PAULO SAVOIA DIAS DA SILVA

Autópsia minimamente invasiva na COVID-19: análise dos achados da tomografia computadorizada *post mortem* do tórax e da histopatologia dos pulmões

Tese apresentada à Faculdade de Medicina de Universidade de São Paulo para obtenção do título de Doutor em Ciências

Programa de Radiologia

Orientador: Prof. Dr. Ellison Fernando Cardoso

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São Paulo

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“É na arena do Coliseu que o gladiador toma conselhos.”

Antigo provérbio difundido por Sêneca (4 a.C. – 65 d.C.)

Dedico:

*Aos meus filhos **Giulia** e **Bruno**, o sentido de tudo.*

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NORMALIZACÃO ADOTADA

Esta tese está de acordo com as seguintes normas, em vigor no momento:

Referências: adaptado de *International Committee of Medical Journals Editors* (Vancouver).

Universidade de São Paulo. Faculdade de Medicina. Divisão de Biblioteca e Documentação. *Guia de apresentação de dissertações, teses e monografias*. Elaborado por Anneliese Carneiro da Cunha, Maria Julia de A. L. Freddi, Maria F. Crestana, Marinalva de Souza Aragão, Suely Campos Cardoso, Valéria Vilhena. 3a ed. São Paulo: Divisão de Biblioteca e Documentação; 2011.

Abreviaturas dos títulos dos periódicos de acordo com *List of Journals Indexed in Index Medicus*.

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

COVID-19: *Coronavirus disease*

SARS-Cov-2: *Severe Acute Respiratory Syndrome Coronavirus* 2

SARS-Cov-1: *Severe Acute Respiratory Syndrome Coronavirus* 1

MERS: *Middle East Respiratory Syndrome*

SARA: Síndrome da Angústia Respiratória Aguda

RT-PCR: Transcrição reversa seguida de reação em cadeia da polimerase

RNA: Ácido Ribonucleico

DNA: Ácido Desoxirribonucleico

FDA: *Food and Drug Administration* (Estados Unidos da América)

N-95: Máscara que filtra cerca de 95% das partículas de transmissão por aerossóis.

FFP2: Máscara *Filtering Face Piece* 2

PAPR: Respirador purificador de ar motorizado

TC: Tomografia computadorizada

DAD: Dano alveolar difuso

CONEP: Comissão Nacional de Ética em Pesquisa

CAAE: Certificado de Apresentação de Apreciação Ética

HCFMUSP: Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

TCLE: Termo de Consentimento Livre e Esclarecido

FMUSP: Faculdade de Medicina da Universidade de São Paulo

PACS: Picture Archiving and Communication System

UTI: Unidade de Terapia Intensiva

HE: Hematoxylin and eosin / hematoxilina e eosina.

LISTA DE FIGURAS

LISTA DE TABELAS

RESUMO

Silva PSD. Autópsia minimamente invasiva na COVID-19: análise dos achados da tomografia computadorizada *post mortem* do tórax e da histopatologia dos pulmões [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2024.

Desde o início da pandemia da COVID-19 no final de 2019 / início de 2020, sentiu-se a necessidade de estudar essa nova doença como um todo, inclusive através de autópsias. Entretanto, pelo risco de contágio das equipes / procedimentos de segurança muito restritos, as autópsias convencionais praticamente não eram realizadas. Para suprir essa lacuna, surge a proposta de um modelo de autópsia minimamente invasiva, através de tomografia *post mortem* do tórax, e correlação com múltiplas biópsias do parênquima pulmonar. Em vivos, as tomografias computadorizadas (TC) de tórax se mostraram um excelente método para avaliação do envolvimento pulmonar e até mesmo do prognóstico da COVID-19, mas se sabe que em mortos os pulmões muitas vezes ficam expirados e, então, primeiramente precisaríamos definir se as alterações inerentes da tomografias *post mortem* realmente teriam limitações significativas para análise da doença, gerando o primeiro objetivo desta tese: de uma amostra de 117 pacientes que morreram de COVID-19 entre março de 2020 e setembro de 2021, e tiveram autópsia solicitada pelo corpo clínico do HCFMUSP, encontramos 5 que tiveram alguma tomografia de tórax feita em até 2 dias antes da morte, mais que isso poderia haver muitas modificações nos achados. Assim, fizemos uma análise descritiva dos achados pré e *post mortem* em uma série de casos, conseguindo determinar um raciocínio lógico de progressão de doença e suspeita da *causa mortis*, encontrar achados adicionais para corroborar ou não a morte e inferir que, sim, a TC *post mortem* pode trazer informações a respeito da COVID-19 fatal, mesmo com as limitações conhecidas, principalmente: pulmões expirados e estase sanguínea. Depois, partimos para o segundo objetivo desta tese, que foi correlacionar os achados de imagem das TCs *post mortem* da análise histopatológica dos pulmões. Para tanto analisamos 29 pacientes que morreram de COVID-19 entre abril e junho de 2020, tiveram autópsia solicitada pelo corpo clínico do HCFMUSP, foram submetidos à TC *post mortem* do tórax e múltiplas amostras histológicas dos pulmões foram colhidas. Excluímos 14 pacientes que apresentaram sinais de pneumonia secundária nas lâminas histopatológicas. Quantificamos os principais achados tomográficos da COVID-19 nos outros 15 pacientes: pulmão normal, vidro fosco, pavimentação em mosaico e consolidações, bem como os principais achados histopatológicos: pulmão normal, DAD agudo / exsudativo e DAD fibroproliferativo. Criamos um índice de gravidade tanto para achados tomográficos como histopatológicos, ponderando tais índices para serem maiores quanto mais achados avançados / graves estiverem presentes. Conseguimos detectar uma correlação positiva ($R=0,66$, $p=0,0078$) entre os dois índices. Ao analisar a porcentagem média de envolvimento pulmonar de cada achado, foram encontradas correlações positivas entre a porcentagem de pulmão normal nos exames de tomografia *post mortem* e histopatologia ($R=0,65$, $p=0,0082$), assim como entre opacidades em vidro fosco na tomografia *post mortem* e pulmões normais na histopatologia ($R=0,65$, $p=0,0086$). No entanto, correlações negativas foram observadas entre a extensão de opacidades em vidro fosco e dano alveolar difuso exsudativo em lâminas histológicas ($R=-0,68$, $p=0,005$). Além disso, foi observada uma tendência de redução na porcentagem de tecido pulmonar normal nas lâminas histológicas à medida que a porcentagem de consolidações nos exames de tomografia *post mortem* aumentou ($R=-0,51$, $p=0,055$). A análise das outras correlações entre a porcentagem de cada achado não mostrou nenhuma correlação significativa ou tendência de correlação ($p \geq 0,10$). Para a avaliação de imagens suspeitas para infartos pulmonares na TC, não obtivemos número de casos significativos para uma análise estatística consistente.

Palavras-chave: COVID-19. Tomografia por raios X. Autópsia. Tórax. Pulmão. Patologia.

ABSTRACT

Silva PSD. Minimally invasive autopsy in COVID-19: analysis of postmortem chest computed tomography findings and histopathology of the lungs [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2024.

Since the onset of the COVID-19 pandemic in late 2019 / early 2020, there has been a need to study this novel disease comprehensively, including through autopsies. However, due to the risk of contagion and stringent safety procedures, conventional autopsies were scarcely performed. To address this gap, the proposal of a minimally invasive autopsy model emerged, involving postmortem chest tomography and correlation with multiple lung parenchyma tissue samples. In living individuals, chest computed tomography (CT) proved to be an excellent method for assessing pulmonary involvement and even predicting the prognosis of COVID-19. Nevertheless, given that lungs become exhaled in most deceased individuals, it was necessary to first establish whether inherent changes in postmortem CTs would indeed have significant limitations for disease analysis. This led to the first objective of this thesis: from a sample of 117 patients who died from COVID-19 between March 2020 and September 2021, with autopsy requested by the clinical staff of HCFMUSP, 5 had a chest tomography performed within 2 days before death, as more time could result in significant modifications to the findings. Consequently, a descriptive analysis of pre and postmortem findings was conducted in a case series, allowing the determination of a logical progression of the disease and suspicion of the cause of death. Additional findings were identified to either support or refute the cause of death, leading to the inference that postmortem CT can provide information about fatal COVID-19, despite known limitations, particularly exhaled lungs and blood stasis. Subsequently, we moved on to the second objective of this thesis, which was to correlate the imaging findings of postmortem CTs with the histopathological analysis of the lungs. For this purpose, 29 patients who died from COVID-19 between April and June 2020, with autopsy requested by the clinical staff of HCFMUSP, underwent postmortem chest CT, and multiple histological samples were collected. Fourteen patients showing signs of secondary pneumonia in histopathological slides were excluded. The main tomographic findings of COVID-19 were quantified in the remaining 15 patients: normal lung, ground-glass opacities, mosaic pattern, and consolidations, as well as the main histopathological findings: normal lung, acute/exudative diffuse alveolar damage (DAD), and fibroproliferative DAD. Severity indices were created for both tomographic and histopathological findings, weighting these indices to be higher with more advanced/severe findings. A positive correlation ($R=0.66$, $p=0.0078$) was detected between the two indices. When analyzing the mean lung involvement percentage of each finding, positive correlations were found between the normal lung percentage in postmortem CT and histopathology ($R=0.65$, $p=0.0082$), as well as between ground-glass opacities in postmortem CT and normal lungs in histopathology ($R=0.65$, $p=0.0086$), but negative correlations were observed between the extension of ground-glass opacities and exudative diffuse alveolar damage in histological slides ($R=-0.68$, $p=0.005$). Additionally, a trend towards a decrease in the percentage of normal lung tissue on histological slides was found as the percentage of consolidations in postmortem CT scans increased ($R=-0.51$, $p=0.055$). The analysis of other correlations between the percentage of each finding did not show any significant correlation or correlation trends ($p \geq 0.10$). For the evaluation of suspected images for pulmonary infarctions in CT, we did not obtain a significant number of cases for a consistent statistical analysis.

Keywords: COVID-19. Tomography, X-ray. Autopsy. Thorax. Lung. Pathology.

1. INTRODUÇÃO

1.1 A Pandemia da COVID-19

No final de 2019, um novo agente coronavírus emergiu na cidade de Wuhan, China e, atingiu humanos provavelmente a partir de morcegos e pangolins, causando de uma doença viral aguda *flu-like*, com casos graves exibindo insuficiência respiratória aguda por pneumonia intersticial. O agente viral e a doença causada por ele ficaram conhecidos como SARS-CoV-2 ou novo coronavírus e COVID-19, respectivamente (1, 2).

A transmissão entre humanos se espalhou rapidamente por diversos países nos cinco continentes, gerando uma pandemia e, até março de 2023, foram reportados quase 677 milhões de casos confirmados e 6,9 milhões de mortes em todo o mundo, sendo cerca de 37 milhões de casos confirmados e 700 mil mortes no Brasil (3).

No Brasil, os primeiros casos foram relatados no final de fevereiro de 2020 e, até o final de dezembro de 2023, após algumas ondas de aumentos e reduções de casos, a mortalidade por 100 mil habitantes chegou a 337,3. O Estado de São Paulo foi o mais acometido em números absolutos de casos confirmados, quase 6,8 milhões, e mais de 182 mil mortes (396,9 mortes por 100 mil habitantes) (4).

O SARS-Cov-2 faz parte de uma família de vírus que causa infecções respiratórias, na maioria das vezes leves, *flu-like*, que podem ser detectados em painéis respiratórios clínicos, utilizando técnicas de biologia molecular. O SARS-Cov-2, que ficou conhecido como o novo coronavírus, é o terceiro coronavírus que pode causar a doença grave em humanos. Os outros dois foram o da SARS-Cov-1 em 2002-2003, e o da MERS, identificada em 2012 (2, 5).

O SARS-Cov-2 é transmitido entre humanos através de secreções respiratórias (pela tosse, espirros, aerossóis, aperto de mãos, contato com superfícies

contaminadas por secreções), a partir de um paciente infectado para outros indivíduos suscetíveis, a partir de membranas mucosas (6). O período de incubação da COVID-19 é de até 12 dias, com média de 5,1 dias (7).

Nas primeiras ondas da pandemia, antes do desenvolvimento e propagação das vacinas, a infecção mostrou ser leve ou assintomática em cerca de 80 a 90% dos casos, e grave em cerca de 10% dos casos, com dispneia, hipoxemia e envolvimento radiológico de mais de 50% dos pulmões. Uma condição crítica se desenvolveu em 5% dos casos, com falência respiratória, pneumonia, choque, falência de múltiplos órgãos e, em casos mais graves, morte (8-10).

Apesar da lesão pulmonar grave ter sido descrita em todas as idades, indivíduos idosos e/ou com comorbidades foram mais propensos a desenvolver pneumonia intersticial grave, SARA, falência de múltiplos órgãos, falência respiratória aguda grave, evoluindo para morte (11).

O diagnóstico pode ser feito através da coleta de secreção da nasofaringe e orofaringe através de *swab*, que por sua vez é submetido à RT-PCR, transformando o RNA do vírus, quando presente, em DNA complementar e amplificando-o, visando identificar alguns dos subtipos genéticos do SARS-Cov-2. A especificidade do teste por vezes se mostrou bem alta, próxima a 100%, embora possam existir falsos positivos por contaminação da amostra. A sensibilidade foi estimada entre 68 e 78% (11-13). Uma outra forma de diagnóstico utilizada na pandemia foi o teste de antígenos para o SARS-Cov-2, conhecido por ser mais rápido (resultados em 15 a 30 minutos), bastante confiável quando positivo, entretanto, menos propenso a detectar o vírus em relação ao teste de RT-PCR, especialmente quando sintomas não estiverem presentes (14). Por isso, o FDA recomendou 2 testes negativos para indivíduos com

sintomas ou 3 testes negativos para indivíduos sem sintomas, intervalados em 48 horas, para descartar a infecção (15).

Mesmo após cerca de 4 anos do início da pandemia, não foi desenvolvido tratamento específico bem estabelecido para a COVID-19. Sintomáticos são indicados para controle da febre, desidratação e náuseas; bem como suporte ventilatório para aqueles em insuficiência respiratória. Corticosteroides ajudam no prognóstico da doença, especialmente nos casos mais graves que evoluem para SARA. Diversos medicamentos com ação anti-SARS-Cov-2 foram testados, entre eles remdesivir, hidroxicloroquina, lopinavir / ritonavir e regimes de interferons, porém pouco ou nenhum efeito significativo sobre o prognóstico ou mortalidade foi observado (11, 16-19).

Medidas preventivas para conter a transmissão da doença incluem precauções padrão, de contato: especialmente a lavagem de mãos; e respiratória: com máscaras cirúrgicas, proteção respiratória para aerossóis com máscaras N-95, FFP2 ou PAPR; proteção para os olhos; e distanciamento social (20).

O desenvolvimento de vacinas em tempo recorde pela comunidade científica internacional, cerca de 1 ano após o início da pandemia, certamente ajudou a reduzir significativamente os casos graves e consequentemente, internações e mortes pela doença, permitindo que as medidas restritivas de locomoção e contato social fossem quase totalmente extintas (21).

1.2 O Papel da Tomografia Computadorizada de Tórax

A TC de tórax se mostrou de grande valor na pandemia da COVID-19 para estudo do envolvimento pulmonar. Os seus achados mais típicos observados foram: opacidades pulmonares em vidro fosco, por vezes associadas a espessamento de

septos inter e intralobulares, e áreas de consolidações pulmonares. Esses achados possuem distribuição predominantemente periférica, são múltiplos e com algum predomínio pelos lobos inferiores (6, 22, 23).

Em até dois dias de sintomas, cerca de 50 a 75% dos pacientes possui TC normal. As primeiras imagens pulmonares a aparecerem geralmente são as opacidades em vidro fosco periféricas e multifocais bilaterais e, conforme a infecção progride, aumentam-se as áreas de vidro fosco, aparece a pavimentação em mosaico (vidro fosco entremeado por espessamento septal) e as consolidações. O pico dessas alterações geralmente ocorre por volta do 9º ao 13º dia de sintomas e, à semelhança da maioria das infecções pulmonares, a melhora radiológica é tardia em relação à melhora clínica (23-25).

São achados tomográficos raros / não típicos da COVID-19: o acometimento exclusivamente central do parênquima, a presença de nódulos, cavidades, linfonodomegalias e derrame pleural. Quando presentes, recomenda-se pensar em outro diagnóstico (23, 25-27).

A tomografia de tórax ganhou tanta importância na COVID-19, que alguns autores até sugerem que um quadro de imagem típico com RT-PCR negativo, seja considerado positivo até que se prove o contrário, uma vez que a sensibilidade do RT-PCR é subótima (11, 12, 23-25, 27).

Por fim, a Sociedade Norte-Americana de Radiologia, o Colégio Americano de Radiologia e a Sociedade de Radiologia da América do Norte elegeram a TC de Tórax como o método de escolha para avaliação do envolvimento pulmonar na COVID-19, podendo estimar seguramente a porcentagem de acometimento pulmonar (28). Esta última informação, por sua vez, foi bastante correlacionada com o prognóstico da doença e utilizada até como critério de internação (29).

1.3. O Papel das Autópsias Convencionais e Minimamente Invasivas

Assim como em outras importantes doenças na história da humanidade, o estudo *post mortem* através de autópsias pode trazer melhor entendimento e caracterização da doença. Entretanto, o número de autópsias convencionais de casos de COVID-19 foi muito inferior ao número de mortes. Por se tratar de uma doença contagiosa e, possivelmente grave, principalmente no início da pandemia, muitos serviços e profissionais de autópsias convencionais não se submeteram a realizá-las para confirmação da *causa mortis* e melhor estudo da COVID-19 fatal, uma vez que os procedimentos de segurança para fazê-las seriam muito rigorosos e, na grande maioria dos serviços, inviáveis (30, 31). Isso gerou uma lacuna de conhecimento importante no contexto da pandemia da COVID-19 (32).

Nesse cenário, ganha importância a chamada autópsia minimamente invasiva, em que o corpo pode ser estudado por imagem, seja por ultrassonografia, tomografia computadorizada ou ressonância magnética, e fragmentos obtidos através de agulhas (guiadas ou não por imagem), para estudo anátomo-patológico, e swabs da naso e orofaringe, para estudo citológico e de biologia molecular. A autópsia minimamente invasiva por ultrassonografia já vem mostrando bons resultados nos últimos anos para estudos de outras doenças e, agora está se mostrando também um bom método para melhor caracterização da COVID-19 fatal (32-35).

No decorrer da pandemia da COVID-19, alguns autores desenvolveram métodos de autópsia minimamente invasiva para melhor estudo da doença. Há publicações referentes a análise histopatológica isolada dos pulmões acometidos (36), análise histopatológica de múltiplos órgãos acometidos (37), análise combinada de ultrassonografia pulmonar *post mortem* e histopatologia dos pulmões (38), até que

começaram a surgir os estudos envolvendo TC do tórax, correlacionado os achados da TC em vida com a análise histopatológica dos pulmões após a morte (39, 40).

Sabe-se que existem limitações na TC *post mortem* do tórax, uma vez que após o óbito os pulmões muitas vezes ficam expirados, diferente dos pulmões inspirados da TC que rotineiramente fazemos em vivos. Além disso, alterações relacionadas a hipóstase supostamente podem prejudicar a análise pulmonar, sobretudo nas regiões mais posteriores e se o exame for realizado muito tempo após a morte (41). Apesar disso, foram publicados alguns relatos de caso e series de relatos de casos, às vezes tentando correlacionar os achados de imagem *post mortem* dos pulmões com os histopatológicos, mais como análises descritivas / relatos de casos (42-45). Outros autores chegaram a usar a TC *post mortem* do tórax a fim de excluir ou confirmar COVID-19 e, caso não houvesse achados pulmonares típicos da doença, encaminhar os corpos para autópsias convencionais, reduzindo o risco de contágio das equipes de necropsia. Tal conduta se mostrou extremamente útil quando o RT-PCR não estava disponível (46, 47).

Do ponto de vista histopatológico, os estudos mostram que as principais alterações das biópsias pulmonares das vítimas fatais da COVID-19 mostram basicamente áreas de pulmão normal, DAD exsudativo, DAD fibroproliferativo, hemorragia alveolar, infarto, broncopneumonia e tromboses (arteriolar e capilar) (36, 38).

1.4 Motivação

Frente a essa lacuna de conhecimento que as necropsias poderiam proporcionar à pandemia da COVID-19, uma vez que as autópsias convencionais praticamente não estavam sendo realizadas, sentimos a necessidade de estudar um

modelo de autópsia minimamente invasiva que analisasse efetivamente a COVID-19 fatal através da TC de tórax *post mortem* e da análise histopatológica dos pulmões, inovando em relação aos modelos disponíveis no momento.

2. OBJETIVOS

1 - Avaliar as limitações e o valor diagnóstico da tomografia computadorizada *post mortem* do tórax na COVID-19 fatal, uma vez que a análise tomográfica pode ser prejudicada pelos pulmões expirados e/ou hipóstase, comuns após a morte.

2 - Estudar a COVID-19 fatal através de um modelo de autópsia minimamente invasiva: realização de TC *post mortem* do tórax e análise da correlação entre achados tomográficos e os achados histopatológicos de múltiplas amostras pulmonares.

3. DESENVOLVIMENTO E RESULTADOS

3.1 Organização do texto de desenvolvimento

As publicações do trabalho foram agrupadas de acordo com os objetivos da tese:

Artigo 1

1. **Título:** *Postmortem Chest Computed Tomography in Fatal COVID-19: A Valuable Diagnostic Tool for Minimally Invasive Autopsy*
2. **Objetivo:** Avaliar as limitações e o valor diagnóstico da tomografia computadorizada post mortem do tórax na COVID-19 fatal, uma vez que a análise tomográfica pode ser prejudicada pelos pulmões expirados e/ou hipóstase, comuns após a morte.
3. **Revista:** Clinics
4. **Estado:** Publicado online em 24/11/2021

Artigo 2

1. **Título:** *Postmortem chest computed tomography in COVID-19: A minimally invasive autopsy method*
2. **Objetivo:** Estudar a COVID-19 fatal através de um modelo de autópsia minimamente invasiva: realização de TC post mortem do tórax e análise da correlação entre achados tomográficos e os achados histopatológicos de múltiplas amostras pulmonares.
3. **Revista:** European Journal of Radiology Open
4. **Estado:** Publicado online em 13/01/2024

3.2.1 Considerações sobre a publicação 1

Conforme mencionado na Introdução da tese, as autópsias convencionais foram restringidas ou suspensas no início da pandemia. No Estado de São Paulo chegaram até mesmo a ser proibidas (48), o que levou a pensarmos em maneiras alternativas de estudarmos a COVID-19 fatal, sendo uma delas a realização de TC *post mortem*. Já era sabido que tal método pode ter suas limitações, pois muitas vezes os pulmões estão expirados e/ou há a presença de estase sanguínea decúbito dependente (hipóstase), o que pode limitar a análise (41).

Nesse contexo resolvemos analisar as tomografias *post mortem* que estavam sendo realizadas nos casos de COVID-19 fatal em nossa instituição, com consentimento dos representantes legais, triar quais desses pacientes fatais teriam feito TC de tórax em vida, e compará-las com as TCs *post mortem* a fim de: verificar os achados tomográficos *post* mortem, relacioná-los com os achados em vida e avaliar se era possível estabelecer algum raciocício lógico de progressão de doença. Entretanto, como sabemos que as alterações pulmonares em quadros infecciosos podem mudar rapidamente, escolhemos empiricamente o corte de 2 dias antes da morte para procurar por TCs de tórax em vida dos pacientes e fazer as análises

O que encontramos resultou no Artigo 1, publicado em revista indexada, disponível na íntegra, a seguir.

3.2.2 Artigo 1

Silva PSDD, Sawamura MVY, Monteiro RAA, Duarte-Neto AN, Martin MDGM, Dolhnikoff M, Mauad T, Saldiva PHN, Leite CC, Silva LFFD, Cardoso EF. Postmortem Chest Computed Tomography in Fatal COVID-19: A Valuable Diagnostic Tool for Minimally Invasive Autopsy. Clinics (Sao Paulo). 2021 Dec 8;76:e3551. doi: 10.6061/clinics/2021/e3551. PMID: 34909914; PMCID: PMC8612301.

Title: Postmortem Chest Computed Tomography in fatal COVID 19: a Valuable Diagnostic Tool in Minimally Invasive Autopsy

Running Title: Minimally Invasive Autopsy for COVID-19

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- Paulo Savoia Dias da Silva, ORCID 0000-0002-0983-1930, designed the study, analyzed chest CTs, wrote the first draft of the manuscript, read and approved the final manuscript.

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Postmortem Chest Computed Tomography in Fatal COVID-19: A Valuable Diagnostic Tool for Minimally Invasive Autopsy

Abstract

Objectives

Postmortem chest Computed Tomography (CT) findings may be obscured due to expiratory images acquisition and hypostasis. Our main aim was to evaluate the limitations and diagnostic value of this technique in patients with fatal COVID-19.

Method

Our sample was composed of five patients with fatal COVID-19 submitted to ultrasound guided minimally invasive autopsy (uMIA). Postmortem chest CT was performed prior to uMIA and compared to pre-mortem chest CT (at most 2 days before death). Data was acquired since the beginning of the COVID-19 pandemic (April 2020). Pre and postmortem chest CT were compared by board certified radiologists sub-specialized in thoracic imaging with more than 5 years of clinical experience.

Results

A descriptive analysis of the cases selected was presented comparing pre and postmortem chest CT images side-by-side. Radiological propaedeutics applied and logical reasoning to interpret findings are brought to discussion. In many cases it was possible to show the progression of the disease until death, to speculate cause of death, and eventually encounter additional findings not related to COVID-19 and by these findings suggest biopsy sites to tissue sample collection. Limitations and artifacts of postmortem chest CT were also discussed, with possible implications.

Conclusion

In all five cases, despite of its known limitations, postmortem chest CT was reliable to logical radiologic propaedeutics and interpretation. When compared to recent pre-mortem study, it helped to understand the progression of the disease. Additionally, postmortem chest CT seems to be an adequate tool to stage lung disease.

Keywords

1. COVID-19, 2. Autopsy, 3. Multidetector Computed Tomography, 4. Radiology, 5. Lung, 6. Pulmonary Medicine

Introduction

The coronavirus disease (COVID-19) pandemic has resulted in more than 4.7 million deaths worldwide (1). Despite the high number of COVID-19 related deaths, published reports on autopsies are scarce, probably because of contagion risk and/or recommended strict protection procedures that restrict autopsies considerably (2, 3). Therefore, to address this postmortem knowledge gap, some authors have studied patients who died because of COVID-19 using minimally invasive autopsy methods, but not chest computed tomography (CT) (4, 5).

Postmortem chest CT has some limitations owing to the inherent characteristics at death such as expired lungs and hypostasis. However, we believe these characteristics do not significantly limit the value of postmortem CT, especially during the COVID-19 pandemic, when traditional autopsies are often avoided or even forbidden.

Few authors have used postmortem chest CT to study fatal COVID-19, but some case reports have been published (6–9). Helmrich et al. presented a case series in which postmortem chest CT was used as a triage tool to refer a body for conventional autopsy when no typical CT characteristics of COVID-19 were found (10). De-Giorgio et al. used postmortem chest CT to confirm or exclude the disease and minimize risks of contagion to the autopsy team (11). Both studies suggest that postmortem CT is especially useful when reverse transcription polymerase chain reaction (RT-PCR) is not feasible.

To validate postmortem chest CT findings, we selected the 5 of 117 patients who had a premortem chest CT performed at most 2 days before death to compare their premortem with their postmortem chest CT and describe findings as well as eventual associated conditions. We hypothesized that a postmortem chest CT could help us understand and stage COVID-19, as well as diagnose other associated conditions, similar to a premortem chest CT, despite changes to the lungs inherent with death.

Patients

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068).

From March 2020 to early September 2021, 117 patients died because of laboratory-confirmed COVID-19 and underwent an autopsy that was requested by our institution’s medical staff, after informed consent was obtained from the next of kin. Of the 117 patients in our convenience sample, only 5 had a chest CT performed up to 2 days before death (4 patients in 2 days and one patient in one day) and a postmortem chest CT performed as part of a minimally invasive autopsy study.

All patients were women, with a mean age of 36±20 years. The mean interval between death and the postmortem chest CT was 14 h 08 min±5 h 28 min. After the postmortem CT was performed, tissues from multiple organs were collected via ultrasound-guided biopsies.

A descriptive analysis is presented. Table 1 shows patient's main data. Afterwards we describe each case with main clinical data and imaging findings.

Case 1 (Figure 1): A 67-year-old female patient was hospitalized for approximately 1 month in the intensive care unit (ICU) before death. The cause of death was acute respiratory distress syndrome (ARDS) caused by COVID-19. Secondary pneumonia was also observed upon lung tissue analysis.

Case 2 (Figure 2): An 11-year-old female patient with rapid progression of COVID-19 was admitted to the hospital 7 days after the onset of respiratory symptoms. She was directly admitted to the ICU and died 1 day later. The causes of death were myocarditis and ARDS caused by COVID-19. Lung tissue analysis revealed no secondary pneumonia.

Case 3 (Figure 3): A 35-year-old female patient was admitted to the hospital 5 days after the onset of respiratory symptoms. She was transferred to the ICU 4 days after admission and died 7 days later. The cause of death was ARDS caused by COVID-19. Secondary pneumonia was observed upon lung tissue analysis.

Case 4 (Figure 4): A 38-year-old female patient was admitted to the ICU 9 days after the onset of respiratory symptoms and died 9 days later. The cause of death was ARDS caused by COVID-19. Lung tissue analysis revealed no secondary pneumonia.

Case 5 (Figure 5): A 31-year-old female patient with a transplanted liver was admitted to the hospital because of hepatic complications. Nine days after admission,

she developed respiratory symptoms and was transferred to the ICU, where she died 1 day later. The cause of death was acute liver failure. Lung tissue analysis revealed neither COVID-19-related pneumonia nor secondary pneumonia.

Table 2 presents the main positive and negative aspects of our analysis.

Discussion

In all five cases, the logical radiological reasoning and interpretation of the main imaging findings showed disease progression until death. Despite the known limitations of postmortem CT, we were able to show that the information obtained can be useful in the appropriate scenario.

This study aimed to elaborate on the use of minimally invasive autopsy techniques, particularly in COVID-19 cases.

The use of postmortem CT to help establish the correct cause of death is not new (12–15). During the COVID-19 pandemic, chest CT has played an important role in diagnosing and staging the disease in patients (16–18). Thus, it is logical to use postmortem CT to study COVID-related deaths, as we have attempted to do since the beginning of the COVID-19 pandemic. The supposed limitations of postmortem chest CT are already known: expired lungs (different from fully inspired lungs of the living); the dead can aspirate during their final moments; hypostasis may be present in the lungs (as in our case 1), depending on the time after death (19); and, of course, lung CT findings can change very quickly, within a few hours or days.

In addition, it is very difficult to identify a COVID-19 patient for whom a premortem chest CT was performed a few days before death, mainly because many of these

patients are in severe clinical condition, with most in ICUs, which limits CT realization. Therefore, despite the relatively small number of cases, our results support the use of postmortem CT in this scenario.

Premortem chest CT findings were important for interpretation of postmortem CT findings. Our diagnostic performance improved when the findings were analyzed together. If we analyzed only postmortem CT findings of our cases, the evolution of the case would not be fully understood. Analysis of cases with almost fully consolidated lungs, such as our case 3, greatly benefits when a recent premortem CT is available for comparison. Furthermore, CT findings can be used to guide small tissue sample biopsies for important histopathologic analysis.

The major limitation of this study is the small number of cases. However, we hope this study inspires others to perform similar studies that add knowledge about minimally invasive autopsies being developed worldwide.

Postmortem chest CT can be useful in minimally invasive autopsies of fatal COVID-19 cases, especially if there is a recent premortem chest CT to compare with the postmortem CT and help interpret the findings. This interpretation can lead to logical diagnostic reasoning of the progression of COVID-19, and even reveal additional findings not related to SARS-CoV-2 infection, help understand the cause of death, and help guide small tissue sample biopsies, if necessary.

Acknowledgements

We thank the autopsy and CT technicians who helped make image acquisition possible. We are also grateful to all involved in the care of patients with COVID-19; to the Coronavirus Crisis Committee of our institution during the worst days of the

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Tables

Table 1. Patient's main data.

Patient	Age (years)	Sex	Body Mass Index (kg/m²)	Time since symptoms onset until death (days)	Hospitalization time (days)	Days between pre and post-mortem CT	Time between death and post-mortem CT	Cause of death	Secondary Pneumonia
1	67	F	32.6	32	29	2	4h47m	ARDS / COVID-19	Yes
2	11	F	22.6	8	1	2	14h03m	Myopericarditis / COVID-19	No
3	35	F	15.6	16	11	2	17h19m	ARDS / COVID-19	Yes
4	38	F	20.4	18	9	1	18h20m	ARDS / COVID-19	No
5	31	F	25.4	1	10	2	16h13m	Acute liver failure	No

Cause of death was determined by histopathological analysis of tissues collected by ultrasound guided biopsy of multiple organs, performed after post-mortem CT. ARDS = acute respiratory distress syndrome.

Table 2 - Main positive and negative aspects of our analysis.

Comparison between pre and postmortem chest CT in COVID-19	
Positive aspects	**Negative aspects**
Confirm typical findings of COVID-19	Hypostasis (when present) may limit posterior lung analysis
Exclude typical findings of COVID-19	Expired lungs may obscure some findings
Determine progression of the disease	
Detect additional chest findings	

Figures

Figure 1 (Case 1):

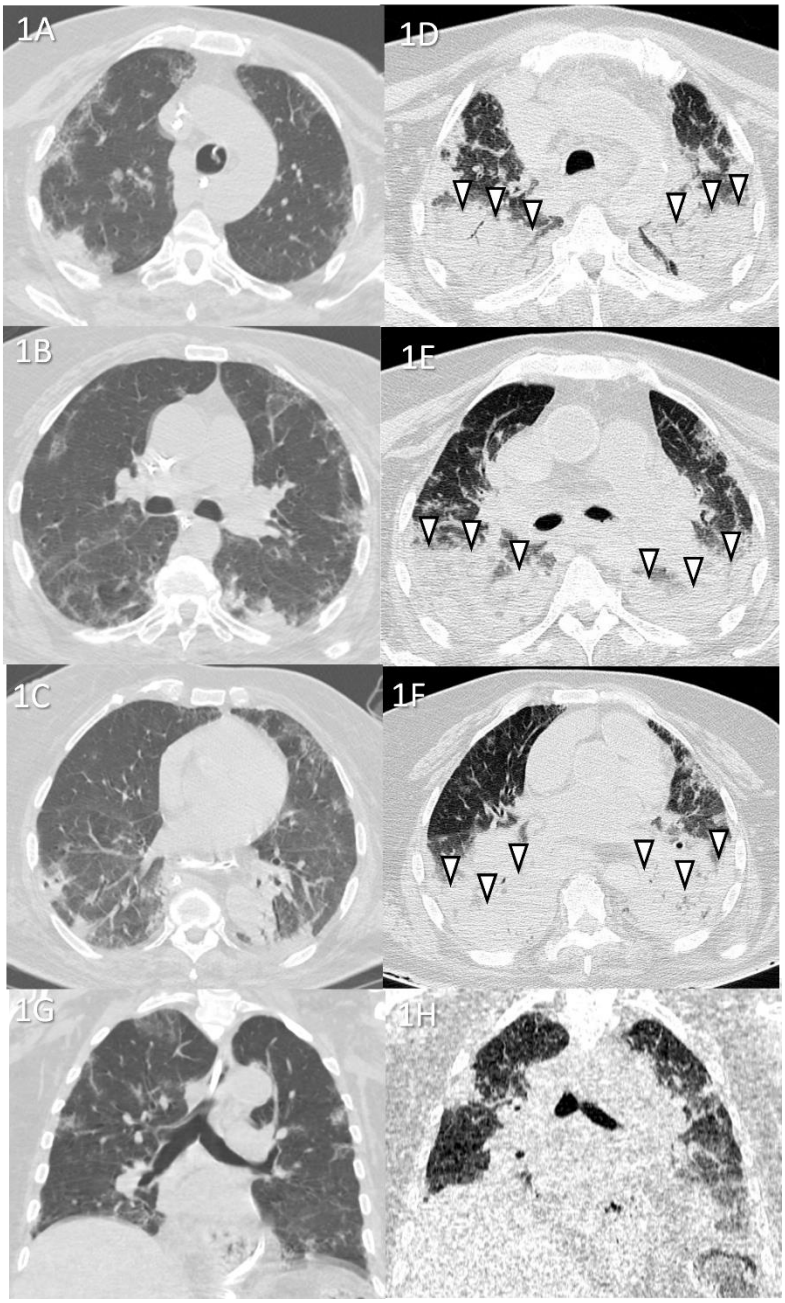

Figure 1 - Case 1: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing peripheral ground-glass opacities, slight consolidations, and interlobular septal thickening. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 4 h 47 min after death showing opacities larger than those on premortem CT, thus demonstrating the progression of the disease until death. Postmortem posterior ‘‘horizontal level forming’’ consolidations because of hypostasis (white arrowheads) are also observed, which limited analysis of the posterior lungs in this case (limitation of the method). Images D and H show pre- and postmortem coronal reformats, respectively.

Figure 2 (Case 2):

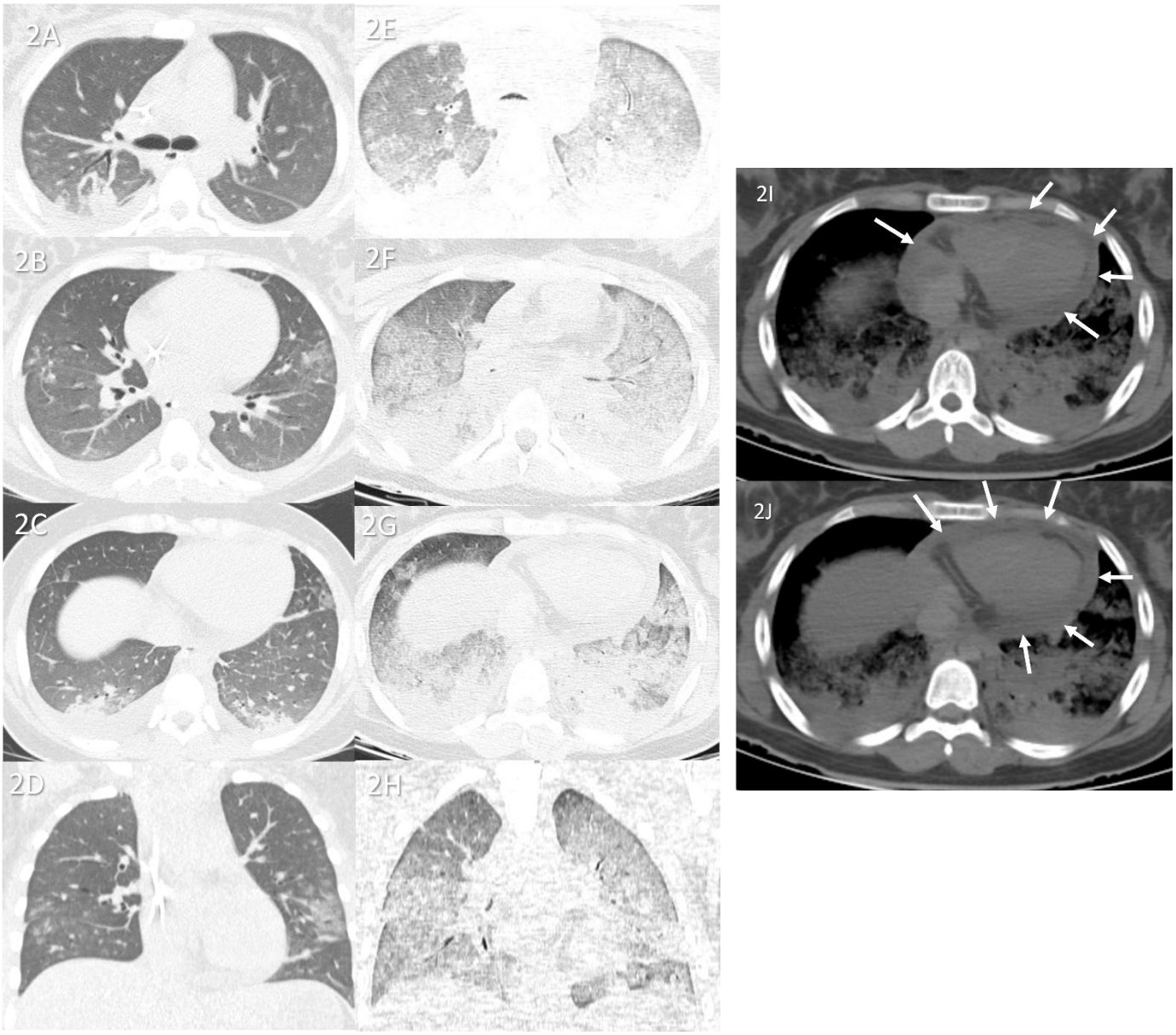

Figure 2 - Case 2: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing peripheral ground-glass opacities, small posterior bilateral consolidations, and right pleural effusion. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 14 h 03 min after death showing a diffuse ‘‘crazy paving’’ pattern, possibly because of acute respiratory distress syndrome and subsequent death. Furthermore, the posterior bilateral consolidations are larger on postmortem CT than on premortem CT, indicating progression of the disease. Images D and H show pre- and postmortem coronal reformats, respectively.
Postmortem axial chest CT (I and J) of the mediastinal window showing pericardial effusion (white arrows) related to myopericarditis (confirmed with the collected tissue sample as the probable cause of death).

Figure 3 (Case 3):

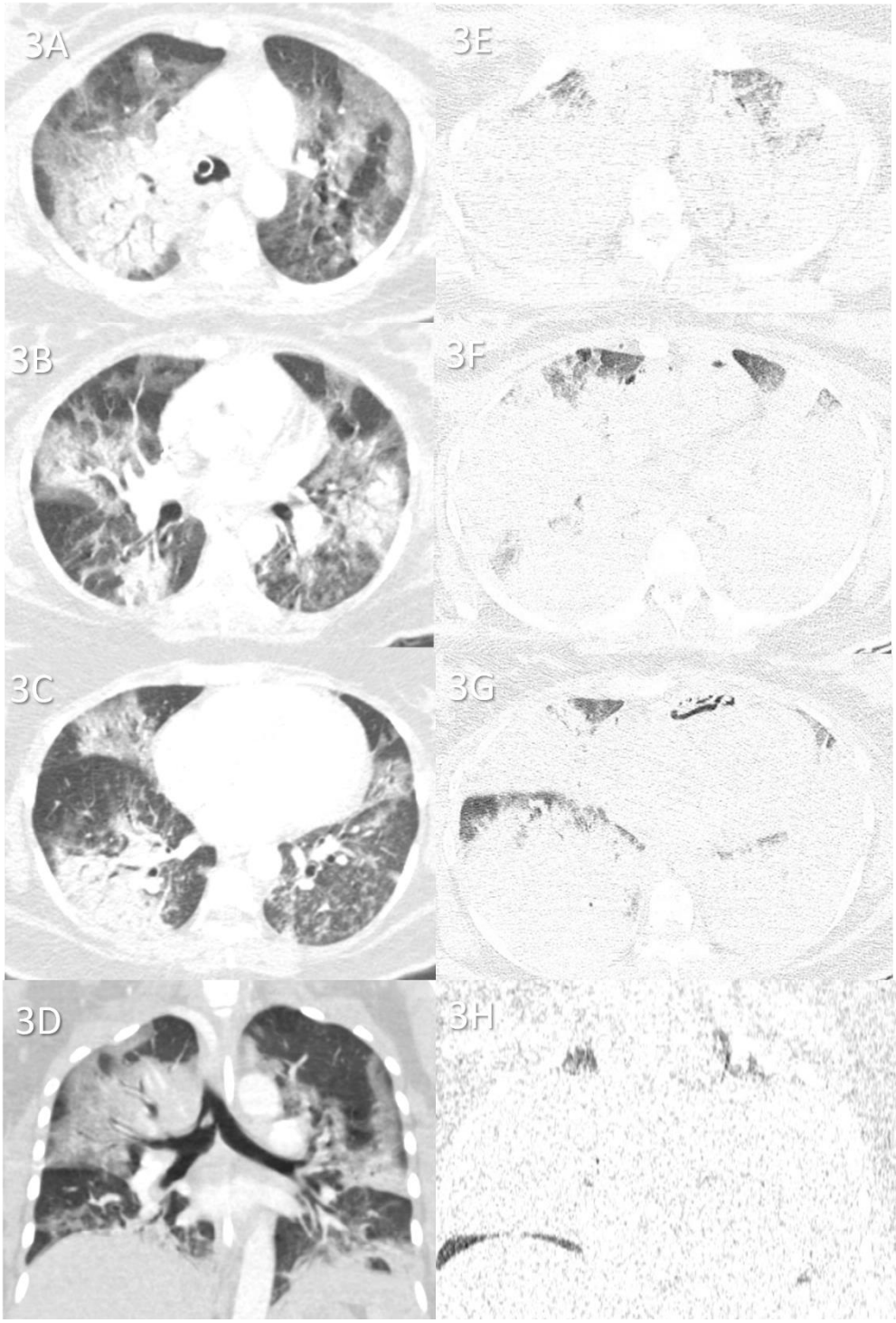

Figure 3 - Case 3: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing patchy peripheral and central ground-glass opacities and bilateral but mainly peripheral consolidations. Postmortem axial chest CT of the upper (E),
mid, (F) and inferior (G) thirds of the lungs obtained 17 h 19 min after death showing rapid progression of the disease, with extensive consolidations in both lungs and a few areas of preserved pulmonary parenchyma. Images D and H show pre and postmortem coronal reformats, respectively.

Figure 4 (Case 4):

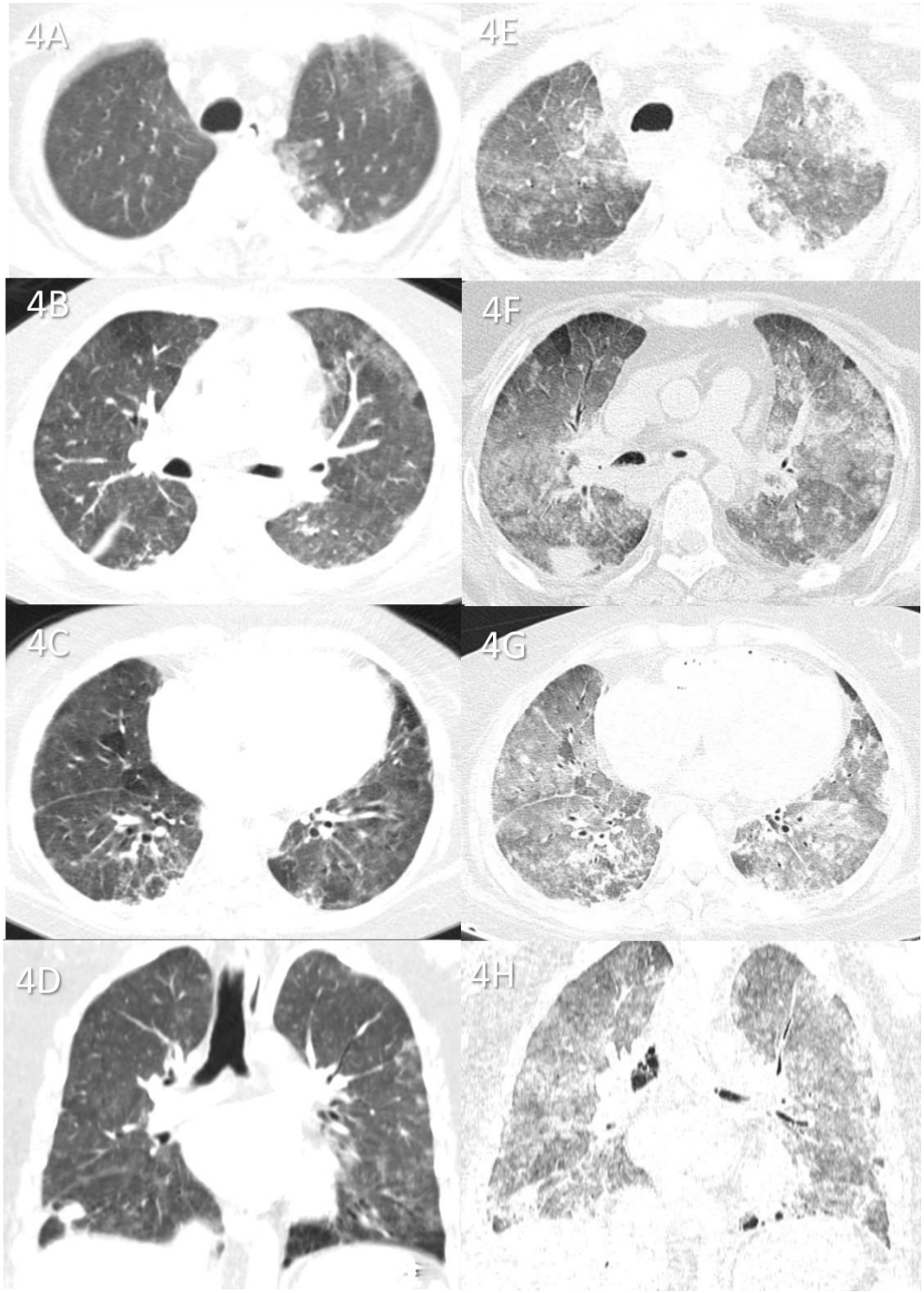

Figure 4 - Case 4: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 1 day before death showing peripheral ground-glass opacities and diffuse pulmonary mosaic attenuation because of ventilation and/or perfusion disturbances (small hypoattenuating areas in the lungs). Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 18 h 20 min after death showing ground-glass opacities larger than those on premortem CT and associated with small consolidations, indicating progression of the disease before death. Because the postmortem CT is of expired lungs, the pulmonary mosaic attenuation is enhanced. This confirms that the small hypoattenuating lung areas on premortem CT are air trapping areas on postmortem CT. Images D and H show pre- and postmortem coronal reformats, respectively.

Figure 5 (Case 5):

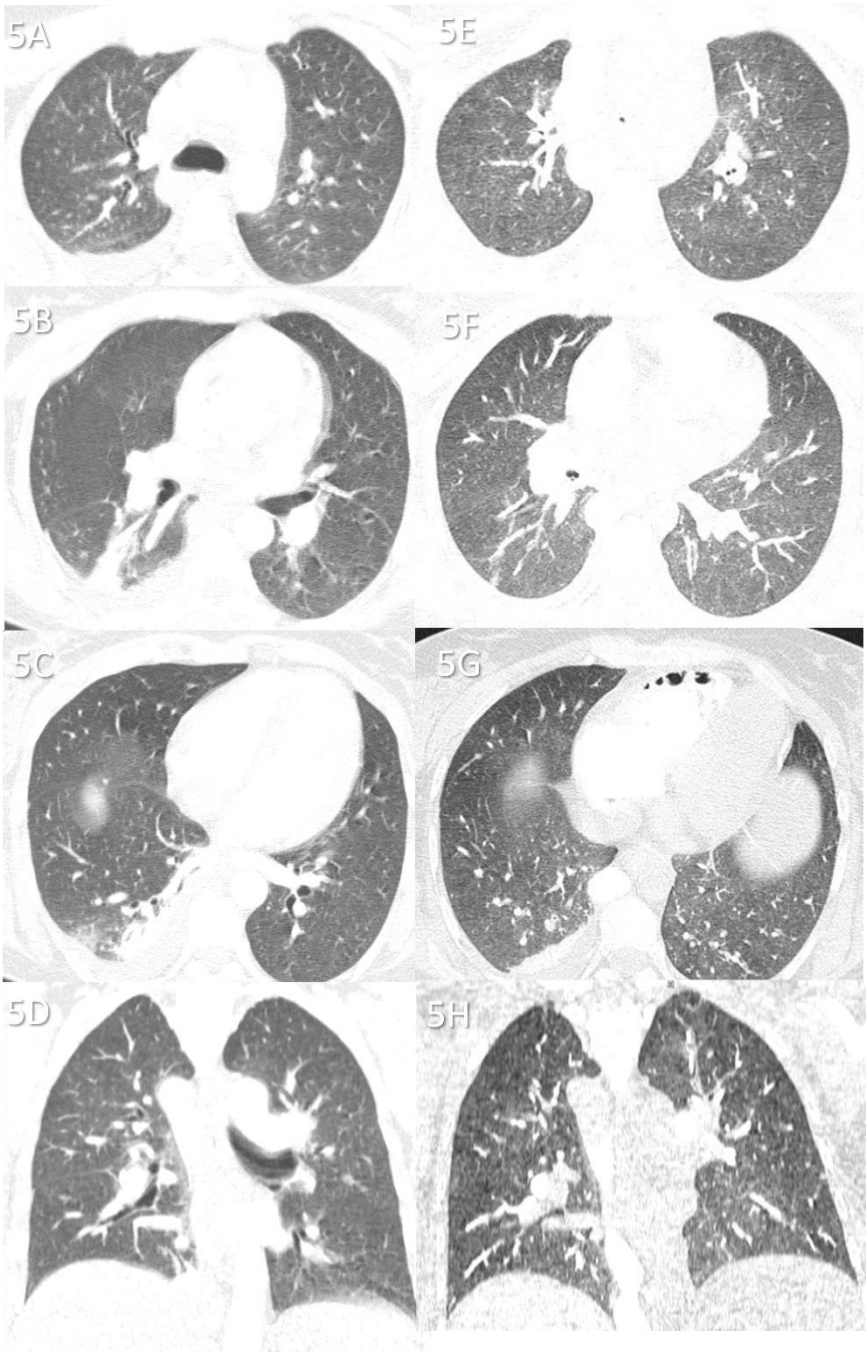

Figure 5 - Case 5: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing a few abnormalities, including a small peripheral and posterior ground-glass opacity in the lower right lobe (C), a small atelectasis in the posterior and medial aspects of the same lobe, and a right pleural effusion. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 16 h 13 min after death showing findings similar to premortem CT findings, except for a diffuse and subtle increase in attenuation of the lungs and thinning of the atelectasis in the right inferior lobe—changes probably because of the expired lungs during postmortem CT. The ground-glass opacity in the small right inferior lobe is not observed on postmortem CT, and the right pleural effusion is stable. Images D and H show pre- and postmortem coronal reformats, respectively. This patient died from liver transplant complications. Pre- and postmortem chest CT showing a normal lung parenchyma, indicating that she died with COVID-19, not from COVID-19.

3.3.1 Considerações sobre a publicação 2

Uma vez que percebemos a utilidade da TC de tórax *post mortem* nos casos de COVID-19 fatal com a experiência que resultou na publicação do artigo 1, o próximo passo seria determinar se os achados de imagem que estávamos encontrando após a morte, mesmo com as limitações levantadas, teriam correlação com os achados histopatológicos da doença.

O trabalho foi pensado, desenhado e executado durante a primeira onda da pandemia da COVID-19 e, portanto, antes do desenvolvimento das vacinas, em um esforço conjunto de radiologistas e patologistas tentarem entender melhor a doença. Pacientes sem patologia pulmonar conhecida prévia à pandemia, que morriam com COVID-19 e tinham autópsia solicitada pelo corpo clínico do HCFMUSP eram candidatos ao estudo. Após a realização da TC *post mortem*, múltiplos fragmentos eram coletados dos pulmões e enviados para análise histopatológica. Pacientes que apresentavam sinais de pneumonia secundária nas lâminas eram excluídos das análises, uma vez que os achados de imagem poderiam estar relacionados a outro agente além do SARS-Cov-2.

O esforço em interpretar e correlacionar os achados de imagem com os histopatológicos resultou no artigo 2, publicado em revista indexada, disponível na íntegra, a seguir.

3.3.2 Artigo 2

Savoia P, Sawamura MVY, Monteiro RAA, Duarte-Neto AN, Martin MGM, Dolhnikoff M, Mauad T, Saldiva PHN, Leite CC, Silva LFF, Cardoso EF. Postmortem chest computed tomography in COVID-19: A minimally invasive autopsy method. Eur. J. Radio. Open 12 (2024) 100546. DOI: https://doi.org/10.1016/j.ejro.2024.100546

Title: Postmortem Chest Computed Tomography in COVID-19: A Minimally Invasive Autopsy Method

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Postmortem Chest Computed Tomography in COVID-19: A Minimally Invasive Autopsy Method with Lung Analysis

ABSTRACT

OBJECTIVES: Performing autopsies in a pandemic scenario is challenging, as the need to understand pathophysiology must be balanced with the contamination risk. A minimally invasive autopsy might be a solution. We present a model that combines radiology and pathology to evaluate postmortem CT lung findings and their correlation with histopathology.

METHODS: Twenty-nine patients with fatal COVID-19 underwent postmortem chest CT, and multiple lung tissue samples were collected. The chest CT scans were analyzed and quantified according to lung involvement in five categories: normal, ground-glass opacities, crazy-paving, small consolidations, and large or lobar consolidations. The lung tissue samples were examined and quantified in three categories: normal lung, exudative diffuse alveolar damage (DAD), and fibroproliferative DAD. A linear index was used to estimate the global severity of involvement by CT and histopathological analysis.

RESULTS: There was a positive correlation between patient mean CT and histopathological severity score indexes - Pearson correlation coefficient (R) = 0.66 (p=0.0078). When analyzing the mean lung involvement percentage of each finding, positive correlations were found between the normal lung percentage between postmortem CT and histopathology (R=0.65, p=0.0082), as well as between ground-glass opacities in postmortem CT and normal lungs in histopathology (R=0.65, p=0.0086), but negative correlations were observed between ground-glass opacities extension and exudative diffuse alveolar damage in histological slides (R=-0.68,

p=0.005). Additionally, it was found is a trend toward a decrease in the percentage of normal lung tissue on the histological slides as the percentage of consolidations in postmortem CT scans increased (R =-0.51, p=0.055). The analysis of the other correlations between the percentage of each finding did not show any significant correlation or correlation trends (p≥0.10).

CONCLUSIONS: A minimally invasive autopsy is valid. As the severity of involvement is increased in CT, more advanced disease is seen on histopathology. However, we cannot state that one specific radiological category represents a specific pathological correspondent. Ground-glass opacities, in the postmortem stage, must be interpreted with caution, as expiratory lungs may overestimate disease.

KEY POINTS

1. Radiologists can read COVID-19 postmortem chest CT aiding pathologists in forensic studies.
2. Normal postmortem chest CT scans correlates to normal lung histopathology (screening tool?).
3. COVID-19 postmortem imaging findings correlate in extension and severity to histopathology.

KEY WORDS

1. Tomography; 2. Thorax; 3. Autopsy; 4. Pathology.

INTRODUCTION

The number of fatal victims of COVID-19 pandemic has reached approximately 6.9 million worldwide (up to March 2023) (1). Despite the elevated number of deaths, literature regarding COVID-19 conventional autopsies was relatively scarce, mostly because of contagion risks and/or the strict protection procedures recommended, which has substantially reduced traditional necropsies (2,3). In our country, conventional autopsies were forbidden when the pandemic broke out (4). This scenario will be observed in the beginning of any pandemic and the need for alternative methods is crucial. Autopsy operational issues drove some authors to develop minimally invasive autopsy methods to study fatal COVID-19: some performed lung, heart, liver and spleen histopathological analysis by using blinded tissue sampling (5), and others used lung ultrasound to guide histopathological analysis (6,7).

Chest CT was proven to be the method of choice to assess COVID-19 lung involvement in living patients, endorsed by the Society of Thoracic Radiology, the American College of Radiology, and the Radiological Society of North America (8). However, the literature about postmortem CT in COVID-19 is limited. Some authors have used postmortem CT as a triage tool to refer a patient to conventional autopsy when there were no typical CT findings of COVID-19 lung involvement to minimize the risks of autopsy team contagion (9,10). A case series analysis has shown that despite postmortem chest CT limitations (mainly expired lungs and hypostasis), most classic COVID-19 findings, as well as eventual additional findings, are present and similar to premortem chest CT. In some cases, authors could even demonstrate disease progression (11).

Our motivation emerged from the fact that autopsies were restricted and occasionally prohibited in the beginning of the COVID-19 pandemic, limiting the study

of a recent novel disease. The development of an alternative method to traditional autopsies, such as a minimally invasive autopsy seemed to be a solution to satisfy the eagerness to comprehend the pathophysiological processes involved in fatal COVID-19 cases.

Our main goal was to evaluate the additional value of postmortem chest CT to minimally invasive autopsy and verify whether postmortem CT lung findings could provide information regarding histopathological involvement.

MATERIALS AND METHODS

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068).

Population

From April to June 2020 (during the 'first wave' of the COVID-19 pandemic), 29 patients died with a laboratory confirmation of COVID-19 and had an autopsy requested by our institution's medical staff after informed consent was obtained from the next of kin.

Minimally Invasive Autopsy Protocol

The deceased bodies were covered by a plastic safety bag and transported to the morgue by nursery staff with adequate personal protective equipment. No inflation of the lungs was performed. The autopsy service performs postmortem studies with a dedicated CT scanner. Two trained technicians prepared the body for the CT scan, wrapping it with an additional plastic bag. The CT operator never had direct contact

with the bodies; in fact, most times, the equipment was operated remotely from another central operation room in the hospital.

Postmortem chest CTs were performed using a Somatom Emotion scanner (Siemens Healthineers, Erlangen Germany). Images were saved in DICOM™ format, with a standard lung filter reconstruction applied. CT Scan parameters are displayed on Table 1. All postmortem studies were sent to an online Picture Archiving and Communication System (PACS), provided by Purview©. All CTs were performed less than 24 hours after death.

Immediately after the postmortem chest CT was performed, multiple tissue samples were collected by ultrasound-guided biopsies, including samples from the brain, lungs, heart, liver, spleen, kidneys, salivary glands, pancreas, testis and striated muscle. This work considers only lung samples. They were ultrasound guided only to certify that lung tissue was sampled.. Furthermore, the biopsies were obtained in a standardized protocol from 4 different regions in each lung (Figure 1): superolateral, superomedial, inferolateral and inferomedial. As postmortem lungs are usually expired, anteriorly, we found the inferior lung limits around the 4^{th} or 5^{th} intercostal space; therefore, the 3^{rd} sternum costal joint was used as the division mark between the superior and inferior regions. Regarding lateral and medial regions, the division marks were the midclavicular lines. The posterior and anterior areas of each region were tissue sampled. We used a portable SonoSite M-Turbo R Ultrasound (Fujifilm, Bothell, WA, USA) with broadband and multifrequency transducers, C60x (5–2 MHz Curved) and HFL38X (13–6 MHz Linear), and semiautomatic coaxial 14G needles, 20 centimeters in length. The needles reached the posterior chest wall to certify that the posterior regions were always included. Approximately 60 pulmonary samples were collected from each lung.

Chest CT analysis

Images were examined independently by two experienced thoracic radiologists (each with more than 10 years of experience in specialized practice) who were blinded to the histopathological findings, and disagreements were resolved by consensus. Each region corresponding to the biopsied regions described above was quantified and classified into the following categories: normal; ground-glass opacities; crazy-paving; small consolidations; and large or lobar consolidations, as shown in Figure 2.

The following radiological criteria were used to define each of the five categories above, based on the Fleischner Society glossary of terms for thoracic imaging (12): (a) normal: preserved parenchyma; (b) ground-glass opacities: hazy increased opacity of lung, with preservation of bronchial and vascular margins; (c) crazy-paving: thickened interlobular septa and intralobular lines superimposed on a background of ground-glass opacity, resembling irregularly shaped paving stones; (d) small consolidations: a homogeneous increase in pulmonary parenchymal attenuation that obscures the margins of vessels and airway walls (up to 1.0 cm); (e) large or lobar consolidations: a homogeneous increase in pulmonary parenchymal attenuation that obscures the margins of vessels and airway walls (larger than 1.0 cm). An air bronchogram may be present.

The radiologists subjectively quantified the proportion of the five different patterns in each region (expressed as 5 percentage ranges – up to 20; 40; 60; 80; and 100%). The most severe diagnosis of each region was also highlighted. A consensus of the analysis was reached.

Other quantitative and qualitative fatal COVID-19 postmortem chest CT findings were also analyzed: percentage of global lung involvement; uni/bilaterality; affected

lobes; posterior or inferior predominance; peripheral, central or mixed distribution; presence of emphysema, fibrosis, mediastinal lymph node enlargement, pleural effusion, pleural thickening, pneumothorax, pericardial effusion, pneumomediastinum, aortic calcifications, coronary calcifications, and subcutaneous emphysema.

Histological analysis

Slides were examined with consensus by five experienced pulmonary pathologists (with a minimum of 6 and a maximum of 30 years of specialized practice experience) blinded to radiological findings and classified into the following categories: normal lung, exudative diffuse alveolar damage (DAD) and fibroproliferative DAD, as illustrated in figure 3.

The following histological criteria were used to define each pattern (13): (a) normal lung: preserved architecture, without inflammation, edema or exudate; (b) exudative DAD: interstitial and/or intraalveolar edema, interstitial inflammation, variable amounts of alveolar hemorrhage and fibrin deposition, intra-alveolar hyaline membranes and type II pneumocyte hyperplasia. Foci of neutrophilic pneumonia were also included in the acute/exudative pattern; and (c) fibroproliferative DAD: any degree of fibroblastic proliferation within the *interstitium* and/or alveolar spaces, including loose aggregates of fibroblasts admixed with scattered inflammatory cells, collagen deposition, squamous metaplasia, and possible remnants of hyaline membranes. The most severe diagnosis of each region was also highlighted, as it was not always the most common.

If the histopathological analysis exhibited any indications of secondary pneumonia, exclusion of the patient was necessary. This precaution ensured the ability

to unequivocally attribute the findings solely to COVID-19, mitigating potential confounding factors.

The pathologists subjectively quantified the proportion of the three different patterns in each region corresponding to the biopsied regions described above (expressed as 5 percentage ranges – up to 20; 40; 60; 80; and 100%). The most severe diagnosis of each region was also highlighted. A consensus of the analysis was reached.

Conventional autopsy of the lungs was not performed because it was forbidden in our country during the development of this study (4).

Statistical analysis

Statistical analysis was performed using R software (http://www.R-project.org). For each region, we calculated the Kappa concordance coefficient for the most severe diagnosis and the categorical percentage estimation between CT and the corresponding histological analysis, as follows: normal CT to normal pathology; ground-glass opacities to exudative DAD; crazy-paving, small consolidations, and large or lobar consolidations to fibroproliferative DAD. In addition, for each region, we calculated the Pearson correlation coefficient between each CT finding and histopathological finding.

To quantify lung pathological involvement in COVID-19, we proposed CT and histopathological severity scores as a linear combination of the percentage of pulmonary involvement weighted by the severity of the change. In the CT severity score (CTSS), the weighted coefficients for larger/confluent consolidations, small consolidations, crazy paving, and ground-glass opacities were 4, 3, 2, and 1,

respectively. In the histological severity score (HSS), the weighted coefficients for fibroproliferative DAD and exudative DAD were 4 and 2, respectively, as follows:

$$CTSS = 4\,(\%\, big\ or\ confluence\ consolidations) + 3\,(\%\, small\ consolidations) + 2\,(\%\, crazy\ paving) + 1\,(\%\, extensive\ ground\ glass\ opacities)$$

$$HSS = 4\,(\%\, fibroproliferative\ DAD) + 2\,(\%\, exudative\ DAD)$$

These scores increase as the severity and extent of pulmonary involvement by COVID-19 increase, ranging from zero to 400. In addition, it enables the calculation of the correlation coefficient between scores. The scores were calculated for each of the 4 regions of each lung, and the mean score of each patient was taken for correlation.

RESULTS

Of the 29 patients who underwent the minimally invasive autopsy protocol described, 14 had signs of secondary pneumonia in the histological analysis and thus had to be excluded once CT findings could be related to an agent other than SARS-CoV-2. Table 2 shows the epidemiological and clinical data of the 29 studied patients. Of the 15 patients without signs of secondary pneumonia in the histopathological examination, not all of them had sufficient and appropriate material from the 4 regions of each lung for the histological analysis. Therefore, in the end, 96 regions of adequate tissue were sampled from these 15 patients (Figure 4). The consensus agreement between the two radiologists about the chest CT analysis was 100% for the most severe diagnosis of each region and 97.66% for the proportion of the five different patterns in each region. The 2.33% disagreement was totally resolved by reanalyzing it together and reaching consensus. The time between death and postmortem CT was 14h36m ± 06h38m (average ± standard deviation).

There was no concordance between the CT and histopathological estimates of extension in all categories evaluated or between the most severe diagnosis for each region in CT and in the histological slides.

There was a positive correlation between the patient mean CT and histopathological severity score indexes. The Pearson correlation coefficient ®, estimated in our sample, was 0.66 ($p = 0.0078$), as shown in Figure 5 (Scatter plot).

Furthermore, we calculated the mean lung involvement percentage in the slides and in the CT for each finding (grouping small and large consolidations in postmortem CT) in each patient and estimated the Pearson correlation coefficient (R) between lung involvement percentages, as shown in a set of graphs (Figure 6). In Figure 6, there is a positive correlation between the percentage of normal lung in postmortem CT and histological slides ($R = 0.65$, $p = 0.0082$) (6A), as well as between the percentage of ground-glass opacities in postmortem CT scans and histological slides ($R = 0.65$, $p = 0.0086$) (6B). Moreover, as the percentage of ground-glass opacities in postmortem CT increased, there was a decrease in the percentage of exudative diffuse alveolar damage on the histological slides (negative correlation – $R = -0.68$, $p = 0.005$) (6F). Additionally, in Figure 6, there is a trend toward a decrease in the percentage of normal lung tissue on the histological slides as the percentage of consolidations in postmortem CT scans increases (negative correlation – $R = -0.51$, $p = 0.055$) (6D). The other subgraphs did not show any significant correlation or correlation trends ($p \geq 0.10$).

Some specific examples of postmortem chest CT lung images and the corresponding histopathological findings of our sample are presented in Figures 7 to 10. The other quantitative and qualitative fatal COVID-19 postmortem chest CT findings of the patients are shown in Table 3.

DISCUSSION

One of the primary factors to be considered pertains to the utility of minimally invasive autopsy in the presented situation. The severity index score created relies on both the percentage of disease involvement and the progression/severity of the disease. As the CT severity score index advances, there is a corresponding progression in the histopathological correlated index.

The utility of chest CT in living patients with COVID-19 was very well established for diagnosis and progression of the disease (8, 14, 15). Regarding postmortem chest CT in COVID-19, since the first case reports were published, attempts have been made to correlate tomographic findings with the progression/severity of the disease, as well as with what would be expected to be found in histopathological findings. Published data suggest that fatal COVID-19 cases exhibit extensive consolidations on CT scans and advanced DADs on histopathological slides, as endorsed by some authors (16-19).

To date, most published studies have been case reports or series of case reports (10, 19-21). This may be due to the difficulty of medical institutions having a dedicated CT scanner for postmortem studies. Additionally, the urgency to study and share information about COVID-19, especially during the early waves of the pandemic, may have limited the conduction of large casuistic studies, which usually requires more time. Despite the known limitations of postmortem chest CT (22), studies comparing COVID-19 pre and postmortem chest CT findings showed that comparison between them is possible (10, 11). A meta-analysis regarding postmortem chest CT in fatal COVID-19 cases was performed and was published in 2022 to try to understand how and what importance that postmortem CT has (16), and it selected a few studies that compared postmortem CT versus histopathological findings (8 out of 20).

The decision to use the mean CT and histopathological severity score indexes for each patient and not for each region was a way to reduce the limitation that it was not always possible to determine the exact limits of each region in both analyses (CT and histopathological), mainly because tissue sample collections were sometimes made with placing the needle with an oblique entry angle, the adjacent region could have been sampled, the fact that the biopsies were sampled under ultrasound guidance and not with CT guidance, and there was a pairwise discordance between CT and histology. This method enabled an increase in the signal-to-noise ratio and demonstrated a positive correlation between the mean CT and Histopathological Severity Score Indexes ($R = 0.66$, $p = 0.0078$ – Figure 5), showing that as the disease progressed with regard to pathology, whether in severity or extent, and that the disease also progressed in postmortem CT, despite the specific findings of the different phases of the disease not showing concordance between CT and histological slides. It is important to consider that a histological slide represents only a small sample of tissue, while the CT analysis includes larger regions of the lung. This “representation” difference imposes additional challenges for direct correlation between pathology and CT in a given region. An exact point-to-point correlation with CT-guided biopsies (which was not the case) could have improved these results, as suggested in other non-COVID correlation studies.

We also found a positive correlation between normal lung in CT and in the slides ($\rho = 0.65$; $p = 0.0082$) and a trend in the correlation between the proportion of normal lung tissue decreasing in histological analysis, as consolidation extended ($\rho = 0.51$; $p = 0.055$). This leads us to believe that the use of postmortem chest CT might be useful for screening cases without lung involvement. In other words, postmortem CT appears to be a good strategy for excluding disease, corroborating on what other

authors had already suggested in initial case reports, as referred to previously in this text. The presence of large consolidations corroborates that the lungs are histologically affected (nonnormal). Future studies might evaluate the probable high negative predictive value of postmortem CT scans.

In addition, our data showed an intriguing relationship between the mean proportion of ground-glass opacities and the histological categories. We found a positive correlation between the mean proportion of ground-glass opacities and the proportion of normal lung tissue and a negative correlation between the proportion of ground-glass opacities and the percentage of disease in the exudative phase. One possible explanation for these findings is that in postmortem cases, the value of ground-glass opacities as a predictor of disease may be questioned once the images are obtained with exhaled lungs and there is more blood stasis, generating nonpathological ground-glass opacities. This is a limitation of the method, as stated previously. Furthermore, as is known in the chest CT of living individuals, when expiration series are performed to evaluate areas of air trapping, we notice that normal areas (without trapping) end up exhibiting high attenuation, simulating ground-glass opacities (12, 23). Perhaps this is the greatest limitation of postmortem chest CTs. Since the lungs are most often in expiration, ground-glass opacities should be interpreted with caution. Some authors have already tried to differentiate postmortem ground-glass opacities attributed to COVID-19 from high attenuation, ground-glass mimicking opacities that would only be related to death itself and not COVID-19 infection (10). They concluded that fatal COVID-19 ground-glass opacities correlate with bilateral, peripheral and multilobar distribution, as in life, despite the small number of cases. In our sample of 15 patients, most of our patients had large consolidations occupying considerable lung volumes, sometimes even encompassing the entire lobe,

and most of our ground-glass/high attenuation opacities did not follow these typical viral disease patterns, probably explaining why we found a positive correlation between ground-glass opacities on CT and normal lungs tissue on the slides. A suggestion would be to suspect ground-glass opacities with the classic distribution of COVID-19, more peripheral, basal and multilobar (8), as pathological, and not those with atypical distribution, which would be more likely related to lung expiration. Another option for future studies regarding these ground-glass opacities confounding factors in postmortem studies could be the use of modern techniques such as CT radiomics analysis. Some authors used it as a supplementary tool for improving specificity for COVID-19 in a living population confounded by ground glass opacity changes from other etiologies (24).

One way to diminish those postmortem chest CT expiration-related issues would be to have patients undergo tracheal intubation or crico-thyrioidotomy (25), insufflate the lungs, and then perform the scan. Two possible issues are known: the difficulty of orotracheal intubation due to head and neck death-related rigor of the soft tissues, as well as possible gas leakage in different body compartments (26). As our study was conducted in the first wave of the pandemic, very little was known about the virus; thus, it was decided to scan patients without intubation to minimize team contagion risks.

The major limitation of minimally invasive autopsy is that other contributors to death (such as acute myocardial infarct or pulmonary thromboembolism) could not be evaluated as they would been evaluated during conventional autopsy. Despite this fact, the method enabled us to improve the understanding of radiological findings and disease progression.

As future lines of studies, larger projects integrating diverse imaging modalities (such as ultrasound and magnetic resonance) also across other parts of the human body, hold promise in enhancing the applicability of minimally autopsy methods, not only in fatal COVID-19. Additionally, the incorporation of artificial intelligence and machine learning in the analysis of postmortem CT images, as it is already being used in living patients (24, 27), has the potential to significantly enhance the precision and interpretation of imaging, especially if a large histopathological dataset is input.

CONCLUSIONS

The use of a minimally invasive autopsy method was useful in accessing the lungs of fatal COVID-19 patients by imaging and histopathology, especially in a context of restricted or suspended conventional autopsies. There is a correlation between the progression and severity of the disease when comparing postmortem CT and histopathology findings. Furthermore, encountering normal lungs in postmortem chest CT might be a good indication that those lungs are also normal in histopathology, and the interpretation of ground-glass opacities should be done with caution.

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ETHICAL STATEMENT

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068). Informed consents were obtained from the next of kin of all deceased patients.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Saldiva Paulo Hilario Nascimento: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mauad Thais**: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Dolhnikoff Marisa**: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Martin Maria da Graça Morais**: Writing – review & editing. **Savoia Paulo**: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cardoso Ellison Fernando**: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **da Silva Luiz Fernando Ferraz**: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Leite Claudia da Costa**: Writing – review & editing, Validation, Supervision, Resources. **Duarte-Neto Amaro Nunes**: Writing – review & editing,

Methodology, Formal analysis. **Monteiro Renata Aparecida Almeida**: Writing – review & editing, Methodology. **Sawamura Marcio Valente Yamada**: Writing – review & editing, Validation, Supervision, Formal analysis.

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TABLES / FIGURES LEGENDS

Table 1. Postmortem Chest CT Scan Parameters

Parameter	Value
kV	130
mA	AEC (78 to 260) or fixed in 128 or 136
Pitch	0.8
Matrix	512 x 512
Detector Rows	16
Detector Thickness	1.2 mm
Collimation	19.2 mm
Slice Thickness	1.5 mm

(AEC = automatic exposure control).

Table 2. Demographics and Clinical Characteristics of patients with fatal COVID-19

	No Secondary Pneumonia	Secondary Pneumonia (excluded)
Patients (number)	15	14
Gender (%)	40 Male/60 Female	57.1 Male/42.9 Female
Age (years)	51.27 ± 16.05	56.79 ± 17.85
Weight (kg)	75.79 ± 15.50	73.10 ± 29.34
Height (m)	1.70 ± 0,07	1.69 ± 0.09
BMI (kg/m²)	25.88 ± 4.15	25.34 ± 9.02
Ethnicity (%)	86.7 White / 6.7 Black / 6.7 Brown	85.7 White / 7.1 Black / 7.1 Brown
Time from Symptoms Onset to Admission (days)	9.13 ± 6.62	6.29 ± 3.79
Time from Symptoms Onset to Death (days)	25.13 ± 9.96	20.29 ± 10.97
Time from Admission to Death (days)	16.0 ± 10.51	14.0 ± 10.10
Time from ICU Admission to Death (days)	11.07 ± 9.07	10.93 ± 9.23

Table 2. Clinical and Demographic Characteristics of our cohort that was composed of 29 patients with fatal COVID-19. Data are displayed as the mean ± standard deviation. ICU: Intensive Care Unit.

Table 3. Other quantitative and qualitative fatal COVID-19 postmortem chest CT findings

Global Lung Involvement	
80 to 100%	11/15 (73.33%)
60 to 80%	3/15 (20.00%)
40 to 60%	1/15 (6.67%)
< 40%	None
Uni/Bilateral	
Bilateral	15/15 (100%)
Unilateral	None
Affected Lobes	
Only lower lobe(s)	None
Lower lobe(s) + at least one other lobe	15/15 (100%)
No lower lobe involvement	None
Predominance	
Inferior	1/15 (6.67%)
Posterior	3/15 (20.00%)
No predominance	11/15 (73.33%)
Distribution	
Peripheral	None
Central	None
Mixed	15/15 (100%)
Pulmonary Emphysema	1/15 (6.67%)
Pulmonary Fibrosis	None
Mediastinal Lymph Node Enlargement	8/15 (53.33%)
Pleural Effusion	
Right	
Large	None
Moderate	2/15 (13.33%)
Small	5/15 (33.33%)
None	8/15 (53.33%)
Left	
Large	None
Moderate	3/15 (20.00%)
Small	5/15 (33.33%)
None	7/15 (46.66%)
Pleural Thickening	3/15 (20.00%)
Pneumothorax	3/15 (20.00%)
Pericardial Effusion	2/15 (13.33%)
Pneumomediastinum	None
Aortic Calcifications	7/15 (46.66%)
Coronary Calcifications	6/15 (40.00%)
Subcutaneous emphysema	None

Figure 1.

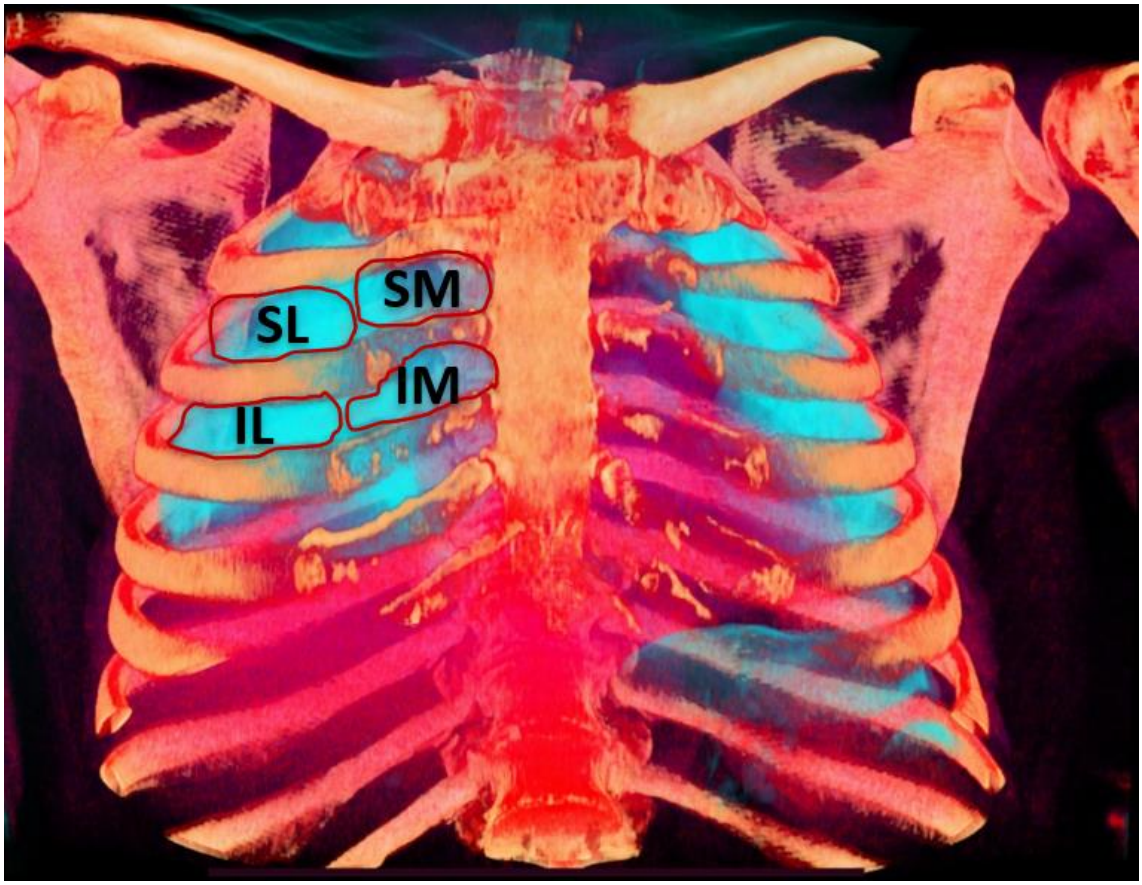

Figure 1. 3D volume rendering reformat from a postmortem chest CT from our study (using RadiAnt™ DICOM Viewer 2022.1 Software – 3D Preset: Bones and Skin 3) shows how the postmortem expired lungs (in blue) have their anterior lower limits around the 4th or 5th intercostal spaces. From this reconstruction, it is possible to understand why the 2nd anterior intercostal space was used for the tissue sample collection of the upper regions and the 3rd anterior intercostal space was used for the tissue sample collection of the inferior regions. The limit between the lateral and medial regions was the midclavicular line. The 4 regions of access to tissue sample collection are also shown: SL = superolateral; SM = superomedial; IL = inferolateral; IM = inferomedial

Figure 2.

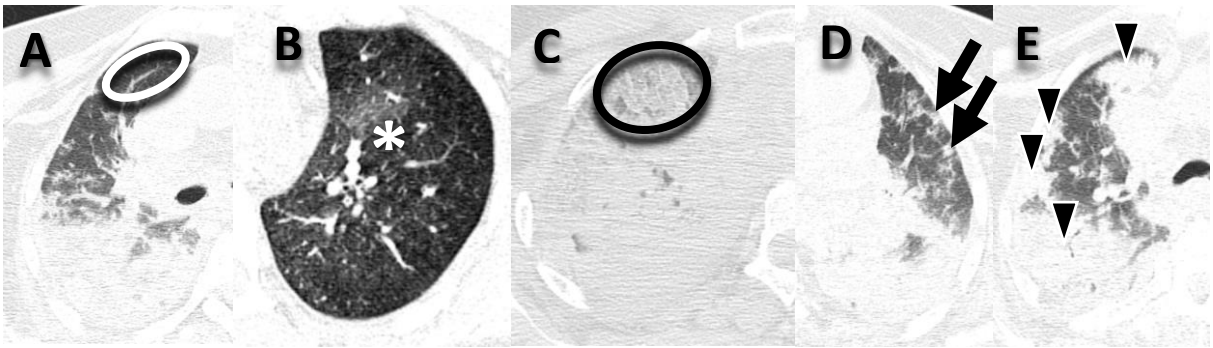

Figure 2. Examples of postmortem CT findings of our sample: A) Normal lung parenchyma (white ellipse). B) Ground-glass opacities (white asterisk). C) Crazy paving (black ellipse). D) Small consolidations (black arrows). E) Large consolidations (black arrowheads).

Figure 3.

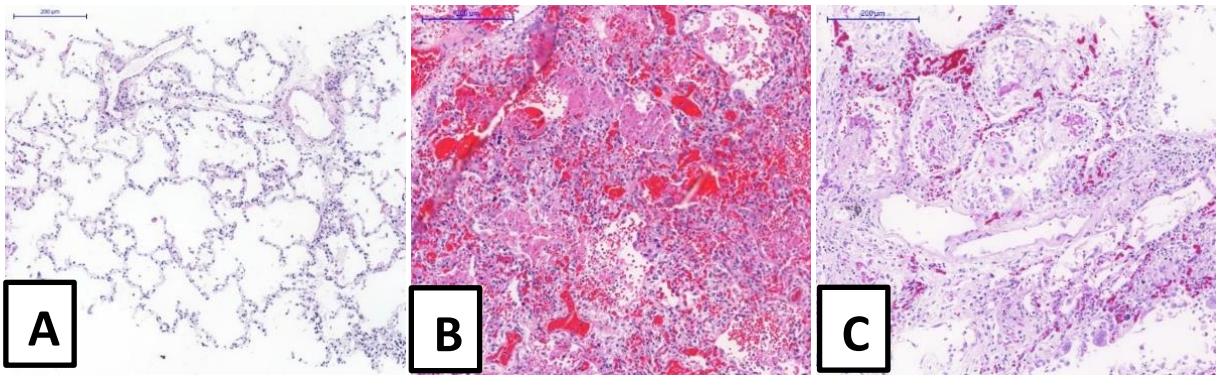

Figure 3. Examples of postmortem histological findings of our sample: A) Normal lung parenchyma. B) Acute/Exudative Diffuse Alveolar Damage. C) Fibroproliferative diffuse alveolar damage.

Figure 4.

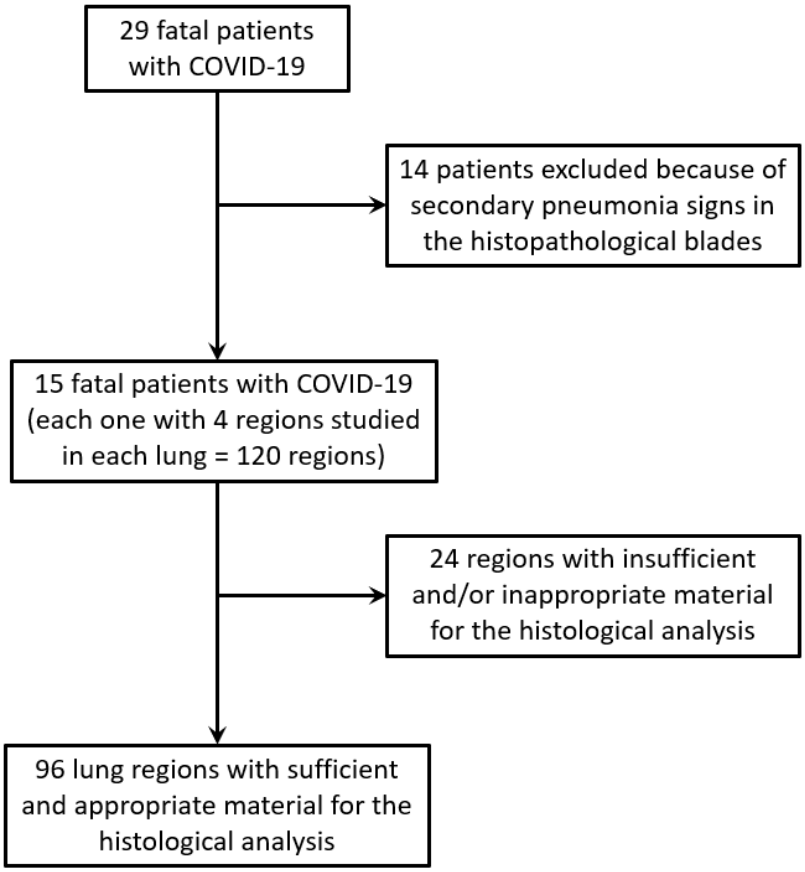

Figure 4. Flow diagram showing the initial number of patients and patients excluded. Afterward, from the patients not excluded, some regions had to be excluded because of insufficient and/or inappropriate material. In the end, 96 lung regions were analyzed.

Figure 5.

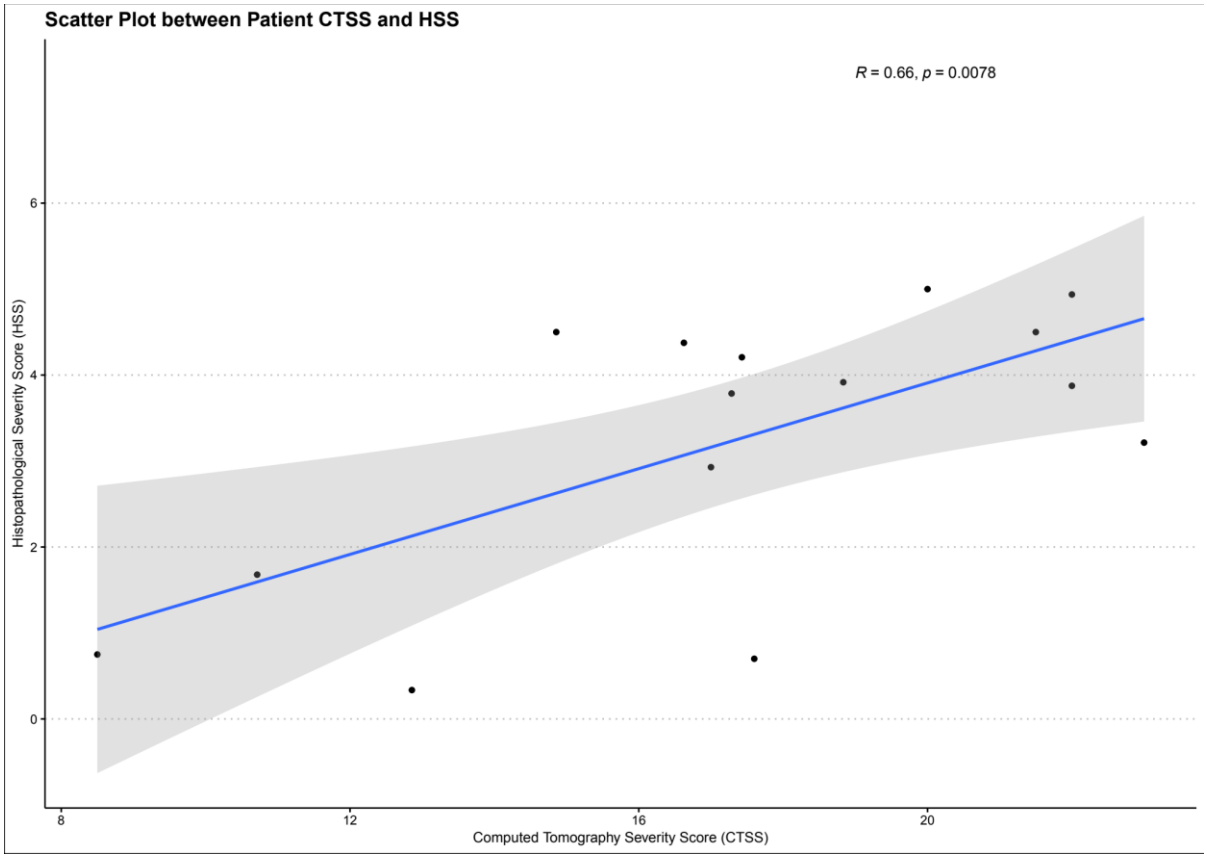

Figure 5. The correlation between CT and Histopathological severity score indexes.

Figure 6.

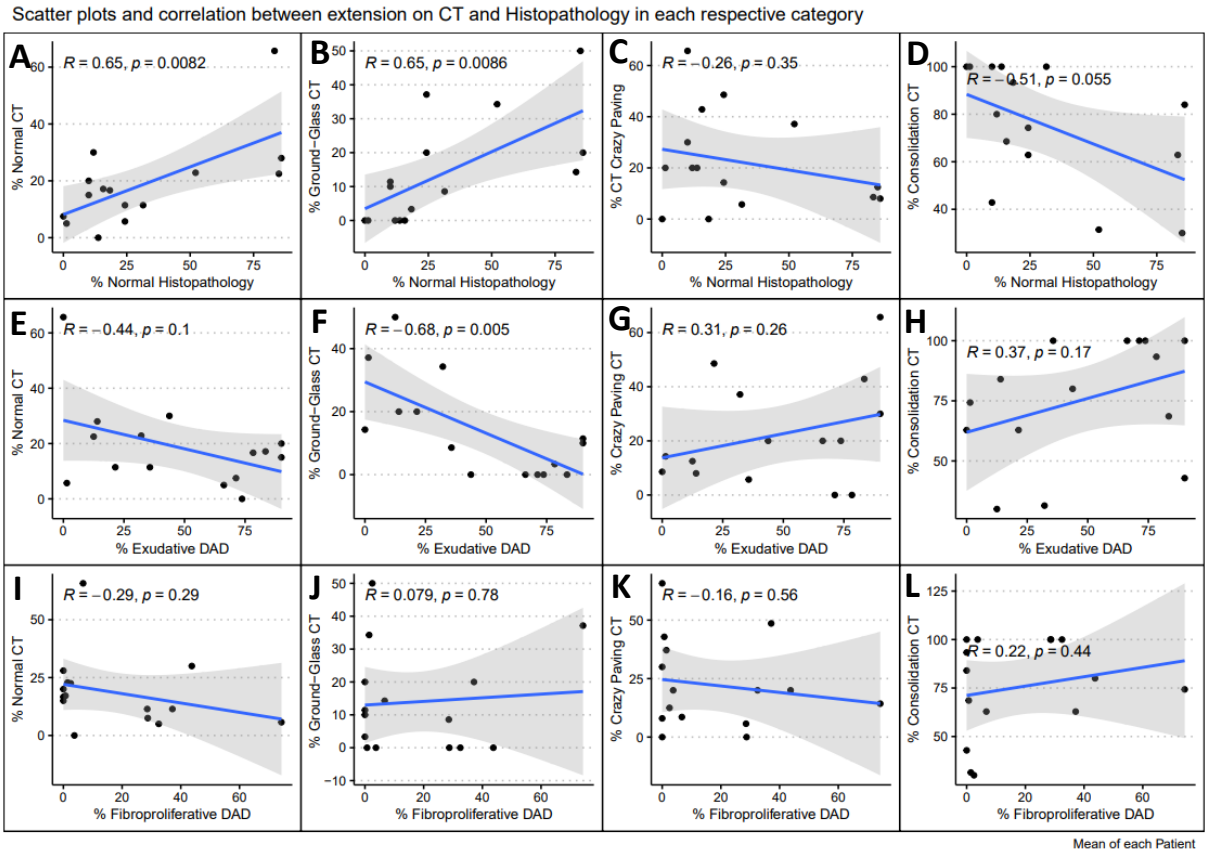

Figure 6. Correlation - Mean lung involvement percentage in the blades and in the CT for each finding.

Figure 7.

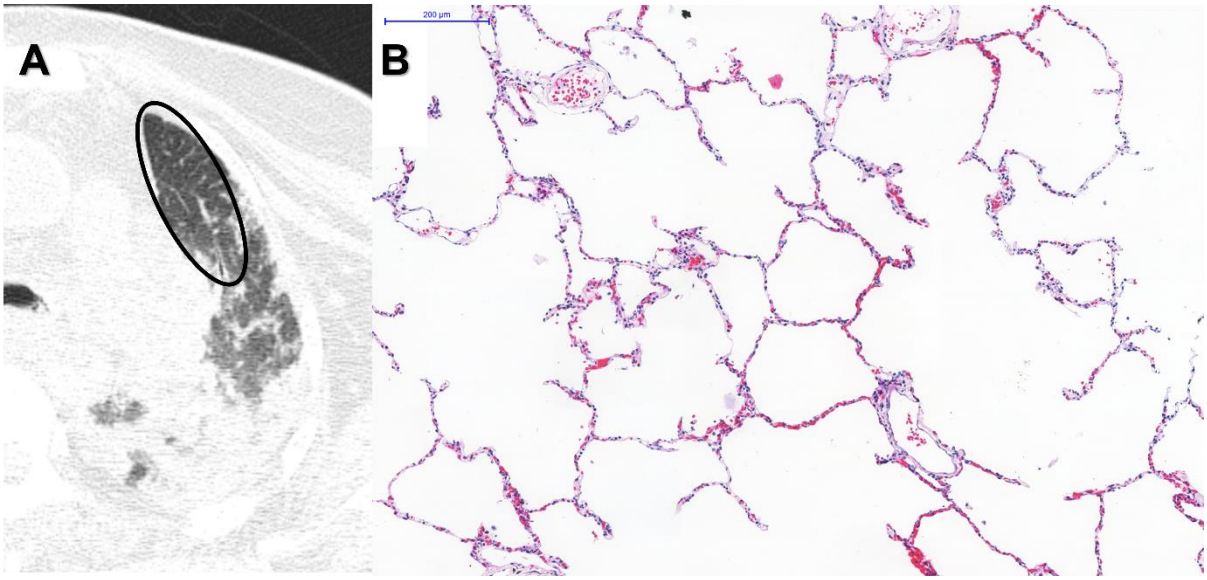

Figure 7. A) Postmortem Chest CT Image, Axial View, Left Upper Regions. There is a large area of normal lung parenchyma (ellipse). B) Histopathological sample of the same regions: Photomicrograph showing normal lung with open airspaces, thin alveolar septa, no inflammation and mild capillary congestion, commonly seen in autopsy specimens - HE Staining. Scale Bar - 200µm - Objective 10x.

Figure 8.

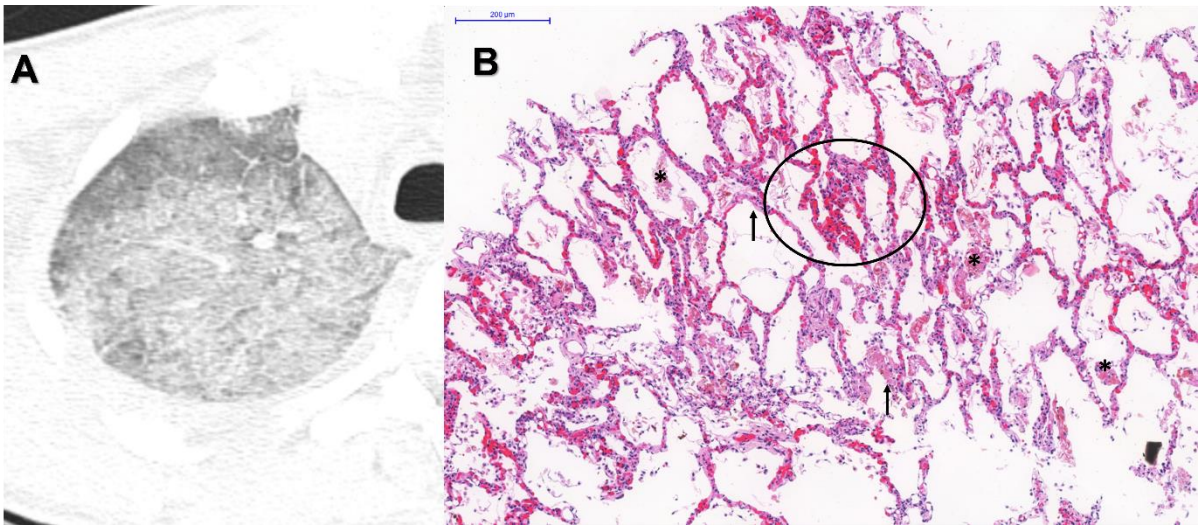

Figure 8. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of ground-glass opacities. B) Histopathological sample of the same regions: Photomicrograph of Acute Exudative DAD showing moderate septal inflammation, intense vascular congestion (ellipse), intra-alveolar fibrin deposition (asterisk) and formation of hyaline membranes (arrows) - HE Staining. Scale Bar - 200µm - Objective 10x.

Figure 9.

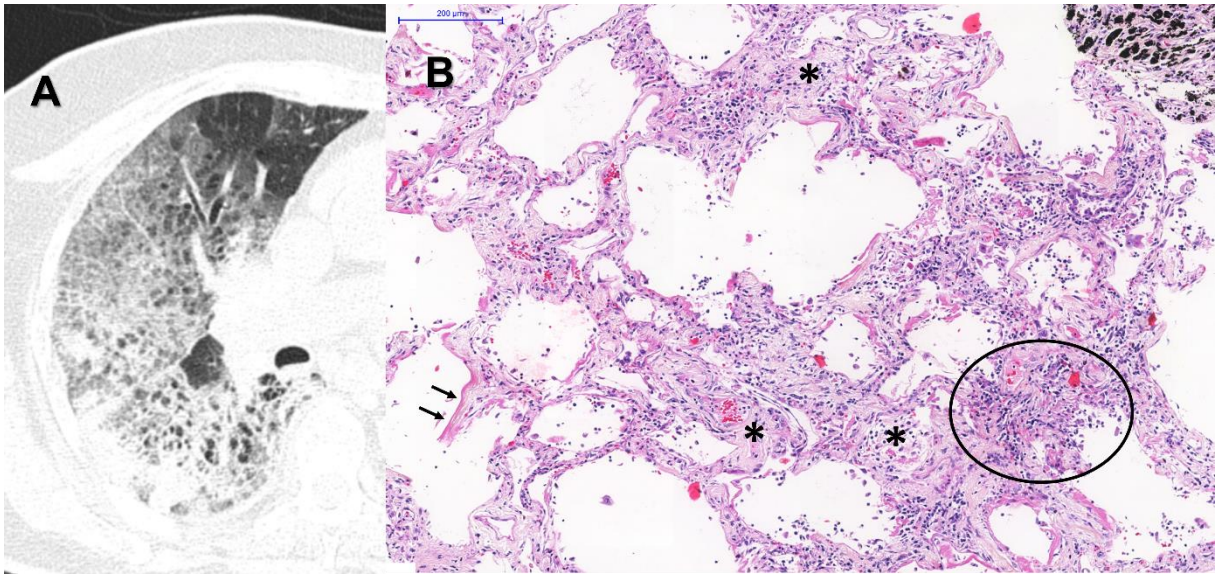

Figure 9. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of 'crazy paving' (thickened interlobular septa and intralobular lines superimposed on a background of ground-glass opacities). B) Histopathological sample of the same regions: Photomicrograph showing mixed DAD with some characteristics of acute DAD such as hyaline membranes (arrows) and abundant inflammatory cells (ellipse), as well as of fibroproliferative DAD with collagen deposition forming intra-alveolar plugs and septal thickening (asterisk) - HE Staining. Scale Bar - 200µm - Objective 10x.

Figure 10

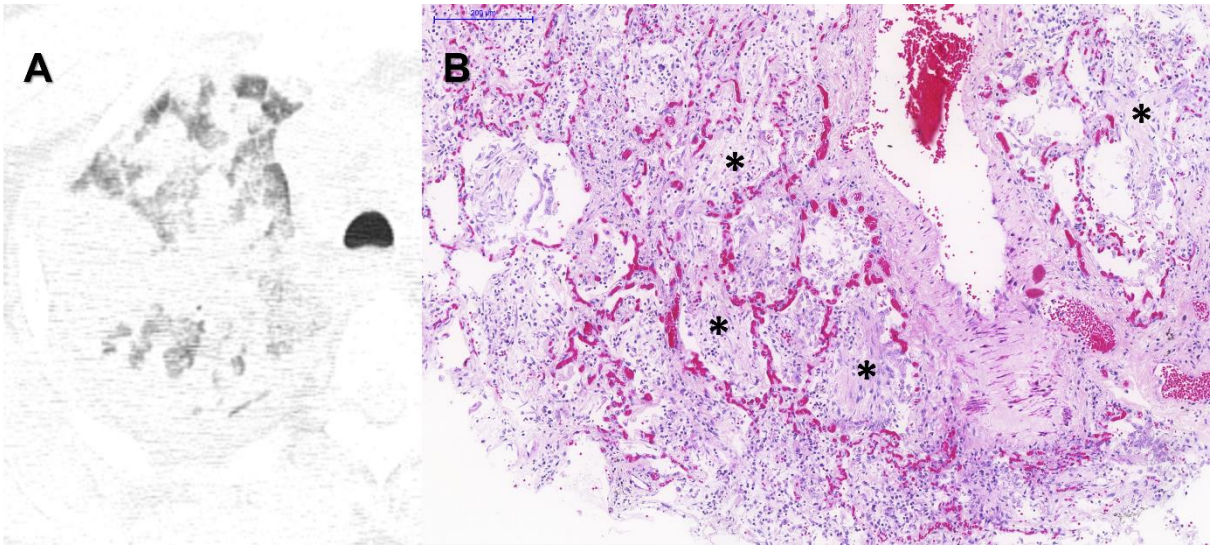

Figure 10. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of lung consolidations – small and large. B) Histopathological sample of the same regions: Photomicrograph of intense fibroproliferative DAD showing collagen deposition forming intra-alveolar plugs throughout the slide (asterisk) - HE Staining. Scale Bar - 200µm - Objective 10x.

3.3.3. Achados e comentários adicionais referentes à publicação 2

Além do exposto no artigo 2, há uma verificação adicional que não obteve dados suficientes para uma análise estatística significativa ou adequada. Trata-se da avaliação de infartos pulmonares em pacientes com COVID-19 fatal. Durante a pandemia foi constatado um aumento na incidência de tromboses vasculares, sobretudo venosa, e tromboembolismo pulmonar (49-52), nos levando a querer estudar infartos pulmonares em nossa amostra.

Na TC *post mortem*, consideramos as áreas suspeitas para infarto pulmonar como as consolidações mais periféricas com opacidades centrais radioluscentes (em vidro fosco ou pavimentação em mosaico), especialmente se apresentassem morfologia algo triangular, com base voltada para a periferia e ápice voltado para o centro como evidente na figura 11 (abaixo). Em vida, tais achados possuem especificidade de 98% e sensibilidade de 46% para infartos pulmonares (53). Tentamos correlacionar tais achados tomográficos com a presenta de infartos pulmonares evidentes nas lâminas histopatológicas de nossa amostra, como exemplificado na figura 12 (abaixo).

Entretanto, dos 15 pacientes com COVID-19 fatal sem pneumonia secundária, suspeitamos de áreas de infarto pulmonar em 5 pacientes, sendo que nas análises histopatológicas apenas 2 dos mesmos 15 pacientes tinham efetivamente infarto pulmonar, um deles que tinha áreas suspeitas na TC post mortem e o outro não. Além disso, mínimas áreas já são consideradas positivas nas lâminas, muitas vezes pequenas para exibirem representação tomográfica. Logo, não pudemos fazer análises satisfatórias ou chegar a uma conclusão em relação aos infartos pulmonares.

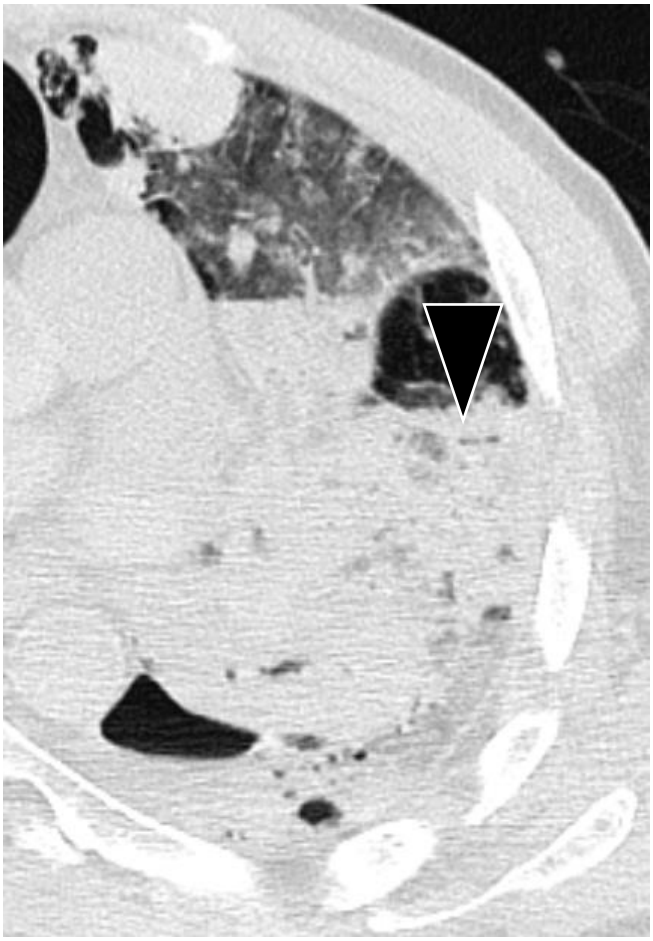

Figura 11 – TC *post mortem* do tórax com área suspeita para infarto pulmonar. Paciente com COVID-19 fatal da nossa amostra apresenta consolidação periférica, algo triangular, com base voltada para a periferia e ápice voltado para o centro, e opacidades centrais radioluscentes (cabeça de seta preta). Tal área foi considerada como suspeita para infarto pulmonar.

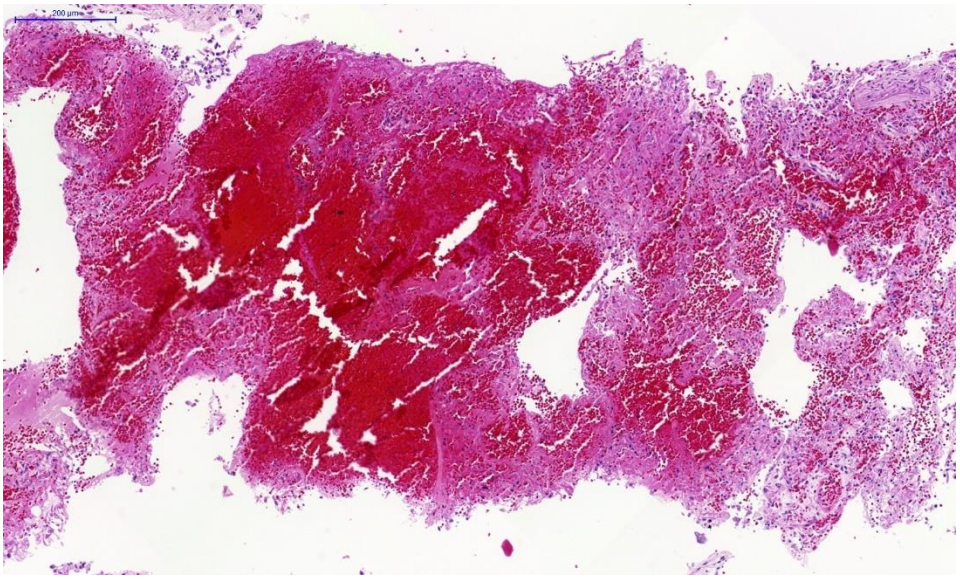

Figura 12 – Lâmina histopatológica da nossa amostra com área de infarto hemorrágico pulmonar. Tecido coletado de pulmões de paciente com COVID-19 fatal mostrando desestruturação alveolar, presença de debris necróticos e hemorragia. Coloração de HE. Barra de Escala - 200µm - Objetiva 10x.

Com relação à correlação encontrada entre as opacidades em vidro fosco nas TCs *post mortem* e os pulmões normais na histopatologia (R = 0.65, p = 0.0086), além do que foi exposto na discussão do artigo, é interessante ilustrar com um exemplo de TC do tórax realizada em vida (Figura 13). Quando a TC é realizada totalmente inspirada (aquisição padrão em vivos) não se observam alterações significativas (pulmões normais), entretanto, após o paciente realizar expiração (manobra eventualmente usada para pesquisar aprisionamento aéreo), a imagem da mesma região evidencia áreas com aumento de atenuação, simulando opacidades em vidro fosco, representando os pulmões normais expirados, permeando áreas de pulmão com menor atenuação, indicando áreas de aprisionamento aéreo, achado comumente observado em alterações de pequenas vias aéreas (54, 55). Como as TCs *post mortem* muitas vezes são realizadas com os pulmões expirados (comum após a morte), o pulmão normal expirado simularia opacidades em vidro fosco, podendo explicar a intrigante correlação encontrada entre opacidades em vidro fosco nas TCs *post mortem* e pulmões normais nas lâminas histopatológicas.

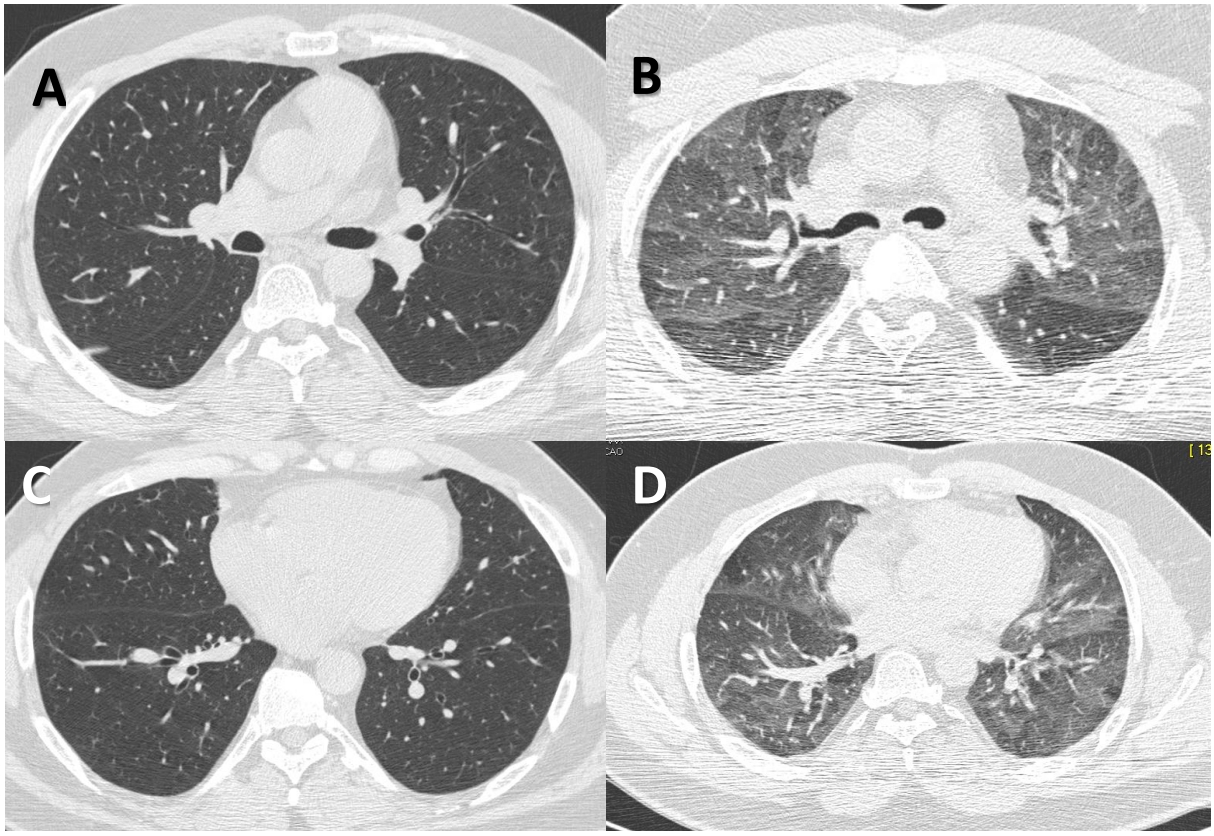

Figura 13 – Exemplo de TC de tórax em vivo (inspiração e expiração) – alta resolução, janela de pulmão. As imagens à esquerda (A e C) foram adquiridas em inspiração máxima (aquisição padrão em vivos) e não se observam alterações pulmonares relevantes. As imagens à direita (B e D) do mesmo paciente e nas mesmas localizações evidenciam áreas de aumento da atenuação pulmonar, simulando opacidades em vidro fosco, representando o pulmão normal expirado, e áreas com menor atenuação pulmonar, indicando áreas de aprisionamento aéreo.

4. CONCLUSÕES

a) Apesar das suas limitações conhecidas, a tomografia computadorizada *post mortem* do tórax foi confiável para propedêutica radiológica lógica e interpretação, com elaboração de raciocínios diagnósticos e de evolução. Quando comparada a estudos pré-morte recentes, ajudou a compreender a progressão da doença. Além disso, a tomografia computadorizada *post mortem* do tórax parece ser uma ferramenta adequada para avaliar o estágio da doença pulmonar.

b) O uso de um método de autópsia minimamente invasiva foi útil para acessar os pulmões de pacientes fatais de COVID-19 por meio de imagens e histopatologia, especialmente em um contexto de autópsias convencionais restritas ou suspensas. Existe uma correlação entre a progressão e a gravidade da doença ao comparar os achados de tomografia computadorizada *post mortem* e histopatologia. Além disso, encontrar pulmões normais em tomografias computadorizadas *post mortem* pode ser um bom indicativo de que esses pulmões também são normais na histopatologia, e a interpretação de opacidades em vidro fosco deve ser feita com cautela.

5. ANEXOS

5.1 Anexo 1 – Parecer CONEP CAAE

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

Plataforma Brasil

PARECER CONSUBSTANCIADO DA CONEP

DADOS DA EMENDA

Título da Pesquisa: Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom

Pesquisador: MARISA DOLHNIKOFF

Área Temática: A critério do CEP

Versão: 5

CAAE: 30364720.0.0000.0068

Instituição Proponente: Hospital das Clínicas da Faculdade de Medicina da USP

Patrocinador Principal: FUNDACAO FACULDADE DE MEDICINA

DADOS DO PARECER

Número do Parecer: 4.104.236

Apresentação do Projeto:

As informações elencadas nos campos "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas das Informações Básicas da Pesquisa, arquivo "PB_INFORMAÇÕES_BÁSICAS_1540567_E1.pdf", gerado na Plataforma Brasil em 26/05/2020.

INTRODUÇÃO

No cenário de uma epidemia por um agente novo e relativamente desconhecido, a autópsia representa ferramenta fundamental para a investigação de fenômenos relacionados à fisiopatogenia da doença e impacto em órgãos-alvo e sistêmico. No caso da COVID-19, raras autópsias foram realizadas até o momento, pelo risco de contágio dos profissionais envolvidos no processo. A autópsia minimamente invasiva (AMI) tem sido proposta como alternativa à autópsia convencional (AC) e se apresenta como ferramenta útil e eficaz neste cenário. O método tem se mostrado eficaz, particularmente para o diagnóstico de doenças infecciosas. Além disso, o procedimento se mostrou adequado para a coleta de amostras para estudos moleculares e para a identificação de microorganismos. Subprojeto 1: Avaliação post-mortem do comprometimento sistêmico na COVID-19 No presente projeto, propomos a realização da AMI-US de pacientes com morte por COVID-19 com coleta de tecido através de punção dos seguintes órgãos e tecido: pulmões, coração, fígado, rins, baço, SNC, músculo esquelético e pele. As autópsias tem o objetivo geral de avaliar o comprometimento pulmonar e sistêmico da COVID-19 em pacientes graves, a

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Telefone: (61)3315-5877 **E-mail:** conep@saude.gov.br

identificação de infecções secundárias associadas ao mau prognóstico, além de criar um biorrepositório para estudos futuros. Os principais objetivos são: Objetivos: a) Descrição dos achados histopatológicos pulmonares e sistêmicos em casos fatais de COVID-19. b) Identificação de infecções secundárias bacterianas e/ou fúngicas por métodos histoquímicos e moleculares. c) Descrição de achados de microscopia eletrônica em amostras do parênquima pulmonar, focando no encontro de vírions do 2019-nCOV, nas alterações da célula endotelial de capilares septais, nas alterações de pneumócitos e no interstício pulmonar. d) Caracterização da imunomarcação de antígenos do 2019-nCOV em diferentes tecidos por meio de imunohistoquímica e) Detecção e quantificação do RNA do vírus 2019-nCOV em amostras de tecido coletadas post mortem, por meio da técnica de PCR. f) Análise por transcriptoma de amostras de tecido pulmonar, coletadas no período post mortem de pacientes com COVID-19 fatal, correlacionando-a com parâmetros bioquímicos, com o aspecto morfológico das lesões à microscopia óptica e com o resultado da imunomarcação de antígenos in situ, por meio de imunohistoquímica, com o intuito de esclarecer processos patogênicos associados à evolução desfavorável da COVID-19. Subprojeto 2. Imunopatologia da COVID-19 fatal em pacientes idosos A maior gravidade e mortalidade da doença em idosos pelo COVID 19 está associada à desregulação imune desta faixa etária, que promove uma reação inflamatória sustentada de maior monta que em jovens. Nós hipotetizamos que nos tecidos pulmonares de pacientes idosos que não morreram de causas pulmonares haverá maior expressão dos marcadores inflamatórios, ligados ao envelhecimento, que se acentuará nos pacientes com SARS- Cov2. Hipotetizamos ainda que a expressão de ACE2 pulmonar será menor nos pacientes idosos que morreram de SARS-2. Os principais objetivos são: Fenotipar a lesão tecidual pulmonar em tecido obtido por MIA, comparando-se com pulmão de faixas etárias semelhantes, Comparar a quantidade de marcadores celulares linfocitários T e B, neutrófilos, células dendríticas, macrófagos e mastócitos em adultos vs adultos idosos (>65 anos) com pulmões normais á autopsia e aquele infectados por COVID2; Comparar a expressão tecidual dos receptores ACE2, e do TMPRSS em adultos vs adultos idosos (>65 anos) com pulmões normais á autopsia e aquele infectados por COVID2, Comparar a expressão de TLR-3, IL-6, TNF-alpha, IFN- gamma , MMPs, TGF beta, IL-1B e IL-17 em tecido pulmonar de adultos vs adultos idosos (>65 anos), Correlacionar achados anátomo-patológicos com marcadores de gravidade clínicos e marcadores inflamatórios séricos nos pacientes SARS-Covid2; Comparar e validar estes dados por dados de transcriptoma/PCR do tecido pulmonar de pacientes com SARS-Cov2.

HIPÓTESE

A maior gravidade e mortalidade da doença em idosos pelo COVID2 está associada à desregulação imune desta faixa etária, que promove uma reação inflamatória sustentada de maior monta que em jovens. Nós hipotetizamos que no tecidos pulmonares de pacientes idosos que não morreram de causas pulmonares haverá maior expressão dos marcadores inflamatórios, ligados ao envelhecimento, que se acentuará nos pacientes com SARS- Cov2. Hipotetizamos ainda expressão de ACE2 pulmonar será menor nos pacientes idosos que morreram de SARS-2.

METODOLOGIA

Autópsia Minimamente Invasiva Guiada Por Ultrassom (AMI-US) O procedimento será realizado em pacientes com confirmação ou suspeita de COVID-19 com óbito no Complexo HCFMUSP-INCOR. A AMI será realizada após solicitação pelo corpo clínico, com autorização dos familiares, utilizando os trâmites já existentes na Instituição. Consentimento livre e esclarecido de familiar ou responsável será obtido para a utilização do tecido coletado para protocolos de pesquisa. A AMI será realizada nas dependências do PISA (Plataforma de Imagem na Sala de Autópsia da FMUSP), por médicos pesquisadores treinados. Os corpos serão embalados com plástico protetor e submetidos a Tomografia Computadorizada de corpo inteiro. Em seguida, coleta de tecido será realizada por punção de órgãos-alvo guiada por ultrassom. Os seguintes órgãos serão biopsiados: pulmões, coração, rins, fígado, baço, cérebro e músculo esquelético. Utiliza-se equipamento portátil de ultrassonografia (US) SonoSite M-TurboR (Fujifilm, Bothell, WA, USA) com transdutores banda larga multifrequenciais C60x (5-2 MHz Convexo) e HFL38X (13-6 MHz Linear) e imagens padrão DICOMR . Os órgãos a serem avaliados por ultrassonografia serão: coração, pulmões, fígado, rins e baço. A avaliação US-modo B dos mesmos coletará informações detalhadas quanto a dimensões, morfologia, ecogenicidade, presença de lesões focais e coleções. Na sequência, as biópsias guiadas pela ultrassonografia serão direcionadas para os órgãos e eventuais alterações focais, por meio de sistema com agulhas Tru-CutR semi-automáticas coaxiais de 14G, com 20 cm de comprimento. Serão coletados um mínimo de 2 fragmentos adequados num total de até 5 tentativas de cada sítio de coleta. Na presença de coleções (derrames pleural e pericárdico, ascite), será realizada a aspiração do material, em quantidade suficiente para a sua análise. Lesões dermatológicas, se presentes, serão biopsiadas por punch. A última estrutura a ser biopsiada, sem o direcionamento pela US, será o cérebro. O corpo será colocado em decúbito lateral com a cabeça hiperfletida e voltada para o ombro ipsilateral, com acesso através do forame magno do osso occipital e trajeto que se estende pelo cerebelo, núcleos da base e lobo frontal. Serão coletados fragmentos a 15 cm, 18 cm e 20 cm do forame magno, mensurados através do guia coaxial. Três

fragmentos de tecido pulmonar congelados serão armazenados para os protocolos moleculares descritos adiante. Todos os demais fragmentos pulmonares coletados, assim como as amostras dos demais órgãos, serão imediatamente colocados em frascos individuais com formol a 10% para posterior análise histopatológica. Para o subprojeto 2, utilizaremos tecido pulmonar de pacientes adultos idosos e não idosos sem patologia pulmonar prévia como controle positivo para as alterações próprias do envelhecimento, coletados no SVOC-USP. A amostra constitui-se de 10 casos em cada braço. Protocolo de análise histológica - As amostras de tecido coletadas serão submetidas a exame histológico de rotina e coradas com hematoxilina-eosina (H&E), analisadas em microscopia óptica. Colorações específicas, como Brown-Brenn, Ziehl-Neelsen, Grocott e PAS serão realizadas para detectar bactérias, bacilos ácido-rápidos e fungos, respectivamente, quando necessário. Microscopia eletrônica - Fragmentos de tecido pulmonar serão fixados em glutaraldeído a 3% para exame microscopia eletrônica. A fixação e o processamento seguiram um protocolo descrito anteriormente. Os cortes finos serão analisados ao microscópio eletrônico de transmissão (Philips Tecnai 10, Hillsboro, OR, EUA, 80 kV) (Duarte et al, 1992). Protocolo de imunohistoquímica e Protocolo do transcriptoma (Wickham H, 2006).

CRITÉRIOS DE INCLUSÃO

1. Pacientes suspeitos ou confirmados para Covid-19 2. Pacientes com óbito no complexo HCFMUSP 2. Pacientes com óbito no complexo HCFMUSP.

CRITÉRIOS DE EXCLUSÃO

Pacientes suspeitos sem confirmação em vida ou post-mortem do diagnóstico de Covid-19.

Objetivo da Pesquisa:

OBJETIVO PRIMÁRIO

1.Descrição dos achados histopatológicos pulmonares e sistêmicos em casos fatais de COVID-19.

2.Identificação de infecções secundárias bacterianas e/ou fúngicas por métodos histoquímicos e moleculares.

3.Descrição de achados de microscopia eletrônica em amostras do parênquima pulmonar, focando no encontro de vírions do 2019-nCOV, nas alterações da célula endotelial de capilares septais, nas alterações de pneumócitos e no interstício pulmonar.

4. Caracterização da imunomarcação de antígenos do 2019-nCOV em diferentes tecidos por meio de imuno-histoquímica

5. Detecção e quantificação do RNA do vírus 2019-nCOV em amostras de tecido coletadas post mortem, por meio da técnica de PCR.
6. Análise por transcriptoma de amostras de tecido pulmonar, coletadas no período post mortem de pacientes com COVID-19 fatal, correlacionando-a com parâmetros bioquímicos, com o aspecto morfológico das lesões à microscopia óptica e com o resultado da imunomarcação de antígenos in situ, por meio de imunohistoquímica, com o intuito de esclarecer processos patogênicos associados à evolução desfavorável da COVID-19.
7. Fenotipar a lesão tecidual pulmonar em tecido obtido por MIA, comparando-se com pulmão de faixas etárias semelhantes Comparar a quantidade de marcadores celulares linfocitários T e B, neutrófilos, células dendríticas, macrófagos e mastócitos em adultos vs adultos idosos (>65 anos) com pulmões normais á autopsia e aquele infectados por COVID2.
8. Comparar a expressão tecidual dos receptores ACE2, e do TMPRSS em adultos vs adultos idosos (>65 anos) com pulmões normais á autopsia e aquele infectados por COVID2.
9. Comparar a expressao de TLR-3, IL-6, TNF-alpha, IFN- gamma , MMPs, TGF beta, IL-1B e IL-17 em tecido pulmonar de adultos vs adultos idosos (>65 anos)
10. Correlacionar achados anátomopatológicos com marcadores de gravidade clínicos e marcadores inflamatórios séricos nos pacientes SARS-Covid2, protocolo desenvolvido pelo grupo do Departamento de Pneumologia.
11.Comparar e validar estes dados por dados de transcriptoma/PCR do tecido pulmonar de pacientes com SARS-Cov2.

OBJETIVO SECUNDÁRIO
Não informado nas Informações Básicas.

Avaliação dos Riscos e Benefícios:
Riscos:
Minimo.

Beneficios:
Embora nao haja beneficios diretos para pessoa, a analise do tecido coletado ira contribuir para o entendimento dos mecanismos envolvidos no desenvolvimento da Covid-19.

Comentários e Considerações sobre a Pesquisa:
EMENDA 02

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

A presente emenda tem por objetivo incluir o médico radiologista Paulo Savoia Dias da Silva na equipe do estudo devido sua experiência prévia em tomografia computadorizada post-mortem, o que auxiliará na realização e avaliação dos exames do estudo.

Considerações sobre os Termos de apresentação obrigatória:

Vide item "Conclusões ou Pendências e Lista de Inadequações".

Recomendações:

vide item "Conclusões ou Pendências e Lista de Inadequações"

Conclusões ou Pendências e Lista de Inadequações:

Não foram encontrados óbices éticos nos documentos da emenda.

Considerações Finais a critério da CONEP:

Diante do exposto, a Comissão Nacional de Ética em Pesquisa - Conep, de acordo com as atribuições definidas na Resolução CNS nº 466 de 2012 e na Norma Operacional nº 001 de 2013 do CNS, manifesta-se pela aprovação da emenda proposta ao projeto de pesquisa.

Situação: Emenda aprovada.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1574235_E2.pdf	09/06/2020 16:20:25		Aceito
Outros	EmendaAdendoInclusPauloSavoia.pdf	09/06/2020 16:19:02	MARISA DOLHNIKOFF	Aceito
Outros	AnuenciaInclusPauloSavoia.pdf	09/06/2020 16:15:55	MARISA DOLHNIKOFF	Aceito
Outros	Doc8declaracaoInsttraduzidav2Carta2.pdf	26/05/2020 18:32:03	MARISA DOLHNIKOFF	Aceito
Outros	Doc7declaracaodaInstoriginaleminglesv1Carta2.pdf	26/05/2020 18:29:22	MARISA DOLHNIKOFF	Aceito
Outros	Doc6Projetcovid19comalteracaogrifadav3.pdf	26/05/2020 18:26:00	MARISA DOLHNIKOFF	Aceito
Outros	Doc5rProjetcovid19semmarcacaov2.	26/05/2020	MARISA	Aceito

Endereço: SRTVN 701, Via W 5 Norte, lote D - Edifício PO 700, 3º andar
Bairro: Asa Norte **CEP:** 70.719-040
UF: DF **Município:** BRASILIA
Telefone: (61)3315-5877 **E-mail:** conep@saude.gov.br

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

Continuação do Parecer: 4.104.236

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Outros	Doc4declaracaotaduzidacolextv2Carta1.pdf	26/05/2020 18:20:56	MARISA DOLHNIKOFF	Aceito
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Outros	CartaRespostaParecerConep.pdf	26/05/2020 17:48:28	MARISA DOLHNIKOFF	Aceito
Outros	Doc8Projetcovid19semmarcacaov2.pdf	22/05/2020 14:06:23	MARISA DOLHNIKOFF	Aceito
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Outros	Formadencolextercovid19.pdf	15/04/2020 11:30:54	MARISA DOLHNIKOFF	Aceito
Declaração de Pesquisadores	DecPesquisRespoJustAdendColExtCovid19.pdf	15/04/2020 11:30:32	MARISA DOLHNIKOFF	Aceito
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Folha de Rosto	FRcovid.pdf	30/03/2020 12:33:10	MARISA DOLHNIKOFF	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEcovidvf.pdf	30/03/2020 12:29:27	MARISA DOLHNIKOFF	Aceito

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Telefone: (61)3315-5877 **E-mail:** conep@saude.gov.br

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

Outros	SGPSistGestdaPesqHCFMUSP.pdf	28/03/2020 21:44:53	MARISA DOLHNIKOFF	Aceito
Outros	ParecerUsodedadosdigitaiscovid19.pdf	28/03/2020 21:41:48	MARISA DOLHNIKOFF	Aceito
Orçamento	Orcamentocovid19.pdf	28/03/2020 21:38:28	MARISA DOLHNIKOFF	Aceito
Outros	SGPCAPPesqResol441covid19.pdf	28/03/2020 21:37:14	MARISA DOLHNIKOFF	Aceito
Projeto Detalhado / Brochura Investigador	Covid19ProjAutop.pdf	28/03/2020 21:31:39	MARISA DOLHNIKOFF	Aceito
Cronograma	CronogramaProjCOVID.pdf	28/03/2020 21:29:07	MARISA DOLHNIKOFF	Aceito
Declaração de Instituição e Infraestrutura	DeclarPatol.pdf	28/03/2020 21:27:37	MARISA DOLHNIKOFF	Aceito
Declaração de Instituição e Infraestrutura	AnuenciaVO.pdf	28/03/2020 21:27:19	MARISA DOLHNIKOFF	Aceito
Declaração de Instituição e Infraestrutura	AnuenciaUTI.pdf	28/03/2020 21:27:00	MARISA DOLHNIKOFF	Aceito
Declaração de Instituição e Infraestrutura	AnuenciaPisa.pdf	28/03/2020 21:26:42	MARISA DOLHNIKOFF	Aceito

Situação do Parecer:
Aprovado

BRASILIA, 23 de Junho de 2020

Assinado por:
Jorge Alves de Almeida Venancio
(Coordenador(a))

Endereço: SRTVN 701, Via W 5 Norte, lote D - Edifício PO 700, 3º andar
Bairro: Asa Norte **CEP:** 70.719-040
UF: DF **Município:** BRASILIA
Telefone: (61)3315-5877 **E-mail:** conep@saude.gov.br

5.2 Anexo 2 – Carta de anuência da Patologia

UNIVERSIDADE DE SÃO PAULO
SERVIÇO DE VERIFICAÇÃO DE ÓBITOS DA CAPITAL

São Paulo, 28 de maio de 2020.

A/C
Departamento de Radiologia da FMUSP

Prezados Senhores

Informamos que o Grupo de Pesquisa de Autópsia Minimamente Invasiva do Departamento de Patologia da FMUSP e o Serviço de Verificação de Óbitos da Capital estão cientes e de acordo com o desenvolvimento do projeto do Dr. Paulo Savoia Dias da Silva em correlação de tomografia post-mortem e autópsia minimamente invasiva na COVID-19 e que dispõem da infraestrutura necessária para desenvolvimento da parte que nos cabe no presente projeto.

Atenciosamente,

Prof. Dr. Luiz Fernando Ferraz da Silva
Diretor do Serviço de Verificação de Óbitos da Capital
Invasiva do Departamento de Patologia da FMUSP
Vice-Coordenador do Grupo de Pesquisa em Autópsia Minimamente

Luiz Fernando Ferraz da Silva
Professor Doutor
Depto Patologia FMUSP

5.3 Anexo 3 – TCLE

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO-HCFMUSP

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DADOS DA PESQUISA

Título da pesquisa - **Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom**
Pesquisador principal – **Marisa Dolhnikoff**
Cargo / Função – **Professora do Departamento de Patologia da FMUSP**
Inscrição Conselho Regional de Medicina (CRM): **54500**
Unidade do HCFMUSP: **FMUSP, Departamento de Patologia**
Departamento/Instituto – **Departamento de Patologia/ Faculdade de Medicina da USP**

Convidamos o(a) Sra. para participar desta pesquisa "Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom".

Uma pessoa de sua família ou sob sua responsabilidade faleceu e os médicos que a atenderam solicitaram a realização de uma autópsia para esclarecer melhor todas as possíveis causas da morte. Atualmente, em alguns países já se realizam autópsias associadas aos exames de imagem, como por exemplo, ultrassonografia e tomografia, os quais têm contribuído para explicar melhor os motivos da morte desta pessoa.

O Hospital das Clínicas-FMUSP está implantando aqui a autópsia minimamente invasiva dirigida pela ultrassonografia (AMI-US), que consiste na coleta de tecido de vários órgãos para avaliação microscópica, utilizando-se uma punção por agulha, sem necessidade de realização de autópsia com abertura do corpo. A avaliação do tecido coletado permitirá complementar os resultados obtidos pelos exames clínico-laboratoriais no diagnóstico da causa da morte e comorbidades e propiciará o estudo da patogenia dessa nova doença (COVID-19). Os tecidos coletados serão armazenados de forma adequada sob minha responsabilidade, e irão fazer parte de um biorrepositórios de covid19. O biorrepositório é uma coleção de material biológico humano, coletado e armazenado ao longo da execução do estudo, conforme o regulamento e normas técnicas, éticas e operacionais pré-definidas, sem fins comerciais.

Por isso, solicitamos sua autorização para o estudo do tecido coletado por punção de agulha pela Autópsia Minimamente Invasiva e do sangue coletado para exames laboratoriais e para o armazenamento dos tecidos em nosso biorrepositório para estudos futuros.

Embora não haja benefícios diretos para a pessoa, a análise do tecido coletado irá contribuir para o entendimento dos mecanismos envolvidos no desenvolvimento da COVID-19.

Rubrica do sujeito de pesquisa ou responsável _____

Rubrica do pesquisador _____

Nome resumido do projeto: **Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom**	Confidencial	
Termo de Consentimento Livre e Esclarecido versão 1 de de de		
Nome do pesquisador: **Marisa Dolhnikoff**		
Hospital das Clínicas da Faculdade De Medicina da USP		
	Rubrica do Participante da Pesquisa /Representante Legal	Rubrica do Investigador Responsável

Em qualquer etapa do estudo, você terá acesso aos profissionais responsáveis para esclarecimento de eventuais dúvidas. A investigadora coordenadora do estudo é a professora Marisa Dolhnikoff do Departamento de Patologia da FMUSP. Ela pode ser encontrada na Av. Dr. Arnaldo, 455 – 1º andar – sala 1155 no telefone (11) 3061-8521. Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) – Rua Ovídio Pires de Campos, 225 – 5º andar – tel: (11) 2661-7585, (11) 2661-1548, (11) 2661-1549, das 7 às 16h de segunda a sexta feira ou por e-mail: cappesq.adm@hc.fm.usp.br

Mesmo tendo fornecido seu consentimento, você poderá retirá-lo a qualquer momento. Nesta situação o tecido coletado não será utilizado para pesquisa.

Todos os dados serão utilizados exclusivamente para fins de pesquisa e as informações de identificação serão mantidas sob sigilo, garantindo a confidencialidade. Os dados serão analisados em conjunto com outros pacientes, não sendo divulgada a identificação de nenhum paciente em particular seja por nome ou imagem que permita sua identificação.

Informamos ainda que não há despesas adicionais para os familiares / responsáveis em qualquer fase do estudo, incluindo os exames realizados. Também não há compensação financeira relacionada à participação.

Os familiares/responsáveis têm direito de serem mantidos atualizados sobre os resultados parciais do estudo.

O material obtido da autópsia minimamente invasivo será armazenado em um biorrepositório e será analisado pelo grupo de pesquisa deste estudo e de seu colaborador no exterior, respeitando as normas éticas previstas em lei *(Resolução CNS nº 441 de 2011, itens 1.II e 2.II)*.

Porém pode acontecer que futuramente seja interessante usar estas amostras para outros trabalhos de pesquisa. Neste caso, há necessidade de consultá-lo para autorizar o uso deste material?

(.....) SIM. Eu quero ser consultado para autorizar ou não cada pesquisa futura com o material.
(....) NÃO. Eu dispenso a autorização para cada pesquisa e estou informado (a) que a Comitê de Ética em Pesquisa do Hospital das Clínicas (CAPPesq) irá examinar a nova pesquisa e decidir sobre a utilização ou não do material.

Acredito ter sido suficientemente informado a respeito das informações que li ou que foram lidas para mim, descrevendo a pesquisa "Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom".

Nome resumido do projeto: **Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom**	Confidencial	
Termo de Consentimento Livre e Esclarecido versão 1 de de de		
Nome do pesquisador: **Marisa Dolhnikoff**		
Hospital das Clínicas da Faculdade De Medicina da USP		
	Rubrica do Participante da Pesquisa /Representante Legal	Rubrica do Investigador Responsável

Eu discuti com o Dra Marisa Dolhnikoff sobre a minha decisão em participar nesse estudo. Ficaram claros para mim quais são os propósitos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que a autorização para a inclusão de meu familiar é isenta de despesas e que tenho o direito de ser mantido atualizado sobre os resultados parciais do estudo.

Concordo voluntariamente em participar deste estudo, assino este termo de consentimento e recebo uma via rubricada pelo pesquisador.

Data ____/____/_____
Assinatura do participante /representante legal

Nome do participante/representante legal

Data ____/____/_____
Assinatura do responsável pelo estudo

Nome resumido do projeto: **Estudos da COVID-19 fatal por meio da autópsia minimamente invasiva guiada por ultrassom**	Confidencial	
Termo de Consentimento Livre e Esclarecido versão 1 de de de		
Nome do pesquisador: **Marisa Dolhnikoff**		
Hospital das Clinicas da Faculdade De Medicina da USP		
	Rubrica do Participante da Pesquisa /Representante Legal	Rubrica do Investigador Responsável

5.4 Anexo 4 - Artigo Publicado 1 – Referente ao Primeiro Objetivo da Tese

CLINICS

COMMENTS

Postmortem Chest Computed Tomography in Fatal COVID-19: A Valuable Diagnostic Tool for Minimally Invasive Autopsy

Paulo Savoia Dias da Silva [I,II,III,*] Marcio Valente Yamada Sawamura [I] Renata Aparecida de Almeida Monteiro [I] Amaro Nunes Duarte-Neto [I] Maria da Graça Morais Martin [I] Marisa Dolhnikoff [I] Thais Mauad [I] Paulo Hilário Nascimento Saldiva [I] Claudia Costa Leite [I] Luiz Fernando Ferraz da Silva [I] Ellison Fernando Cardoso [I,II]

[I]Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, BR. [II]Hospital Israelita Albert Einstein, Sao Paulo, SP, BR. [III]Fleury Group, Sao Paulo, SP, BR.

Silva PSD, Sawamura MVY, Monteiro RAA, Duarte-Neto AN, Martin MGM, Dolhnikoff M, et al. Postmortem Chest Computed Tomography in Fatal COVID-19: A Valuable Diagnostic Tool for Minimally Invasive Autopsy. Clinics (Sao Paulo). 2021;76:e3551

*Corresponding author. E-mail: paulosavoia@gmail.com

The coronavirus disease (COVID-19) pandemic has resulted in more than 4.7 million deaths worldwide (1). Despite the high number of COVID-19-related deaths, published reports on autopsies are scarce, probably because of contagion risk and/or recommended strict protection procedures that restrict autopsies considerably (2,3). Therefore, to address this postmortem knowledge gap, some authors have studied patients who died because of COVID-19 using minimally invasive autopsy methods but not chest computed tomography (CT) (4,5).

Postmortem chest CT has some limitations owing to the inherent characteristics at death such as expired lungs and hypostasis. However, we believe these characteristics do not significantly limit the value of postmortem CT, especially during the COVID-19 pandemic, when traditional autopsies are often avoided or even forbidden.

Few authors have used postmortem chest CT to study fatal COVID-19, but some case reports have been published (6–9). Helmrich et al. presented a case series in which postmortem chest CT was used as a triage tool to refer a body for conventional autopsy when no typical CT characteristics of COVID-19 were found (10). De-Giorgio et al. used postmortem chest CT to confirm or exclude the disease and minimize risks of contagion to the autopsy team (11). Both studies suggest that postmortem CT is especially useful when reverse transcription polymerase chain reaction (RT-PCR) is not feasible.

To validate postmortem chest CT findings, we selected the 5 of 117 patients who had a premortem chest CT performed at most 2 days before death to compare their premortem with their postmortem chest CT and describe findings as well as eventual associated conditions. We hypothesized that a postmortem chest CT could help us understand and stage COVID-19, as well as diagnose other associated conditions, similar to a premortem chest CT, despite changes to the lungs inherent with death.

Patients

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068).

From March 2020 to early September 2021, 117 patients died because of laboratory-confirmed COVID-19 and underwent an autopsy that was requested by our institution's medical staff, after informed consent was obtained from the next of kin. Of the 117 patients in our convenience sample, only 5 had a chest CT performed up to 2 days before death (4 patients in 2 days and one patient in one day) and a postmortem chest CT performed as part of a minimally invasive autopsy study. All patients were women, with a mean age of 36 ± 20 years. The mean interval between death and the postmortem chest CT was 14 h 08 min ± 5 h 28 min. After the postmortem CT was performed, tissues from multiple organs were collected via ultrasound-guided biopsies.

A descriptive analysis is presented. Table 1 shows patient's main data. Afterwards we describe each case with main clinical data and imaging findings.

Case 1 (Figure 1): A 67-year-old female patient was hospitalized for approximately 1 month in the intensive care unit (ICU) before death. The cause of death was acute respiratory distress syndrome (ARDS) caused by COVID-19. Secondary pneumonia was also observed upon lung tissue analysis.

Case 2 (Figure 2): An 11-year-old female patient with rapid progression of COVID-19 was admitted to the hospital 7 days after the onset of respiratory symptoms. She was directly admitted to the ICU and died 1 day later. The causes of death were myocarditis and ARDS caused by COVID-19. Lung tissue analysis revealed no secondary pneumonia.

Case 3 (Figure 3): A 35-year-old female patient was admitted to the hospital 5 days after the onset of respiratory symptoms. She was transferred to the ICU 4 days after

No potential conflict of interest was reported.

DOI: 10.6061/clinics/2021/e3551

Table 1 - Main data of each patient.

Patient	Age (years)	Sex	Body mass index (kg/m^2)	Time since symptoms onset until death (days)	Hospitalization time (days)	Days between pre- and postmortem CT	Time between death and postmortem CT	Cause of death[a]	Secondary pneumonia
1	67	F	32.6	32	29	2	4 h 47 min	ARDS/COVID-19	Yes
2	11	F	22.6	8	1	2	14 h 03 min	Myopericarditis/ COVID-19	No
3	35	F	15.6	16	11	2	17 h 19 min	ARDS/COVID-19	Yes
4	38	F	20.4	18	9	1	18 h 20 min	ARDS/COVID-19	No
5	31	F	25.4	1	10	2	16 h 13 min	Acute liver failure	No

[a]Cause of death was determined via an histopathologic analysis of tissues collected through ultrasound-guided biopsy of multiple organs, performed after postmortem CT. ARDS, acute respiratory distress syndrome.

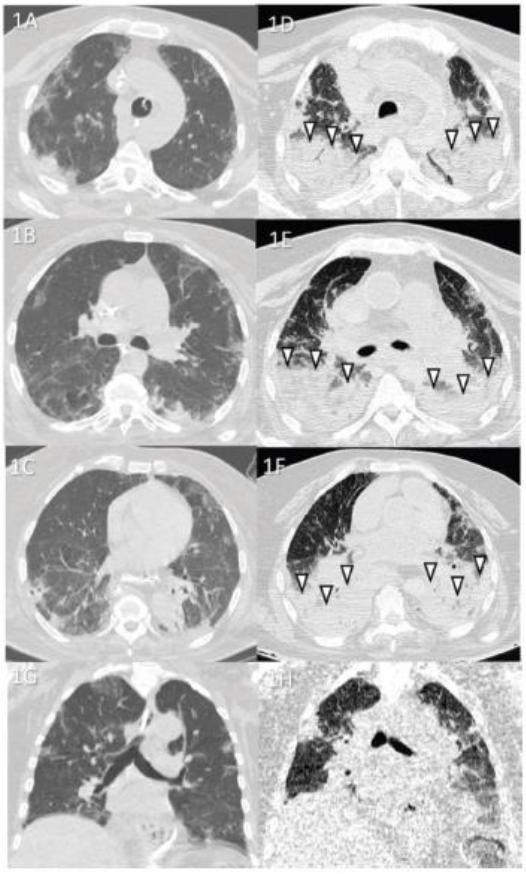

Figure 1 - Case 1: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing peripheral ground-glass opacities, slight consolidations, and interlobular septal thickening. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 4 h 47 min after death showing opacities larger than those on premortem CT, thus demonstrating the progression of the disease until death. Postmortem posterior "horizontal level forming" consolidations because of hypostasis (white arrowheads) are also observed, which limited analysis of the posterior lungs in this case (limitation of the method). Images D and H show pre- and postmortem coronal reformats, respectively.

admission and died 7 days later. The cause of death was ARDS caused by COVID-19. Secondary pneumonia was observed upon lung tissue analysis.

Case 4 (Figure 4): A 38-year-old female patient was admitted to the ICU 9 days after the onset of respiratory symptoms and died 9 days later. The cause of death was ARDS caused by COVID-19. Lung tissue analysis revealed no secondary pneumonia.

Case 5 (Figure 5): A 31-year-old female patient with a transplanted liver was admitted to the hospital because of hepatic complications. Nine days after admission, she developed respiratory symptoms and was transferred to the ICU, where she died 1 day later. The cause of death was acute liver failure. Lung tissue analysis revealed neither COVID-19-related pneumonia nor secondary pneumonia.

Table 2 presents the main positive and negative aspects of our analysis.

In all five cases, the logical radiological reasoning and interpretation of the main imaging findings showed disease progression until death. Despite the known limitations of postmortem CT, we were able to show that the information obtained can be useful in the appropriate scenario.

This study aimed to elaborate on the use of minimally invasive autopsy techniques, particularly in COVID-19 cases. The use of postmortem CT to help establish the correct cause of death is not new (12–15). During the COVID-19 pandemic, chest CT has played an important role in diagnosing and staging the disease in patients (16–18). Thus, it is logical to use postmortem CT to study COVID-related deaths, as we have attempted to do since the beginning of the COVID-19 pandemic. The supposed limitations of postmortem chest CT are already known: expired lungs (different from fully inspired lungs of the living); the dead can aspirate during their final moments; hypostasis may be present in the lungs (as in our case 1), depending on the time after death (19); and, of course, lung CT findings can change very quickly, within a few hours or days.

In addition, it is very difficult to identify a COVID-19 patient for whom a premortem chest CT was performed a few days before death, mainly because many of these patients are in severe clinical condition, with most in ICUs, which limits CT realization. Therefore, despite the relatively small number of cases, our results support the use of postmortem CT in this scenario.

Premortem chest CT findings were important for interpretation of postmortem CT findings. Our diagnostic performance improved when the findings were analyzed

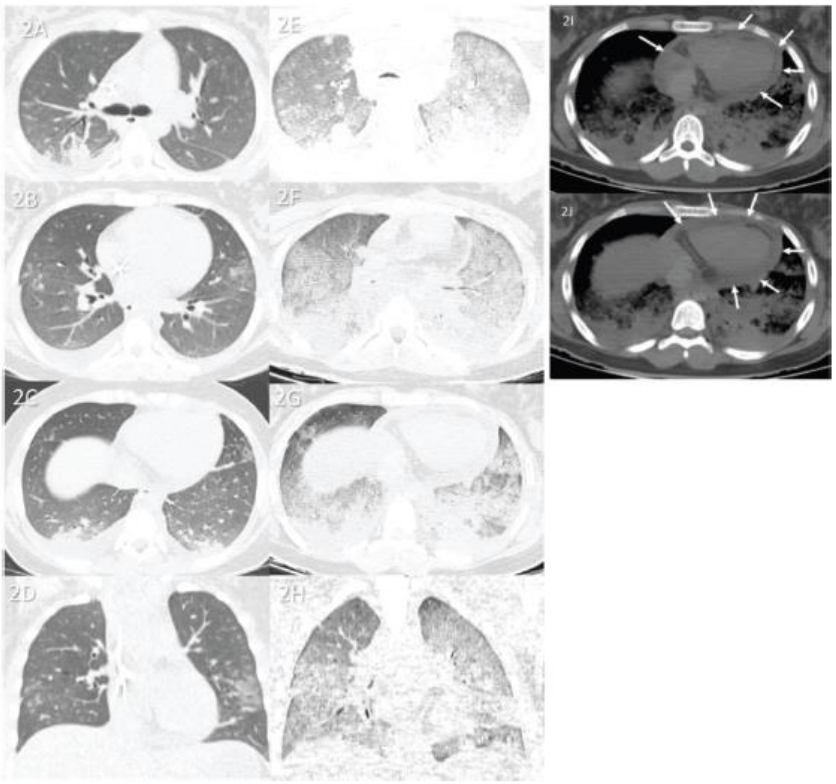

Figure 2 - Case 2: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing peripheral ground-glass opacities, small posterior bilateral consolidations, and right pleural effusion. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 14 h 03 min after death showing a diffuse "crazy paving" pattern, possibly because of acute respiratory distress syndrome and subsequent death. Furthermore, the posterior bilateral consolidations are larger on postmortem CT than on premortem CT, indicating progression of the disease. Images D and H show pre- and postmortem coronal reformats, respectively. Postmortem axial chest CT (I and J) of the mediastinal window showing pericardial effusion (white arrows) related to myopericarditis (confirmed with the collected tissue sample as the probable cause of death).

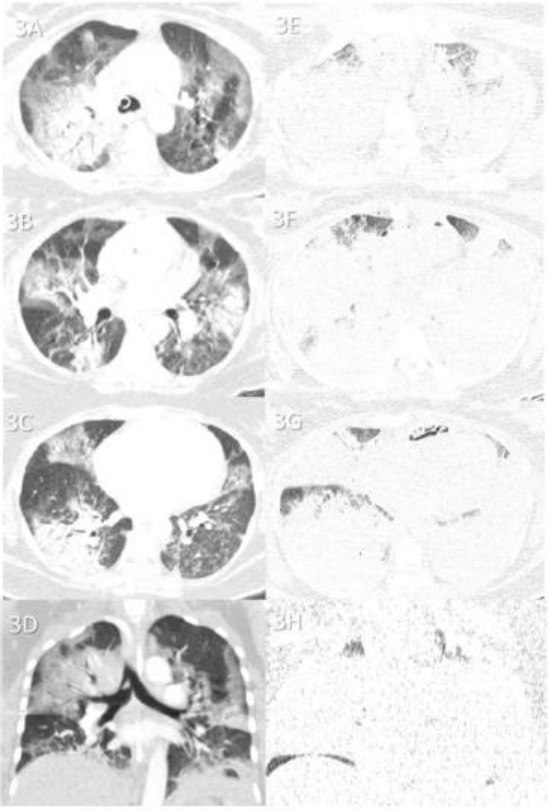

Figure 3 - Case 3: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing patchy peripheral and central ground-glass opacities and bilateral but mainly peripheral consolidations. Postmortem axial chest CT of the upper (E), mid, (F) and inferior (G) thirds of the lungs obtained 17 h 19 min after death showing rapid progression of the disease, with extensive consolidations in both lungs and a few areas of preserved pulmonary parenchyma. Images D and H show pre- and postmortem coronal reformats, respectively.

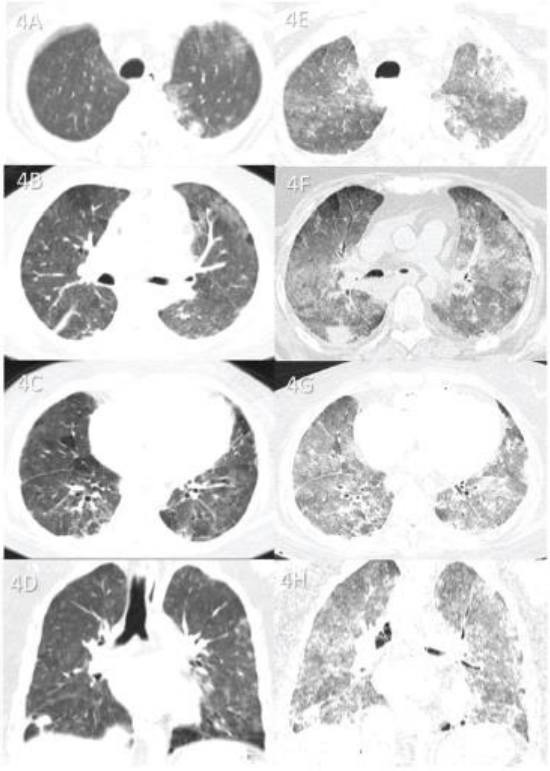

Figure 4 - Case 4: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 1 day before death showing peripheral ground-glass opacities and diffuse pulmonary mosaic attenuation because of ventilation and/or perfusion disturbances (small hypoattenuating areas in the lungs). Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 18 h 20 min after death showing ground-glass opacities larger than those on premortem CT and associated with small consolidations, indicating progression of the disease before death. Because the postmortem CT is of expired lungs, the pulmonary mosaic attenuation is enhanced. This confirms that the small hypoattenuating lung areas on premortem CT are air-trapping areas on postmortem CT. Images D and H show pre- and postmortem coronal reformats, respectively.

together. If we analyzed only postmortem CT findings of our cases, the evolution of the case would not be fully understood. Analysis of cases with almost fully consolidated lungs, such as our case 3, greatly benefits when a recent premortem CT is available for comparison. Furthermore, CT findings can be used to guide small tissue sample biopsies for important histopathologic analysis.

The major limitation of this study is the small number of cases. However, we hope this study inspires others to perform similar studies that add knowledge about minimally invasive autopsies being developed worldwide.

Postmortem chest CT can be useful in minimally invasive autopsies of fatal COVID-19 cases, especially if there is a recent premortem chest CT to compare with the postmortem CT and help interpret the findings. This interpretation can lead to logical diagnostic reasoning of the progression of COVID-19, and even reveal additional findings not related to SARS-CoV-2 infection, help under-

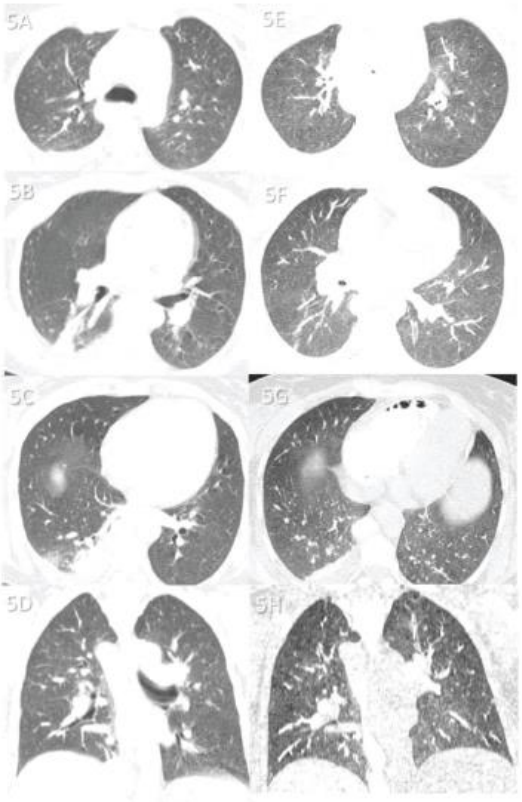

Figure 5 - Case 5: Premortem axial chest computed tomography (CT) of the upper (A), mid (B), and inferior (C) thirds of the lungs obtained 2 days before death showing a few abnormalities, including a small peripheral and posterior ground-glass opacity in the lower right lobe (C), a small atelectasis in the posterior and medial aspects of the same lobe, and a right pleural effusion. Postmortem axial chest CT of the upper (E), mid (F), and inferior (G) thirds of the lungs obtained 16 h 13 min after death showing findings similar to premortem CT findings, except for a diffuse and subtle increase in attenuation of the lungs and thinning of the atelectasis in the right inferior lobe—changes probably because of the expired lungs during postmortem CT. The ground-glass opacity in the small right inferior lobe is not observed on postmortem CT, and the right pleural effusion is stable. Images D and H show pre- and postmortem coronal reformats, respectively. This patient died from liver transplant complications. Pre- and postmortem chest CT showing a normal lung parenchyma, indicating that she died with COVID-19, not from COVID-19.

stand the cause of death, and help guide small tissue sample biopsies, if necessary.

■ ACKNOWLEDGMENTS

We thank the autopsy and CT technicians who helped make image acquisition possible. We are also grateful to all involved in the care of patients with COVID-19; to the Coronavirus Crisis Committee of our institution during the worst days of the pandemic; and, of course, to all legal representatives of patients with COVID-19 who agreed to the postmortem examination. Funding: This work was supported by the Bill and Melinda Gates Foundation [INV-002396]; Conselho Nacional de Desenvolvimento Científico e Tecnológico [401825/2020-5, 304987/2017-4 to M.D. and 304277/2019-3 to T.M.]; Fundação de Amparo à Pesquisa do Estado de São Paulo 2013/17159-2; and Hospital das Clinicas da Faculdade de Medicina da University of São Paulo – HC Convida [HC-01.18/2020, HC-01.29/2020, and HC-02.18/2020].

Table 2 - Main positive and negative aspects of our analysis.

Positive aspects	Negative aspects
Confirms typical findings of COVID-19	Hypostasis (when present) may limit posterior lung analysis
Excludes typical findings of COVID-19	Expired lungs may obscure some findings
Determines progression of the disease	
Detects additional chest findings	

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5.4 Anexo 4 - Artigo Publicado 2 – Referente ao Segundo Objetivo da Tese

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Postmortem chest computed tomography in COVID-19: A minimally invasive autopsy method

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HIGHLIGHTS

- Radiologists can read COVID-19 postmortem chest CT aiding pathologists in forensic studies.
- Normal postmortem chest CT scans correlates to normal lung histopathology (screening tool?).
- COVID-19 postmortem imaging findings correlate in extension and severity to histopathology.

ARTICLE INFO

Keywords:
Tomography
Thorax
Autopsy
Pathology

ABSTRACT

Objectives: Performing autopsies in a pandemic scenario is challenging, as the need to understand pathophysiology must be balanced with the contamination risk. A minimally invasive autopsy might be a solution. We present a model that combines radiology and pathology to evaluate postmortem CT lung findings and their correlation with histopathology.

Methods: Twenty-nine patients with fatal COVID-19 underwent postmortem chest CT, and multiple lung tissue samples were collected. The chest CT scans were analyzed and quantified according to lung involvement in five categories: normal, ground-glass opacities, crazy-paving, small consolidations, and large or lobar consolidations. The lung tissue samples were examined and quantified in three categories: normal lung, exudative diffuse alveolar damage (DAD), and fibroproliferative DAD. A linear index was used to estimate the global severity of involvement by CT and histopathological analysis.

Results: There was a positive correlation between patient mean CT and histopathological severity score indexes - Pearson correlation coefficient (R) = 0.66 (p = 0.0078). When analyzing the mean lung involvement percentage

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of each finding, positive correlations were found between the normal lung percentage between postmortem CT and histopathology (R=0.65, p = 0.0082), as well as between ground-glass opacities in postmortem CT and normal lungs in histopathology (R=0.65, p = 0.0086), but negative correlations were observed between ground-glass opacities extension and exudative diffuse alveolar damage in histological slides (R=−0.68, p = 0.005). Additionally, it was found is a trend toward a decrease in the percentage of normal lung tissue on the histological slides as the percentage of consolidations in postmortem CT scans increased (R = −0.51, p = 0.055). The analysis of the other correlations between the percentage of each finding did not show any significant correlation or correlation trends (p ≥ 0.10).

Conclusions: A minimally invasive autopsy is valid. As the severity of involvement is increased in CT, more advanced disease is seen on histopathology. However, we cannot state that one specific radiological category represents a specific pathological correspondent. Ground-glass opacities, in the postmortem stage, must be interpreted with caution, as expiratory lungs may overestimate disease.

1. Introduction

The number of fatal victims of COVID-19 pandemic has reached approximately 6.9 million worldwide (up to March 2023) [1]. Despite the elevated number of deaths, literature regarding COVID-19 conventional autopsies was relatively scarce, mostly because of contagion risks and/or the strict protection procedures recommended, which has substantially reduced traditional necropsies [2,3]. In our country, conventional autopsies were forbidden when the pandemic broke out [4]. This scenario will be observed in the beginning of any pandemic and the need for alternative methods is crucial. Autopsy operational issues drove some authors to develop minimally invasive autopsy methods to study fatal COVID-19: some performed lung, heart, liver and spleen histopathological analysis by using blinded tissue sampling [5], and others used lung ultrasound to guide histopathological analysis [6,7].

Chest CT was proven to be the method of choice to assess COVID-19 lung involvement in living patients, endorsed by the Society of Thoracic Radiology, the American College of Radiology, and the Radiological Society of North America [8]. However, the literature about postmortem CT in COVID-19 is limited. Some authors have used postmortem CT as a triage tool to refer a patient to conventional autopsy when there were no typical CT findings of COVID-19 lung involvement to minimize the risks of autopsy team contagion [9,10]. A case series analysis has shown that despite postmortem chest CT limitations (mainly expired lungs and hypostasis), most classic COVID-19 findings, as well as eventual additional findings, are present and similar to premortem chest CT. In some cases, authors could even demonstrate disease progression [11].

Our motivation emerged from the fact that autopsies were restricted and occasionally prohibited in the beginning of the COVID-19 pandemic, limiting the study of a recent novel disease. The development of an alternative method to traditional autopsies, such as a minimally invasive autopsy seemed to be a solution to satisfy the eagerness to comprehend the pathophysiological processes involved in fatal COVID-19 cases.

Our main goal was to evaluate the additional value of postmortem chest CT to minimally invasive autopsy and verify whether postmortem CT lung findings could provide information regarding histopathological involvement.

2. Materials and methods

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068).

2.1. Population

From April to June 2020 (during the 'first wave' of the COVID-19 pandemic), 29 patients died with a laboratory confirmation of COVID-19 and had an autopsy requested by our institution's medical staff after informed consent was obtained from the next of kin.

2.2. Minimally invasive autopsy protocol

The deceased bodies were covered by a plastic safety bag and transported to the morgue by nursery staff with adequate personal protective equipment. No inflation of the lungs was performed. The autopsy service performs postmortem studies with a dedicated CT scanner. Two trained technicians prepared the body for the CT scan, wrapping it with an additional plastic bag. The CT operator never had direct contact with the bodies; in fact, most times, the equipment was operated remotely from another central operation room in the hospital.

Postmortem chest CTs were performed using a Somatom Emotion scanner (Siemens Healthineers, Erlangen Germany). Images were saved in DICOM™ format, with a standard lung filter reconstruction applied. CT Scan parameters are displayed on Table 1. All postmortem studies were sent to an online Picture Archiving and Communication System (PACS), provided by Purview©. All CTs were performed less than 24 h after death.

Immediately after the postmortem chest CT was performed, multiple tissue samples were collected by ultrasound-guided biopsies, including samples from the brain, lungs, heart, liver, spleen, kidneys, salivary glands, pancreas, testis and striated muscle. This work considers only lung samples. They were ultrasound guided only to certify that lung tissue was sampled. Furthermore, the biopsies were obtained in a standardized protocol from 4 different regions in each lung (Fig. 1): superolateral, superomedial, inferolateral and inferomedial. As postmortem lungs are usually expired, anteriorly, we found the inferior lung limits around the 4th or 5th intercostal space; therefore, the 3rd sternum costal joint was used as the division mark between the superior and inferior regions. Regarding lateral and medial regions, the division marks were the midclavicular lines. The posterior and anterior areas of each region were tissue sampled. We used a portable SonoSite M-Turbo R Ultrasound (Fujifilm, Bothell, WA, USA) with broadband and multi-frequency transducers, C60x (5–2 MHz Curved) and HFL38X (13–6 MHz Linear), and semiautomatic coaxial 14 G needles, 20 centimeters in length. The needles reached the posterior chest wall to certify that the posterior regions were always included. Approximately 60 pulmonary samples were collected from each lung.

Table 1
Postmortem Chest CT Scan Parameters.

Parameter	Value
kV	130
mA	AEC (78 to 260) or fixed in 128 or 136
Pitch	0.8
Matrix	512 × 512
Detector Rows	16
Detector Thickness	1.2 mm
Collimation	19.2 mm
Slice Thickness	1.5 mm

(AEC = automatic exposure control).

2.3. Chest CT analysis

Images were examined independently by two experienced thoracic radiologists (each with more than 10 years of experience in specialized practice) who were blinded to the histopathological findings, and disagreements were resolved by consensus. Each region corresponding to the biopsied regions described above was quantified and classified into the following categories: normal; ground-glass opacities; crazy-paving; small consolidations; and large or lobar consolidations, as shown in Fig. 2.

The following radiological criteria were used to define each of the five categories above, based on the Fleischner Society glossary of terms for thoracic imaging [12]: (a) normal: preserved parenchyma; (b) ground-glass opacities: hazy increased opacity of lung, with preservation of bronchial and vascular margins; (c) crazy-paving: thickened interlobular septa and intralobular lines superimposed on a background of ground-glass opacity, resembling irregularly shaped paving stones; (d) small consolidations: a homogeneous increase in pulmonary parenchymal attenuation that obscures the margins of vessels and airway walls (up to 1.0 cm); (e) large or lobar consolidations: a homogeneous increase in pulmonary parenchymal attenuation that obscures the margins of vessels and airway walls (larger than 1.0 cm). An air bronchogram may be present.

The radiologists subjectively quantified the proportion of the five different patterns in each region (expressed as 5 % ranges – up to 20; 40; 60; 80; and 100 %). The most severe diagnosis of each region was also highlighted. A consensus of the analysis was reached.

Other quantitative and qualitative fatal COVID-19 postmortem chest CT findings were also analyzed: percentage of global lung involvement; uni/bilaterality; affected lobes; posterior or inferior predominance; peripheral, central or mixed distribution; presence of emphysema, fibrosis, mediastinal lymph node enlargement, pleural effusion, pleural thickening, pneumothorax, pericardial effusion, pneumomediastinum, aortic calcifications, coronary calcifications, and subcutaneous emphysema.

2.4. Histological analysis

Slides were examined with consensus by five experienced pulmonary pathologists (with a minimum of 6 and a maximum of 30 years of specialized practice experience) blinded to radiological findings and classified into the following categories: normal lung, exudative diffuse alveolar damage (DAD) and fibroproliferative DAD, as illustrated in Fig. 3.

The following histological criteria were used to define each pattern [13]: (a) normal lung: preserved architecture, without inflammation, edema or exudate; (b) exudative DAD: interstitial and/or intraalveolar edema, interstitial inflammation, variable amounts of alveolar hemorrhage and fibrin deposition, intra-alveolar hyaline membranes and type II pneumocyte hyperplasia. Foci of neutrophilic pneumonia were also included in the acute/exudative pattern; and (c) fibroproliferative DAD: any degree of fibroblastic proliferation within the *interstitium* and/or alveolar spaces, including loose aggregates of fibroblasts admixed with scattered inflammatory cells, collagen deposition, squamous metaplasia, and possible remnants of hyaline membranes. The most severe diagnosis of each region was also highlighted, as it was not always the most common.

If the histopathological analysis exhibited any indications of secondary pneumonia, exclusion of the patient was necessary. This precaution ensured the ability to unequivocally attribute the findings solely to COVID-19, mitigating potential confounding factors.

The pathologists subjectively quantified the proportion of the three different patterns in each region corresponding to the biopsied regions described above (expressed as 5 % ranges – up to 20; 40; 60; 80; and 100 %). The most severe diagnosis of each region was also highlighted. A consensus of the analysis was reached.

Conventional autopsy of the lungs was not performed because it was forbidden in our country during the development of this study [4].

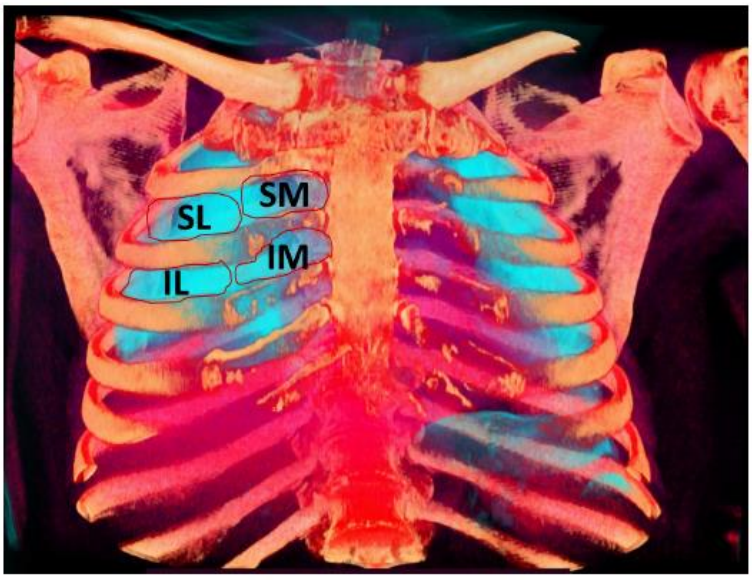

Fig. 1. 3D volume rendering reformat from a postmortem chest CT from our study (using RadiAnt™ DICOM Viewer 2022.1 Software – 3D Preset: Bones and Skin 3) shows how the postmortem expired lungs (in blue) have their anterior lower limits around the 4th or 5th intercostal spaces. From this reconstruction, it is possible to understand why the 2nd anterior intercostal space was used for the tissue sample collection of the upper regions and the 3rd anterior intercostal space was used for the tissue sample collection of the inferior regions. The limit between the lateral and medial regions was the midclavicular line. The 4 regions of access to tissue sample collection are also shown: SL = superolateral; SM = superomedial; IL = inferolateral; IM = inferomedial.

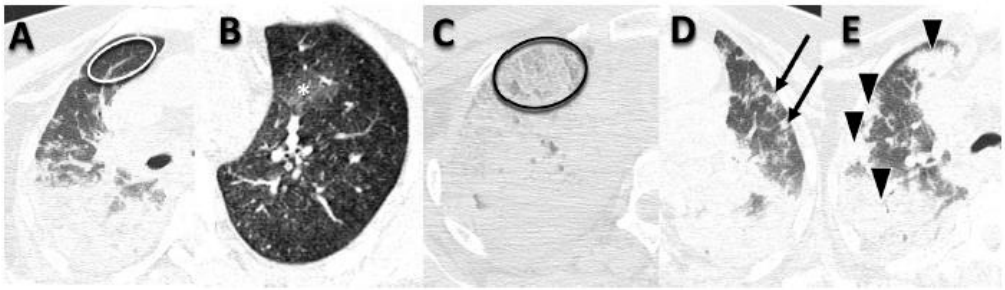

Fig. 2. Examples of postmortem CT findings of our sample: A) Normal lung parenchyma (white ellipse). B) Ground-glass opacities (white asterisk). C) Crazy paving (black ellipse). D) Small consolidations (black arrows). E) Large consolidations (black arrowheads).

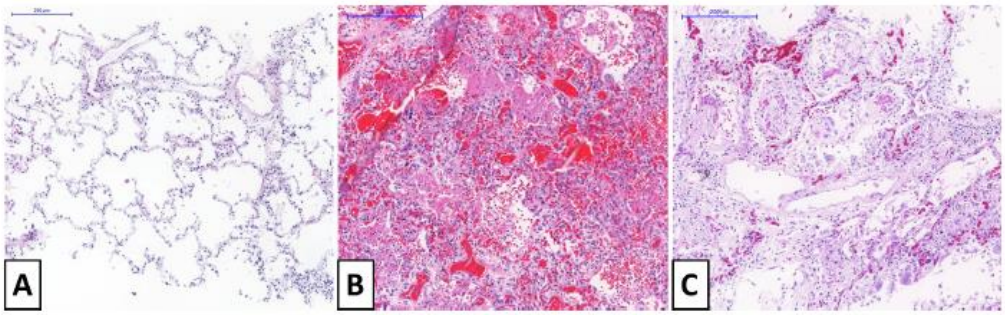

Fig. 3. Examples of postmortem histological findings of our sample: A) Normal lung parenchyma. B) Acute/Exudative Diffuse Alveolar Damage. C) Fibroproliferative diffuse alveolar damage.

2.5. Statistical analysis

Statistical analysis was performed using R software (http://www.R-project.org). For each region, we calculated the Kappa concordance coefficient for the most severe diagnosis and the categorical percentage estimation between CT and the corresponding histological analysis, as follows: normal CT to normal pathology; ground-glass opacities to exudative DAD; crazy-paving, small consolidations, and large or lobar consolidations to fibroproliferative DAD. In addition, for each region, we calculated the Pearson correlation coefficient between each CT finding and histopathological finding.

To quantify lung pathological involvement in COVID-19, we proposed CT and histopathological severity scores as a linear combination of the percentage of pulmonary involvement weighted by the severity of the change. In the CT severity score (CTSS), the weighted coefficients for larger/confluent consolidations, small consolidations, crazy paving, and ground-glass opacities were 4, 3, 2, and 1, respectively. In the histological severity score (HSS), the weighted coefficients for fibroproliferative DAD and exudative DAD were 4 and 2, respectively, as follows:

$$CTSS = 4(\%big \quad or \quad confluence \quad consolidations) + 3(\%small \quad consolidations) + 2(\%crazy \quad paving) + 1(\%extensive \quad ground \quad glass \quad opacities)$$

$$HSS = 4(\%fibroproliferative \quad DAD) + 2(\%exudative \quad DAD)$$

These scores increase as the severity and extent of pulmonary involvement by COVID-19 increase, ranging from zero to 400. In addition, it enables the calculation of the correlation coefficient between scores. The scores were calculated for each of the 4 regions of each lung, and the mean score of each patient was taken for correlation.

3. Results

Of the 29 patients who underwent the minimally invasive autopsy protocol described, 14 had signs of secondary pneumonia in the histological analysis and thus had to be excluded once CT findings could be related to an agent other than SARS-CoV-2. Table 2 shows the epidemiological and clinical data of the 29 studied patients. Of the 15 patients without signs of secondary pneumonia in the histopathological examination, not all of them had sufficient and appropriate material from the 4 regions of each lung for the histological analysis. Therefore, in the end,

Table 2
Clinical and Demographic Characteristics of our cohort that was composed of 29 patients with fatal COVID-19. Data are displayed as the mean ± standard deviation. ICU: Intensive Care Unit.

Demographics and Clinical Characteristics of patients with fatal COVID-19		
	No Secondary Pneumonia	**Secondary Pneumonia (excluded)**
Patients (number)	15	14
Gender (%)	40 Male/60 Female	57.1 Male/42.9 Female
Age (years)	51.27 ± 16.05	56.79 ± 17.85
Weight (kg)	75.79 ± 15.50	73.10 ± 29.34
Height (m)	1.70 ± 0,07	1.69 ± 0.09
BMI (kg/m^2)	25.88 ± 4.15	25.34 ± 9.02
Ethnicity (%)	86.7 White/6.7 Black/ 6.7 Brown	85.7 White/7.1 Black/ 7.1 Brown
Time from Symptoms Onset to Admission (days)	9.13 ± 6.62	6.29 ± 3.79
Time from Symptoms Onset to Death (days)	25.13 ± 9.96	20.29 ± 10.97
Time from Admission to Death (days)	16.0 ± 10.51	14.0 ± 10.10
Time from ICU Admission to Death (days)	11.07 ± 9.07	10.93 ± 9.23

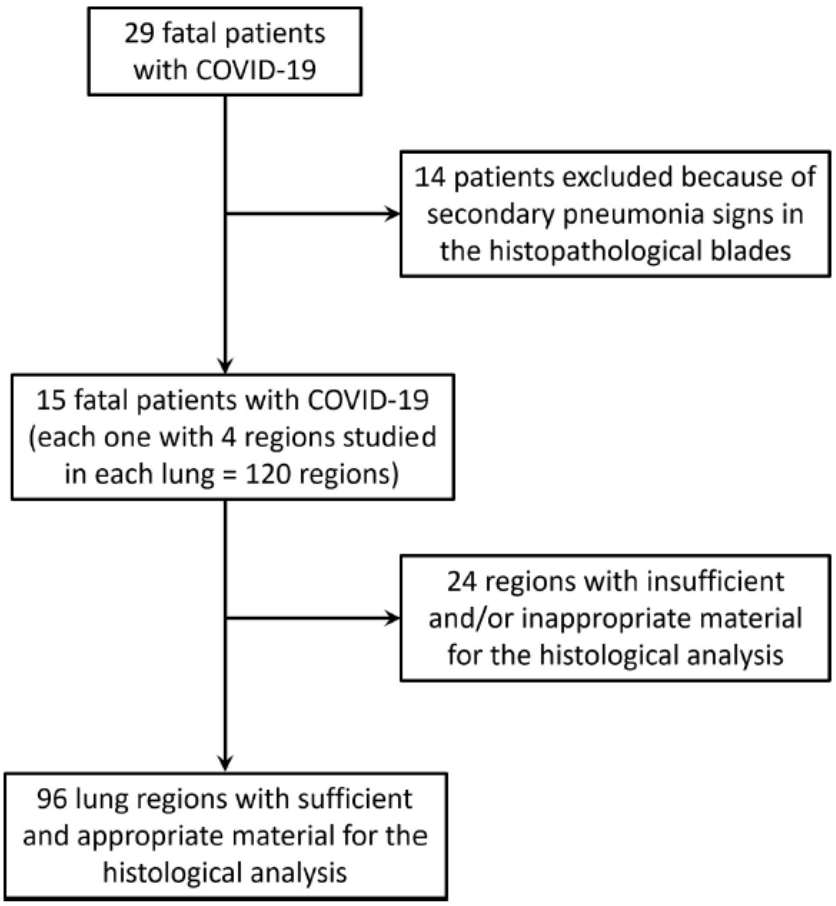

Fig. 4. Flow diagram showing the initial number of patients and patients excluded. Afterward, from the patients not excluded, some regions had to be excluded because of insufficient and/or inappropriate material. In the end, 96 lung regions were analyzed.

96 regions of adequate tissue were sampled from these 15 patients (Fig. 4). The consensus agreement between the two radiologists about the chest CT analysis was 100 % for the most severe diagnosis of each region and 97.66 % for the proportion of the five different patterns in each region. The 2.33 % disagreement was totally resolved by reanalyzing it together and reaching consensus. The time between death and postmortem CT was 14h36m ± 06h38m (average ± standard deviation).

There was no concordance between the CT and histopathological estimates of extension in all categories evaluated or between the most severe diagnosis for each region in CT and in the histological slides.

There was a positive correlation between the patient mean CT and histopathological severity score indexes. The Pearson correlation coefficient ®, estimated in our sample, was 0.66 ($p = 0.0078$), as shown in Fig. 5 (Scatter plot).

Furthermore, we calculated the mean lung involvement percentage in the slides and in the CT for each finding (grouping small and large consolidations in postmortem CT) in each patient and estimated the Pearson correlation coefficient (R) between lung involvement percentages, as shown in a set of graphs (Fig. 6). In Fig. 6, there is a positive correlation between the percentage of normal lung in postmortem CT and histological slides ($R = 0.65$, $p = 0.0082$) (6 A), as well as between the percentage of ground-glass opacities in postmortem CT scans and histological slides ($R = 0.65$, $p = 0.0086$) (6B). Moreover, as the

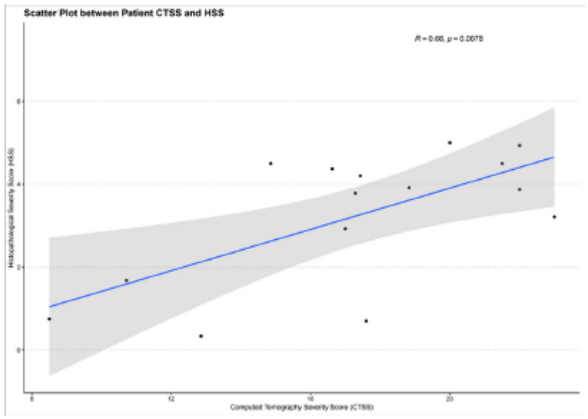

Fig. 5. : The correlation between CT and Histopathological severity score indexes.

percentage of ground-glass opacities in postmortem CT increased, there was a decrease in the percentage of exudative diffuse alveolar damage on the histological slides (negative correlation – $R = -0.68$, $p = 0.005$)

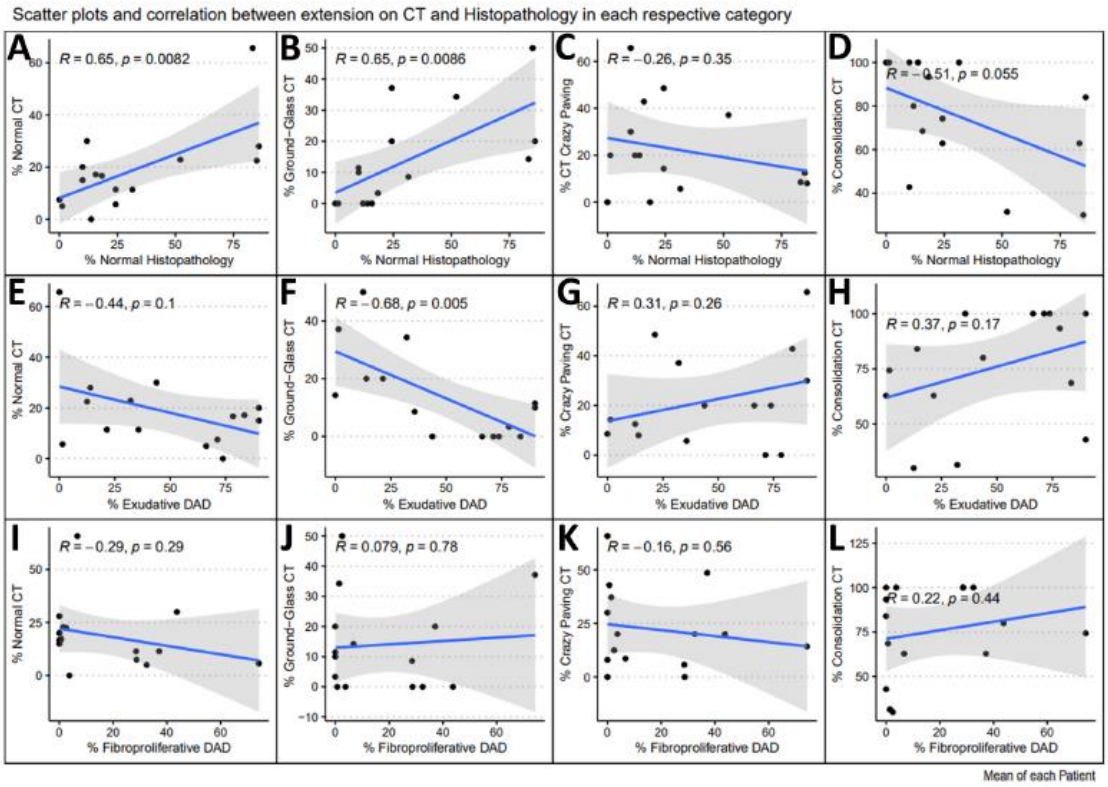

Fig. 6. : Correlation - Mean lung involvement percentage in the blades and in the CT for each finding.

(6 F). Additionally, in Fig. 6, there is a trend toward a decrease in the percentage of normal lung tissue on the histological slides as the percentage of consolidations in postmortem CT scans increases (negative correlation – R = −0.51, p = 0.055) (6D). The other subgraphs did not show any significant correlation or correlation trends ($p \geq 0.10$).

Some specific examples of postmortem chest CT lung images and the corresponding histopathological findings of our sample are presented in Figs. 7–10. The other quantitative and qualitative fatal COVID-19 postmortem chest CT findings of the patients are shown in Table 3.

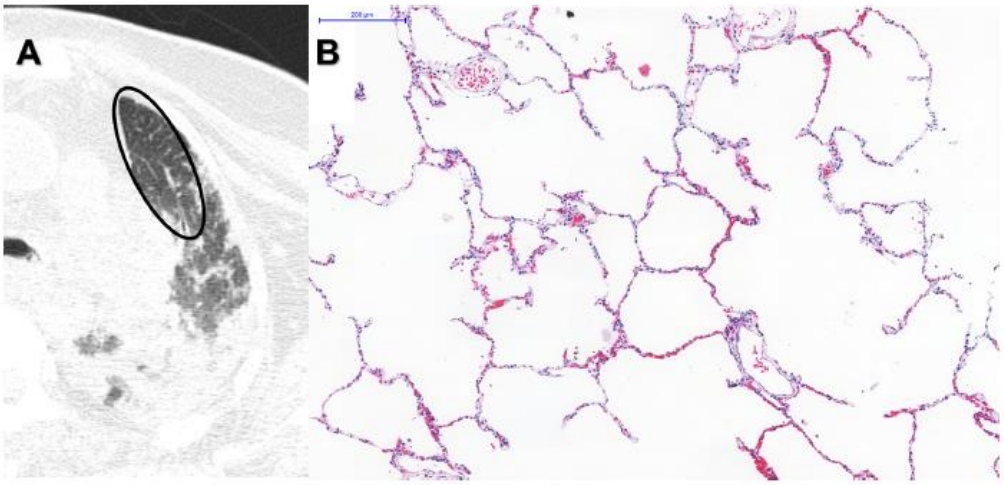

Fig. 7. A) Postmortem Chest CT Image, Axial View, Left Upper Regions. There is a large area of normal lung parenchyma (ellipse). B) Histopathological sample of the same regions: Photomicrograph showing normal lung with open airspaces, thin alveolar septa, no inflammation and mild capillary congestion, commonly seen in autopsy specimens - HE Staining. Scale Bar - 200 μm - Objective 10x.

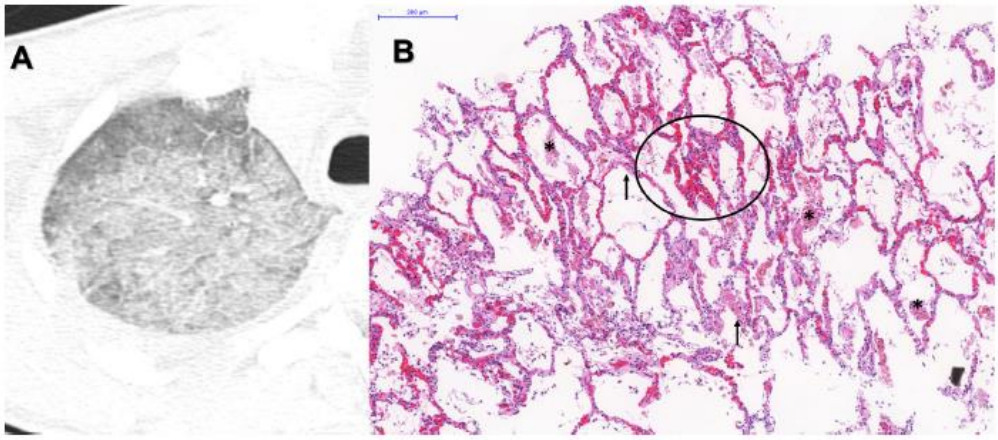

Fig. 8. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of ground-glass opacities. B) Histopathological sample of the same regions: Photomicrograph of Acute Exudative DAD showing moderate septal inflammation, intense vascular congestion (ellipse), intra-alveolar fibrin deposition (asterisk) and formation of hyaline membranes (arrows) - HE Staining. Scale Bar - 200 μm - Objective 10x.

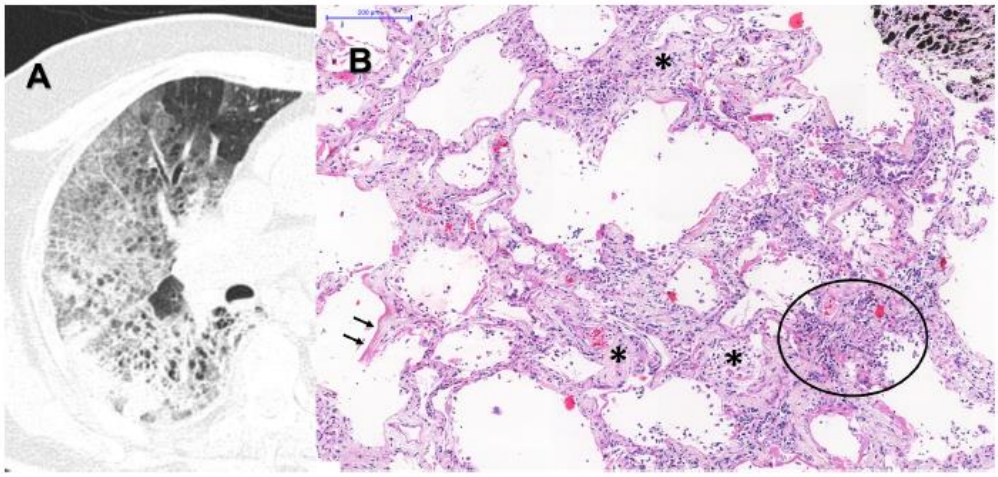

Fig. 9. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of 'crazy paving' (thickened interlobular septa and intralobular lines superimposed on a background of ground-glass opacities). B) Histopathological sample of the same regions: Photomicrograph showing mixed DAD with some characteristics of acute DAD such as hyaline membranes (arrows) and abundant inflammatory cells (ellipse), as well as of fibroproliferative DAD with collagen deposition forming intra-alveolar plugs and septal thickening (asterisk) - HE Staining. Scale Bar - 200 μm - Objective 10x.

4. Discussion

One of the primary factors to be considered pertains to the utility of minimally invasive autopsy in the presented situation. The severity index score created relies on both the percentage of disease involvement and the progression/severity of the disease. As the CT severity score index advances, there is a corresponding progression in the histopathological correlated index.

The utility of chest CT in living patients with COVID-19 was very well established for diagnosis and progression of the disease [8, 14, 15]. Regarding postmortem chest CT in COVID-19, since the first case reports were published, attempts have been made to correlate tomographic findings with the progression/severity of the disease, as well as with what would be expected to be found in histopathological findings. Published data suggest that fatal COVID-19 cases exhibit extensive consolidations on CT scans and advanced DADs on histopathological slides, as endorsed by some authors [16–19].

To date, most published studies have been case reports or series of case reports [10, 19–21]. This may be due to the difficulty of medical institutions having a dedicated CT scanner for postmortem studies. Additionally, the urgency to study and share information about COVID-19, especially during the early waves of the pandemic, may have limited the conduction of large casuistic studies, which usually requires more time. Despite the known limitations of postmortem chest CT [22], studies comparing COVID-19 pre and postmortem chest CT findings showed that comparison between them is possible [10,11]. A meta-analysis regarding postmortem chest CT in fatal COVID-19 cases was performed and was published in 2022 to try to understand how and what importance that postmortem CT has [16], and it selected a few studies that compared postmortem CT versus histopathological findings (8 out of 20).

The decision to use the mean CT and histopathological severity score indexes for each patient and not for each region was a way to reduce the limitation that it was not always possible to determine the exact limits of

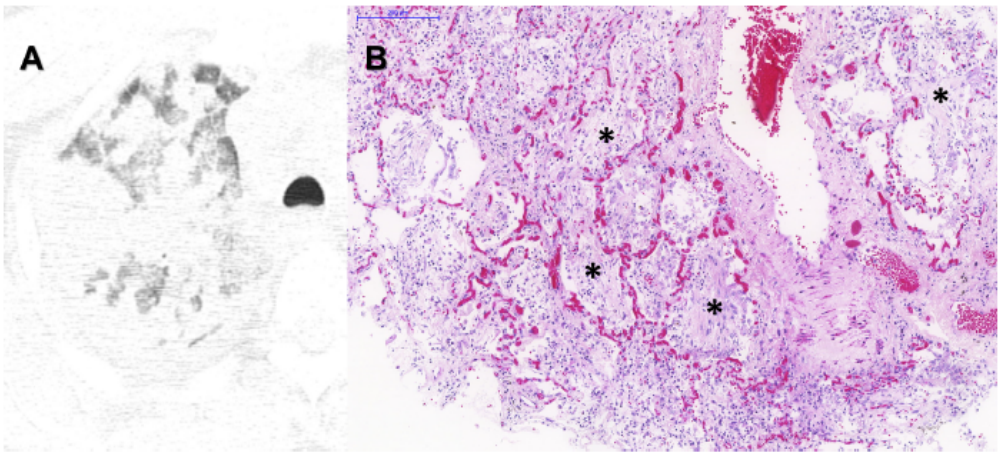

Fig. 10. A) Postmortem Chest CT Image, Axial View, Right Upper Regions: There is a considerable amount of lung consolidations – small and large. B) Histopathological sample of the same regions: Photomicrograph of intense fibroproliferative DAD showing collagen deposition forming intra-alveolar plugs throughout the slide (asterisk) - HE Staining. Scale Bar - 200 μm - Objective 10x.

each region in both analyses (CT and histopathological), mainly because tissue sample collections were sometimes made with placing the needle with an oblique entry angle, the adjacent region could have been sampled, the fact that the biopsies were sampled under ultrasound guidance and not with CT guidance, and there was a pairwise discordance between CT and histology. This method enabled an increase in the signal-to-noise ratio and demonstrated a positive correlation between the mean CT and Histopathological Severity Score Indexes ($R = 0.66$, $p = 0.0078$ – Fig. 5), showing that as the disease progressed with regard to pathology, whether in severity or extent, and that the disease also progressed in postmortem CT, despite the specific findings of the different phases of the disease not showing concordance between CT and histological slides. It is important to consider that a histological slide represents only a small sample of tissue, while the CT analysis includes larger regions of the lung. This "representation" difference imposes additional challenges for direct correlation between pathology and CT in a given region. An exact point-to-point correlation with CT-guided biopsies (which was not the case) could have improved these results, as suggested in other non-COVID correlation studies.

We also found a positive correlation between normal lung in CT and in the slides ($\rho = 0.65$; $p = 0.0082$) and a trend in the correlation between the proportion of normal lung tissue decreasing in histological analysis, as consolidation extended ($\rho = 0.51$; $p = 0.055$). This leads us to believe that the use of postmortem chest CT might be useful for screening cases without lung involvement. In other words, postmortem CT appears to be a good strategy for excluding disease, corroborating on what other authors had already suggested in initial case reports, as referred to previously in this text. The presence of large consolidations corroborates that the lungs are histologically affected (nonnormal). Future studies might evaluate the probable high negative predictive value of postmortem CT scans.

In addition, our data showed an intriguing relationship between the mean proportion of ground-glass opacities and the histological categories. We found a positive correlation between the mean proportion of ground-glass opacities and the proportion of normal lung tissue and a negative correlation between the proportion of ground-glass opacities and the percentage of disease in the exudative phase. One possible explanation for these findings is that in postmortem cases, the value of ground-glass opacities as a predictor of disease may be questioned once the images are obtained with exhaled lungs and there is more blood stasis, generating nonpathological ground-glass opacities. This is a limitation of the method, as stated previously. Furthermore, as is known in the chest CT of living individuals, when expiration series are performed to evaluate areas of air trapping, we notice that normal areas (without trapping) end up exhibiting high attenuation, simulating ground-glass opacities [12,23]. Perhaps this is the greatest limitation of postmortem chest CTs. Since the lungs are most often in expiration, ground-glass opacities should be interpreted with caution. Some authors have already tried to differentiate postmortem ground-glass opacities attributed to COVID-19 from high attenuation, ground-glass mimicking opacities that would only be related to death itself and not COVID-19 infection [10]. They concluded that fatal COVID-19 ground-glass opacities correlate with bilateral, peripheral and multilobar distribution, as in life, despite the small number of cases. In our sample of 15 patients, most of our patients had large consolidations occupying considerable lung volumes, sometimes even encompassing the entire lobe, and most of our ground-glass/high attenuation opacities did not follow these typical viral disease patterns, probably explaining why we found a positive correlation between ground-glass opacities on CT and normal lungs tissue on the slides. A suggestion would be to suspect ground-glass opacities with the classic distribution of COVID-19, more peripheral, basal and multilobar [8], as pathological, and not those with atypical distribution, which would be more likely related to lung expiration. Another option for future studies regarding these ground-glass opacities confounding factors in postmortem studies could be the use of modern techniques such as CT radiomics analysis. Some authors used it as a supplementary tool for improving specificity for COVID-19 in a living population confounded by ground glass opacity changes from other etiologies [24].

One way to diminish those postmortem chest CT expiration-related issues would be to have patients undergo tracheal intubation or cricothyrioidotomy [25], insufflate the lungs, and then perform the scan. Two possible issues are known: the difficulty of orotracheal intubation due to head and neck death-related rigor of the soft tissues, as well as possible gas leakage in different body compartments [26]. As our study was conducted in the first wave of the pandemic, very little was known about the virus; thus, it was decided to scan patients without intubation to minimize team contagion risks.

The major limitation of minimally invasive autopsy is that other contributors to death (such as acute myocardial infarct or pulmonary thromboembolism) could not be evaluated as they would been evaluated during conventional autopsy. Despite this fact, the method enabled us to improve the understanding of radiological findings and disease progression.

Table 3
Other quantitative and qualitative fatal COVID-19 postmortem chest CT findings.

Global Lung Involvement	
80 to 100 %	11/15 (73.33 %)
60 to 80 %	3/15 (20.00 %)
40 to 60 %	1/15 (6.67 %)
< 40 %	None
Uni/Bilateral	
Bilateral	15/15 (100 %)
Unilateral	None
Affected Lobes	
Only lower lobe(s)	None
Lower lobe(s) + at least one other lobe	15/15 (100 %)
No lower lobe involvement	None
Predominance	
Inferior	1/15 (6.67 %)
Posterior	3/15 (20.00 %)
No predominance	11/15 (73.33 %)
Distribution	
Peripheral	None
Central	None
Mixed	15/15 (100 %)
Pulmonary Emphysema	1/15 (6.67 %)
Pulmonary Fibrosis	None
Mediastinal Lymph Node Enlargement	8/15 (53.33 %)
Pleural Effusion	
Right	
Large	None
Moderate	2/15 (13.33 %)
Small	5/15 (33.33 %)
None	8/15 (53.33 %)
Left	
Large	None
Moderate	3/15 (20.00 %)
Small	5/15 (33.33 %)
None	7/15 (46.66 %)
Pleural Thickening	3/15 (20.00 %)
Pneumothorax	3/15 (20.00 %)
Pericardial Effusion	2/15 (13.33 %)
Pneumomediastinum	None
Aortic Calcifications	7/15 (46.66 %)
Coronary Calcifications	6/15 (40.00 %)
Subcutaneous emphysema	None

As future lines of studies, larger projects integrating diverse imaging modalities (such as ultrasound and magnetic resonance) also across other parts of the human body, hold promise in enhancing the applicability of minimally autopsy methods, not only in fatal COVID-19. Additionally, the incorporation of artificial intelligence and machine learning in the analysis of postmortem CT images, as it is already being used in living patients [24,27], has the potential to significantly enhance the precision and interpretation of imaging, especially if a large histopathological dataset is input.

5. Conclusions

The use of a minimally invasive autopsy method was useful in accessing the lungs of fatal COVID-19 patients by imaging and histopathology, especially in a context of restricted or suspended conventional autopsies. There is a correlation between the progression and severity of the disease when comparing postmortem CT and histopathology findings. Furthermore, encountering normal lungs in postmortem chest CT might be a good indication that those lungs are also normal in histopathology, and the interpretation of ground-glass opacities should be done with caution.

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Ethical statement

This study was approved by our National Research Ethical Committee (CONEP CAAE 30364720.0.0000.0068). Informed consents were obtained from the next of kin of all deceased patients.

CRediT authorship contribution statement

Saldiva Paulo Hilário Nascimento: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mauad Thais:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Dolhnikoff Marisa:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Martin Maria da Graça Morais:** Writing – review & editing. **Savoia Paulo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Cardoso Ellison Fernando:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **da Silva Luiz Fernando Ferraz:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Leite Claudia da Costa:** Writing – review & editing, Validation, Supervision, Resources. **Duarte-Neto Amaro Nunes:** Writing – review & editing, Methodology, Formal analysis. **Monteiro Renata Aparecida Almeida:** Writing – review & editing, Methodology. **Sawamura Marcio Valente Yamada:** Writing – review & editing, Validation, Supervision, Formal analysis.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marisa Dolhnikoff reports financial support was provided by Bill and Melinda Gates Foundation. Marisa Dolhnikoff reports financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico. Thais Mauad reports financial support was provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico. Marisa Dolhnikoff reports financial support was provided by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). Ellison Fernando

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