effect of vaccination on the disease outcome. In a separate

analysis, we will assess the association between vaccination and hospitalization and/or death among individuals who have tested positive for SARS-CoV-2.

2. IRB and Ethics Statement: The protocol has been submitted to the Ethical Committee for Research of Federal University of Mato Grosso do Sul (CAAE: 43289221.5.0000.0021). The work of investigators at the University of Florida, Yale University, Stanford University, and Barcelona Institute for Global Health was conducted to inform the public health response and was therefore covered under Public Health Response Authorization under the US Common Rule.

Study Details

Study Site: The State of São Paulo (23°3′S, 46°4'W) is the most populous state in Brazil: an estimated population of 46,289,333 in 2020. São Paulo State has 645 municipalities and its capital, São Paulo city, has 12 million inhabitants. São Paulo State reported 2,827,833 COVID-19 cases (cumulative incidence rate: 6,109 per 100,000 population) and 92,548 deaths (cumulative mortality: 200 per 100,000 population), by 24/04/2021. The State Secretary of Health of Sao Paulo (SES-SP) initiated its COVID-19 vaccination campaign on 17 January 2021 and is administering two vaccines, CoronaVac and ChAdOx1. As of 24 April 2021, 10.7 million doses (6.9 million first doses and 3.8 million second doses) have been administered in the State.

Data Sources and Integration: We will identify eligible cases and controls from the State of São Paulo who test positive and negative, respectively, from the *state laboratory testing registry* of public health laboratory network; 2) Determine vaccination status from *state vaccination registries*; and 3) Extract information from *national healthcare and surveillance databases* that will be used to define outcomes, match controls to cases, determine vaccination status, serve as covariates for post-stratification and provide a source for cross-validation of information from databases. Registries are not available which enables constructing a cohort of people eligible for vaccination in the general population. Data sources for this study will include:

- State laboratory testing registry (**GAL**) of the network of public health laboratories
- State COVID-19 vaccination registry (**Vacina Já**)
- National surveillance database of severe acute respiratory illnesses (**SIVEP-Gripe**) created by Ministry of Health Brazil in 2009
- National surveillance system of suspected cases of COVID-19 (**e-SUS**) from mild to moderate "influenza like illness", created by the Ministry of Health Brazil in 2020

The databases will be integrated by the São Paulo State Government – PRODESP - using CPF numbers (Brazilian citizens' unique identifier code) and send to the VEBRA-COVID group anonymized. The database will be updated on a bi-weekly basis.

Study Population

Inclusion criteria:

- Has a residential address in the State of São Paulo,
- Eligible to receive a COVID-19 vaccine based on age,
- With complete information, which is consistent between databases, on age, sex, and residential address
- With consistent vaccination status and dates for those who were vaccinated.

Exclusion criteria:

- Does not have a residential address in the State of São Paulo,
- Not eligible to receive a COVID-19 vaccine based on age,
- With missing or inconsistent information on age, sex, or city of residence
- With existing but inconsistent vaccination status or dates.

Case definition and eligibility: We will use information from integrated GAL/SIVEP-Gripe/e-SUS databases to identify cases that are defined as eligible members of the study population (as defined above, Study Population) who:

- Had a sample with a positive SARS-CoV-2 RT-PCR, which was collected between January 17, 2021 and 7 days prior to database extraction of information
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 RT-PCR test results

Control definition and eligibility: We will use integrated GAL/SIVEP-Gripe/e-SUS databases to identify eligible controls. Controls are defined as eligible members of the study population who:

- Had a sample with a negative SARS-CoV-2 RT-PCR result, which was collected after January 17, 2021,
- Did not have a positive RT-PCR test in the 90 day period preceding the index positive RT-PCR result
- Did not have a subsequent positive RT-PCR test in the 7-day period following the index positive RT-PCR result
- Have complete and consistent data on SARS-CoV-2 PCR test result

When studying each vaccine, individuals that received another vaccine are eligible for selection as a case and/or control until the day they receive their vaccine, i.e. we will consider test positive and test negative cases for RT-PCR collected before the day of receipt of the other vaccine.

Matching: Test-negative controls will be matched 1:1 to the cases. We chose the matching factors to balance the ability to reduce bias and to enroll sufficient case-control pairs. Matching factors will include variables that are anticipated to be causes of the likelihood of receiving the vaccine, risk of infection and likelihood of receiving PCR testing for SARS-CoV-2 (see Figures 1-5):

- Age, categorized as 5-years age bands (e.g., 70-74, 75-79 years),
- Sex,
- Municipality,
- Self-reported race,
- Window of ±3 days between collection of RT-PCR positive respiratory sample for cases and collection of RT-PCR negative respiratory sample for controls. If the date of respiratory sample collection is missing, the date of notification of testing result will be used,
- Prior ARI (defined as the number of previous symptomatic events that were reported to surveillance systems between February 1, 2020, and January 16, 2021, categorized as any vs. none).

We will use the standard algorithms to conduct matching which include: 1) setting a seed, 2) locking the database, 4) creating a unique identifier for matching after random ordering, 5) implementing exact matching based on matching variables, sampling controls at random if more than one available per case within strata.

An individual who fulfils the control definition and eligibility and later has a sample tested that fulfils the case definition and eligibility can be included in the study as both a case and a control. An individual who fulfils the control definition for multiple different sample collection dates can be included in the study as a control for each collection date, up to a maximum of three times.

Exposure definition:
CoronaVac vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days
- Received the second dose in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days

ChAdOx1vaccination:

- Received the first vaccine dose, and not having received a second dose, in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - 14-27 days
    - ≥28 days

- Received the second dose in the following time periods relative to sample collection for their PCR test:
    - 0-13 days
    - ≥14 days

In a secondary analysis, we will also consider the following time periods relative to sample collection after the first dose: 0-13 days, 14-27 days, 28-41 days, 42-55 days, and ≥56 days.

Statistical Analyses: We will evaluate the effectiveness of CoronaVac and ChAdOx1for the following SARS-CoV-2 infection outcomes:

- Primary: Symptomatic COVID-19, defined as one or more reported COVID-19 related symptom with onset within 0-10 days before the date of their positive RT-PCR test

- Secondary:
    - COVID-19 associated hospitalization
    - COVID-19 associated ICU admission
    - COVID-19 associated respiratory support
    - COVID-19 associated death

We will evaluate vaccine effectiveness for the primary outcome according to the test-negative design. Table 1 shows a list of all planned analyses in the test-negative design.

Our initial analyses will focus on estimating vaccine effectiveness in the population with age ≥70 years of age who were the initial priority group of the COVID-19 vaccination campaign. Our initial analysis of ChAdOx1 will include individuals with age ≥60 years, who were eligible for vaccination at the time of the analysis.

Case-control analysis: Analyses of the primary outcome will be restricted to case and control pairs who are matched based on the presence of a COVID-19 related symptom before or at the time of testing.

We will use conditional logistic regression to estimate the odds ratio (OR) of vaccination among cases and controls, accounting for the matched design, where 1-OR provides an estimate of vaccine effectiveness under the standard assumptions of a test-negative design. For the CoronaVac analysis, the reference group will be individuals who have not received a first dose of CoronaVac by the date of respiratory sample collection. For the ChAdOx1 analysis, the reference group will be individuals who have not received a first dose of ChAdOx1by the date of respiratory sample collection. Date of notification of the testing result will be used if the date of respiratory sample collection is missing. To evaluate potential biases and the timing of vaccine effectiveness after administration, we will evaluate the windows of vaccination status corresponding: A) 0-13 days and ≥14 days after the 1st dose and 0-13 days and ≥14 days after the 2nd dose of CoronaVac; and B) 0-13 days, 14-27 days and ≥28 after the 1st dose and0-13 days and ≥14 days after the 2nd dose of ChAdOx1.

We will include the following covariates in the adjusted model, which we hypothesize are predictive of vaccination, the risk of SARS-CoV-2 infection and COVID-19 severity and healthcare access and utilization:

- Age as continuous variable
- Comorbidities (None, 1-2, ≥3 comorbidities)
- Evidence of prior SARS-CoV-2 infection (defined as positive PCR test, antigen test or rapid antibody test)

Although data on comorbidities is available through e-SUS and SIVEP-Gripe, this data may have different degrees of missingness between databases and between cases and control groups. Adjusting for comorbidities using complete case data will likely introduce bias. We will explore the feasibility of multiple imputation of comorbidity in a sensitivity analysis. Additional sensitivity analyses will evaluate potential effect modification of the vaccine

effectiveness by history of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign and age subgroups. Finally, we will perform subgroup analyses by sex, presence of comorbidities (any vs. none), presence of cardiovascular disease, presence of diabetes, and region of residence ("Grande São Paulo" health region vs. others).

Case-control analysis of severe clinical outcomes: We will perform additional analyses for hospitalization, ICU admission, respiratory support, and death among cases and controls. To perform these analyses, we will restrict the analysis data set to matched case-control pairs in which the case has the outcome of interest, and perform the analysis as described above. For these outcomes, we will consider a restricted polynomial spline term for continuous age, as the risk of these outcomes is highly dependent on age, and compare models using a likelihood ratio test.

Survival/logistic regression analysis of hospitalization, ICU, respiratory support and death: As an exploratory analysis, we will perform additional analyses for hospitalization and death amongst individuals who test positive and estimate the hazards according to vaccination status at the date of positive test, adjusting for covariates described in the case-control analyses. Sensitivity analyses will be conducted to evaluate the association of influence of a positive RT-PCR, antigen or serological test result prior to the vaccination campaign.

Sample size calculations and timing of analyses: The power of a matched case-control study depends on the assumed odds ratio and the number of discordant pairs (i.e. pairs in which the case is exposed and the control is unexposed, or vice versa), which is a function of the assumed odds ratio and the expected prevalence of exposure among controls. Moreover, the estimate of the odds ratio for one level of a categorical variable compared to baseline is determined by the distribution of all discordant pairs. As vaccine coverage and incidence are changing over time, the latter in ways we cannot predict, and there is no power formula for this analysis, we will simulate power and enroll individuals until we have reached a target power, which we can assess without analyzing the data. In particular, after determining the number of discordant case-control pairs for each combination of exposure categories, we will randomly assign one of each pair to each relevant exposure type according to a Bernoulli distribution, with the probability determined by the assumed odds ratio comparing the two categories. We will run an unadjusted conditional logistic regression on the simulated dataset to determine the p-value, and estimate the power as the proportion of N=1,000 simulations that return $p<0.05$. Code to perform the power calculation can be found at https://github.com/mhitchings/VEBRA_COVID-19.

Timing of final analyses: We will perform an analysis of the primary outcome upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose for the CoronaVac. For the ChAdOx1, we will perform an analysis of effectiveness of at least one dose upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥28 days after the first dose. In addition, we will perform an analysis of effectiveness of two doses upon reaching simulated 80% power to detect vaccine effectiveness of 40% ≥14 days after the second dose. For ChAdOx1, we will perform an analysis of effectiveness of two doses upon reaching simulated 80% power to detect vaccine effectiveness of 50% ≥14 days after the second dose. We chose a vaccine effectiveness of 40% to address the question of whether vaccination with CoronaVac and ChAdOx achieved a threshold of real-world effectiveness, below which the public health value of vaccination may need to be reconsidered. We chose a threshold of 50% for the two-dose schedule of ChAdOx1 based on results from randomized trials and existing real-world effectiveness studies.

Privacy: Only SES-SP, São Paulo State data management had access to the identified dataset to linkage the datasets by name, date of birth, mother's name and CPF. After the linkage, the CPF was encrypted and the de-identified dataset was sent to the team for analysis.

Working group: Matt Hitchings, Otavio T. Ranzani, Julio Croda, Albert I. Ko, Derek Adam Cummings, Wildo Navegantes de Araujo, Jason R. Andrews, Roberto Dias de Oliveira, Patricia Vieira da Silva, Mario Sergio Sacaramuzzini Torres, Wade Schulz, Tatiana Lang D Agostini, Edlaine Faria de Moura Villela, Regiane A. Cardoso de Paulo, Olivia Ferreira Pereira de Paula, Jean Carlo Gorinchteyn

References

1 Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* 2020; **20**: 533–4.

2 de Souza WM, Buss LF, Candido D da S, *et al.* Epidemiological and clinical characteristics of the COVID-19 epidemic in Brazil. *Nat Hum Behav* 2020; **4**: 856–65.

3 Ranzani OT, Bastos LSL, Gelli JGM, *et al.* Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *The Lancet Respiratory Medicine* 2021; **9**: 407–18.

4 SEADE Foundation - São Paulo State. CORONAVIRUS - CASOS EM SP. Fundação SEADE. https://www.seade.gov.br/coronavirus/ (accessed April 25, 2021).

5 Naveca F, Nascimento V, Souza V, *et al.* COVID-19 epidemic in the Brazilian state of Amazonas was driven by long-term persistence of endemic SARS-CoV-2 lineages and the recent emergence of the new Variant of Concern P.1. In Review, 2021 DOI:10.21203/rs.3.rs-275494/v1.

6 Faria NR, Mellan TA, Whittaker C, *et al.* Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. *medRxiv* 2021; published online March 3. DOI:10.1101/2021.02.26.21252554.

7 Fujino T, Nomoto H, Kutsuna S, *et al.* Novel SARS-CoV-2 Variant Identified in Travelers from Brazil to Japan. *Emerg Infect Dis* 2021; **27**. DOI:10.3201/eid2704.210138.

8 Nonaka CKV, Franco MM, Gräf T, *et al.* Genomic Evidence of SARS-CoV-2 Reinfection Involving E484K Spike Mutation, Brazil. *Emerg Infect Dis* 2021; **27**. DOI:10.3201/eid2705.210191.

9 Jangra S, Ye C, Rathnasinghe R, *et al.* The E484K mutation in the SARS-CoV-2 spike protein reduces but does not abolish neutralizing activity of human convalescent and post-vaccination sera. *medRxiv* 2021; published online Jan 29. DOI:10.1101/2021.01.26.21250543.

10 Wu Z, Hu Y, Xu M, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *The Lancet Infectious Diseases* 2021; : S1473309920309877.

11 Zhang Y, Zeng G, Pan H, *et al.* Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis* 2021; **21**: 181–92.

12 Palacios R, Batista AP, Albuquerque CSN, *et al.* Efficacy and Safety of a COVID-19 Inactivated Vaccine in Healthcare Professionals in Brazil: The PROFISCOV Study. *SSRN Journal* 2021. DOI:10.2139/ssrn.3822780.

13 Folegatti PM, Ewer KJ, Aley PK, *et al.* Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020; **396**: 467–78.

14 Voysey M, Clemens SAC, Madhi SA, *et al.* Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet* 2021; **397**: 99–111.

15 Dagan N, Barda N, Kepten E, *et al.* BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. *N Engl J Med* 2021; : NEJMoa2101765.

16 Verani JR, Baqui AH, Broome CV, *et al.* Case-control vaccine effectiveness studies: Preparation, design, and enrollment of cases and controls. *Vaccine* 2017; **35**: 3295–302.

17 Verani JR, Baqui AH, Broome CV, *et al.* Case-control vaccine effectiveness studies: Data collection, analysis and reporting results. *Vaccine* 2017; **35**: 3303–8.

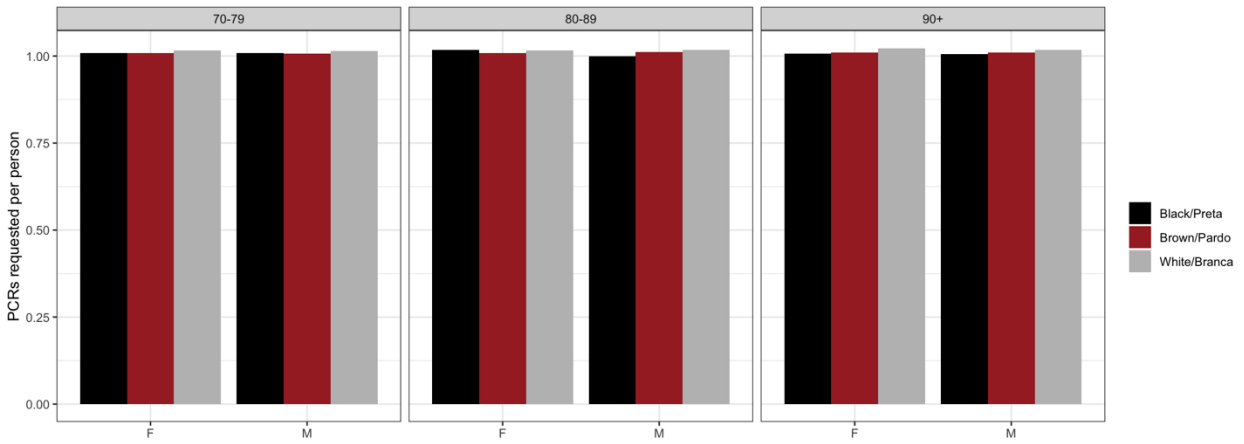


Figure 1: PCR testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

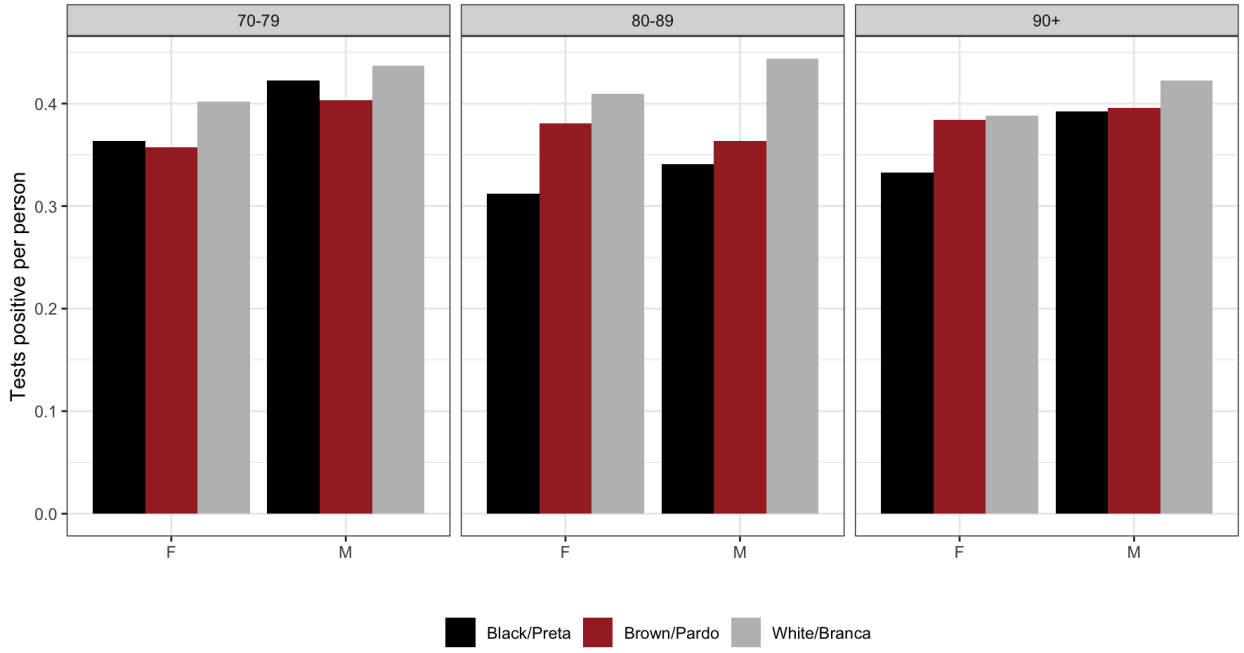


Figure 2: PCR positive testing rate by age, sex and self-reported race (from data extracted on April 07, 2021)

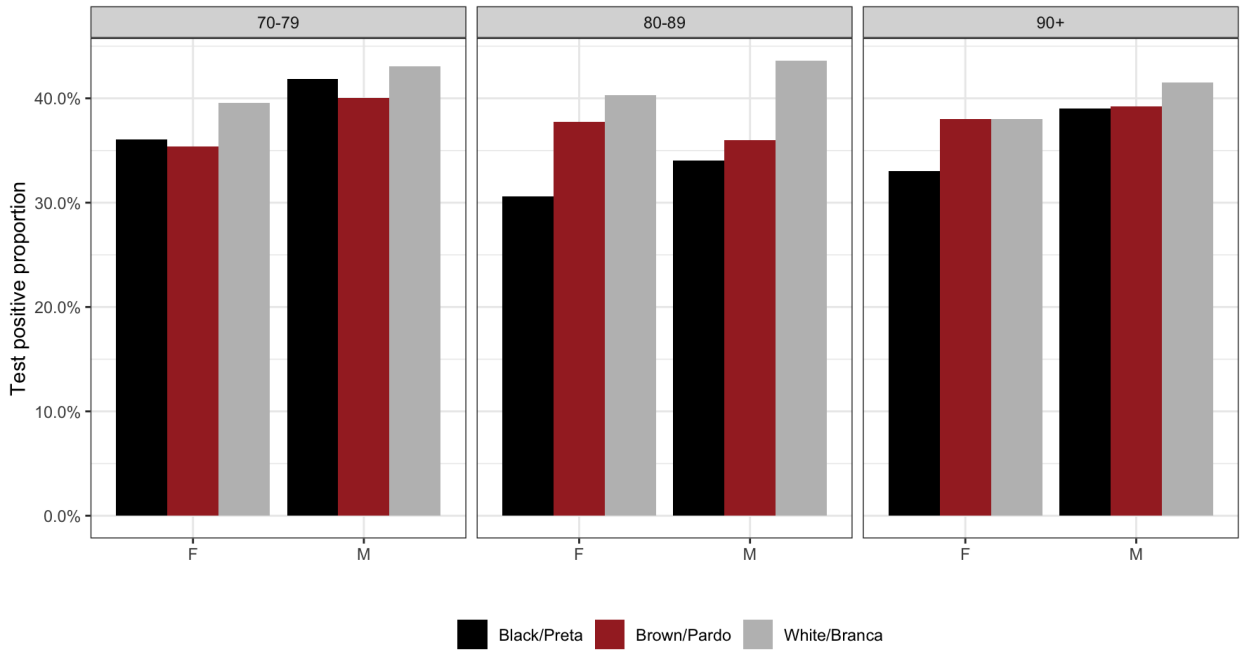


Figure 3: PCR positive proportion by age, sex and self-reported race (from data extracted on April 07, 2021)

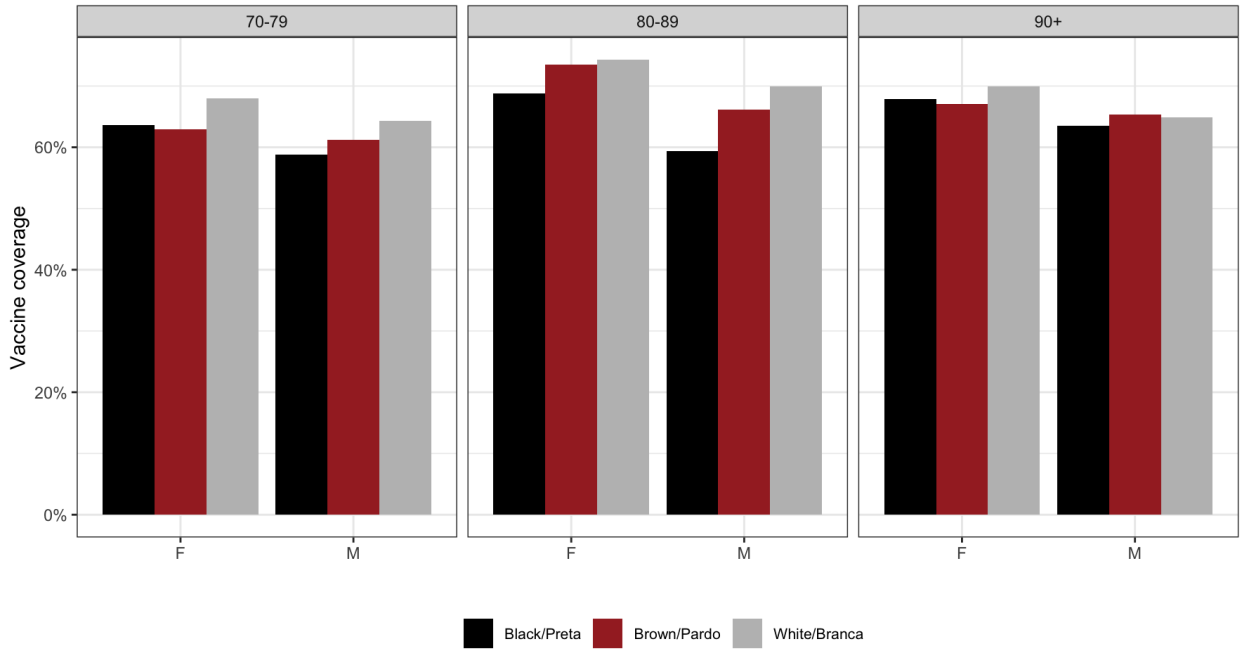
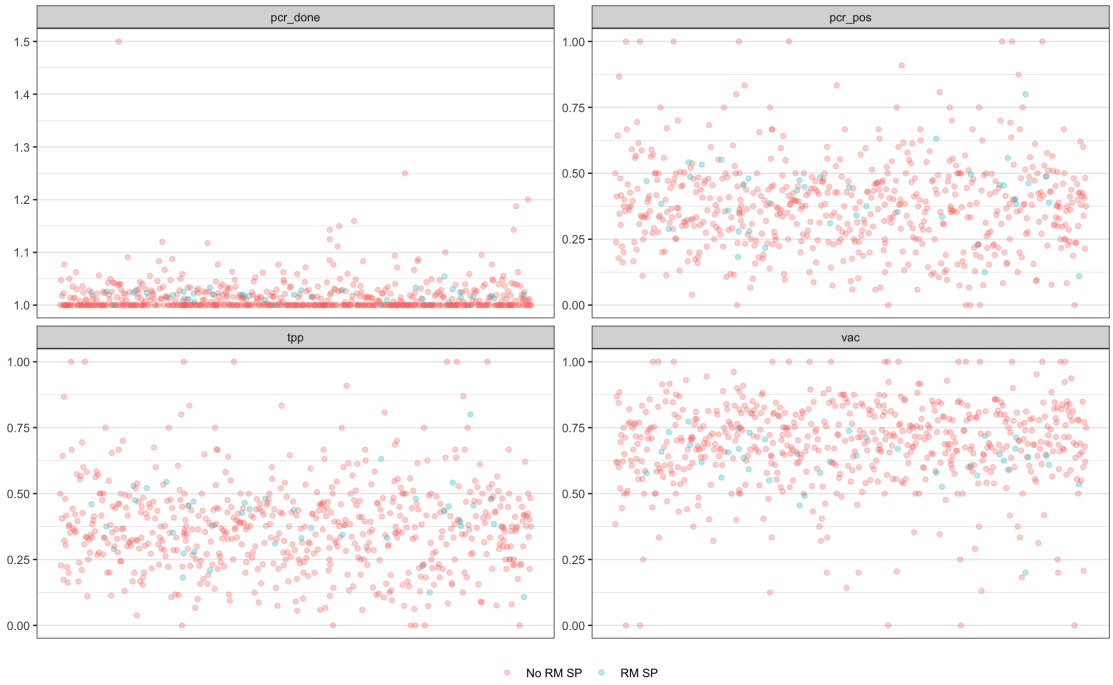


Figure 4: Vaccine coverage by age, sex and self-reported race (from data extracted on April 07, 2021)

Panel A. Indicators by Municipality



Panel B. Indicators by Municipality and Race

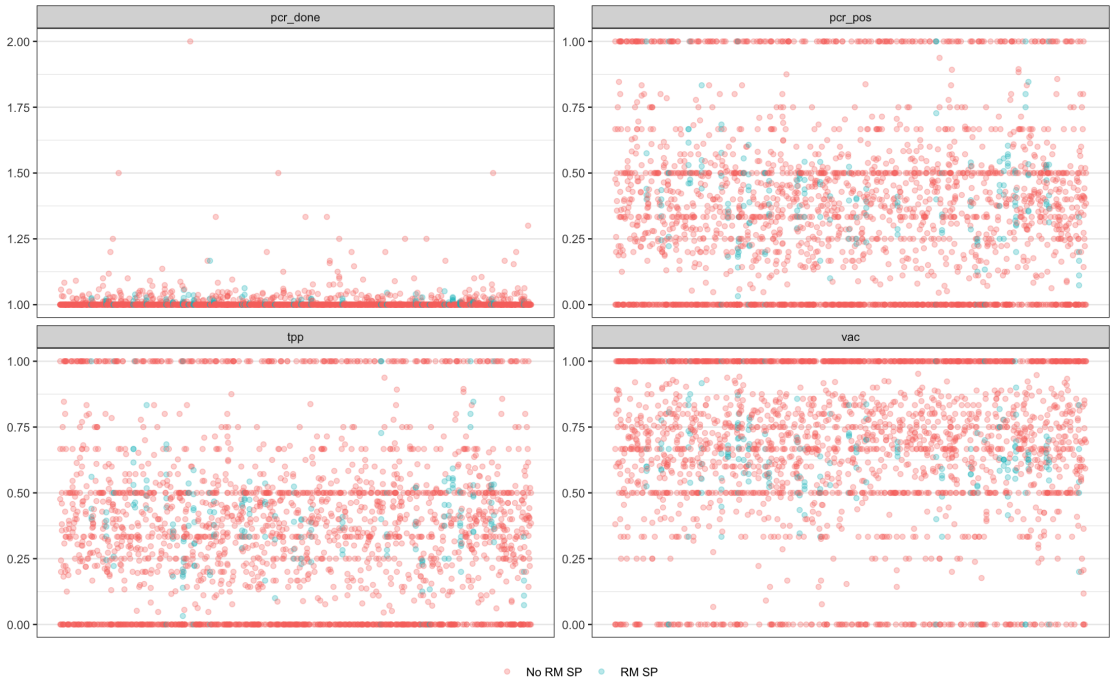
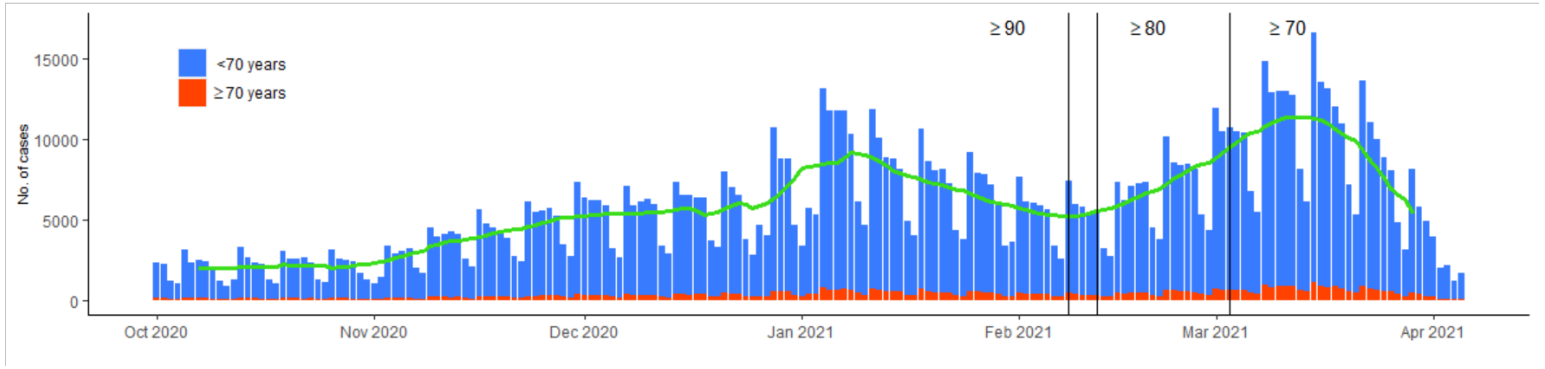


Figure 5: PCR testing rate (pcr_done), PCR positive testing rate (pcr_pos), positivity proportion (tpp) and vaccine coverage (vac) by each municipality (A) and municipality and race (B). RM SP denotes metropolitan area of São Paulo city (from data extracted on April 07, 2021)

Supplementary Figure 1. Reported RT-PCR or Antigen confirmed COVID-19 in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict moving 14-day averages for case. Vertical lines represent vaccine eligibility by age.



Supplementary Figure 2. Reported RT-PCR or Antigen confirmed COVID-19 rates in the general population of the São Paulo State, Brazil from October 2020 to April 7, 2021. Lines depict rolling averages. Vertical lines represent vaccine eligibility by age.

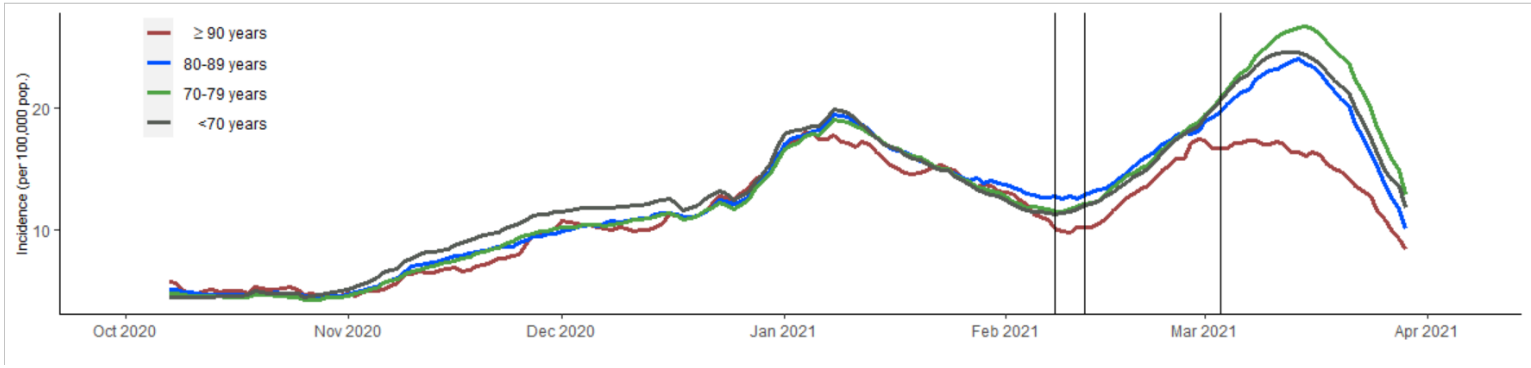


Table 1: Table of planned analyses

Analysis	Exposure	Outcome
CoronaVac		
Primary outcome, primary exposure	Two-dose regimen of CoronaVac in the period starting 14 days after administration of the 2nd dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of CoronaVac in the period 0-13 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of CoronaVac, in the period starting 14 days after administration of the 1st dose	
Primary outcome, bias indicator	One-dose regimen of CoronaVac, in the period 0-13 days after administration of the 1st dose	
ChAdOx1		
Primary outcome, primary exposure	One-dose regimen of ChAdOx1 in the period starting 28 days after administration of the 1st dose	Positive test for SARS-CoV-2, with at least one COVID-19 symptom reported 0-10 days before sample collection date
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1 in the period ≥14 days after administration of the 2nd dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1 in the period 0-13 days after administration of the 1st dose	
Primary outcome, secondary exposure (1-dose)	One-dose regimen of ChAdOx1, in the period starting 14-27 days after administration of the 1st dose	
Primary outcome, secondary exposure (2-dose)	Two-dose regimen of ChAdOx1, in the period starting 0-13 days after administration of the 2nd dose	
Primary outcome, bias indicator	One-dose regimen of ChAdOx1, in the period 0-13 days after administration of the 1st dose	