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UNIVERZITA KARLOVA
1. lékařská fakulta

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Prevence a včasná predikce rejekce a primární dysfunkce štěpu po transplantaci plic

Prevention and early prediction of rejection and primary graft dysfunction after lung
transplantation

Disertační práce

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Seznam zkratk

ACR	akutní celulární rejekce
AMR	akutní humorální rejekce
ARDS	syndrom akutní dechové tísně (acute respiratory distress syndrome)
BAL	bronchoalveolární laváž
BLAD	baseline lung allograft dysfunction
BMI	body mass index
BOS	syndrom obliterující bronchiolitidy (bronchiolitis obliterans syndrom)
CHS	kontrolovaná hypotermie (controlled hypothermic storage)
CLAD	chronická dysfunkce plicního štěpu
COVID-19	coronavirus disease 2019
CPB	mimotělní oběh (cardiopulmonary bypass)
CT	výpočetní tomografie
CTLA-4	cytotoxic T-lymphocyte antigen 4
DBD	dárce po smrti mozku (donor after brain death)
DCD	dárce po oběhové zástavě (donor after circulatory death)
ECMO	extrakorporální membránová oxygenace
EVLP	ex-vivo perfuze a ventilace dárcovských plic
FEV1	objem usilovně vydechnutého vzduchu za první sekundu (forced expiratory volume in 1 second)
FN	fakultní nemocnice
HLA	human leukocyte antigen
IC	imunitní kontrolní body (immune checkpoints)
IRI	ischemicko-reperfuzní poškození (ischemia-reperfusion injury)

ISHLT	the International Society for Heart and Lung Transplantation
LAS	lung allocation score
LF	lékařská fakulta
NPTP	Národní program transplantace plic pro Českou a Slovenskou republiku
PAH	plicní arteriální hypertenze
PD-1	programmed death-receptor 1
PD-L1	programmed death-ligand 1
PECAM-1	platelet–endothelial-cell adhesion molecule-1
PEEP	pozitivní tlak v dýchacích cestách na konci výdechu (positive end-expiratory pressure)
PGD	primární dysfunkce štěpu
POC	point-of-care
RAS	restriktivní syndrom alograftu (restrictive allograft syndrome)
ROTEM	rotační tromboelastometrie
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TLC	celková plicní kapacita (total lung capacity)
UFH	nefrakcionovaný heparin
UK	Univerzita Karlova

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Abstrakt

Transplantace plic je život zachraňující operační výkon vyhrazený pro pacienty s postižením plic v terminálním stadiu onemocnění. Principem je nahrazení nemocné plíce nebo obou plic příjemce orgánem od zemřelého dárce. Transplantace plic je vysoce specializovaná multidisciplinární metoda prováděná ve Fakultní nemocnici v Motole jako jediném centru pro Českou a Slovenskou republiku. Nejčastější indikace k transplantaci jsou chronická obstrukční plicní nemoc, plicní fibróza a plicní arteriální hypertenze. S rostoucím počtem transplantací stoupá i počet re-transplantací provedených pro chronickou dysfunkci plicního štěpu.

Medián přežití po transplantaci plic dle mezinárodního registru International Society for Heart and Lung Transplantation lehce přesahuje 6 let. Dožití po transplantaci plic je limitováno zejména infekčními komplikacemi a chronickou dysfunkcí plicního štěpu (CLAD). Mezi významné rizikové faktory pro její rozvoj patří kromě jiných akutní rejekce, primární dysfunkce štěpu (PGD) a některé infekční komplikace.

Cílem tohoto výzkumného projektu bylo experimentální testování tkáňové exprese a sérových hladin jednotlivých molekul, u kterých jsme předpokládali možnou souvislost s akutní celulární rejekcí (ACR). Dále bylo cílem retrospektivně analyzovat několik různých perioperačních faktorů a jejich vliv na rozvoj PGD nebo ACR a rozbor problematiky SARS-CoV-2 pozitivních potenciálních dárců a očkování transplantovaných pacientů proti nemoci COVID-19.

V naší experimentální práci jsme analyzovali expresi vybraných imunitních markerů u 60 pacientů s cílem identifikovat fenotypy T lymfocytů, posoudit vyčerpání imunitního systému a určit zastoupení makrofágů a B lymfocytů ve vzorcích plicní tkáně získaných kryobiopsií. Hodnotili jsme také funkčnost T buněk prostřednictvím měření vybraných

cytokinů. Zaznamenali jsme vyšší tkáňovou expresi PD-L1 a PECAM-1/CD31 u pacientů s ACR, což podporuje využití imunohistochemie v diagnostice a zdůrazňuje potenciál těchto molekul jako možných budoucích terapeutických cílů.

Ve dvou studiích ve spolupráci se zahraničními transplantačními centry jsme hodnotili výsledky prezervace dárcovských plic v kontrolované hypotermii. Zásadním parametrem pro nás byl výskyt těžké formy PGD, který byl v obou kohortách minimální. Naše publikace popisující zkušenosti s prodlouženou ischemií má potenciál být zásadní prací pro budoucí rozvoj transplantačního programu ve světě.

Retrospektivní observační studie pojednávající o antikoagulaci při použití extrakorporální membránové oxygenace (ECMO) během transplantace hodnotila výsledky 109 transplantovaných pacientů, u nichž byla transplantace provedena s venoarteriální ECMO podporou s centrální kanylací a ukončením podpory na konci výkonu. Sledovali jsme dávku heparinu, krevní ztráty, spotřebu krevních derivátů a komplikace během operace a do 48 hodin po přijetí na jednotku intenzivní péče. Nižší dávky antikoagulace vedly ke snížení krevních ztrát, spotřeby krevních derivátů a počtu chirurgických revizí pro hemotorax. Navíc ve skupině s nižší dávkou heparinu nebyl v prvních 72 hodinách po transplantaci zaznamenán výskyt těžké formy PGD.

Analyzovali jsme také dva případy potenciálních dárců plic s nejasnými výsledky testů na COVID-19 a doporučili zohlednění prahového cyklu (Ct) polymerázové řetězové reakce při rozhodování o akceptaci. Rozhodnutí by mělo zahrnovat i radiologické nálezy a anamnézu. Jelikož přítomnost virové RNA nemusí znamenat infekčnost, zdůraznili jsme potřebu určit hodnoty Ct korelující s viabilitou viru. Detekce anti-SARS-CoV-2 protilátek a kvantifikace virového antigenu v séru mohou poskytnout cenné doplňující informace. Chybný výběr dárce může totiž vést k těžké PGD a závažným komplikacím ovlivňujícím výsledek transplantace.

Poslední přehledový článek se zabývá problematikou očkování proti viru SARS-CoV-2 u pacientů po transplantaci plic. Shrnutí poznatků o imunogenicitě, účinnosti a bezpečnosti vakcinace proti COVID-19 ukazuje, že tato vysoce riziková skupina nereaguje na očkování imunitní odpovědí srovnatelnou se zbytkem populace. Byla proto doporučena aplikace třetí posilovací dávky vakcíny jako prevence těžkého průběhu infekce. V dostupných studiích jsme hledali odpověď na otázku, zda zvýšená imunitní odpověď po další dávce nezvýší riziko akutní rejekce nebo jiných komplikací. Práce zdůrazňuje klíčové aspekty personalizované strategie očkování, která minimalizuje riziko komplikací a zároveň zajistí dostatečnou ochranu proti onemocnění COVID-19.

Náš výzkum přinesl nové poznatky v diagnostice, prevenci komplikací a optimalizaci perioperační péče při transplantaci plic, včetně antikoagulačních strategií a prezervace dárcovských orgánů. Tyto výsledky přispějí k přesnější diagnostice rejekce, lepšímu pochopení rizik a možnostem jejich minimalizace.

Klíčová slova: transplantace plic, primární dysfunkce štěpu, akutní celulární rejekce, checkpoint molekuly, kontrolovaná hypotermie, ECMO antikoagulace, SARS-CoV-2

Abstract

Lung transplantation is a life-saving surgical procedure reserved for patients with end-stage lung disease. It involves replacing the diseased lung or both lungs of the recipient with an organ from a deceased donor. Lung transplantation is a highly specialized, multidisciplinary method performed exclusively at Motol University Hospital for both the Czech Republic and Slovak Republic. The most common indications include chronic obstructive pulmonary disease, pulmonary fibrosis, and pulmonary arterial hypertension. With the increasing number of transplants, the number of re-transplantations performed due to chronic lung allograft dysfunction has also risen.

The median survival after lung transplantation, according to the International Society for Heart and Lung Transplantation registry, slightly exceeds six years. Survival is primarily limited by infectious complications and chronic lung allograft dysfunction. Acute rejection, primary graft dysfunction (PGD), and certain infections are significant risk factors for its development.

The aim of this research project was to experimentally test the tissue expression and serum levels of specific molecules suspected of being associated with acute cellular rejection (ACR). Another goal was to retrospectively analyze various perioperative factors and their impact on the development of PGD or ACR, alongside exploring the challenges of SARS-CoV-2-positive potential donors and vaccination in lung transplant recipients against COVID-19 disease.

In our experimental work, we analyzed the expression of selected immune markers in 60 patients to identify T lymphocyte phenotypes, assess immune exhaustion, and determine the presence of macrophages and B lymphocytes in lung tissue samples obtained via cryobiopsy. We also evaluated T cell functionality by measuring selected cytokines. We observed increased tissue expression of PD-L1 and PECAM-1/CD31 in patients with ACR,

supporting the use of immunohistochemistry in diagnostics and highlighting these molecules as potential future therapeutic targets.

In collaboration with international transplant centers, two studies evaluated the initial experiences and outcomes of donor lung preservation under controlled hypothermic storage. The incidence of severe PGD in both cohorts was minimal. Our publication on prolonged ischemic time has the potential to become a cornerstone for the future development of global transplant programs.

The retrospective observational study on anticoagulation during transplantation examined 109 patients who underwent lung transplantation using venoarterial extracorporeal membrane oxygenation (ECMO) with central cannulation, with ECMO support terminated at the end of the procedure. We monitored heparin dosage, blood loss, blood product usage, and complications during surgery and up to 48 hours postoperatively in the intensive care unit. Lower doses of anticoagulation reduced blood loss, blood product usage, and the number of surgical revisions for hemothorax. Additionally, no cases of severe PGD were recorded within 72 hours post-transplantation in the low-heparin group.

We also analyzed two cases of potential lung donors with ambiguous COVID-19 test results, recommending the consideration of polymerase chain reaction cycle threshold (Ct) values in donor acceptance decisions. Such decisions should also include radiological findings and medical history. Since the presence of viral RNA does not necessarily indicate infectivity, we emphasized the need to determine Ct values correlating with viral viability. The detection of anti-SARS-CoV-2 antibodies and quantification of viral antigens in serum may provide valuable additional information. Incorrect donor selection can lead to severe PGD and significant complications affecting transplant outcomes.

Our final review article focused on SARS-CoV-2 vaccination in lung transplant recipients. The summary of findings on immunogenicity, efficacy, and safety highlighted that

this high-risk group often fails to mount immune responses comparable to the general population. Consequently, a third booster dose of the vaccine was recommended to prevent severe infection. However, questions remain regarding whether an enhanced immune response from an additional dose might increase the risk of acute rejection or other complications. This work emphasizes the importance of personalized vaccination strategies to minimize risks and ensure sufficient protection against COVID-19.

Our research has brought new insights into diagnostics, complication prevention, and perioperative care optimization in lung transplantation, including anticoagulation strategies and donor organ preservation. These findings contribute to more precise rejection diagnostics, a better understanding of risks, and opportunities for their minimization.

Keywords: lung transplantation, primary graft dysfunction, acute cellular rejection, checkpoint molecules, controlled hypothermic storage, ECMO anticoagulation, SARS-CoV-2

1. Úvod

1.1. Transplantace plic jako léčebná modalita

Transplantace plic je komplexní, život zachraňující chirurgický výkon, určený pacientům v terminálním stadiu plicního onemocnění. Principem transplantace je náhrada nemocné jedné plíce nebo obou plic zdravým orgánem od zemřelého dárce (Hardy et al., 1963; Patterson et al., 1988).

1.1.1. Organizace péče v České a Slovenské republice

Transplantace plic se provádí výhradně ve specializovaných centrech. Tato centra disponují multidisciplinárním týmem, jehož součástí jsou chirurgové, pneumologové, anesteziologové, rehabilitační lékaři, fyzioterapeuti, specializované sestry, perfuzionisté a transplantační koordinátoři. S narůstajícími nároky na péči a pokrokem v oboru se navíc zvyšuje potřeba zapojení dalších specialistů, jako jsou kardiologové, kardiochirurgové, mikrobiologové a další odborníci s relevantními zkušenostmi v péči o pacienty před a po transplantaci plic za účelem zajištění komplexní multidisciplinární péče a optimalizace dlouhodobých výsledků transplantace. V České republice je takovým centrem Fakultní nemocnice v Motole (FN Motol), kde III. chirurgická klinika 1. lékařské fakulty Univerzity Karlovy (1. LF UK) a FN Motol zastřešuje Národní program transplantace plic pro Českou a Slovenskou republiku (NPTP). Předtransplantační vyšetření a vedení čekací listiny, jako i interní/pneumologickou potransplantační péči zajišťuje Pneumologická klinika 2. LF UK a FN Motol. Potransplantační péči dále provádí Klinika plicních nemocí a tuberkulózy FN a LF

Univerzity Palackého, Olomouc. Předtransplantační vyšetření a referenci pacientů do FN Motol a potransplantační péči ve Slovenské republice zajišťuje Klinika pneumologie, fteziologie a funkční diagnostiky Slovenskej zdravotníckej univerzity a Univerzitnej nemocnice Bratislava.

1.1.2. Indikace a výběr příjemce

Mezi nejčastější diagnózy vedoucí k transplantaci plic patří chronická obstrukční plicní nemoc, intersticiální plicní procesy a plicní arteriální hypertenze (PAH) (Son a Shin, 2022). V důsledku pokroků v moderní modulátorové léčbě dochází k poklesu počtu transplantací pro cystickou fibrózu (Milinic et al., 2024). Méně časté diagnózy vedoucí k transplantaci jsou lymfangioleiomyomatóza nebo vzácná nádorová onemocnění, jako je například plicní epitelioidní hemangioendoteliom (Desie et al., 2015; Khawar et al., 2019; Novysedlak et al., 2024). Naopak se stále častěji provádí retransplantace plic, přičemž hlavní diagnózou vedoucí k tomuto zákroku je chronická dysfunkce plicního štěpu (CLAD) (Harhay et al., 2022; Novysedlak, Vachtenheim Jr., et al., 2022).

Seznam absolutních kontraindikací k transplantaci plic dle aktuálně platného konsenzu International Society for Heart and Lung Transplantation (ISHLT) (Leard et al., 2021) je uveden v **Tabulce 1**. Pozoruhodné je, že ani věk příjemce nad 70 let nebo body mass index (BMI) $> 35 \text{ kg/m}^2$ není absolutní kontraindikací k transplantaci. Retransplantace pro restriktivní fenotyp (RAS) CLAD (blíže viz kapitola 1.3.3) nebo retransplantace provedená dříve, než za rok od primární transplantace jsou spojeny s významným rizikem (Leard et al., 2021), přesto jsou tyto indikace v některých situacích odůvodněné.

Seznam absolutních kontraindikací k transplantaci plic. <i>přeloženo z Consensus document for the selection of lung transplant candidates: An update from the International Society for Heart and Lung Transplantation</i> (Leard et al., 2021)	
1	Nesouhlas pacienta s transplantací.
2	Zhoubné onemocnění s vysokým rizikem recidivy nebo úmrtí.
3	Glomerulární filtrace < 40 ml/min/1,73 m ² , pokud není zvažována multiorgánová transplantace.
4	Akutní koronární syndrom nebo infarkt myokardu v posledních 30 dnech (s výjimkou zátěžové ischemie).
5	Cévní mozková příhoda v posledních 30 dnech.
6	Jaterní cirhóza s portální hypertenzí nebo syntetickou dysfunkcí, pokud není zvažována multiorgánová transplantace.
7	Akutní selhání jater.
8	Akutní selhání ledvin se stoupajícím kreatininem nebo pacient na dialýze s nízkou pravděpodobností zotavení.
9	Septický šok.
10	Aktivní extrapulmonální nebo diseminovaná infekce.
11	Aktivní tuberkulóza.
12	Infekce HIV s detekovatelnou virovou náloží.
13	Omezený funkční stav (např. nepohyblivý pacient) s nízkým rehabilitačním potenciálem po transplantaci.
14	Progredující kognitivní porucha.

15	Opakované epizody non-compliance bez zlepšení (Poznámka: U pediatrických pacientů nejde o absolutní kontraindikaci a mělo by být prováděné průběžné hodnocení adherence k léčbě v průběhu vývojových stádií.)
16	Aktivní užívání nebo závislost na návykových látkách včetně aktivního užívání tabáku, vapování, kouření marihuany nebo užívání intravenózních drog.
17	Jiný závažný nekontrolovaný zdravotní stav, který bude pravděpodobně limitovat přežití po transplantaci.

Tabulka 1: *Seznam absolutních kontraindikací k transplantaci plic. Přeloženo z angličtiny z Consensus document for the selection of lung transplant candidates: An update from the International Society for Heart and Lung Transplantation (Leard et al., 2021)*

Alokace dárcovských plic je založená zejména na shodě v krevní skupině (AB0 systému). Uplatňují se přitom stejná pravidla jako pro krevní transfuze, velikostním poměru mezi dárce a příjemcem, imunologické shodě a urgentnosti čekatele (Barac et al., 2019). Ta může být vyjádřena i pomocí tzv. „lung allocation score“ (LAS) (Egan et al., 2006). V rámci NPTP je výběr konkrétního příjemce a mnohdy i několika náhradníků prováděn transplantčním chirurgem ve spolupráci s pneumologem a na základě konsenzu celého týmu.

1.2. Odběr a implantace dárcovských plic

Transplantační odběr je prováděn specializovaným chirurgem v dárcovské nemocnici, kam na výzvu cestuje odběrový tým z NPTP ve složení transplantační koordinátor, chirurg a

sálová sestra. Implantace probíhá ve FN Motol, v případě transplantace bloku srdce a plic pak v Institutu klinické a experimentální medicíny.

1.2.1. Klinická anatomie a významné variace

Odkysličená krev je do plic na každé straně přiváděná plicní tepnou a okysličená krev je vrácená do levé síně cestou plicních žil. Kmen plicnice (truncus pulmonalis) navazuje na pravou komoru a po přibližně 5 cm se větví na dvě plicní tepny. Plicní tepna je vpravo o něco delší než vlevo (Carter a Abbott, 2013). Většinou obliterovaný ductus arteriosus propojuje kmen plicnice zhruba v místě bifurkace s obloukem aorty (Gournay, 2011). Další větvení plicních tepen, které vykazuje jistou variabilitu, je zásadní pro případ lobární transplantace, kdy je prováděná anatomická redukce parenchymu lobektomií nebo bilobektomií za účelem zmenšení objemu dárcovských plic (Kandathil a Chamarchy, 2018). Typické uspořádání čtyř plicních žil, které ústí do horní posterolaterální části levé síně, dvě na každé straně, je přítomno pouze u 74 % případů (Ho et al., 2012). Tato anatomická variabilita musí být odběrovým chirurgem pečlivě zvážena, protože může ovlivnit zajištění dostatečného množství okolní stěny levé síně během odběru.

Vzduch proudí tracheou, která se větví v úrovni obratle Th4-Th5 na pravý a levý hlavní bronchus. Pravý hlavní bronchus měří zhruba 2 - 2,5 cm. Levý hlavní bronchus je o něco delší (4-6 cm) a jeho průběh je více horizontální (Drevet et al., 2016). Jednou z nejvýznamnějších anatomických variant, kterou lze pozorovat na CT dárce, pokud je dostupné, a někdy až při bronchoskopii v dárcovské nemocnici, je tzv. tracheální bronchus (nazýván také bronchus suis). Tento pravý horní lobární bronchus se odděluje od trachey nad její bifurkací a může být příčinou technických komplikací jak při odběru, tak především při implantaci dárcovských plic příjemci (Mendogni et al., 2019).

1.2.2. Dárce, odběr a prezervace dárcovských plic

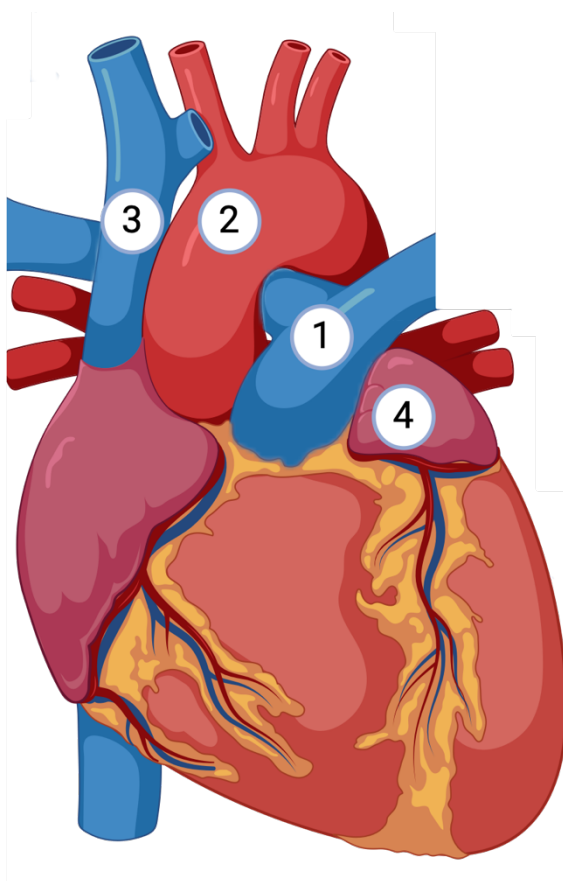
V ČR a SR je podle zákona (č. 285/2002 Sb. o darování, odběrech a transplantacích tkání) potenciálním dárce každý, kdo za svého života nevyjádří nesouhlas s dárcovstvím. V ČR je definováno dárcovství po smrti mozku (donor after brain death, DBD) a dárcovství po smrti způsobené oběhovou zástavou (donor after circulatory death, DCD). Typy DCD dárce blíže specifikuje modifikovaná Maastrichtská klasifikace, shrnuta v **Tabulce 2** (Thuong et al., 2016). V České republice se nyní provádí jen odběry od DCD dárce kategorie III.

Kategorie	Definice a subkategorie	Typ
I	dárce nalezen po smrti IA - mimo nemocnici IB - v nemocnici	nekontrolovaná
II	zástava oběhu před svědky IIA - mimo nemocnici IIB - v nemocnici	nekontrolovaná
III	odnětí terapie	kontrolovaná
IV	srdeční zástava při prokázané smrti mozku	nekontrolovaná

Tabulka 2: Modifikovaná Maastrichtská kritéria dle New classification of donation after circulatory death donors definitions and terminology (Thuong et al., 2016).

Při odběru plic v režimu DBD probíhá nejdříve bronchoskopická a makroskopická evaluace orgánu, potvrzení akceptace a následně preparace a zajištění vena cava superior, případně vena cava inferior a zavedení proplachové kanyly do truncus pulmonalis. Po

nezbytné přípravě všech odebíraných orgánů a aplikaci epoprostenolu do plicní tepny odběrový chirurg naloží svorku na aortu a spustí se perfúze jednotlivých odebíraných orgánů vhodným studeným roztokem (plíce se v NPTP proplachují izotonickým roztokem s nízkým obsahem draslíku). Díky tomuto postupu se lze teplé ischemii úplně vyhnout nebo ji minimalizovat na vteřiny. Proplach plic je možné spustit až po nastřížení ouška levé síně, v opačném případě jsou plíce ohroženy edémem způsobeným proplachem (Donahoe et al., 2018).



Obrázek 1: Schéma chirurgického postupu při transplantačním odběru plic u DBD dárce. 1 - umístění zavedené kanyly v truncus pulmonalis, preferenčně v místě bifurkace; 2 - naložení svorky na aortu (poloha svorky může být modifikována a v případě, že srdce není určeno k transplantaci, může být naložena jen pod bránici; 3 - naložení svorky nebo ligatury na horní

dutou žílu; 4 - proplach plic může být spuštěn až po nastřížení ouška levé síně. Schéma vytvořeno v BioRender.com.

Dárci, jehož orgány byly odebrány po zástavě oběhu, je nejprve ukončena terapie (nejčastěji jde o extubaci a zastavení oběhové podpory). V našem centru jako dárce plic zvažujeme takového DCD dárce, u kterého dojde ke zástavě oběhu do hodiny od ukončení terapie. DCD odběr se tedy vyznačuje teplou ischemií, která trvá od začátku hypoperfuze plic (za teplou ischemií považujeme pokles saturace kyslíkem pod 75% a/nebo systolického krevního tlaku pod 50 mmHg) do začátku proplachu studeným roztokem (Levvey et al., 2008). Před ukončením terapie nebo po zástavě oběhu je provedeno bronchoskopické vyšetření odběrovým chirurgem. Makroskopické zhodnocení a samotná akceptace dárcovských plic nastává až po odebrání a retrográdním proplachu na backtable.

Po odběru jsou plíce uloženy buď do boxu s ledem, což byla dlouhou dobu standardní metoda uchovávání, která se vyznačuje teplotami blízkými nule a rizikem poškození dárcovského orgánu v důsledku mrazu (freezing injury), což je spojeno s vyšším výskytem primární dysfunkce štěpu (PGD) a ischemicko-reperfuzního poškození. V posledních letech se však stále častěji používá metoda kontrolované hypotermie (CHS, 4–10 °C) (Cenik et al., 2024). Prodloužení prezervace dárcovských plic v kontrolované hypotermii bez negativního dopadu na krátkodobé výsledky bylo předmětem jedné ze zde prezentovaných publikací.

Další možností překlenutí doby od odběru do implantace je ex-vivo perfuze a ventilace dárcovských plic (EVLP). Randomizované studie potvrdily bezpečnost metody EVLP, avšak nepřinesly přesvědčivé důkazy o jejích výhodách ve srovnání se standardní prezervací na ledu (Van Raemdonck et al., 2022). Tato modalita by v budoucnu mohla umožnit terapeutické působení na dárcovský orgán s potenciálem rozšířit počet akceptovatelných plic (Ali et al., 2022; Van Raemdonck et al., 2023). Dle Nykanen et al. budou budoucí centra pro orgánové

inženýrství využívat genovou editaci a epigenetické inženýrství k vytvoření kvalitnějších orgánů, které budou fungovat lépe a déle (Nykanen et al., 2024).

1.2.3. Chirurgické postupy při implantaci

Implantaci dárcovských plic příjemci předchází explantace původních, nemocí postižených plic příjemce. Ta může být komplikována velkým množstvím adhezí v pleurální dutině a s tím spojenou významnou krevní ztrátou (Loor et al., 2022). Hilové struktury jsou nejdříve vypreparovány a následně jsou větve plicní tepny a plicních žil pomocí cévních staplerů přerušeny. Dále je přerušen bronchus elektrokoagulací. Po přípravě hilových struktur příjemce a také dárce (na bočním sterilním stolku) je v našem centru nejdříve prováděná bronchiální anastomóza následována anastomózou levé síně a plicní tepny. Všechny anastomózy jsou konstruovány jedním pokračujícím stehem (PDS 4/0 pro bronchiální anastomózu, Prolene 4/0 pro anastomózu levé síně a Prolene 5/0 pro anastomózu plicní tepny). Následuje reperfuze štěpu, kterou se ukončí ischemie (Aigner a Klepetko, 2012).

1.2.4. Využití mechanické podpory oběhu při transplantaci plic

Mechanická podpora oběhu je neodmyslitelnou součástí programu transplantace plic. U kriticky nemocných pacientů na čekací listině může dojít k progresi stavu do takové míry, že na vhodný orgán čekají se zavedenou mimotělní podporou krevního oběhu. Nejčastěji jde o podporu extrakorporální membránovou oxygenací (ECMO) v různých konfiguracích (tzv. ECMO-bridge). Nicméně jsou možné i alternativní přístupy, zejména typu "PA-LA shunt" (Balik et al., 2022). Transplantace těchto "superurgentních" čekatelů nejsou výjimečné. Méně časté jsou re-transplantace z ECMO-bridge, u kterých je indikací nutno zvažovat s velikou pečlivostí (Novysedlak, Vachtenheim Jr., et al., 2022). Výhodou ECMO podpory u

probuzeného pacienta bez intubace (tzv. awake ECMO) je možnost udržovat nebo dokonce vylepšit rehabilitační potenciál čekatele (Biscotti et al., 2017).

Protokoly transplantačních center ohledně perioperačního využití ECMO jsou různá. Preferenční postup v NPTP je standardní perioperační použití ECMO s centrální kanylací (nasávací kanyla do pravého ouška a návratová kanyla do kořene aorty) s nebo bez clamshell torakotomie. Kanylace probíhá po provedení torakotomie a alespoň částečné přípravě hilových struktur dle tolerance příjemce. Využití ECMO i v případech, kdy by transplantace bez podpory byla proveditelná minimalizuje riziko IRI a PGD.

V jiných centrech, ze kterých je nejzásadovějším zastáncem „no-ECMO“ standardu UZ Leuven v Belgii, je ECMO indikováno jen v případech, kdy transplantaci bez oběhové podpory provést nelze. Orlitova et al. ve své práci přiznávají komplikace související s použitím ECMO až v 67 % případů. Nejčastější z komplikací přitom bylo potransplantační krvácení do pleurální dutiny. (Orlitova et al., 2023). U transplantace bez ECMO je vhodnější začít implantací pravé plíce. Tento postup snižuje riziko urgentní ECMO kanylace (Van Slambrouck, Decaluwe, et al., 2024).

ISHLT ve svých doporučených postupech v případě transplantace s ECMO doporučuje zvážit protokol s nízkou dávkou heparinu, pokud lze zajistit adekvátní průtoky (Martin et al., 2024). Ke snížení perioperačního krvácení a spotřeby krevních derivátů je vhodné použít „point-of-care“ (POC) přístup využívající rotační tromboelastometrii (ROTEM). Ve studii Durila et al. autoři prokázali signifikantně nižší krevní ztrátu ($p = 0.013$) a nižší spotřebu krevních derivátů ($p = 0.009$ pro erytrocyty a $p < 0.0001$ pro čerstvě zmraženou plasmu) bez negativních dopadů na tvorbu krevní sraženiny při primární a sekundární hemostáze v ROTEM vs. no-ROTEM skupině (Durila et al., 2021).

Transplantace příjemců s PAH je mimořádně riziková vzhledem ke komplikacím spojeným se selháváním pravé komory srdeční. U těchto příjemců je ECMO zásadní pro

bezpečný průběh a dobrý výsledek operace. Vzhledem k potřebě zajištění adekvátní hemodynamické a respirační podpory v pooperačním období je často již před začátkem samotné transplantace provedena periferní kanylace v lokální anestezii (Savale et al., 2024; Stacel et al., 2024).

American Association for Thoracic Surgery doporučuje ECMO v situacích, kdy je mechanicky podpora oběhu nezbytná použít VA-ECMO (Hartwig et al., 2023). Ve výjimečných případech ale může být vhodnější plný mimotělní oběh (CPB). Kromě jednoznačné indikace při provádění transplantace bloku srdce a plic jde o situace, ve kterých je součástí operace výkon na srdci (například sutura defektu septa síní) nebo transplantována jedna plíce po kontralaterální pneumonektomii (Le Pimpec-Barthes et al., 2009). Analýza kohorty o více než 1000 transplantovaných pacientů ukázala, že delší využití CPB (více než 3 hodiny) je spojena s těžkou formou PGD (Ayyat et al., 2021).

1.3. Role imunitních buněk v transplantaci orgánů

Imunitní systém je tvořen buňkami, molekulami a tkáněmi, jejichž hlavním úkolem je chránit organismus před potenciálně škodlivými antigeny. Tradičně se dělí na přirozenou (vrozenou) imunitu a imunitu adaptivní (Marshall et al., 2018). Přirozená imunita, která je evolučně starší, zahrnuje buňky jako makrofágy a neutrofilní leukocyty, které hrají klíčovou roli při vzniku a udržování zánětu, ale také v procesu hojení tkání. Mezi další složky přirozené imunity patří například NK buňky, dendritické buňky a další (Spahn et al., 2014). Adaptivní imunitní systém je založen na aktivitě B lymfocytů, které produkují protilátky, a T lymfocytů, které se vyznačují buněčnou cytotoxicitou. Tuto cytotoxicitu zprostředkovávají pomocí perforinů a granzymů nebo prostřednictvím produkce cytokinů, jako je TNF- α či IFN- γ (Ang a Ting, 2019; Choy, 2010).

Ačkoli je imunitní systém zásadní pro ochranu proti infekcím a nádorovým buňkám, v kontextu transplantací představuje hlavní překážku v podobě rizika rejekce transplantované tkáně (Phillips a Callaghan, 2023). Je proto zásadní pečlivé vyvážení a pravidelná monitorace hladin imunosupresivní medikace (Schuurmans et al., 2023).

1.4. Potransplantační péče a časté neinfekční komplikace

Pacienti jsou po transplantaci plic směřováni na anesteziologicko-resuscitační oddělení, kde dochází ke stabilizaci stavu se snahou o časnou extubaci a rehabilitaci (Song et al., 2018). Pacienti, u kterých není možná časná extubace nebo kteří vyžadují reintubaci či tracheostomii, vykazují delší hospitalizaci, potřebu delší rehabilitace a horší šestiměsíční přežití, přičemž se selháním extubace jsou asociovány faktory jako kouření dárce, PGD a větší objem podaných erytrocytárních koncentrátů (Chapin et al., 2024).

Medián přežití po transplantaci plic u dospělých pacientů mírně přesahuje 6 let (van der Mark et al., 2020). Dožití je omezeno především infekčními komplikacemi, maligním onemocněním a CLAD (Ehram et al., 2021; Studer et al., 2004). Mezi klíčové rizikové faktory pro rozvoj CLAD patří kromě jiných PGD, akutní rejekce a některé infekční nemoci (Daud et al., 2007; Koutsokera et al., 2018; Royer et al., 2016).

1.4.1. Primární dysfunkce štěpu

PGD je akutní poškození plic vznikající během prvních 72 hodin po transplantaci. Je vyvolaná ischemicko-reperfúzním poškozením (IRI) plic a má výrazně negativní dopad na krátkodobé i dlouhodobé přežití (Roesel et al., 2022). Definice PGD je založena na hypoxemii a nálezů alveolárních infiltrátů na předozadním rentgenovém snímku hrudníku (Snell et al., 2017).

Stupeň	Známky edému na RTG snímku hrudníku	PaO ₂ /FiO ₂
PGD 0	ne	> 300
PGD 1	ano	> 300
PGD 2	ano	200 - 300
PGD 3	ano	< 200

Tabulka 3: *Hodnocení tíže primární dysfunkce štěpu po transplantaci plic. Přeloženo z angličtiny z Report of the ISHLT Working Group on Primary Lung Graft Dysfunction, part I: Definition and grading-A 2016 Consensus Group statement of the International Society for Heart and Lung Transplantation (Snell et al., 2017). PaO₂ - parciální tlak kyslíku v arteriální krvi; FiO₂ - frakce vdechovaného kyslíku; PGD - primární dysfunkce štěpu. Hodnota PaO₂/FiO₂ by měla být měřena u pacientů na umělé plicní ventilaci při PEEP 5 cm H₂O a FiO₂ 1,0 u pacientů na mechanické ventilaci.*

Tabulka 3 shrnuje diagnostická kritéria pro jednotlivé stupně PGD. Jakýkoliv poměr PaO₂/FiO₂ má odpovídat PGD 0, pokud na rentgenovém snímku hrudníku není přítomný difuzní plicní edém. V případě bilaterální transplantace plic musí být plicní edém patrný na obou transplantovaných plicích (Christie et al., 2008).

Etiologie rozvoje PGD je multifaktoriální. Vychází z těžkého IRI, během kterého tkáňové makrofágy zásadně přispívají k sekundární infiltraci neutrofilů a lymfocytů, která následně vede k závažnému poškození plicní tkáně (Morrison et al., 2017). Roli zde sehrává řada faktorů spojených s dárce, příjemcem i operačním výkonem samotným, mezi nimiž významnou úlohu hraje například prodloužená doba studené ischemie nebo doba implantace.

(Porteous a Lee, 2017; Vandervelde et al., 2022). Podmínkám a délce prezervace ve vztahu k PGD se blíže věnujeme v této disertační práci ve dvou dále uvedených publikacích.

Podle starších studií, která vycházela z dřívější definice CLAD, byla PGD identifikována jako faktor asociovaný s rozvojem CLAD (Daud et al., 2007; Kreisel et al., 2011). Navíc podle dvou studií Christie et al. PGD negativně ovlivňuje délku umělé plicní ventilace, dobu hospitalizace i celkové přežití, a to bez ohledu na přítomnost CLAD (Christie, Kotloff, et al., 2005; Christie, Sager, et al., 2005). Recentní studie ovšem tuto asociaci neprokázala. PGD se zdá být rizikovým faktorem pro stav označovaný jako „baseline lung allograft dysfunction“ (BLAD), avšak ne pro rozvoj CLAD (Li et al., 2021). V každém případě, PGD indukuje produkci prozánětlivých cytokinů, které mohou zvýšit expresi dárcovských HLA-II (human leukocyte antigen) na transplantovaném štěpu. Zvýšená exprese dárcovských HLA-II spolu s probíhajícím zánětem podporuje rozvoj dárcovsky specifické aloreaktivity (Bharat et al., 2008). PGD může rovněž negativně ovlivňovat telomery, čímž zesílí imunitní odpovědi a naruší reparační procesy. Dysfunkce telomer epiteliálních buněk by mohla představovat jeden z klíčových mechanismů spojujících PGD s rozvojem CLAD (Greenland et al., 2023). Asociace mezi PGD 3 a CLAD byla popsána i v populaci dětských příjemců. Incidence těžké formy PGD v čase 48 nebo 72 hodin po transplantaci dosáhla ve zkoumané kohortě 33 % (Wong et al., 2023).

1.4.2. Akutní rejekce štěpu

Akutní rejekce transplantovaných plic může mít podobu akutní celulární rejekce (ACR), zprostředkovanou T-lymfocyty, nebo akutní humorální rejekce (AMR), kterou řídí plazmatické buňky vytvářející protilátky (Martinu et al., 2011). Přibližně polovina transplantovaných pacientů je léčená pro rejekci během prvního roku (Martinu et al., 2009).

Častější ACR je diagnostikována ze vzorku plicní tkáně získaného ve většině případů transbronchiální biopsií. **Tabulka 4** shrnuje kritéria histopatologického hodnocení přítomnosti ACR a lymfocytické bronchiolitidy v bioptickém vzorku plicní tkáně (Stewart et al., 2007).

A: akutní rejekce	
0 - bez rejekce	normální parenchym
1 - minimální rejekce	roztroušené, ojedinělé perivaskulární mononukleární infiltráty o tloušťce 2–3 buněk
2 - lehká rejekce	častější perivaskulární mononukleární infiltráty snadno rozeznatelné při nízkém zvětšení; infiltráty mohou obsahovat lymfocyty, makrofágy a eozinofily
3 - střední rejekce	husté perivaskulární mononukleární infiltráty často spojené s endotelitidou; rozšíření zánětlivých infiltrátů do alveolárních sept, respiračních bronchiolů, alveolárních chodbiček a alveolů; běžný je nález eozinofilů a občasné se vyskytující neutrofilů
4 - těžká rejekce	difúzní infiltráty mononukleárních buněk perivaskulárně, v intersticiu a v respiračních bronchiolech, alveolárních chodbičkách a alveolách; poškození alveolárních pneumocytů a endotelitida
B: zánět dýchacích cest (lymfocytická bronchiolitida)	
0 - bez známek zánětu	bez známek zánětu bronchiolů
1R - nízký stupeň zánětu	mononukleární buňky v submukóze bronchiolů, občasná přítomnost eozinofilů
2R - vysoký stupeň zánětu	velké a aktivované mononukleární buňky, eozinofily a plazmocytoidní dendritické buňky v submukóze; známky poškození epitelu s nekrózou, metaplazií a intraepiteliální infiltrací lymfocytů
X - vzorek nehodnotitelný	bronchiolární tkáň nezastižena ve vzorku

Tabulka 4: ISHLT klasifikace histopatologického hodnocení akutní rejekce plicního štěpu - A a B klasifikace. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection (Stewart et al., 2007). Upraveno podle (Parulekar a Kao, 2019).

Většina světových pracovišť upřednostňuje méně rizikovou klíčkovou biopsii před invazivnější a potenciálně rizikovější kryobiopsií. V NPTP je díky mnohaleté zkušenosti preferovanou metodou kryobiopsie, která umožňuje odběr menšího množství větších vzorků (Steinack et al., 2022). Při klíčkové biopsii se doporučuje odebrat alespoň 5 vzorků (Stewart et al., 2007). Při kryobiopsii v NPTP odebíráme standardně vzorky dva. Preferenčním místem odběru jsou bazální segmenty levého dolního laloku (Martinu et al., 2009). Bronchoskopie s biopsií může být provedena akutně při zhoršení stavu nebo v rámci sledování (surveillance).

Mnoho epizod ACR je diagnostikováno u asymptomatických pacientů právě při těchto surveillance biopsiích. U klinicky stabilních pacientů není první epizoda A1 rejekce během prvního roku po transplantaci, pokud zůstane neléčená, spojena se zvýšeným rizikem CLAD nebo úmrtí (Levy et al., 2020). Většina center léčí ACR podáním vysokých dávek intravenózního metylprednisolonu v rozmezí 500–1000 mg denně po dobu 3 dnů. Následně může být přechodně zvýšena dávka perorálního prednisonu. Léčba kortikorezistentní ACR je mnohem složitější a často zahrnuje antithymocytární globulin, alemtuzumab nebo extrakorporální fotoforézu (Parulekar a Kao, 2019).

Plicní AMR má klinickou a subklinickou formu, které se liší přítomností dysfunkce štěpu. Diagnostika AMR je výrazně složitější a spočívá v kombinaci detekce protilátek proti dárcovským HLA a necharakteristických histologických změn v plicní tkáni (marginace neutrofilů, neutrofilní kapilaritida a arteritida). Depozita složky komplementu C4d přitom mohou, ale nemusí být ve štěpu přítomná (Levine et al., 2016). Léčba AMR spočívá zejména v podávání intravenózních imunoglobulinů, rituximabu a plazmaferéze a to samostatně nebo v kombinaci (Parulekar a Kao, 2019).

Obě formy akutní rejekce a také lymfocytická bronchiolitida jsou rizikovými faktory pro následný rozvoj CLAD (Bos et al., 2022).

1.4.3. Chronická dysfunkce plicního štěpu

CLAD zůstává hlavním faktorem limitujícím dlouhodobé přežití po transplantaci plic. Vyvine se přibližně u 50 % příjemců během 5 let (Bos et al., 2020). ISHLT definovala CLAD jako významný a trvalý pokles objemu usilovně vydechnutého vzduchu za první sekundu (FEV1) o 20 % nebo více oproti výchozím hodnotám po transplantaci, přičemž byly vyloučeny alternativní příčiny snížené funkce štěpu. Výchozí hodnota je definována jako průměr dvou nejvyšších naměřených hodnot FEV1 po transplantaci plic, mezi nimiž je časový odstup alespoň tři týdnů (Verleden et al., 2019). **Tabulka 5** uvádí jednotlivé stupně CLAD dle míry poklesu FEV1.

Stupeň	Výsledek spirometrického vyšetření
CLAD 0	aktuální FEV1 > 80 % výchozí hodnoty FEV1
CLAD 1	aktuální FEV1 > 65–80 % výchozí hodnoty FEV1
CLAD 2	aktuální FEV1 > 50–65 % výchozí hodnoty FEV1
CLAD 3	aktuální FEV1 > 35–50 % výchozí hodnoty FEV1
CLAD 4	aktuální FEV1 ≤ 35 % výchozí hodnoty FEV1

Tabulka 5: *Stadia CLAD dle Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment—A consensus report from the Pulmonary Council of the ISHLT (Verleden et al., 2019).*

CLAD zahrnuje nehomogenní skupinu fenotypů s různými patologickými charakteristikami, histologickými nálezy a odlišnou mírou odpovědi na léčbu (Silva et al., 2022). V současnosti rozlišujeme tři fenotypy: syndrom obliterující bronchiolitidy (BOS),

restriktivní syndrom alograftu (RAS) a smíšenou formu těchto dvou fenotypů. Diagnostickou komplikací představuje tzv. nedefinovaný fenotyp, u kterého není pochyb o probíhající CLAD, nicméně kombinace nálezů ztěžuje jeho zařazení do tří základních fenotypů (Verleden et al., 2019). V **Tabulce 6** jsou uvedena diagnostická kritéria pro jednotlivé fenotypy CLAD.

	Obstrukce	Restrikce	CT opacity
BOS	ano	ne	ne
RAS	ne	ano	ano
smíšený	ano	ano	ano
nedefinovaný	ano	ne	ano
	ano	ano	ne

Tabulka 6: *Diagnostická kritéria jednotlivých fenotypů CLAD dle Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment—A consensus report from the Pulmonary Council of the ISHLT (Verleden et al., 2019). Obstrukce je definována poklesem FEV1 (jak je uvedeno v textu) a je spojena s dalšími ukazateli omezení průtoku vzduchu, jako je poměr FEV1/FVC < 0,70. Restrikce je definována jako ≥ 10 % snížení výchozí hodnoty celkové plicní kapacity (TLC). BOS - syndrom obliterující bronchiolitidy, RAD - restriktivní syndrom alograftu, CT - výpočetní tomografie.*

Morfologickým podkladem BOS je obliterující bronchiolitida. Rané stadium zahrnuje subepiteliální fibrózu způsobenou organizací fibrinu s následnou jizevnatou fibrotizací bronchiolární stěny, zatímco pozdní stadium je charakterizováno fibrotickými zátkami, které obliterují bronchioly (Meyer et al., 2014). Ty mohou být někdy nahrazené jizevnatým

vazivem. RAS je spojen s progresivní fibrózou pleury a/nebo intersticia, zpočátku připomínající difuzní alveolární poškození nebo akutní fibrinózní a organizující se pneumonii, později vedoucí k difuzní plicní fibróze. Histologicky může zahrnovat tři vzorce: intraalveolární fibroelastózu, obraz podobný nespecifické intersticiální pneumonii nebo trakční emfyzém, který je spojován s lepší prognózou. RAS může být kombinován s obstrukčními rysy obliterující bronchiolitidy, což vysvětluje smíšený fenotyp CLAD (Glanville et al., 2019; Novysedlak et al., 2023).

Existuje celá řada neinvazivních postupů, které mají za cíl zabránit rozvoji CLAD nebo zastavit progresi. Jde zejména o farmakologické metody nebo extrakorporální fotoforézu. Nicméně přesvědčivé důkazy o účinnosti a jasné doporučení chybí (Novysedlak et al., 2023). Vzhledem k prokázané roli mikroaspirací v rozvoji BOS probíhá odborná diskuse stran vhodnosti provádění antirefluxní plastiky u transplantovaných pacientů před nebo po transplantaci (D'Ovidio et al., 2005). Recentní studie zahrnující 132 pacientů ukázala, že navzdory antirefluxní plastice nedošlo k významnému zpomalení poklesu funkce transplantovaných plic (Frankel et al., 2023). Jedinou kauzální léčbou CLAD tedy zůstává retransplantace plic (Novysedlak et al., 2023; Novysedlak, Vachtenheim, et al., 2022).

1.5. Infekční komplikace po transplantaci plic

Pacienti po transplantaci plic jsou vysoce náchylní k infekčním komplikacím zejména kvůli kombinaci imunosuprese a neustálé komunikaci plic s vnějším prostředím (Trachuk et al., 2020). Za přibližně 50 % všech infekčních onemocnění u pacientů po transplantaci plic jsou odpovědné bakteriální patogeny. Virové infekce tvoří druhou nejčastější příčinu, přičemž představují přibližně třetinu všech případů. Invazivní plísňové infekce se během prvního roku po transplantaci rozvinou u téměř 10 % pacientů (Dettori et al., 2024).

Infekční komplikace jsou zodpovědné až za 37 % úmrtí v období od 30 dnů do 1 roku po transplantaci (Yusen et al., 2016). Některé z infekcí pravděpodobně také přispívají k akutní rejekci nebo rozvoji CLAD. Organizující se pneumonie je považována za nezávislý rizikový faktor pro AMR, avšak nikoliv pro CLAD (Keller et al., 2024). Naproti tomu kolonizace plísní rodu *Aspergillus* byla identifikována jako rizikový faktor pro rozvoj CLAD (Weigt et al., 2009). Mezi virové patogeny s pro-rejekčním rizikem patří zejména cytomegalovirus a komunitně získané respirační virové infekce, jako jsou například virus chřipky, respirační syncytiální virus či koronaviry. (Nosotti et al., 2018).

1.5.1. Infekce SARS-CoV-2 v kontextu transplantace plic

Pandemie nemoci „coronavirus disease 2019“ COVID-19 způsobené virem „severe acute respiratory syndrome coronavirus 2“ SARS-CoV-2 v letech 2019-2023 byla zásadní výzvou pro programy transplantace plic po celém světě. Během pandemie došlo ve většině transplantačních center k výraznému poklesu počtu výkonů, přičemž transplantace byly omezeny na urgentní případy. Roli sehrál také nedostatek kapacit na jednotkách intenzivní péče a nedostatek dárců zvýšil úmrtnost na čekacích listinách (Vachtenheim et al., 2022).

COVID-19 představuje komplexní a dynamické onemocnění s pleiotropními účinky. Virové poškození a následné metabolické adaptace buněk vykazují značné rozdíly v závislosti na typu buňky, genetické výbavě hostitele a vlivech prostředí. V optimálním případě pro virus SARS-CoV-2 dochází po infekci k sérii intracelulárních adaptací, které zahrnují rozsáhlé modifikace buněčných procesů, včetně transkripce, metabolismu a signalizačních drah. Tyto změny transformují infikovanou buňku na systém podporující replikaci viru SARS-CoV-2, což vede k produkci a uvolňování nových virových částic a signálních molekul. Následné patologické procesy pak spouštějí kaskádu událostí, které zasahují buňky i orgánové systémy v celém organismu (Jamison et al., 2022). Virus SARS-CoV-2 efektivně infikuje buňky dolních dýchacích cest a vyvolává promptní imunitní odpověď (Paces et al., 2020).

Obavy z přenosu viru od dárce na druhou stranu vyvolaly otázky o možných zbytečných ztrátách kvalitních orgánů (Ceulemans, Van Slambrouck, et al., 2021; Van Slambrouck et al., 2022). Navíc se ukázalo, že virová RNA může v plicích asymptomaticky přetrvávat i desítky dnů po překonání nemoci (Ceulemans, Khan, et al., 2021). Mezi efektivní a cenově dostupné metody patří POC testy, které umožňují detekci virové RNA nebo antigenu ve vzorcích získaných bronchoalveolární laváží (BAL). Tyto testy mohou poskytnout cenné doplňující informace při rozhodování o akceptaci dárcovských plic (Van Slambrouck, Schoenaers, et al., 2024). Za účelem prevence přenosu onemocnění z dárce na příjemce bylo navrženo několik strategií a problematice se věnujeme také v jedné z našich dále předložených publikací.

Samotné poškození plic v důsledku nemoci COVID-19 se také stalo indikací pro transplantaci plic (Naar et al., 2022; Tasoudis et al., 2023). Ta je jedinou možností léčby pro nevratný syndrom akutní dechové tísně (ARDS) a plicní fibrózu vzniklou v důsledku onemocnění COVID-19 (Cerier et al., 2023). V období od srpna 2020 do září 2021 bylo ve Spojených státech amerických až 7 % transplantací plic provedeno pro respirační selhání

způsobené onemocněním COVID-19 (Roach et al., 2022). V rakouských transplantačních centrech bylo v období od 1. ledna 2020 do 30. května 2021 provedeno celkem 19 transplantací plic u pacientů s ARDS spojeným s onemocněním COVID-19. Třicet dní po transplantaci nebyla zaznamenána žádná úmrtnost. Při délce sledování 134 dní (medián, rozmezí 47–450 dní) bylo naživu 14 z 19 pacientů. (Lang et al., 2022). **Tabulka 7** shrnuje klíčové faktory, které je třeba zohlednit při posuzování zařazení pacienta s COVID-19 asociovaným ARDS na čekací listinu pro transplantaci plic.

Přestože randomizované i observační studie naznačují dobrou účinnost a bezpečnost vakcín proti COVID-19, jejich dlouhodobý efekt a bezpečnost jsou stále předmětem zkoumání (Milota et al., 2021). U transplantovaných pacientů je problém ochrany před infekcí vakcinací mnohem složitější vzhledem k užívané imunosupresi. U žádného z očkováných pacientů, kteří předtím neprodělali infekci COVID-19, nebyla po podání dvou dávek mRNA vakcíny BNT162b2 detekována přítomnost IgG protilátek proti SARS-CoV-2 (Havlin et al., 2021). Nicméně mRNA vakcíny proti COVID-19 prokazují imunogenicitu u předtím imunizovaných transplantovaných pacientů, přičemž protilátková a T-buněčná odpověď zůstávají stabilní a dlouhodobě detekovatelné až po dobu 6 měsíců od vakcinace (Liu et al., 2024). Třetí dávka očkování u příjemců solidních orgánů byla předmětem naší přehledové publikace, kterou komentujeme v této disertační práci.

Klíčové faktory při posuzování zařazení pacienta s COVID-19 asociovaným ARDS na čekací listinu k transplantaci plic <i>dle How COVID-19 Affects Lung Transplantation: A Comprehensive Review</i> <i>(Vachtenheim et al., 2022)</i>	
1	Věk kandidáta: Potenciální kandidáti by měli být mladší než 65 let. Použití ECMO jako metody na překlenutí období do transplantaci plic (ECMO bridge) u starších pacientů prokázalo horší výsledky.
2	Zachovaná dobrá funkce ostatních orgánových systémů: Kandidáti by neměli vykazovat selhání jiných orgánů a neměli by trpět neřešitelnými komorbiditami.
3	Čas na zotavení nativních plic: Před zvažováním transplantace by mělo uplynout dostatečné období, které umožní adekvátní posouzení potenciálu zotavení nativních plic. Transplantace by neměla být indikována dříve než 4–8 týdnů od nástupu prvních klinických příznaků respiračního selhání. V případě jasně patrného pokračujícího zlepšení plicních funkcí by transplantace neměla být indikována, bez ohledu na dobu, která od začátku onemocnění uplynula.
4	Radiologický důkaz nevratného plicního poškození: Měli by být přítomné jasné radiologické známky nevratného poškození plic (například bulózní destrukce, plicní fibróza, rozsáhlá parenchymová konsolidace nebo pneumofluidotorax).
5	Preference pro awake ECMO po dobu čekání na transplantaci: Ideální kandidát na ECMO by měl být při vědomí, bez orotracheální intubace, aktivně rehabilitovat a být schopen poskytnout informovaný souhlas. V případech, kdy pacient není při vědomí, může být udělena výjimka, pokud má velký potenciál na zotavení po transplantaci. V takových situacích by měl být zajištěn informovaný souhlas rodiny nebo právního zástupce.

6	Negativní test na SARS-CoV-2: Je nezbytné opakovaně potvrdit negativní RT-PCR test z dolních dýchacích cest. U pacientů, kteří nejsou závislí na umělé plicní ventilace a nemají tracheostomii, lze provést RT-PCR vyšetření z nasofaryngeálního stěru. Lze také provést kultivaci viru a zhodnotit přítomnost protilátek.
7	Zkušenosti transplantačního centra: Transplantace plic u pacientů s ARDS a ECMO podporou patří k nejsložitějším. Transplantační centra provádějící tyto operace by proto měla mít značné zkušenosti s vysoce rizikovými pacienty a komplexními klinickými situacemi.
8	Etické aspekty: Transplantační centrum by mělo mít přístup k dostatečnému počtu dárců a prokazovat nízkou mortalitu na čekací listině, aby byla zajištěna spravedlivá alokace orgánů.
9	Pravidelná evaluace: Zdravotní stav pacienta a jeho transplantabilita by měly být kontinuálně a systematicky přehodnocovány multidisciplinárním týmem.
10	Bilaterální transplantace plic: Měla by být zvažována výhradně transplantace obou plic vzhledem k častému výskytu konkomitantní plicní hypertenze a sekundárních nozokomiálních infekcím, které mohou na imunosupresivní léčbě vyústit v těžkou pneumonii.

Tabulka 7: *Klíčové faktory při posuzování zařazení pacienta s COVID-19 asociovaným ARDS na čekací listinu k transplantaci plic dle How COVID-19 Affects Lung Transplantation: A Comprehensive Review (Vachtenheim et al., 2022). RT-PCR - real-time polymerázová řetězová reakce;*

1.6. Checkpoint molekuly a jejich potenciál v transplantologii

Imunitní kontrolní body (immune checkpoints, IC) jsou molekulární mechanismy zabudované do imunitního systému, které modulují charakter imunitní odpovědi při prezentaci antigenu za fyziologických i patologických podmínek (Righi et al., 2024). IC molekuly se dělí do dvou skupin. Kostimulační molekuly aktivují T lymfocyty, zatímco inhibiční molekuly vysílají tlumivé signály, které zabraňují aktivaci T lymfocytů, čímž předcházejí nadměrnému zánětu a autoimunitní reakci (Taefehshokr et al., 2020).

V moderní onkologii jsou IC dráhy T buněk považovány za významný mechanismus rezistence vůči imunitní odpovědi (Taefehshokr et al., 2020). Objev inhibičních IC molekul, jako jsou PD-1/PD-L1 a „cytotoxic T-lymphocyte antigen 4“ (CTLA-4), předznamenal významný průlom v oblasti imunoterapie nádorových onemocnění (Shiravand et al., 2022). Blokace funkce inhibičních IC molekul prostřednictvím tzv. checkpoint inhibitorů našla uplatnění v terapii řady onemocnění, včetně karcinomu plic. (Lahiri et al., 2023).

Naopak stimulace inhibičních IC molekul může být prospěšná při prevenci rejekce v kontextu transplantace solidních orgánů (Righi et al., 2024).

PD-1 je exprimován jako monomer na aktivovaných T a B lymfocytech, tymocytech a NK buňkách (Righi et al., 2024). PD-1 po vazbě na PD-L1 potlačuje proliferaci buněk, blokuje průběh buněčného cyklu a snižuje produkci prozánětlivých cytokinů (Freeman et al., 2000; Ishida et al., 1992). Mechanismem účinku CTLA-4 je dynamická kompetice s CD28 o ligand ze skupiny B7 na antigen prezentujících buňkách (Rowshanravan et al., 2018). Belatacept je monoklonální protilátka, která napodobuje přirozený receptor CTLA-4. (Larsen et al., 2005). V současnosti je schválen jako léková profylaxe rejekce u transplantace ledviny (Vincenti et al., 2010).

Adhezivní molekula platelet-endothelial-cell adhesion molecule-1 (PECAM-1) hraje klíčovou roli v udržování těsných spojení mezi endoteliálními buňkami, migraci leukocytů a regulaci imunitní odpovědi, přičemž využívá jak homofilní, tak heterofilní vazby. Tyto interakce, které umožňují přenos endogenních a exogenních signálů, jsou zprostředkovány extracelulárními imunoglobulinovými podobnými doménami a cytoplazmatickými tyrosinovými inhibičními motivy (Hu et al., 2016). Je vhodné zmínit, že PECAM-1 (neboli také CD31) byť není typickou IC molekulou, má funkci typickou pro checkpoint molekuly. Konkrétně se ukazuje, že působí jako kontrolní receptor monocytů pro aktivaci receptorů pro Fc doménu imunoglobulinů G. Downregulace PECAM-1 tedy vede ke zvýšení fagocytární aktivity monocytů (Merchand-Reyes et al., 2019).

2. Hypotézy a cíle

Cílem této práce bylo detailně analyzovat imunologické procesy existující na pozadí ACR a identifikovat potenciální doposud neznámé faktory ovlivňující rozvoj PGD a ACR. Na vlastní kohortě pacientů jsme analyzovali imunologické procesy spojené s ACR a možné dosud nepopsané markery rejekce, abychom lépe porozuměli patofyziologii tohoto stavu a přispěli k jeho včasné diagnostice. V tomto ohledu jsme se zaměřili na checkpoint molekuly a molekuly asociované s transendoteliální migrací, které nebyly v kontextu transplantace plic literaturou dosud popsány. Dále jsme zkoumali dopady prezervace dárcovských plic v kontrolované hypotermii a použití snížených dávek heparinu jako ECMO antikoagulace na rozvoj PGD. Věnovali jsme se také problematice dárců plic s COVID-19 pozitivním testem a významu očkování proti COVID-19 u pacientů na čekací listině a po transplantaci, s cílem optimalizovat preventivní opatření a prohloubit porozumění potenciálním rizikům, která může očkování představovat pro rozvoj akutní rejekce štěpu.

Úvodní hypotéza k vybraným publikacím:

2.1. Vybrané checkpoint molekuly hrají roli v patogenezi akutní celulární rejekce plicního štěpu a mohou sloužit jako přídavný diagnostický marker

V této publikaci jsme se zaměřili na experimentální identifikaci nových markerů akutní celulární rejekce. Předpokládali jsme, že analýza imunitních buněk a checkpoint molekul ve vzorcích plicní tkáně získaných kryobiopsií a v konkomitantním krevním séru

může objasnit klíčové mechanismy rozvoje ACR. Zabývali jsme se zastoupením a funkcí T lymfocytů, makrofágů a B lymfocytů a expresí checkpoint molekul s cílem vytvořit komplexní profil imunitní odpovědi a lépe pochopit patofyziologii tohoto procesu.

2.2. Vhodně zvolená metoda prezervace dárcovských plic umožňuje prodloužení studené ischemie bez negativních dopadů na krátkodobé výsledky

Cílem zde prezentovaných prací bylo zhodnotit dopad kontrolované hypotermie na krátkodobé výsledky po transplantaci. Dle naší hypotézy vhodné teplotní podmínky prezervace dárcovských plic umožňují prodloužení studené ischemie po dobu delší než 15 hodin, aniž by to mělo negativní dopad na krátkodobé výsledky po transplantaci.

2.3. Vhodné perioperační dávkování heparinu snižuje riziko vzniku primární dysfunkce plicního štěpu

V této publikaci bylo cílem porovnat dopady různých dávek antikoagulace při transplantaci plic s využitím ECMO na výsledky transplantace. Předpokládali jsme, že nižší dávky heparinu vedou k nižšímu riziku těžké formy PGD, menší krevní ztrátě a menšímu počtu chirurgických revizí.

2.4. Možnosti prevence infekce SARS-CoV-2 u příjemců transplantace plic (vakcinace příjemce a evaluace virové nálože pozitivního dárce)

Cílem dvou publikací v této části bylo v přehledné podobě shrnout zkušenosti s očkováním pacientů po transplantaci a čekatelů na čekací listině a rozptýlit tak obavy z negativních dopadů na transplantované pacienty. Dále jsme měli za cíl zdůraznit, že pouhá přítomnost virové RNA SARS-CoV-2 v nazofaryngeálním stěru a bronchoalveolární laváži neznamená viabilitu viru a neměla by tedy automaticky zabránit dárčovství.

3. Metodika

Detailní popis konkrétní metodiky je součástí publikovaných prací. Každý výzkum s využitím dat nebo vzorků pacientů probíhal se souhlasem Etické komise FN Motol. Klinická data byla ve všech pracích anonymizována a identifikovatelné odkazy na jednotlivce byly odstraněny. Všechny předložené studie se řídily principy Helsinské Deklarace a jsou v souladu s the ISHLT Ethics Statement.

4. Výsledky a diskuse

4.1. Molekuly PD-L1 a PECAM-1 jako diagnostické biomarkery akutní rejekce

Novysedlak R, Balko J, Tavandzis J, Tovazhnianska V, Slavcev A, Vychytilova K, Smetanova J, Bohyn A, Vajter J, Borcinova M, Vanaudenaerde BM, Lischke R, Vachtenheim J Jr, Ceulemans LJ, Ozaniak Strizova Z. Elevated PD-L1 and PECAM-1 as Diagnostic Biomarkers of Acute Rejection in Lung Transplantation. Transpl Int. 2024 Nov;37:13796. doi: 10.3389/ti.2024.13796.

Za účelem lepšího porozumění roli imunitních buněk a checkpoint molekul v kontextu ACR jsme v této studii analyzovali na kohortě 60 pacientů expresi povrchových molekul CD3, CD4, CD8, CD20, CD68, CD47, PD-1, PD-L1 a CD31/PECAM-1. Cílem bylo identifikovat poměry a fenotypy jednotlivých subpopulací T lymfocytů, posoudit míru vyčerpání imunitního systému, pochopit jeho dynamiku a určit zastoupení makrofágů a B lymfocytů ve vzorcích plicní tkáně získaných pomocí kryobiopsie.

Dále jsme pomocí metody Luminex měřili funkčnost T buněk prostřednictvím hodnocení exprese PD-L1, interleukinu-2 (IL-2), granzymu B, tumor nekrotizujícího faktoru alfa (TNF α) a interferonu gama (IFN γ), abychom získali informace o aktivaci či inhibici imunitních buněk v transplantovaném orgánu.

Zaznamenali jsme signifikantně vyšší tkáňovou expresi PECAM-1/CD31 a PD-L1 v rejekční kohortě oproti pacientům bez rejekce. Tento výsledek nejen podporuje využití imunohistochemie jako podpůrné metody v diagnostice ACR, ale zároveň může představovat

jeden ze základních kamenů pro další výzkum role checkpoint molekul v patofyziologii rejekce a jejich potenciálního terapeutického využití.



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Elevated PD-L1 and PECAM-1 as Diagnostic Biomarkers of Acute Rejection in Lung Transplantation

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Acute cellular rejection (ACR) frequently occurs following lung transplantation (LuTx) and represents a risk factor for the development of chronic lung allograft dysfunction (CLAD) as well as long-term survival. The histopathological diagnosis of ACR carries a burden of interobserver variability. The widespread utilization and cost-effectiveness of immunohistochemistry (IHC) was proven beneficial in diagnosing rejection in human kidney transplantations and LuTx rat models. However, its potential for ACR detection in patients remains unexplored. We analyzed surface markers (CD3, CD4, CD8, CD20, CD68, CD47, PD-1, PD-L1, and CD31/PECAM-1) on lung tissue cryobiopsy samples collected within 6 months post-LuTx from 60 LuTx recipients, 48 of whom were diagnosed with ACR. Additionally, serum samples from 51 patients were analyzed using a multiplex bead-based Luminex assay. The cytokines and markers included PD-L1, IL2, TNF α , IFN γ , and Granzyme B. We observed a significant increase in PD-L1 tissue expression within the rejection group, suggesting a concerted effort to suppress immune responses, especially those mediated by T-cells. Furthermore, we noted significant differences in PECAM-1 levels between ACR/non-ACR. Additionally, peripheral blood C-reactive-protein levels

Abbreviations: ACR, acute cellular rejection; AMR, acute humoral rejection; APCs, antigen presenting cells; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DBD, donation after brain death; IHC, immunohistochemistry; IFN γ , interferon gamma; ILD, interstitial lung disease; ISHLT, the International Society for Heart and Lung Transplantation; PAH, pulmonary arterial hypertension; PD-1, programmed death-receptor 1; PD-L1, programmed death-ligand 1; LuTx, lung transplantation; RAS, restrictive allograft syndrome; ROC, receiver operating characteristics; TNF α , tumor necrosis factor alpha; WBC, white blood cells.

tended to be higher in the ACR group, while Luminex serum analyses did not reveal any significant differences between groups. In conclusion, our findings suggest the potential value of PECAM-1 and PD-L1 markers in diagnosing ACR.

Keywords: lung transplantation, acute cellular rejection, immunohistochemistry, luminex, checkpoint inhibitors

INTRODUCTION

Long-term allograft survival has always been significantly challenged by the persistent risk of transplantation rejection [1–4]. During transplantation, both ischemia-reperfusion and mechanical injury as well as inadequate organ storage conditions prompt immune system reactions through the local release of cytokines, chemokines, adhesion molecules, damage-associated molecular patterns, and other signaling molecules [5–7]. These events trigger an influx of innate immune cells to the graft, which is followed by the presentation of allogeneic antigens by antigen-presenting cells (APCs) to adaptive immune cells [8].

Acute organ rejection involves acute cellular rejection (ACR) orchestrated by T-cells and acute humoral rejection (AMR) driven by antibody-producing plasma cells [9].

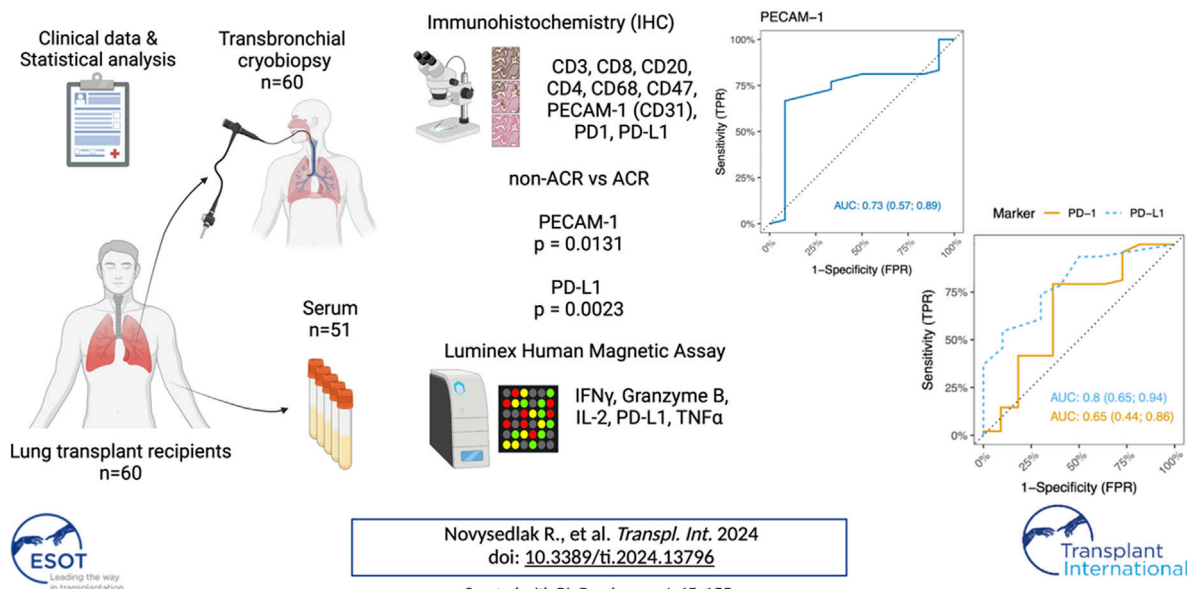
Antibody-mediated rejection (AMR) is a profoundly studied phenomenon particularly in kidney transplants, leading to standardized nomenclature and diagnostic criteria. However, its applicability in lung transplants is limited, emphasizing the significance of T-cell-mediated rejection in lung allografts [10].

T-cell-mediated ACR in lung transplants, impacting small airways and vasculature, represents a significant clinical challenge [11–13]. The incidence of ACR is highest early post-lung transplantation, with 27% of adult patients experiencing at least one treated episode within the first year. ACR is associated with bronchiolitis obliterans syndrome (BOS), a main phenotype of chronic lung allograft dysfunction (CLAD), with late ACR episodes (after 180 days post-transplant) linked to an elevated risk of BOS [1, 14–18].

Diagnostic assessment of ACR faces interobserver variability, particularly in lower-grade rejection, and understanding of the specific traits and phenotypic patterns of infiltrating T-cells during ACR remains limited [11]. Therefore, ACR demands attention from researchers to pinpoint potential biomarkers that could help to understand immune responses and strengthen the diagnostic process and early detection of rejection.

Immune checkpoint molecules have been extensively studied in the oncological context [19]. However, their role and potential use in solid organ transplantation is far from being understood. Several studies have shown that the interaction between Programmed Death-Receptor 1 (PD-1) and Programmed Death-Ligand 1 (PD-L1) is essential for both initiating and sustaining tolerance to the graft [20].

Elevated PD-L1 and PECAM-1 as Diagnostic Biomarkers of Acute Rejection in Lung Transplantation



GRAPHICAL ABSTRACT

PD-1 is a key inhibitory receptor involved in both adaptive and innate immune responses. It is expressed on various immune cells, including activated T cells, natural killer cells, B lymphocytes, macrophages, dendritic cells, and monocytes. PD-1 plays a crucial role in dampening autoimmune reactions and thus, preserving immune tolerance [21, 22]. As a PD-1 ligand, PD-L1 is typically found on macrophages, activated T and B cells, dendritic cells, and various epithelial cells, with its expression being elevated under inflammatory conditions. PD-L1 is often found in immune environments characterized by high loads of CD8⁺ T cells and the production of Th1 cytokines and interferons [21].

In contrast to other costimulatory molecules, PD-L1 expression extends beyond hematopoietic cells, as it can also be detected on endothelial cells, placental trophoblasts, and even pancreatic islet cells [23].

In the context of transplantation, the PD-1/PD-L1 pathway has been primarily investigated in animal models, with limited research was conducted in humans, particularly among lung transplant recipients [24, 25].

PD-L1 expression was shown to be significantly upregulated following transplantation on endothelial cells within heart allografts [26].

This increased expression within the vasculature indicates that PD-L1 may play a crucial role at the interface between immune cells and the transplanted organ, highlighting its potential importance in regulating the alloimmune response. In this regard, molecules involved in endothelial-immune cell interactions warrant particular attention.

Platelet/endothelial cell adhesion molecule-1 (PECAM-1 or CD31) is a key regulator of leukocyte transmigration across the endothelium and has been shown to be essential for transendothelial migration. PECAM-1-mediated leukocyte migration can be effectively inhibited by PECAM-1-specific blocking antibodies or by downregulating PECAM-1 expression [27].

Thus, examining PD-1/PD-L1 coinhibitory signals could provide valuable insights into the regulation of the alloimmune response, while clarifying the involvement of PECAM-1 in transplant rejection could highlight its potential as a novel therapeutic target in transplantation.

Immunohistochemistry (IHC), a cost-effective technique, has proven useful in diagnosing ACR in human kidney transplants [28–30]. Although in rat models, IHC aimed specifically at CD4⁺ and CD8⁺ T-cell proportions and distribution, improved the sensitivity and specificity of lung rejection diagnosis and grading, the same approach in human lung ACR is insufficient [31].

To address these gaps, and to better understand the role of immune checkpoint molecules in transplantation, our study explores multiple IHC biomarkers, including CD3, CD4, CD8, CD20, CD68, CD47, PD-1, PD-L1, and CD31/PECAM-1 within a large cohort. We aimed to identify T-cell subtype proportions and phenotypes, assess immune exhaustion levels, understand immune system dynamics, examine leukocyte transendothelial migration patterns, evaluate “don’t eat me” signals expression, and determine macrophage and B-cell proportions within lung allograft specimens.

Additionally, to obtain a detailed understanding of the immune landscape in LuTx recipients, we have extended our analyses by measuring T cell functionality via a multiplex assay. To provide a comprehensive profile of the immune status and functionality of T cells, which are critical in the context of transplantation, PD-L1, IL-2, Granzyme B, Tumor Necrosis Factor alpha (TNFα) and interferon gamma (IFNγ), were evaluated.

PD-L1 was included due to its role in immune regulation, whereas IL-2 provided insights into the activation status and responsiveness of T cells. Granzyme B, TNFα, and IFNγ are integral to the effector functions of T cells. Together, they provide comprehensive insights into T cells’ cytotoxic potential, inflammatory responses, and the regulatory balance of immune activation, all of which are crucial for graft survival and effective immune defense.

Understanding not just the phenotype but also the function of T cells is vital for developing strategies to enhance graft survival and reduce the risk of rejection.

This comprehensive analysis aimed to provide crucial insights into immune events within lung allografts.

MATERIALS AND METHODS

Study Design

This retrospective study includes 171 adult patients (≥18 years) who underwent bilateral lung transplantation (LuTx) at Motol University Hospital in Prague between 1 January 2018, and 31 December 2021. Excluded were single, lobar, and multiorgan LuTx, as well as re-transplants. Patients without cryobiopsy within the initial 6 months post-transplant, lacking cryopreserved samples, or tissue samples for research were also excluded.

Routine and on-demand cryobiopsies were collectively analyzed, with routine samples taken after one, three, or six post-transplant months. Demographics and clinical data were obtained from patient files, and only laboratory results before ACR treatment initiation were considered. Serum samples taken prior to the initiation of potential rejection treatment were analyzed for selected cytokines using a customized Luminex Human Magnetic Assay. Tissue samples were evaluated using IHC. The study, approved by the Ethics Committee of Motol University Hospital (EK-530/21), received written informed consent from all patients at transplantation listing. Follow-up was censored on 24 September 2023.

Study Population

Donor and preservation variables included: age, gender, weight, height, BMI, donor type [donation after brain death (DBD) vs. donation after circulatory death], cytomegalovirus (CMV) status and times of ischemia for both lungs.

Recipient variables included: age, gender, weight, height, BMI, CMV status, underlying comorbidities, indication for transplant, immunosuppression regimen used, date of the first post-transplant lung tissue cryobiopsy, acute cellular rejection

TABLE 1 | Immunohistochemistry staining specifications.

Antibody	Clone; manufacturer	Dilution	Pre-treatment
anti-CD3	RBT-CD3 [BioSB]	1:20	Heating in a buffer solution of pH9 in a water bath
anti-CD8	C8/144B [Dako]	1:200	Heating in a buffer solution of pH9 in a water bath
anti-CD20	L26 [Dako]	1:300	Heating in a buffer solution of pH6 in a water bath
anti-CD4	4B12 [BioGenex]	1:250	Heating in a buffer solution of pH9 in a water bath
anti-CD68	PG-M1 [Dako]	1:100	Heating in a buffer solution of pH9 in a water bath
anti-CD47	PA5-80435 [ThermoFisher Scientific]	1:200	Heating in a buffer solution of pH6 in a water bath
anti-CD31 (anti-PECAM-1)	JC70A [Dako]	1:40	Heating in a buffer solution of pH6 in a water bath
anti-PD1	polyclonal serum [Abd Serotec]	1:200	Without antigen retrieval
anti-PD-L1	22C3 [Dako]	Certified kit	Processed according to the certified Autostainer Link 48 protocol

(grades A and B), infection status, peripheral blood levels of C-reactive protein (mg/L), peripheral blood levels of white blood cells ($\times 10^9/L$) and percentage and count ($\times 10^9/L$) of its subtypes, namely, lymphocytes, monocytes, neutrophils, eosinophils, basophils and immature granulocytes.

Immunohistochemistry

Sixty formalin-fixed paraffin-embedded tissue samples were retrospectively analyzed, evaluating the expression of CD3, CD8, CD20, CD4, CD68, CD47, PECAM-1 (CD31), PD1, and PD-L1. Histologic sections (3 μ m thick) underwent staining with specific antibodies, including Anti-CD3, Anti-CD8, Anti-CD20, Anti-CD4, Anti-CD68, Anti-CD47, Anti-CD31, Anti-PD1, and Anti-PD-L1. Staining protocols involved various pre-treatments and dilutions (Table 1), with detection using a micropolymeric non-biotin system, except for PD-L1. Manual scoring by an experienced pathologist assessed the absolute count of immune cells positive for selected markers per 1 mm², starting from “hot spots” within each sample. Our study focused on immune cells and evaluated cytoplasmic and membranous staining. Specifically, PECAM-1 showed membranous staining and PECAM-1-positive endothelial cells were excluded from scoring. Differentiation of PECAM-positive immune cells (specifically intraalveolar macrophages) from endothelial cells was enabled by distinct characteristics of the macrophages, such as their morphology, intra-alveolar location, and lower staining intensity, as shown in Figure 4. The nuclei were counterstained with hematoxylin.

Luminex Assay

Our sample preparation procedures were followed in accordance with the manufacturer's guidelines to ensure accuracy and reproducibility. Specifically, we focused on blood sera derived from 51 patients and analyzed a panel of cytokines and markers, including IFN γ , Granzyme B, IL-2, PD-L1 and TNF α . A customized Luminex Human Magnetic Assay, sourced from Biotechne, R&D Systems s.r.o. in Prague, was used. The assay enabled precise detection of cytokines and chemokines in serum from lung transplant samples. Data were acquired using the Bio-Plex 200 system.

Cryobiopsies

Transbronchial cryobiopsy was the method of lung tissue sample collection, facilitated through flexible bronchoscopy

targeting primarily the left lower lobe when possible. This procedure, conducted under total anesthesia, adhered to standard medical protocols. Cryobiopsies were evaluated according to ISHLT guidelines and scored for acute cellular rejection (ACR) (Grade A) and lymphocytic bronchiolitis (Grade B) [32]. Both tissue samples and peripheral blood were meticulously preserved at a stable temperature of -80°C until the analysis was performed.

Statistical Analyses

Continuous variables were standardly reported as median (interquartile range) and categorical variables as number (percentage). Data were grouped into two main groups – control group (only grade A0 = non-ACR) and rejection group (ACR grade A1-3). Fisher's exact test was used to compare categorical variables between groups. Spearman correlations (ρ) and Mann-Whitney U tests were used to evaluate relations between clinical, IHC and Luminex variables and ACR. Kruskal-Wallis test was used to evaluate relations between all A0-A3 groups (Supplementary Table S1). Values falling below the lower limit of quantification were subjected to a halving procedure in the analytical process.

To evaluate the predictive capacity of IHC markers for graft acceptance or rejection (non-ACR vs. ACR), individual Receiver Operating Characteristic (ROC) curves were constructed for each marker, and the corresponding Area Under the Curve (AUC) was calculated. The Youden's Index and Euclidian distance were computed to find the ideal cut-off values. This part of the analysis was performed by an experienced biostatistician (A.B.).

RESULTS

Study Population and Baseline Characteristics

Immunohistochemical analyses were performed on a cohort of 60 tissue samples obtained from 60 bilateral lung transplant (LuTx) recipients. However, for subsequent Luminex analyses, samples from 9 patients were unavailable. Recipient age was 53 (42–60) years. Among recipients, 40 (67%) were male and 20 (33%) female. Indications for transplantation included chronic obstructive

TABLE 2 | Cohort donor, preservation, and recipient characteristics.

Cohort characteristics	Results
Donor and preservation	
Age at donation, years	44 (31–53)
Sex, n (%)	
Male	32 (53)
Female	28 (47)
Body mass index, kg/m ²	24 (22–26)
CMV status, n (%)	
Positive	39 (65)
Negative	21 (35)
Total ischemic time (longest time of two lungs), min	345 (294–390)
Total ischemic time (mean of two lungs), min	293 (245–330)
Recipient	
Age at transplant, years	53 (43–60)
Sex, n (%)	
Male	40 (67)
Female	20 (33)
Body mass index, kg/m ²	26 (19–28)
Indication for transplant, n (%)	
Chronic obstructive pulmonary disease	19 (32)
Interstitial lung disease	26 (43)
Cystic fibrosis	8 (13)
Pulmonary arterial hypertension	7 (12)
Time spent on waiting-list, days	183 (85–354)
CMV status, n (%)	
Positive	37 (62)
Negative	20 (33)

Data are expressed as median (interquartile range) if not otherwise indicated.

Abbreviation: CMV, cytomegalovirus.

pulmonary disease (COPD) in 19 (32%) patients, interstitial lung disease (ILD) in 26 (43%), cystic fibrosis in 8 (13%) and pulmonary arterial hypertension (PAH) in 7 (12%) patients. Only DBD donors were reported in this cohort. Donor age was 44 (31–53) years. Two (3%) donors were older than 70 and one (2%) was younger than 18. Among donors, 32 (53%) were male, 28 (47%) female. **Tables 2, 3** summarize the baseline characteristics of the study cohort. No

differences in baseline characteristics were observed between control and rejection group. **Supplementary Table S1** presents the distribution of acute cellular rejection grades in the study cohort. Standard induction immunosuppression at our center consists of basiliximab, tacrolimus, mycophenolate, and corticosteroids. For selected patients, an alternative strategy to basiliximab is employed. **Supplementary Table S2** presents the percentage of patients in whom basiliximab and each alternative modality to it was used, either alone or in combination. For maintenance immunosuppression tacrolimus, mycophenolate, and corticosteroids are used. No significant difference was observed when comparing induction immunosuppressive regimens ($p = 0.3341$) and infection status ($p = 0.7191$) between the groups (**Supplementary Tables S2, S3**). A description of underlying immunological conditions is provided in **Supplementary Table S4**.

The Levels of C-Reactive Protein Were Associated With Acute Cellular Rejection

Our study cohort was initially stratified into two subgroups based on the presence or absence of ACR. These subgroups were subsequently compared in relation to differential white blood cell counts (WBC) and acute-phase proteins, specifically C-reactive protein (CRP). **Figure 1** shows scatter plots with median and interquartile range of the measured values. Interestingly, no difference was observed in total WBC count, and percentages and counts of neutrophils, monocytes, lymphocytes, and eosinophils (**Table 4**), suggesting the limited efficacy of basic leukocyte parameters in predicting ACR within this context.

On the other hand, CRP, an acute-phase protein synthesized in the liver due to interleukin-6 secretion by macrophages and T-cells, displayed variations between the observed groups. As shown in **Figure 1**, peripheral blood levels of CRP tended to be lower in the non-ACR group [8.15 (2.4–17.8)] as compared to the ACR group 17.5 (8.45–36.83) $p = 0.055$.

TABLE 3 | Baseline characteristics of control and rejection group.

	no ACR (A0) n = 12	ACR (A1-3) n = 48	p-value
Donor age, years	45 (36–54)	43 (31–50)	0.54
Male donors	8	24	0.35
Female donors	4	24	
Donor BMI, kg/m ²	24 (23–26)	24 (22–27)	1.0
Total ischemic time (longest time of two lungs), min	299 (281–364)	351 (304–396)	0.12
Total ischemic time (mean of two lungs), min	255 (239–314)	297 (256–331)	0.13
Recipient age, years	58 (48–61)	52 (38–59)	0.39
Male recipients	9	31	0.73
Female recipients	3	17	
Recipient BMI, kg/m ²	26 (22–28)	26 (19–27)	0.64
Indication for transplant		—	
Chronic obstructive pulmonary disease	5	14	0.59
Interstitial lung disease	6	20	
Cystic fibrosis	1	7	
Pulmonary arterial hypertension	0	7	

Data are expressed as simple count (categorical variables) or median (range), respectively. Abbreviation: BMI, body mass index.

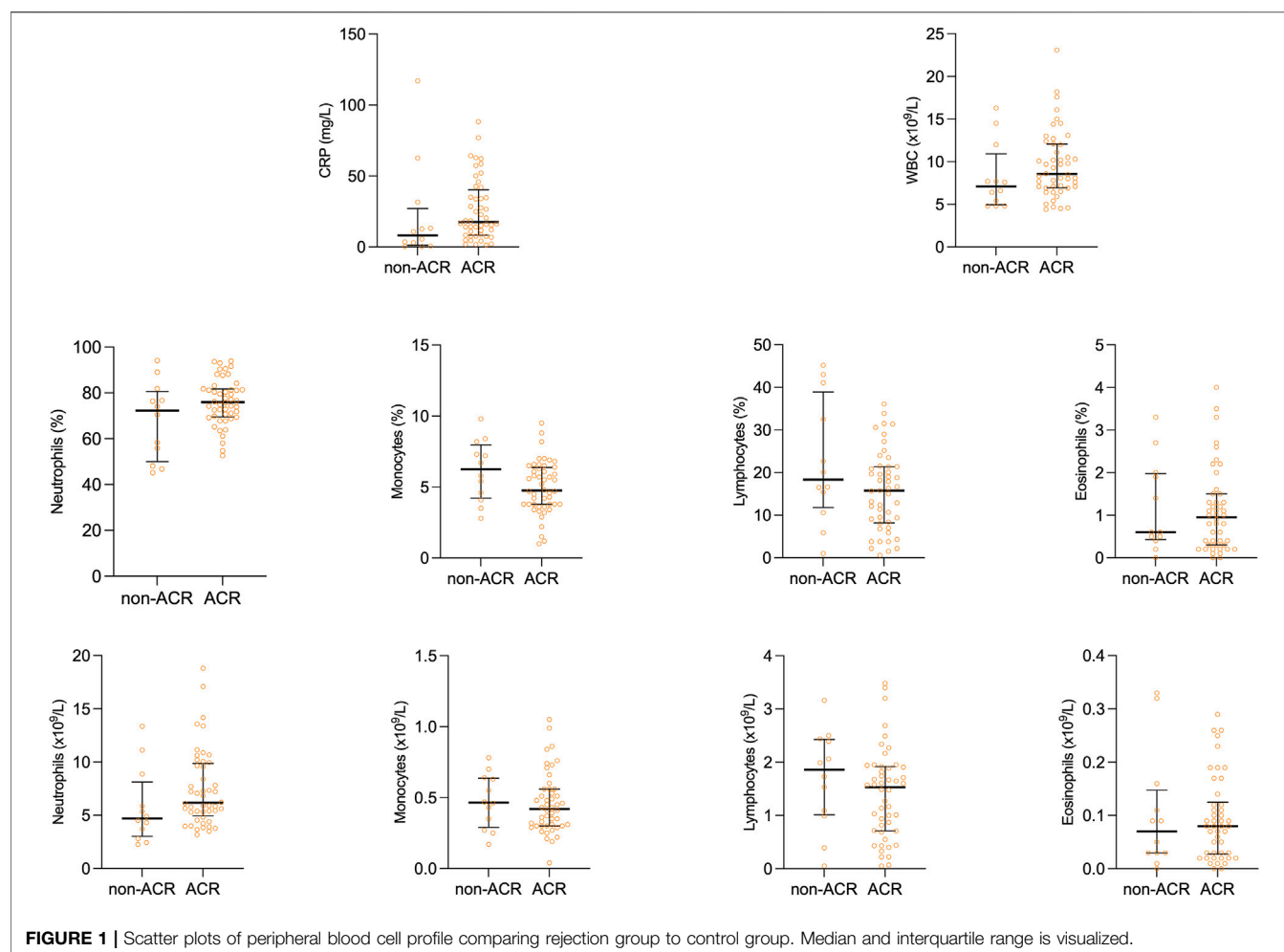


FIGURE 1 | Scatter plots of peripheral blood cell profile comparing rejection group to control group. Median and interquartile range is visualized.

TABLE 4 | Peripheral blood cell profile.

	no ACR (A0) n = 12	ACR (A1-3) n = 48	p-value
CRP (mg/L)	8.15 (2.4–17.8)	17.5 (8.45–36.83)	0.06
WBC count ($\times 10^9/L$)	7.1 (5.25–8.78)	8.55 (7.05–12.03)	0.15
Neutrophils (%)	72.25 (53.95–78)	75.95 (69.70–81.50)	0.19
Neutrophils ($\times 10^9/L$)	4.70 (3.48–6.63)	6.17 (5.17–9.73)	0.07
Monocytes (%)	6.25 (4.48–7.53)	4.75 (3.8–6.33)	0.09
Monocytes ($\times 10^9/L$)	0.46 (0.33–0.63)	0.42 (0.30–0.56)	0.70
Lymphocytes (%)	18.35 (14.20–34.65)	15.75 (8.33–21.4)	0.18
Lymphocytes ($\times 10^9/L$)	1.86 (1.06–2.40)	1.53 (0.72–1.91)	0.25
Eosinophils (%)	0.6 (0.48–1.93)	0.95 (0.3–1.45)	0.83
Eosinophils ($\times 10^9/L$)	0.07 (0.03–0.12)	0.08 (0.03–0.12)	0.97

Data are expressed as median (interquartile range). Abbreviations: CPR, C-reactive protein; WBC, white blood cells.

TABLE 5 | Positive immune cell counts per 1 mm² in lung tissue were determined for specific IHC markers in the study groups, excluding endothelial cells in PECAM-1 from the scoring system.

	no ACR (A0) n = 12	ACR (A1-3) n = 48	p-value
CD3	82 (38.5–97.75)	94.5 (56–136)	0.10
CD4	0 (0–5.25)	0 (0–6)	0.99
CD8	4.5 (0–30.25)	0 (0–19)	0.25
CD20	2 (0–10.25)	2.5 (0–18)	0.98
PD1	14 (6.5–42)	32 (19.75–59.75)	0.12
PD-L1	1.5 (0–5.25)	9.5 (3.25–18)	0.0023
CD68	37.5 (20.25–102.75)	77 (23.75–92.50)	0.61
PECAM-1	36.5 (35.25–43.25)	58 (40.75–84)	0.0131
CD47	275.5 (202–381.5)	338.5 (235.5–418)	0.29

Data are expressed as median (interquartile range). Two samples for PD-L1 were missing in both groups, and one sample for PD-1 was missing in the non-ACR group. Bold values indicate statistical significance at the level of $p < 0.05$.

Neither T-Cell Subsets Nor B-Cells and Macrophages Exhibited Significant Elevation in Patients With Acute Cellular Rejection

In the course of our investigations, our primary objective revolved around elucidating the potential impact of ACR on the

proportions of critical immune cell types (T-cells, B-cells, and macrophages) and demonstrating whether ACR elicits substantial changes in the abundance or distribution of these cell populations. Table 5 outlines the counts of positive immune cells per 1 mm² for selected IHC markers. We hypothesized that examining the specific surface markers, such as CD3, CD4, CD8,

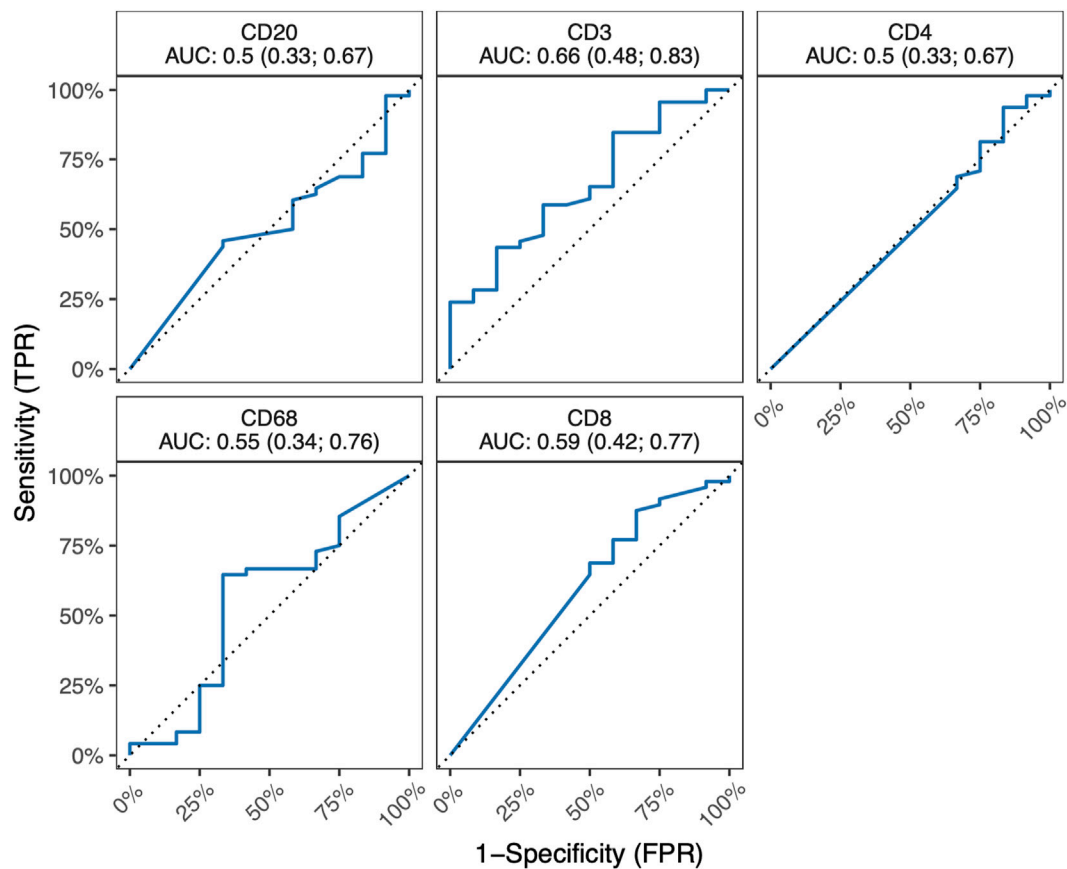


FIGURE 2 | ROC curves for IHC markers CD3, CD4, CD8, CD20 and CD68, along with corresponding AUC values and their 95% confidence interval. All 95% confidence intervals include 0.5, which shows that none of the markers are good predictors.

CD20, and CD68, might offer a viable means of detecting the initial stages of ACR. Unfortunately, these markers did not show any differences between the ACR and non-ACR groups. As depicted in **Figure 2**, neither T-cell subsets nor B-cells and macrophages exhibited significant elevation in patients with ACR.

PD-L1 Is Significantly Increased in Lung Transplant Recipients Exhibiting Acute Cellular Rejection

In the subsequent array of analyses, we examined immune checkpoints in lung tissue samples to understand the balance of activation/inhibition signals transmitted through immune receptors. Our primary focus was on the most prominent immune checkpoint pathway, predominantly occurring in T-cells, which involves the interaction between PD-1 and PD-L1 [33]. Following this, our attention shifted to exploring the novel potent macrophage checkpoint CD47, known as the “don’t eat me” signal [34]. While CD47 displayed no significant variations between the ACR and non-ACR group, striking differences were observed when analyzing the PD-L1 expression within lung allografts.

As shown in **Figure 3A**, PD-L1 exhibited significant increase in the rejection group (PD-L1 $p = 0.0023$), indicating an ongoing attempt to inhibit immune responses, particularly those involving T-cells. On the other hand, while the increase in PD-L1 levels might imply an effort to foster peripheral immune tolerance through its interaction with the PD-1 receptor, there was no observed increase in PD-1 receptor within the ACR cohort when compared to the non-ACR group. **Figure 3B** shows areas under ROC curves, and associated 95% confidence intervals, based on marked values for PD-L1 (0.80 confidence interval [0.65; 0.94]) and PD-1 (0.65 confidence interval [0.44; 0.86]). PD-L1 does not include 0.5 in its 95% confidence interval, therefore it can be a good ACR predictor. **Supplementary Figure S1** shows Youden’s Index and Euclidian distance for PD-L1. **Table 6, 7** show confusion matrices for PD-L1. PD-L1 remained significant ($p = 0.0112$) when analyzed across all ACR A grades collectively, as shown in **Supplementary Table S5**.

In Human Lung Allografts, Leukocytes Exhibit Dynamic Transendothelial Migration

PECAM-1, also known as CD31, plays a crucial role in facilitating the movement of leukocytes across the

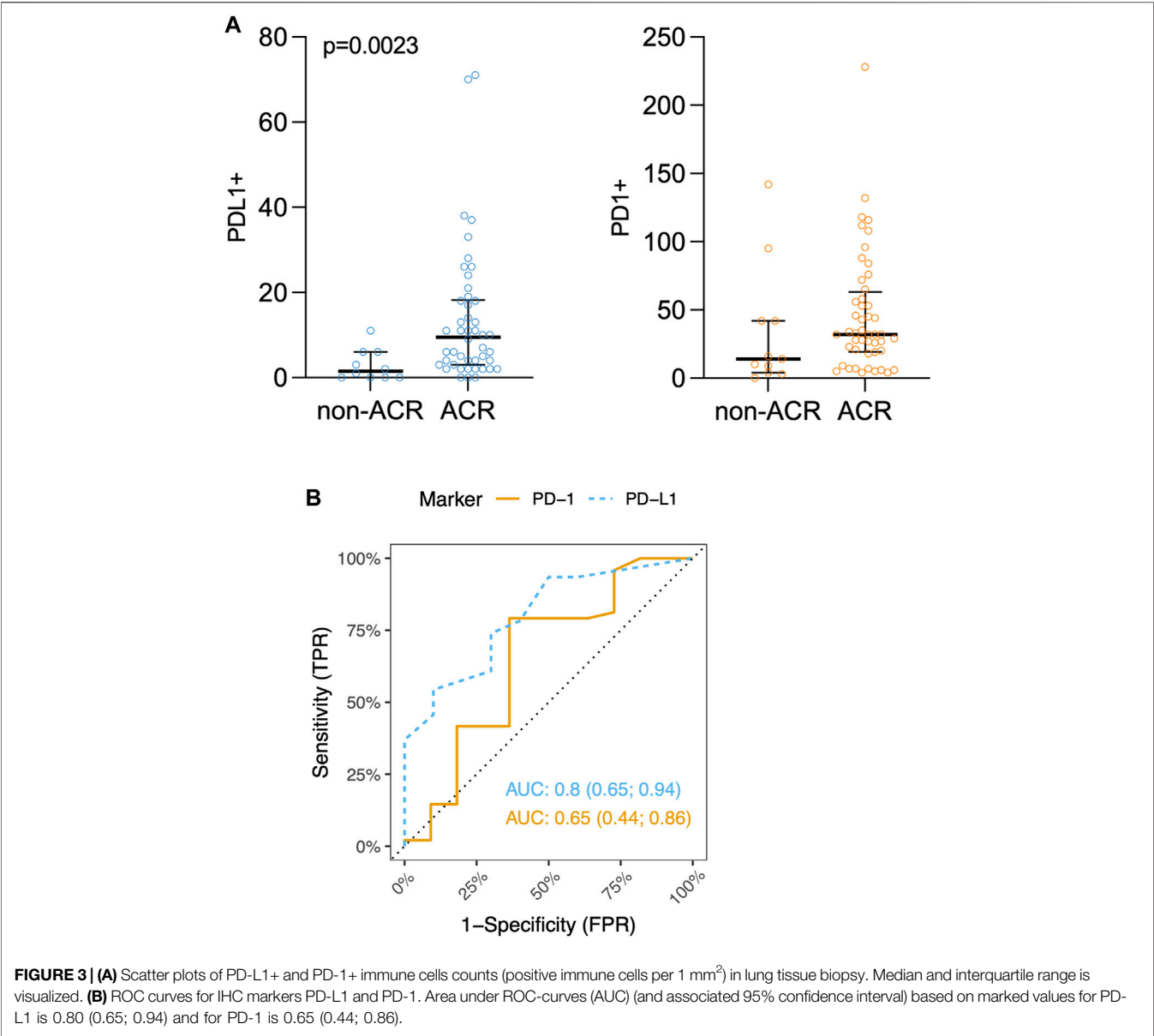


TABLE 6 | Confusion matrix showing Youden's Index for PD-L1 with cut-off value of 7 positive cells/mm².

PD-L1 Actual	Predicted	
	Rejected	Non-rejected
Rejected	25	21
Non-rejected	1	9

intercellular junctions of vascular endothelial cells during the process of leukocyte transmigration [35]. Given the increased scientific interest in anti-PECAM-1 therapies blocking transendothelial migration of leukocytes, our aim was to investigate the potential involvement of PECAM-1 in ACR

TABLE 7 | Confusion matrix showing Euclidian distance for PD-L1 with cut-off value of 4 positive cells/mm².

PD-L1 Actual	Predicted	
	Rejected	Non-rejected
Rejected	34	12
Non-rejected	3	7

of lung allografts. **Figures 4A–C** show the PECAM-1 IHC staining of the samples. Interestingly, PECAM-1 expression, assessed via IHC, demonstrated a trend towards significance ($p = 0.0874$) when analyzed across all ACR A grades collectively (**Supplementary**

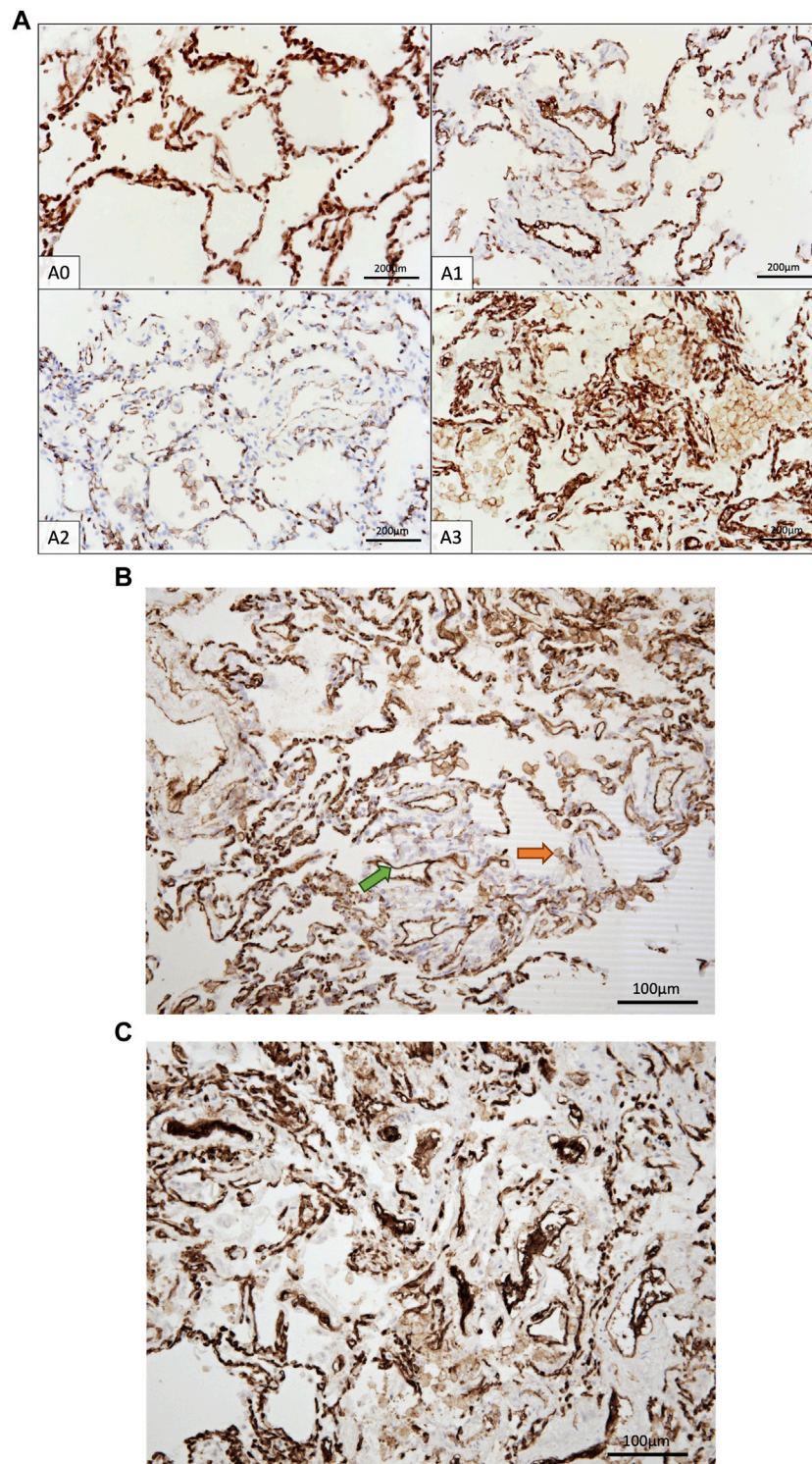


FIGURE 4 | (A) IHC staining of CD31⁺ cells in control group and A1-A3 rejection groups. **(B)** CD31⁺ immune cells (orange arrow) and endothelial cells (green arrow). Only immune cells were counted. **(C)** Endothelium exhibiting CD31 positivity, presumably indicative of endothelial swelling associated with endothelitis, a characteristic frequently observed in A3.

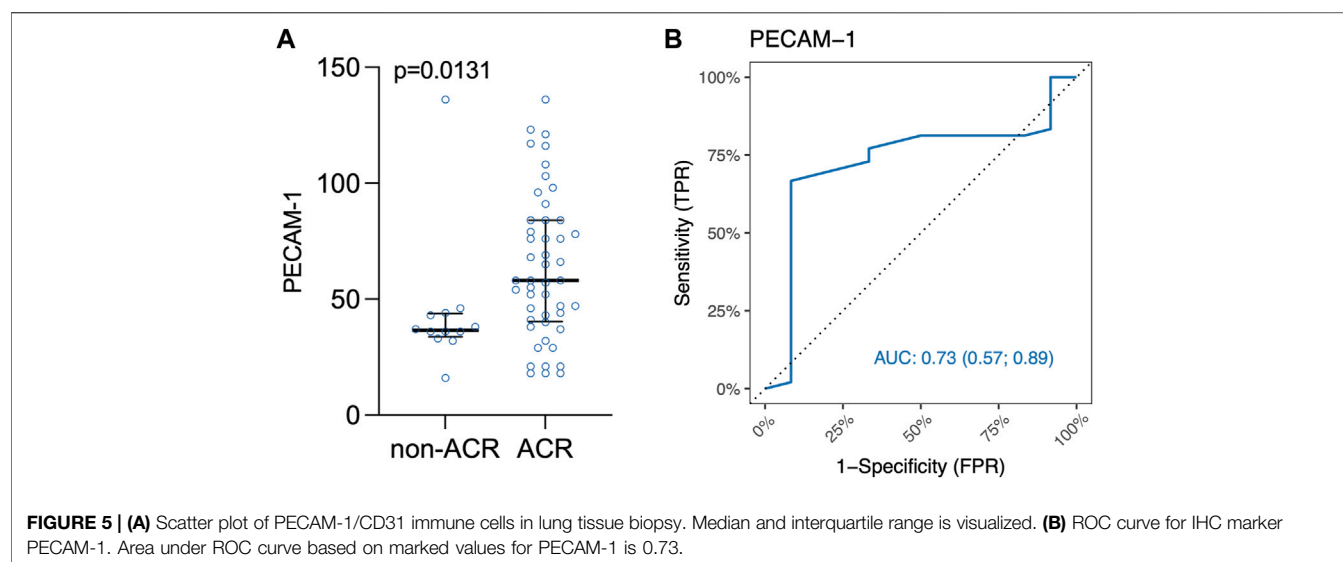


TABLE 8 | Confusion matrix showing Youden's Index and Euclidian distance for PECAM-1/CD31 with cut-off value of 47 positive cells/mm².

PECAM-1		
Actual	Predicted	
	Rejected	Non-rejected
Rejected	32	16
Non-rejected	1	11

Table S5). PECAM-1 was significantly elevated in LuTx patients diagnosed with ACR ($p = 0.0131$) compared to those without ACR (**Figure 5A**). This finding suggests that PECAM-1 may have promising potential as a biomarker for ACR detection. **Figure 5B** shows area under ROC curve based on marked value for PECAM-1 (0.73).

Both Youden's Index and Euclidian distance cut-off point value based on the ranked values for PECAM-1 was 47 positive immune cells/mm² (**Supplementary Figure S2**) indicating a threshold for distinguishing between samples that are positive or negative for PECAM-1 expression. **Table 8** shows confusion matrix for PECAM-1.

T Cell Functional Capacities Were Unaffected in the Rejection Group

To gain a deeper understanding of T cell functionality in LuTx, we conducted a multiplex bead-based immunoassay using Luminex technology on serum samples to evaluate key molecules reflecting T cytotoxic and proliferative function. These molecules included PD-L1, IL-2, Granzyme B, TNF α , and IFN γ .

This multi-faceted approach allowed us to assess the cytotoxic capacities of T cells, and the inflammatory environment in the context of graft survival.

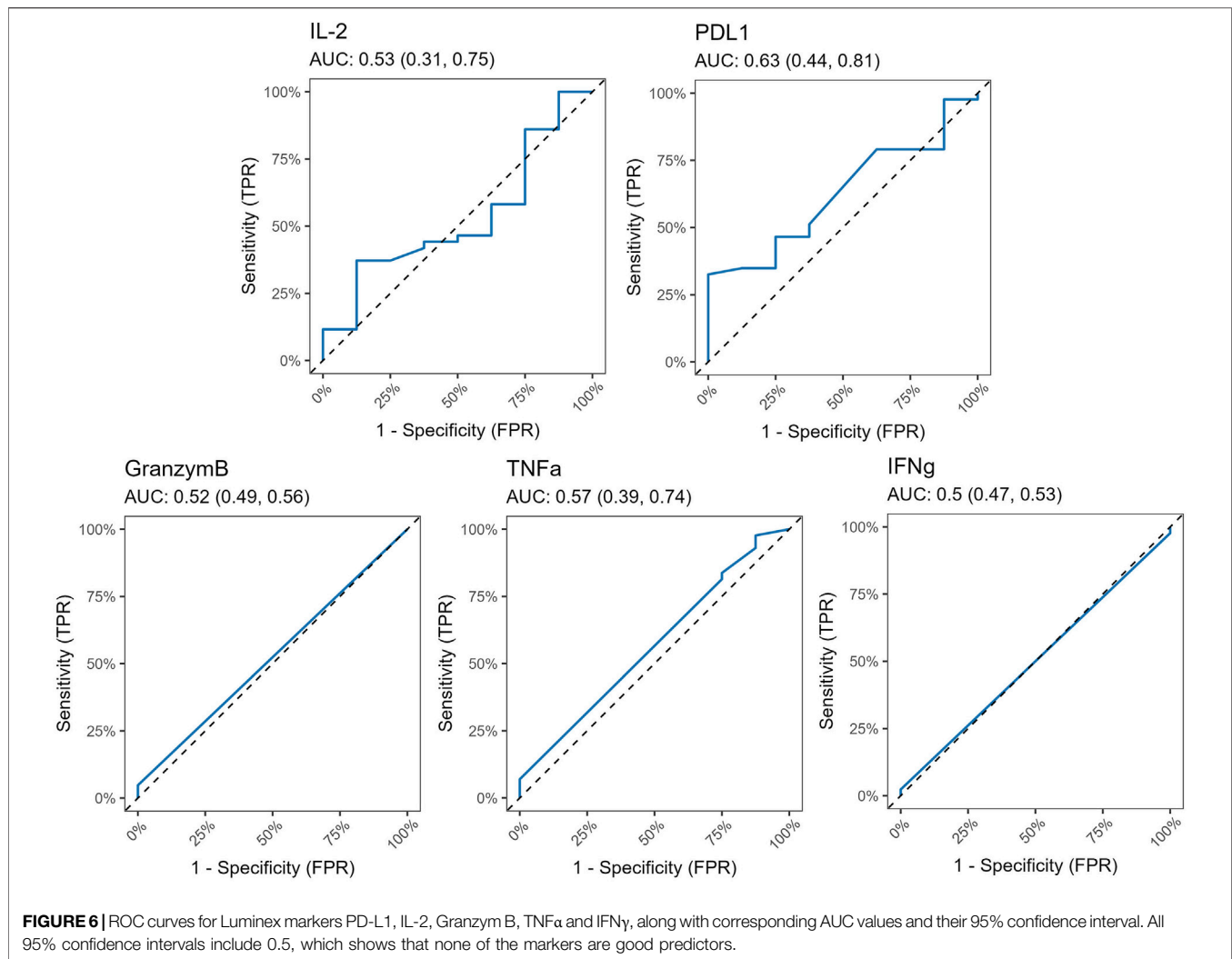
In the rejection group, our analysis revealed an increase in serum levels of IL-2, a cytokine that plays a critical role in T cell

proliferation and immune regulation, alongside a decrease in PD-L1 levels. However, these changes did not reach statistical significance, with p -values of 0.8046 for IL-2 and 0.1224 for PD-L1, respectively. This suggests that while there may be a trend in these biomarkers, the observed variations are not strong enough to draw definitive conclusions about their roles in rejection processes.

Furthermore, no significant differences were detected in the levels of granzyme B, TNF α , and IFN γ , indicating that these immune markers may not be associated with rejection in this study population. **Figure 6** shows areas under ROC curves, and associated 95% confidence intervals, based on marked values for analyzed molecules. All 95% confidence intervals include 0.5, which shows that none of the markers are good predictors.

DISCUSSION

LuTx patients frequently face ACR complications, impacting lung function and contributing to CLAD. Our study analyzed ACR and non-ACR groups, focusing on variations in WBC counts and acute-phase proteins. Since these variables may be affected by immunosuppressive treatment, our study included only patients treated at our center, where the standard maintenance therapy consists of tacrolimus, mycophenolate, and corticosteroids. The induction immunosuppressive regimens varied among individual patients; however, there were no statistically significant differences between the ACR and non-ACR groups. Similar investigations by Vos et al. linked systemic inflammation, CRP levels, and graft failure, aligning with elevated CRP during acute heart rejection observed by Eisenberg et al [36–38]. In our study, despite no significant differences in total WBC count or cell percentages, CRP levels tended to be higher in the ACR group, indicating a potential association. Although the infection status of our patients did not statistically differ between ACR and non-ACR groups, changes in



CRP levels should always be interpreted with caution, as not only accompanying infections, but also stress, inflammatory conditions, and other factors, may influence CRP [39, 40].

Next, we employed IHC to assess specific surface markers, aiming to uncover how ACR might influence the ratios of crucial immune cell (T-cells, B-cells, macrophages). Unfortunately, no significant differences were found between ACR and non-ACR groups.

Upon examining checkpoint molecules, it became apparent that CD47 did not seem a feasible marker for ACR. However, our focus shifted towards exploring the PD-1 and PD-L1 inhibitory pathway. To date, several studies have explored the functions of PD-1 and PD-L1 in transplantation. Wang et al. underscored their vital role in establishing cardiac allograft tolerance in mouse models [41]. Tanaka et al. highlighted PD-L1's pivotal role in both inducing and maintaining peripheral tolerance following heart transplantation by modulating the equilibrium among T-cell subsets [42]. Additionally, Choudhary et al. observed an upregulation of PD-L1 within cardiomyocytes, demonstrating a correlation with the severity of ACR after transplantation [43].

Righi et al., focusing on 24 LuTx patients, revealed the importance of PD-1 in acute rejection and its progression into CLAD. They proposed evaluating PD-1-expressing lymphocytes in transbronchial biopsies for prognostic monitoring [44]. Subsequently, Kaiho et al. investigated PD-1/PD-L1 in acute rejection using a mouse tracheal transplantation model, finding a PD-L1-mediated immune checkpoint association with rejection, suggesting a potential immunotherapy target in LuTx [45].

Our study contributes to the understanding of the involvement of the PD-1/PD-L1 axis in LuTx by demonstrating a significant increase of tissue PD-L1 levels within the ACR group. While it remains uncertain which cells produce PD-L1 in lung allografts *in vivo*, this increase indicates an active effort to suppress immune responses, particularly those associated with T-cells. However, we did not detect a concurrent rise in the PD-1 receptor among the ACR cohort when compared to the non-ACR group.

We hypothesize that this phenomenon may result from PD-L1 production by various non-immune cells within the lung tissue,

such as epithelial and endothelial cells, representing a localized immune suppression effort within the graft, primarily mediated by tissue-specific responses rather than T-cell-mediated modulation [46]. Furthermore, animal transplant models have shown that blocking PD-L1 leads to rejection, while blocking PD-1 and PD-L2 has no effect on graft survival. This indicates that PD-L1 and PD-L2 may play distinct roles in promoting tolerance, with PD-L1 expression, rather than PD-1 expression, emerging as the more reliable marker of immune regulation in transplantation [20].

Thus, the role of the PD-1/PD-L1 mechanism in acute rejection after lung transplantation has not yet been elucidated. These data, in accordance with previous studies, may imply the impairment of peripheral tolerance in LuTx recipients experiencing ACR.

In contemporary oncology, checkpoint molecules have emerged as pivotal targets in the therapeutic landscape, particularly within the realm of cancer treatment. This prominence arises from their capacity to modulate immune responses, a feature notably exploited to counteract the immunosuppressive microenvironment characteristics of malignancies [47]. Conversely, in the context of transplantation, the immune system often experiences heightened activation, resulting in the potential rejection of the transplanted organ. Hence, the contrasting immunological dynamics observed between cancer and transplantation underscore the likelihood of checkpoint molecules assuming a significant role in the latter scenario as well.

Khan et al. showed that the CTLA4, combined with the Fc portion of human immunoglobulin G1 (CTLA4-Ig) used as monotherapy immunosuppressant in mouse airway transplants promoted a favorable phase of immunotolerance, which facilitated microvascular and tissue repair [48].

The TIM family, notably TIM-1 and TIM-3, are pivotal regulators of the immune response and have been investigated in experimental transplant models. Murine studies reveal that inhibiting TIM-1 and boosting TIM-3 signaling enhances allograft outcomes [49]. The consistent findings across acute and chronic rejection models underscore the potential of TIM-3 interaction in mitigating detrimental immune responses [49]. The administration of stable galectin-9 in murine skin and cardiac transplants prolongs allograft survival by decreasing Th1 and Th17 cytokines and fostering Tregs [50–52]. To date, studies evaluating the role of LAG-3 in lung transplantation are lacking.

While there is speculation regarding the therapeutic utility of checkpoint molecules in modulating immune responses to prevent rejection, it is imperative to consider the potential adverse effects associated with such therapies. Of particular concern is the development of autoimmunity, a consequence that is undesirable across various clinical context. Moreover, according to Cui et al., immune checkpoint inhibitors were significantly associated with rejection in solid organ transplant recipients [53]. Therefore, any exploration of checkpoint inhibitor therapy in transplantation must carefully weigh the benefits of immune modulation against the risk of inducing autoimmune phenomena.

PECAM-1 plays a pivotal role in facilitating the migration of leukocytes across intercellular junctions within vascular endothelial cells during the transmigration process [35, 54]. The protective role of PECAM-1 in acute rejection has been demonstrated in various studies, yet its expression has not been previously analyzed by IHC in lung tissue during rejection episodes [55]. In 2022, Tran-Dinh et al. introduced an AI model evaluating CD31 cleavage for early ACR detection post LuTx [55]. We assessed the immunohistochemical surface expression of PECAM-1 in leukocytes from two distinct groups of LuTx recipients: individuals experiencing ACR and those without such complications. To our surprise, LuTx patients diagnosed with ACR showed a significant increase in PECAM-1 expression (IHC), prompting us to hypothesize that inhibiting transendothelial migration might represent a therapeutic approach for ACR.

In oncology, endothelial-immune cell interactions within the tumor microenvironment influence immune infiltration and function, highlighting the critical role of endothelial cells in immune response [56]. There is no reason to believe this would be any different in transplantation. Notably, endothelial cells in the donor lung are among the first to encounter the recipient's immune system.

PECAM-1 is involved in a wide array of processes related to inflammation, vascular biology, and various immune functions [57]. It has several splice variants, each capable of exhibiting distinct adhesive properties, which may subsequently impact its ligand-binding characteristics and functional role in leukocyte transmigration [58, 59]. The functional role of PECAM-1 is influenced by multiple factors, including the nature and tissue localization of the inflammatory response, as well as genetic determinants [57].

PECAM-1 possess both pro- and anti-inflammatory roles. Besides facilitating transendothelial leukocyte migration, it also plays a role in dampening leukocyte activation and reducing pro-inflammatory cytokine production [60]. In the context of ACR, macrophages expressing PECAM-1 may polarize into M2 subset which exhibit anti-inflammatory and graft-protective effects [61]. Thus, high PECAM-1 expression in the graft may reflect immune cell infiltration as well as active repair processes and endothelial resilience.

This study shows the potential of IHC in ACR diagnosis. Despite the additional cost, time, and effort required to perform IHC, its application could be advantageous in borderline cases as a supplementary technique to traditional histopathology. To improve assessments, the ISHLT recommends obtaining at least five adequate samples, reducing variability [32]. This is particularly crucial in cases of ACR, especially when confronting lower-grade rejection. This variability not only presents challenges in individual patient management but also hinders efforts to achieve standardization in multicenter trials [11, 62]. Identifying appropriate IHC markers could help tackle these issues and our data suggests that both PD-L1 and PECAM-1 need further exploration in ACR.

In order to better understand T cell functionality in LuTx, we also employed a multiplex bead-based immunoassay using Luminex technology to assess key molecules that reflect T cell

cytotoxicity and proliferation. The molecules analyzed were PD-L1, IL-2, Granzyme B, TNF α , and IFN γ . This approach aimed to provide insights into the cytotoxic potential of T cells and the surrounding inflammatory environment, which are crucial for improving graft survival, formulating targeted therapies, and enhancing outcomes for transplant patients.

The role of IL-2 in acute lung rejection has been previously reported [63–65]. Luminex analysis in our study cohort did not yield significant results. However, elevated levels of IL-2 in patients with ACR is in line with the older work of Jordan et al. [66]. Our Luminex analyses were constrained by the small size of the study cohort. To this we also attribute the inconclusive results of other biomarkers investigated by the Luminex method. Future studies with larger sample sizes and the inclusion of more relevant biomarkers, such as PECAM-1, could provide more insightful findings.

In our center, cryobiopsies are the standard of care. The Zurich group has demonstrated that cryobiopsies offer a superior diagnostic yield for ACR compared to forceps biopsies, leading to reclassification and treatment strategy changes in 28.6% of cases [67]. Our findings show that nearly half of the samples in this cohort exhibit an A1 rejection grade. Notably, identifying A1 rejection in a clinically stable patient through biopsy may not necessitate therapeutic intervention.

This study adopts a retrospective design, encompassing solely double lung transplant patients with samples available in archives in a single high-volume transplant center. Observational design, limited cohort size and group size imbalances are notable. However, the cohorts, aside from size differences, exhibit consistent characteristics. Our selection might indeed introduce bias and therefore, a prospective study would be imperative to also ascertain an accurate representation of the prevalence within our patient cohort. Despite widespread use of IHC, interpretational variability also remains a challenge.

Conducting larger studies is essential for evaluating immunohistochemistry (IHC), with a specific focus on PD-L1/PECAM-1 markers, in the diagnosis of ACR.

CONCLUSION

IHC investigations of PECAM-1 and PD-L1 markers might be valuable for diagnosing ACR. Further research is required to enhance our understanding of the role of immune checkpoint inhibitors in lung transplantation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by the Ethics Committee of Motol University Hospital (EK-530/21). The

studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conceptualization: RN and ZOS; Methodology: RN, ZOS, JB, JT, VT, JS, AB, and MB; Formal analysis and investigation: RN, JB, JT, and ZOS; Data acquisition: RN, JB, VT, AS, KV, JS, and ZOS; Statistical analysis: AB, MB, and RN; Writing - original draft preparation: RN and ZOS; Writing - review and editing: RN, JB, JT, VT, AS, KV, JS, AB, JaV, MB, BV, RL, JiV, LC, and ZOS; Funding acquisition: RL, ZOS, and LC; Resources: RL, ZOS, and LC; Supervision: ZOS and LC. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2024.13796/full#supplementary-material>

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4.2. Prezervace v kontrolované hypotermii umožňuje prodloužení studené ischemie dárcovských plic bez negativního dopadu na krátkodobé výsledky

4.2.1. Prodloužená doba ischemie (>15 hodin) dárcovských plic při prezervaci v kontrolované hypotermii: multicentrická zkušenost

Novysedlak R, Provoost AL, Langer NB, Van Slambrouck J, Barbarossa A, Cenik I, Van Raemdonck D, Vos R, Vanaudenaerde BM, Rabi SA, Keller BC, Svorcova M, Ozaniak Strizova Z, Vachtenheim J Jr, Lischke R, Ceulemans LJ. Extended ischemic time (>15 hours) using controlled hypothermic storage in lung transplantation: A multicenter experience. J Heart Lung Transplant. 2024 Jun;43(6):999-1004. doi: 10.1016/j.healun.2024.02.006. Epub 2024 Feb 13. PMID: 38360161.

Tato retrospektivní práce vznikla ve spolupráci s transplantačními centry světového významu - UZ Leuven/KU Leuven v Belgii a Massachusetts General Hospital/Harvard Medical School v Bostonu, USA. Analyzovali jsme výsledky 13 dospělých pacientů, u kterých byla transplantace provedena po studené ischemii v podmínkách CHS delší než 15 hodin pro druhou implantovanou plíci. Sledované parametry kromě jiných zahrnovaly výskyt PGD 3 během prvních 72 hodin, použití ECMO po operaci, dobu pooperační umělé plicní ventilace, délku pobytu na JIP, celkovou délku hospitalizace a výskyt akutní buněčné rejekce (ACR) v prvním měsíci.

Dárcovské plíce byly uchovávány v přenosném CHS zařízení při teplotě 7 (5–9,3) °C. Indikací bylo překlenutí noci a/nebo transport na dlouhou vzdálenost. Medián doby ischemie

druhé implantované plíce činil 17,3 hodin. Nejdelší studená ischemie v této kohortě o velikosti 14 pacientů přesáhla 22 hodin. U 4 z 13 pacientů jsme pozorovali PGD 3 během prvních 72 hodin, avšak u žádného pacienta v čase 72 hodin. Medián trvání umělé plicní ventilace po transplantaci představoval 29 hodin. Pobyt na JIP trval 9 dní a hospitalizace 30 dní (obojí medián). Čtyři pacienti potřebovali pooperační ECMO. Jeden pacient zemřel 7. den po výkonu v důsledku technické chyby při zavádění ECMO kanyly.

Předkládaná práce je jednou z prvních publikací v historii transplantací plic popisujících takto dlouhé časy ischemie s příznivými krátkodobými výsledky. Implementace CHS do klinické praxe se pojí s mnohými benefity, z kterých nejvýraznější je možnost transformace transplantace plic jako akutního výkonu na výkon poloakutní. To neznamena jen možnost posunu do denních hodin, ale také získání časového prostoru k provedení nezbytných imunologických křížových zkoušek, přípravě příjemce nebo transportování plic urgentnímu příjemce na vzdálenosti dříve nepředstavitelné.

BRIEF COMMUNICATION

Extended ischemic time (> 15 hours) using controlled hypothermic storage in lung transplantation: A multicenter experience



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KEYWORDS:

controlled hypothermic storage;
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lung transplantation;
ischemic time;
primary graft dysfunction

Static ice storage has long been the standard-of-care for lung preservation, although freezing injury limits ischemic time (IT). Controlled hypothermic storage (CHS) at elevated temperature could safely extend IT. This retrospective analysis assesses feasibility and safety of CHS with IT > 15 hours. Three lung transplant (LuTx) centers (April-October 2023) included demographics, storage details, IT, and short-term outcome from 13 LuTx recipients (8 male, 59 years old). Donor lungs were preserved in a portable CHS device at 7 (5-9.3)°C. Indication was overnight bridging and/or long-distance transport. IT of second-implanted lung was 17.3 (15.1-22) hours. LuTx were successful, 4/13 exhibited primary graft dysfunction grade 3 within 72 hours and 0/13 at 72 hours. Post-LuTx mechanical ventilation was 29 (7-442) hours. Intensive care unit stay was 9 (5-28) and hospital stay 30 (16-90) days. Four patients needed postoperative extracorporeal membrane oxygenation (ECMO). One patient died (day 7) following malpositioning of an ECMO cannula. This multicenter experience demonstrates the possibility of safely extending IT > 15 hours by CHS.

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The standard-of-care in donor lung preservation is static ice storage (SIS) in a *camping box*, with a recommended maximum cold ischemic time (IT) of 8 to 10 hours to mitigate the risk of primary graft dysfunction (PGD).^{1,2} However, SIS may result in an organ's temperature drop to 0°C. This increases the freezing injury and limits IT. Recent evidence suggests that controlled hypothermic storage (CHS) at an increased temperature (6-10°C) better protects the organ quality and mitochondrial health and introduces time flexibility without compromising outcome.^{3,4} Clinical data on systematically extending IT (≥15 hours) with CHS are lacking. Our study investigates the feasibility and safety of extended lung preservation and transplantation using CHS, analyzing short-term outcomes.

Methods

Retrospective multicenter cohort analysis was performed at University Hospitals Leuven (Belgium), Motol University Hospital (Prague, Czech Republic), and Massachusetts General Hospital (Boston, USA). Adult patients (≥18-years-old) who underwent bilateral lung transplantation (LuTx) (April-October, 2023) with total IT (flush-reperfusion) of the second-implanted lung > 15 hours were reported. This cut-off best reflects the concept of overnight bridging. The follow-up was October 31, 2023. Donor lungs were stored in the LUNGguard (Paragonix, Boston, USA) (Figure S1). Table S1 outlines differences in procurement and implantation protocols between centers.

Data were extracted from patient files. Approval from local institutional review boards was granted. Recipients provided written consent for the publication of medical data. The study is compliant with the International Society for Heart and Lung Transplantation Ethics statement. Parameters were selected based on prior studies.^{5,6}

Donor/preservation variables included: age, sex, duration of intensive care unit hospitalization, donor type (donation after brain death, donation after circulatory death), last donor PaO₂/FiO₂ (P/F, FiO₂ = 100%), CHS indication, donor-recipient center distance, average storage temperature, ex-vivo lung perfusion (EVLP) parameters, preservation time, and total IT.

Recipient variables were age, sex, transplant indication, waiting-list time, high-urgency status, preoperative ventilation and extracorporeal membrane oxygenation (ECMO), surgical approach, intraoperative ECMO indication, blood products, and implantation time.

Primary end-points were IT of second lung > 15 hours, PGD3 within and at 72 hours, postoperative ECMO use, time of postoperative ventilation, length of intensive care unit/hospital stay, and patient survival at final follow-up. Secondary end-points were Clavien-Dindo, complications during hospital stay and 30 days after discharge and acute cellular rejection (ACR) at the first month.

PGD was assessed by X-ray and partial oxygen pressure divided by the fraction of inspired oxygen in arterial blood gases (PaO₂/FiO₂) at 0-24-48-72 hours post-transplant. Grade 3 was assigned when the X-ray revealed pulmonary edema with a PaO₂/FiO₂ < 200 or when the combination of ECMO with bilateral pulmonary edema on X-ray occurred. Two physicians evaluated X-rays, blinded to outcome. ACR diagnosis relied on transbronchial biopsy samples evaluated by an experienced pathologist.

Continuous variables were reported as median (minimum-maximum) and categorical variables as number (percentage). GraphPad-Prism10 was used for analyses and visualization.

Results

In the study period, 113 LuTx were performed (Leuven *n* = 43, Prague *n* = 40, Boston *n* = 30), of which 13 (12%) (8 male, 59 years) were preserved by CHS device with a total IT > 15 hours of the second lung.

Table 1 summarizes donor/preservation data. Donor-recipient center distance reached 138 (15-5,420) km. In the long-distance case, the lungs were first stored with CHS (602 minutes), which was then interrupted by EVLP (221 minutes) followed by CHS (256 minutes). Storage temperature was 7 (5-9.3)°C.

Table 2 describes recipient pre-/intraoperative characteristics. Three (23%) were high urgent. No patient was bridged to transplant. In 8 (62%) transplants, intraoperative ECMO was used. Five (38%) as routine protocol, one (8%)

Table 1 Donor and Lung Preservation Characteristics

No	Donor age (years)	Donor sex	Donor ICU hospitalization (days)	Donor type	Last donor P/F ratio	LUNGguard Indication	Average temperature (°C)	Preservation time of second lung (minutes)		Total ischemic time of first lung (minutes)	Total ischemic time of second lung (minutes)
								first lung	of second lung		
1	54	F	2	DCD-III	486	Overnight bridging	6.1	762	938	953	1,135
2	68	F	1	DBD	448	Overnight bridging	6.3	855	1,103	1,063	1,322
3	59	F	5	DCD-III	376	Overnight bridging	8.8	735	920	838	1,038
4	54	M	9	DCD-IV	264	Overnight bridging	9.3	732	860	899	1,011
5	71	F	4	DBD	471	Overnight bridging	7.0	520	718	723	923
6	46	M	2	DBD	321	Overnight bridging	7.6	760	944	941	1,133
7	49	M	4	DBD	463	Overnight bridging	6.4	832	1,019	1,000	1,185
8	75	F	3	DBD	513	Overnight bridging	5.0	851	968	1,009	1,203
9	45	M	1	DBD	545	Overnight bridging	9.0	815	950	985	1,108
10	65	F	2	DBD	327	Overnight bridging	6.9	705	815	840	948
11	48	M	3	DCD-III	427	Overnight bridging	6.4	739	835	877	952
12	28	M	4	DBD	479	Long-distance transport (5,420 km)	N/A	^a 858	(CHS 1 - 602, EVLP - 221, CHS 2 - 256)	897	972
13	59	F	1	DBD	363	Overnight bridging	9.0	650	758	799	904
MV	54	-	3	-	448	-	7.0	760	920	899	1,038

Abbreviations: CHS, controlled hypothermic storage; DBD, donation after brain death; DCD-III, donation after cardiac death (Maastricht classification III); DCD-IV, donation after cardiac death (Maastricht classification IV); EVLP, ex-vivo lung perfusion; F, female; ICU, intensive care unit; P/F, PaO2/FiO2 (O2 100%); M, male; MV, median value; N/A, not available.

Bold values indicate highest observed preservation and ischemic times in our cohort.

^aSame LUNGguard for CHS 1 and CHS 2. For EVLP, the XVIVO System (XPS) was used.

Table 2 Lung Transplant Recipient Intraoperative Characteristics

No	Rec. age (years)	Rec. sex	Indication for LuTx	Surgical approach	Implantation time (first, minutes)	Implantation time (second, minutes)	Intraoperative				
							ECMO	ECMO indication	RBC (units)	FFP (units)	Platelets (units)
1	66	F	COPD	BT	73	81	No	N/A	2	1	0
2	53	F	PAH	BT	96	113	VA central	Small chest cavity with limited exposure	1	0	0
3	64	F	COPD	BT	72	58	No	N/A	2	0	0
4	54	M	ILD	BT	51	39	No	N/A	0	0	0
5	64	F	COPD	BT	88	86	VV peripheral	First (right) lung edema	3	0	0
6	63	M	COPD	BT	57	57	No	N/A	0	0	0
7	22	M	PAH	BT	68	64	VA peripheral	Cardiac arrest	0	4	1
8	59	F	COPD	BT	48	71	No	N/A	0	0	0
9	61	M	COPD	CST	80	68	VA central	Routine use	2	2	0
10	49	M	ILD	CST	67	65	VA central	Routine use	0	0	0
11	47	M	COPD	CST	59	38	VA central	Routine use	0	2	0
12	58	M	COPD	CST	39	72	VA central	Routine use	8	3	1
13	68	M	ILD	CST	68	65	VA central	Routine use	0	0	0
MV	59	-	-	-	68	65	-	-	0	0	0

Abbreviations: BT, bilateral thoracotomy; COPD, chronic obstructive pulmonary disease; CRP - C-reactive protein; CST, clamshell thoracotomy; ECMO, extracorporeal life support; F, female; FFP, fresh frozen plasma; ILD, interstitial lung disease; LuTx, lung transplantation; M, male; MV, median value; N/A, not applicable; RBC, red blood cells; Rec., recipient; VA, veno-arterial; VV, veno-venous.

for PAH, one (8%) for hemodynamic collapse during anesthesia-induction with successful cardiopulmonary resuscitation, and one (8%) due to first-lung syndrome.

Table 3 shows short-term outcome. Postoperative ECMO was used in 4 (31%) patients, two (15%) due to lung edema, and two (15%) because of non-hypoxic cardiac reasons. One (8%) patient required tracheostomy. Four (31%) cases of PGD3 within 72 hours were documented. No PGD3 at 72 hours was reported (Figure S2). After 1 month, 11 (85%) patients underwent transbronchial biopsy, revealing ACR A2 in two (15%), and airway inflammation B1R in one (8%). One (8%) patient died on day 7 following a malpositioning of a peripheral ECMO cannula resulting in hemodynamic collapse and brain death. All other (92%) patients are alive at follow-up.

Discussion

This initial report from experienced LuTx centers shifts the established paradigm on lung preservation time. Our findings highlight CHS as a safe and feasible method for IT extension beyond 15 hours, although this cohort captures several high-risk recipients and extended criteria donors.

Previously, extended preservation was only possible with EVLP.⁷ However, based on our clinical observations, long EVLP may induce lung edema and come with substantial costs regarding finances and human resources.

Already 30 years ago, animal research showed favorable graft reperfusion physiology when lungs were preserved at controlled temperatures close to 10°C.^{8,9} In 2021, the Toronto group re-initiated this idea and showed the first successful clinical cases after 10°C preservation for 12.1 hours (second lung).³ A clinical trial comparing 10°C (transport in SIS) versus SIS, with safe overnight bridging as objective, showed 5.7% of the 10°C group patients developed PGD3 at 72 hours, versus 9.3% in the SIS group.⁴

The GUARDIAN-Lung Registry collects clinical data on LuTx following SIS versus CHS using LUNGguard. The report on 86 CHS versus 90 SIS LuTx-cases at the 2023 International Society for Heart and Lung Transplantation meeting showed a 54% reduction in PGD3 at 72 hours and higher 1-year survival. Total IT in CHS group exhibited a mean of 7.4 hours.¹⁰

CHS has the potential to increase the donor pool and overcome time pressure without compromising the outcome. This facilitates comprehensive immunological cross-match tests, allows long-distance transportation, and turns LuTx in a semielective procedure. Improvement in work-life balance of transplant teams could enhance attractiveness to the field.

However, data on clinical outcome after CHS are still limited. Before the adoption of CHS as the new gold standard, data of a large randomized clinical trial (lead by Toronto) on SIS versus CHS (allowing overnight bridging) (NCT05898776) are awaited as well as an update of the propensity-matched analysis of the GUARDIAN registry

Table 3 Recipient Short-term Outcomes

No	Postoperative ventilation (hours)	PGD 0 hour	PGD 24 hours	PGD 48 hours	PGD 72 hours	ICU stay (days)	Hospital stay (days)	C-D score ^a	Complications until 30 days after hospital discharge	Follow-up (days)
1	48	0	0	0	0	9	30	3b	Pleural effusion, atrial fibrillation	197
2	108	UG	UG	UG	0	16	32	4a	Atrial fibrillation, acute kidney injury, delirium	179
3	N/A	3	3	3	2	7 (dead POD7)	7 (dead POD7)	5	Retropertoneal bleeding due to ECMO cannula malposition, hemodynamic collapse, severe encephalopathy, cerebral edema, brain death	7 (died)
4	29	3	2	0	0	8	16	3b	Unilateral hemothorax, atrial fibrillation, pericarditis	75
5	86	3	3	3	0	15	45	3a	Bilateral pleural effusion, delirium	46
6	22	0	0	1	0	5	30	3a	Unilateral pneumothorax	34
7	64	UG	UG	UG	0	14	^b	4a	Pericardial effusion, failure of bronchial anastomosis with pneumomediastinum, bilateral wound dehiscence	28
8	20	3	2	2	2	8	^b	2	Pericarditis, atrial fibrillation	22
9	7	0	0	0	0	11	34	3b	Repeated pneumothorax	118
10	17	0	0	0	0	7	20	2	None	54
11	21	0	0	0	0	8	19	2	None	48
12	442	0	0	2	2	28	90	4b	Stroke, acute kidney injury	208
13	41	0	0	0	0	^b	^b	3b	Atrial fibrillation, left lower lobe atelectasis due to mucus plug	10
MV	29	0	0	0	0	9	30	-	-	48

Abbreviations: C-D, Clavien-Dindo; ICU, intensive care unit; MV, median value; N/A, not applicable; PGD, primary graft dysfunction; POD, postoperative day; UG, ungradable.

^aDuring post-transplant hospitalization.^bOngoing hospitalization on the follow-up day.

(1,000 patients). Additional research is required to identify the optimal temperature and maximal time of preservation.

Conclusion

CHS with IT > 15 hours appears to be safe, with favorable short-term outcomes. This highlights potential of CHS to facilitate LuTx logistics and expand the donor pool.

CRedit authorship contribution statement

Conceptualization: RN, ALP, NBL, RL, LJC. Data curation: RN, ALP, NBL, BCK, MS, LJC. Formal analysis: RN, LJC. Methodology: RN, ALP, NBL, JVS, AB, IC, DVR, RV, BMV, SAR, BCK, MS, ZOS, JV Jr, RL, LJC. Supervision: LJC. Visualization: RN, LJC. Writing - original draft: RN, LJC. Writing - review & editing: ALP, NBL, JVS, IC, DVR, RV, BMV, BCK, ZOS, JV Jr. All authors read and approved the final manuscript.

Disclosure statement

In relation to this manuscript, we disclose that Paragonix granted 5 LUNGguard devices to the authors to test feasibility. No financial compensation was granted.

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Supplementary data

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.healun.2024.02.006](https://doi.org/10.1016/j.healun.2024.02.006).

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4.2.2. Transplantace plic po prezervaci v kontrolované hypotermii pomocí přenosného zařízení: první multicentrická evropská zkušenost.

Provoost AL, Novysedlak R, Van Raemdonck D, Van Slambrouck J, Prisciandaro E, Vandervelde CM, Barbarossa A, Jin X, Denaux K, De Leyn P, Van Veer H, Depypere L, Jansen Y, Pirenne J, Neyrinck A, Bouneb S, Ingels C, Jacobs B, Godinas L, De Sadeleer L, Vos R, Svorcova M, Vajter J, Kolarik J, Tavandzis J, Havlin J, Ozaniak Strizova Z, Pozniak J, Simonek J, Vachtenheim J Jr, Lischke R, Ceulemans LJ. Lung transplantation following controlled hypothermic storage with a portable lung preservation device: first multicenter European experience. Front Cardiovasc Med. 2024 Jun 6;11:1370543. doi: 10.3389/fcvm.2024.1370543. PMID: 38903974; PMCID: PMC11187339.

Tato publikace popisuje implementaci přenosného CHS zařízení během 14měsíčního období ve dvou velkoobjemových evropských centrech - FN Motol a UZ Leuven. Studie pomocí deskriptivní statistiky retrospektivně hodnotí výsledky 36 pacientů po transplantaci plic, u kterých byla k prezervaci dárcovských plic zvolená kontrolovaná hypotermie (3,7 - 9,3°C). Indikace pro použití CHS zahrnovaly překlenutí noci (26 případů; 72,2 %), vzdálený odběr (4 případy; 11,1 %), urgentní alokaci (2 případy; 5,6 %), logistické důvody (2 případy; 5,6 %), překlenutí doby potřebné k ověření transplantability příjemce (1 případ; 2,8 %) a marginálního dárce (1 případ; 2,8 %). Konkrétně šlo o dárce vysokého věku (94 let). I přes poměrně rizikový profil dárců a příjemců byla těžká forma PGD během prvních 72 hodin zaznamenána u 12 (33,3%) pacientů a v čas 72 hodin jen u jednoho (2,8%) pacienta. Hospitalizace na JIP trvala 8 dní, zatímco celková délka pobytu v nemocnici po výkonu 28 dní (obojí medián).



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Lung transplantation following controlled hypothermic storage with a portable lung preservation device: first multicenter European experience

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Introduction: Compared with traditional static ice storage, controlled hypothermic storage (CHS) at 4–10°C may attenuate cold-induced lung injury between procurement and implantation. In this study, we describe the first European lung transplant (LTx) experience with a portable CHS device.

Abbreviations

BMI, body mass index; BREATHE, Laboratory of Respiratory Diseases and Thoracic Surgery; CF, cystic fibrosis; CHS, controlled hypothermic storage; CLAD-BOS, chronic lung allograft dysfunction bronchiolitis obliterans syndrome; COPD, chronic obstructive pulmonary disease; DBD, donation after brain death; DCD, donation after circulatory death; ECMO, extracorporeal membrane oxygenation; FiO₂, fraction of inspired oxygen; GUARDIAN-LUNG, Global Utilization And Registry Database for Improved preservaTion of doNor LUNGS; HU, high-urgency; ICU, intensive care unit; ILD, interstitial lung disease; IQR, interquartile range; ISHLT, International Society for Heart and Lung Transplantation; LTx, lung transplantation; NA, not applicable; PaO₂, partial oxygen pressure; PGD, primary graft dysfunction; PGD3, primary graft dysfunction grade 3; POD, postoperative day; PVD, pulmonary vascular disease; RCT, randomized controlled trial; SIS, static ice storage; UG, ungradable; VA, venoarterial; VV, venovenous.

Methods: A prospective observational study was conducted of all consecutively performed LTx following CHS (11 November 2022 and 31 January 2024) at two European high-volume centers. The LUNGguard device was used for CHS. The preservation details, total ischemic time, and early postoperative outcomes are described. The data are presented as median (range: minimum–maximum) values.

Results: A total of 36 patients underwent LTx (i.e., 33 bilateral, 2 single LTx, and 1 lobar). The median age was 61 (15–68) years; 58% of the patients were male; 28% of the transplantations had high-urgency status; and 22% were indicated as donation after circulatory death. In 47% of the patients, extracorporeal membrane oxygenation (ECMO) was used for perioperative support. The indications for using the CHS device were overnight bridging ($n = 26$), remote procurement ($n = 4$), rescue allocation ($n = 2$), logistics ($n = 2$), feasibility ($n = 1$), and extended-criteria donor ($n = 1$). The CHS temperature was 6.5°C (3.7°C–9.3°C). The preservation times were 11 h 18 (2 h 42–17 h 9) and 13 h 40 (4 h 5–19 h 36) for the first and second implanted lungs, respectively, whereas the total ischemic times were 13 h 38 (4 h 51–19 h 44) and 15 h 41 (5 h 54–22 h 48), respectively. The primary graft dysfunction grade 3 (PGD3) incidence rates were 33.3% within 72 h and 2.8% at 72 h. Intensive care unit stay was 8 (4–62) days, and the hospital stay was 28 (13–87) days. At the last follow-up [139 (7–446) days], three patients were still hospitalized. One patient died on postoperative day 7 due to ECMO failure. In-hospital Clavien–Dindo complications of 3b were observed in six (17%) patients, and 4a in seven (19%).

Conclusion: CHS seems safe and feasible despite the high-risk recipient and donor profiles, as well as extended preservation times. PGD3 at 72 h was observed in 2.8% of the patients. This technology could postpone LTx to daytime working hours. Larger cohorts and longer-term outcomes are required to confirm these observations.

KEYWORDS

controlled hypothermic storage, lung preservation, overnight bridging, preservation temperature, preservation time, primary graft dysfunction, total ischemic time

1 Introduction

Optimal donor lung preservation is a critical determinant of successful lung transplantation (LTx). For decades, static ice storage (SIS) has been the standard for organ preservation. However, the vulnerability of lung tissue to cold-induced injury caused by near-freezing temperatures may increase the risk of severe primary graft dysfunction (PGD) (1–3). Moreover, preservation on ice is limited to a maximum of 8 h. Therefore, SIS constrains the donor pool and frequently necessitates LTx to be performed overnight under suboptimal working conditions with limited staff. The flaws of SIS have fueled a renewed interest in optimizing donor lung preservation to further improve outcomes after LTx (3–10). Pioneering animal research in the 1990s, followed by the first recent clinical application in Toronto, showed that controlled hypothermic storage (CHS) at 10°C better preserves mitochondrial integrity (3, 4, 6, 8, 11–14). This approach effectively maintains tissue metabolism and mitigates lung injury during the interval between procurement and implantation. Furthermore, CHS enables the prolongation of preservation times, facilitating a shift to planned semi-elective transplant procedures and expanding the donor pool by increasing geographic reach and optimizing donor–recipient matching.

The portable device for CHS (LUNGguard™) maintains the temperature between 4 and 8°C (15). The system was used for the first time in North America in February 2021 (at Duke University Hospital, North Carolina, USA), and introduced in Europe on 11 November 2022 (with the first European LTx performed using the device at the University Hospitals Leuven, Belgium) (16, 17). No manuscripts on the clinical outcome have been published, to our knowledge. The aim of this study is to provide a descriptive cohort analysis of the first European experience on lung CHS with this device by reporting the perioperative characteristics and short-term outcome.

2 Methods

2.1 Study design

A prospective observational study of all consecutive cases that underwent LTx between 11 November 2022 and 31 January 2024 was conducted at two European high-volume centers: (1) the University Hospitals Leuven (Leuven, Belgium) and (2) the Motol University Hospital (Prague, Czech Republic). The sole inclusion criterion was lung preservation with the CHS device, and there were no exclusion criteria. Data were collected from written and electronic patient files, as well as donor data prospectively

collected by Eurotransplant. The study was approved by the research Ethics Committees of Leuven (S67697) and Prague (EK387/23). Written informed consent was obtained from each patient. The follow-ups were reported until 31 January 2024.

2.2 Controlled hypothermic storage

All lungs were stored in a portable CHS device (LUNGguard) developed by Paragonix Technologies (Waltham, MA, USA). The SherpaCool phase-changing technology enables CHS by maintaining preservation temperatures at 4–8°C for 40 h. The system received the Food and Drug Administration (FDA) clearance in the United States, and the CE (for Conformité Européenne or European Conformity) mark in Europe. A smartphone application connected to a logger and thermometer in the CHS device permits remote continuous real-time monitoring of location and preservation temperature (15).

Routinely, during procurement the lungs were flushed in an antegrade fashion: in Leuven, 4 L OCS™ lung solution (TransMedics, Inc., Andover, MA, USA) was used, whereas in Prague, 6 L Perfadex™ (XVIVO AB, Göteborg, Sweden). After procurement the lungs were split at the donor center and additionally flushed in a retrograde fashion with 0.5–1 L per lung. A maximum of 250 g of ice was used on the bench table, and in Leuven the donor lung surface temperature was controlled by infrared thermal camera prior to storage. Next, the lungs were packed separately in three plastic bags as in our standard approach, and stored in the CHS device: the first bag included the organ itself immersed in 1 L of preservation solution, the second bag was filled with 1 L saline and the first bag, and the third bag contained the first two bags without additional solution. The preservation solution and saline were stored in a fridge at 6°C at the recipient center, and were placed in the CHS device for transport to the donor center, and only removed from the CHS device just prior to its utilization. Finally, after packing, the lungs were stored simultaneously in the CHS device, and the storage temperature was measured continuously through the built-in thermometer of the device.

The clinical protocol for overnight bridging gradually changed with growing experience. At first, lung preservation was only extended with the CHS device for cases with expected cold-flush after 10:00 PM and with recipient anesthesia at 7:30 AM. Eventually, the window of extended preservation was prolonged to cases with expected cold-flush after 6:00 PM.

2.3 Recipient, donor, and procedural variables

The recipient characteristics included the recipient center (Leuven, Prague), sex (male, female), age, body mass index (BMI), indication for LTx [chronic lung allograft dysfunction bronchiolitis obliterans syndrome (CLAD-BOS), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), interstitial lung disease (ILD), pulmonary vascular disease (PVD)], time on waiting list, high-urgency (HU) listing, duration

of preoperative hospitalization, and need for preoperative extracorporeal membrane oxygenation (ECMO).

Donor characteristics were sex (male, female), age, BMI, type of donation [donation after brain death (DBD), donation after circulatory death (DCD)], cause of brain injury (cardiac arrest, cerebral ischemia, intracerebral hemorrhage, status epilepticus, suicide, trauma), intensive care unit (ICU) stay, partial arterial oxygen pressure over the fraction of inspired oxygen [partial oxygen pressure (PaO₂)/fraction of inspired oxygen (FiO₂)], Oto-score for secretions (0 = none, 1 = minor, 2 = moderate, 3 = major), and Oto-score for chest x-ray findings (0 = clear, 1 = minor, 2 = opacity 1 ≤ lobe, 3 = opacity > 1 lobe) (18).

Lung preservation variables included indication for CHS, preservation temperature, preservation time for the first and second implanted lung, distance between donor and recipient center, and mode of transport. Preservation time was defined as the interval between the moment the lungs were inserted and removed from the CHS device.

The surgical variables were type of LTx (single, bilateral, lobar), surgical approach (anterolateral thoracotomy, clamshell), need and indication for intraoperative ECMO, blood product transfusion (packed cells, fresh frozen plasma, platelets), total ischemic time of the first and second implanted lung, and surgical time. The total ischemic time was defined as the interval between cardiac arrest for DCD, or cross-clamp for DBD, until lung reperfusion in the recipient, hence including both cold and warm ischemic times. Surgical time was defined as the time from the initial incision to final skin closure.

The postoperative outcomes were as follows: need for ECMO, time on the ventilator, extubation status (successful first extubation, reintubation, tracheostomy, death before extubation), PGD at 0/24/48/72 h after LTx, Clavien–Dindo score, ICU stay, and hospital stay. PGD was based on the International Society for Heart and Lung Transplantation (ISHLT) consensus definition and was assessed by pulmonary edema on chest x-ray and PaO₂/FiO₂ at 0/24/48/72 h post-LTx (19). PGD grade 3 (PGD3) was assigned when the chest x-ray revealed pulmonary edema with a PaO₂/FiO₂ <200 or when the combination of ECMO with bilateral pulmonary edema on chest x-ray occurred. Data on arterial blood gases were acquired by automated extraction of electronic patient files. The chest x-rays were evaluated retrospectively by two experienced physicians. Postoperative surgical complications were graded according to the Clavien–Dindo classification (20). The longer-term outcomes included follow-up and patient survival.

2.4 Lung transplant procedure and immunosuppression protocol

In Leuven, a routine LTx is performed via bilateral anterolateral thoracotomy in a sequential single LTx fashion, with selective use of ECMO to anticipate and overcome hemodynamic and respiratory instabilities (hypercapnia, PGD, pulmonary hypertension, ventilatory limitations). In Prague, the surgical approach involves a clamshell thoracotomy with sequential single LTx, and protocol use of intraoperative central venoarterial (VA) ECMO. In case of a

single LTx, in both centers a unilateral anterolateral thoracotomy is performed without ECMO.

Immunosuppression consisted of triple therapy with tacrolimus, mycophenolate mofetil, and steroids. The induction immunosuppression used was rabbit antithymocyte globulin (Leuven) or basiliximab (Prague).

2.5 Statistical analysis

Descriptive statistical analyses were performed using Microsoft 365 Excel (Windows). The graphs were plotted with GraphPad Prism10 (San Diego, CA, USA). The continuous variables were summarized as median (range: minimum–maximum) values, and the categorical variables as observed frequencies and percentages.

3 Results

3.1 Recipient and donor characteristics

A total of 160 patients underwent LTx in Leuven ($n = 85$; 53.1%) or Prague ($n = 75$; 46.9%). CHS storage was carried out in 36 LTx cases: 24 (66.7%) in Leuven and 12 (33.3%) in Prague. Most of the patients were men ($n = 21$; 58.3%) aged 61 (15–68) years. The BMI was 26.2 (13.5–29.9) kg/m². Indications for LTx were as follows: 17 (47.2%) COPD, 14 (38.9%) ILD, 3 (8.3%) PVD, 1 (2.8%) CF, and 1 (2.8%) CLAD-BOS after LTx. Time on the waiting list was 97.5 (1–826) days. A total of 10 (27.8%) patients were transplanted in a HU setting, following pretransplant hospitalization of 9.5 (2–106) days, with 3 (8.3%) preoperative venovenous (VV) ECMO.

The donor population was predominantly female ($n = 20$; 55.6%), aged 56 (29–94) years, with a BMI of 25.6 (18.0–34.9) kg/m². There were 28 DBD (77.8%) and 8 (22.2%) DCD procedures, of which 7 were DCD class 3 (DCD-III) and 1 DCD-IV. The causes of death were varied, with 19 (52.8%) patients dying because of intracerebral hemorrhage, 6 (16.7%) trauma [head injury ($n = 1$) and falling ($n = 5$)], 5 (13.9%) cardiac arrests, 3 (8.3%) cerebral ischemia, 2 (5.6%) suicide [drug intoxication ($n = 1$) and gunshot ($n = 1$)], and 1 (2.8%) status epilepticus. Preoperative ICU stay was 3 (1–12) days. PaO₂/FiO₂ was 413.5 (264–545). Oto-score for secretions was 1 and chest x-ray findings 0, indicating minor secretions and absence of opacities, respectively. The recipient and donor characteristics are presented in [Table 1](#) and the [Supplementary Material](#).

3.2 Controlled hypothermic storage

Indications for using the CHS device were overnight bridging ($n = 26$; 72.2%), remote procurement ($n = 4$; 11.1%), rescue allocation ($n = 2$; 5.6%), logistics ($n = 2$; 5.6%), feasibility ($n = 1$; 2.8%), and extended-criteria donor ($n = 1$; 2.8%) (advanced donor age: 94 years). Preservation temperature was 6.5°C (3.7°C–

9.3°C), and CHS preservation time was 11 h 18 (02 h 42–17 h 09) and 13 h 40 (04 h 05–19 h 36) for the first and second implanted lungs, respectively. The distance between donor and recipient center was 148 (0–980) km, with air transport in 10 (27.8%) cases. The details on lung preservation are summarized in [Table 2](#).

3.3 Surgical variables

Most recipients underwent a full-size bilateral LTx ($n = 33$; 91.7%), while two (5.6%) a single LTx, and one (2.8%) a lobar bilateral LTx (pediatric CF). Bilateral anterolateral thoracotomy was the surgical approach in 25 (69.4%) patients, clamshell in 9 (25%), and a unilateral anterolateral thoracotomy in 2 (5.6%). In 18 (50%) patients intraoperative ECMO was used, of which 1 (2.8%) was due to reperfusion edema of the first implanted lung. Altogether 19 (52.8%) patients required blood products intraoperatively [0.5 (0–20) units]. Total ischemic time was 13 h 38 (04 h 51–19 h 44) and 15 h 41 (05 h 54–22 h 48) for the first and second implanted lungs, respectively. The surgical time was 07 h 00 (02 h 57–13 h 19). The surgical variables are listed in [Table 2](#) and the [Supplementary Material](#).

3.4 Short-term outcomes

Five (13.9%) patients required ECMO postoperatively of which two (5.6%) for suboptimal oxygenation and ventilation caused by lung edema, and three (8.3%) for non-hypoxic reasons. One (2.8%) patient was switched from VV ECMO to VA ECMO due to cardiogenic shock. She died at POD7 after withdrawal of supportive therapy because of irreversible ischemic-hypoxic encephalopathy. Postoperative time on ventilator was 25.5 (6–526) h. Two (5.6%) patients required tracheostomy due to failure from weaning. Within and at 72 h PGD3 was present in 12 (33.3%) patients and one (2.8%) patient, respectively ([Figure 1A](#)). ICU stay was 8 (4–62) days, while hospital stay was 28 (13–87) days. During hospitalization, six (17%) patients suffered from a Clavien–Dindo 3b scoring, and seven (19%) from a 4a classification ([Figure 1B](#)). Follow-up was 139 (7–446) days. At the final date of follow-up, three (8.3%) patients were still hospitalized, and patient survival was 97.2% ($n = 35$). The postoperative outcomes are outlined in [Table 3](#) and the [Supplementary Material](#).

4 Discussion

This first European experience with the portable LUNGuard shows safe and good short-term outcome for preservation through CHS, and the possibility of converting the transplant procedure to a diurnal activity. The median preservation temperature was 6.5°C. The main indication for using CHS was overnight bridging ($n = 26$; 72.2%). Although maximum

TABLE 1 Recipient and donor characteristics.

	Recipient					Donor				
	Sex (M/F)	Age (years)	Indication for LTx	Time on waiting list (days)	High urgency	Sex (M/F)	Age (years)	Type of donation	ICU stay (days)	PaO2/FiO2
1	M	55	ILD	121	Yes	M	44	DCD-III	12	457
2	F	15	CF	77	No	M	37	DBD	4	374
3	M	66	COPD	515	No	M	35	DCD-III	6	326
4	M	63	ILD	277	Yes	F	63	DBD	2	420
5	F	66	COPD	826	No	F	54	DCD-III	2	486
6	F	53	PVD ^a	500	Yes	F	68	DBD	1	448
7	M	66	ILD	56	No	M	44	DBD	3	361
8	F	64	COPD	651	No	F	59	DCD-III	5	376
9	M	54	ILD	297	No	M	54	DCD-IV	9	264
10	F	64	ILD	235	No	F	74	DBD	4	339
11	F	62	COPD	57	No	F	56	DBD	2	340
12	M	61	COPD	460	No	M	94	DCD-III	8	440
13	F	64	COPD	261	No	F	71	DBD	4	471
14	M	63	COPD	20	No	M	46	DBD	2	321
15	M	22	PVD ^{ab}	27	No	M	49	DBD	4	463
16	F	59	COPD	507	No	F	75	DBD	3	513
17	F	63	COPD	161	No	F	30	DBD	2	442
18	F	52	ILD	18	No	F	87	DBD	3	486
19	F	61	COPD	168	No	F	70	DBD	2	276
20	M	59	PVD ^{ac}	2	Yes	M	69	DBD	7	345
21	F	64	COPD	7	No	F	67	DBD	2	396
22	M	48	ILD	6	Yes	M	60	DBD	3	391
23	M	53	ILD	11	Yes	M	68	DBD	1	515
24	M	55	COPD	320	No	M	71	DCD-III	8	421
25	M	56	ILD	227	No	F	56	DBD	4	435
26	M	61	COPD	9	No	M	45	DBD	1	545
27	M	68	ILD	483	No	F	35	DBD	1	407
28	M	49	ILD	1	Yes	F	65	DBD	2	327
29	M	65	COPD	88	No	M	29	DBD	3	377
30	M	47	COPD	107	Yes	M	48	DCD-III	3	427
31	M	68	ILD	26	Yes	F	59	DBD	1	363
32	F	56	ILD	113	No	F	66	DBD	5	465
33	F	60	CLAD-BOS	48	Yes	F	44	DBD	2	390
34	F	65	COPD	72	No	F	36	DBD	7	431
35	M	57	ILD	3	No	F	54	DBD	4	510
36	M	67	COPD	60	No	M	40	DBD	12	337
MV	—	61	—	97.5	—	—	56	—	3	413.5

CF, cystic fibrosis; CLAD-BOS, chronic lung allograft dysfunction bronchiolitis obliterans syndrome; COPD, chronic obstructive pulmonary disease; DBD, donation after brain death; DCD, donation after circulatory death; F, female; FiO2, fraction of inspired oxygen; ICU, intensive care unit; ILD, interstitial lung disease; LTx, lung transplantation; M, male; MV, median value; PaO2, partial oxygen pressure; PVD, pulmonary vascular disease.

^aChronic thromboembolic pulmonary hypertension.

^bPulmonary capillary hemangiomatosis.

^cEnd-stage sarcoidosis with secondary pulmonary hypertension.

preservation (19 h 36) and total ischemic times (22 h 48) importantly exceeded the current limits of 8 and 10 h, respectively, PGD3 incidence at 72 h was 2.8%.

The hypothesis suggesting CHS is associated with improved post-reperfusion outcome compared with SIS was first proposed by the group of Joel Cooper (Toronto) between 1989 and 1993, based on animal research on donor lung preservation temperature (11–14, 21–23). The conclusion of this preclinical research was that the optimal lung preservation temperature was around 8–10°C, allowing the option of extended preservation up to 24 h (11, 21). Three decades later, in 2021, there was a reinstated interest in preservation temperature by Ali et al. and

Cypel et al. from the Toronto group, with a first clinical evaluation of five patients receiving LTx after a CHS at 10°C (4). The purpose was overnight bridging and starting the LTx procedure in the morning. Preservation time was 10 h 24 (09 h 55–14 h 48) and 12 h 06 (10 h 54–16 h 30) for the first and second implanted lung, respectively. There was no PGD3 at 72 h, median time on the ventilator was 2 (0–7) days, median hospital stay was 17 (14–26) days, and 30-day survival was 100%. Based on these promising results, the research group led by Cypel set up a prospective non-randomized clinical trial assessing the extension of the static donor lung preservation at 10°C (*n* = 70) vs. SIS (*n* = 140) (9). The lungs were procured and transported in

TABLE 2 Controlled hypothermic storage device and lung transplantation characteristics.

	Controlled hypothermic storage device			Lung transplantation details					
	Indication for CHS device	Preservation temperature (°C)	Preservation time CHS device first/second lung (h)	Type of LTx	Surgical approach	Intraoperative ECMO	Blood products (units)	Total ischemic time first/second lung (h)	Surgical time (h)
1	Feasibility	6.9	04 h 30/06 h 44	BLTx	BAT	No	0	07 h 31/09 h 17	07 h 27
2	Overnight bridging	5.9	07 h 56/11 h 20	Lobar BLTx	BAT	No	4	11 h 26/14 h 29	08 h 25
3	Overnight bridging	5.8	09 h 13/12 h 05	BLTx	BAT	Yes	6	11 h 35/14 h 40	07 h 20
4	Remote procurement	4.0	07 h 46/10 h 06	BLTx	BAT	Yes	20	10 h 42/13 h 12	11 h 01
5	Overnight bridging	6.1	12 h 42/15 h 38	BLTx	BAT	No	3	15 h 53/18 h 55	06 h 36
6	Overnight bridging	6.3	14 h 15/18 h 23	BLTx	BAT	Yes	1	17 h 43/22 h 02	12 h 12
7	Rescue allocation	6.4	06 h 03/NA	Single LTx	UAT	No	0	09 h 11/NA	03 h 23
8	Overnight bridging	8.8	12 h 15/15 h 20	BLTx	BAT	No	2	13 h 58/17 h 18	13 h 19
9	Overnight bridging	9.3	12 h 12/14 h 20	BLTx	BAT	No	0	14 h 59/16 h 51	05 h 39
10	Overnight bridging	5.7	07 h 43/09 h 07	BLTx	BAT	No	0	09 h 41/10 h 59	04 h 43
11	Remote procurement	8.9	04 h 36/06 h 32	BLTx	BAT	No	0	06 h 59/08 h 39	04 h 31
12	Extended-criteria donor ^a	6.9	02 h 42/04 h 20	BLTx	BAT	No	1	05 h 05/06 h 52	05 h 07
13	Overnight bridging	7.0	08 h 40/11 h 58	BLTx	BAT	Yes	3	12 h 03/15 h 23	08 h 35
14	Overnight bridging	7.6	12 h 40/15 h 44	BLTx	BAT	No	0	15 h 41/18 h 53	07 h 10
15	Overnight bridging	6.4	13 h 52/16 h 59	BLTx	BAT	Yes	5	16 h 40/19 h 45	07 h 39
16	Overnight bridging	5.0	14 h 11/16 h 08	BLTx	BAT	No	0	16 h 49/20 h 03	07 h 04
17	Logistics	9.0	04 h 14/05 h 38	BLTx	BAT	No	0	06 h 32/08 h 50	05 h 00
18	Overnight bridging	4.2	15 h 59/17 h 36	BLTx	BAT	No	0	18 h 05/19 h 48	06 h 09
19	Overnight bridging	5.4	13 h 14/16 h 57	BLTx	BAT	No	1	16 h 11/20 h 15	09 h 48
20	Overnight bridging	8.7	11 h 42/16 h 42	BLTx	BAT	No	0	15 h 27/20 h 24	12 h 29
21	Overnight bridging	8.6	13 h 27/15 h 01	BLTx	BAT	Yes	0	15 h 40/17 h 55	05 h 27
22	Overnight bridging	6.6	17 h 09/19 h 36	BLTx	BAT	Yes	14	19 h 44/22 h 48	08 h 21
23	Overnight bridging	6.5	11 h 43/15 h 47	BLTx	BAT	No	6	15 h 05/18 h 59	09 h 01
24	Overnight bridging	5.5	15 h 40/18 h 02	BLTx	BAT	No	0	18 h 03/21 h 16	07 h 23
25	Overnight bridging	8.1	10 h 30/12 h 32	BLTx	Clamshell	Yes	0	12 h 35/14 h 32	06 h 30
26	Overnight bridging	9.0	13 h 35/15 h 50	BLTx	Clamshell	Yes	4	16 h 25/18 h 28	08 h 11
27	Rescue allocation	5.5	10 h 50/NA	Single LTx	UAT	No	0	13 h 32/NA	02 h 57
28	Overnight bridging	6.9	11 h 45/13 h 35	BLTx	Clamshell	Yes	0	14 h 00/15 h 48	07 h 00
29	Remote procurement	3.7	03 h 07/04 h 05	BLTx	Clamshell	Yes	2	04 h 51/05 h 54	05 h 05
30	Overnight bridging	6.4	12 h 19/13 h 55	BLTx	Clamshell	Yes	2	14 h 37/15 h 52	04 h 02
31	Overnight bridging	9.0	10 h 50/12 h 38	BLTx	Clamshell	Yes	0	13 h 19/15 h 04	06 h 40
32	Overnight bridging	5.6	09 h 49/11 h 26	BLTx	Clamshell	No	0	11 h 50/13 h 45	05 h 55
33	Remote procurement	5.9	11 h 45/13 h 45	BLTx	Clamshell	Yes	20	13 h 44/15 h 35	08 h 00
34	Logistics	7.6	10 h 34/12 h 27	BLTx	Clamshell	Yes	0	13 h 14/14 h 16	05 h 20
35	Overnight bridging	6.7	08 h 32/11 h 50	BLTx	BAT	Yes	2	11 h 38/14 h 00	07 h 00
36	Overnight bridging	6.0	10 h 55/12 h 27	BLTx	BAT	Yes	2	12 h 53/14 h 37	05 h 45
MV	—	6.5	11 h 18/13 h 40	—	—	—	0.5	13 h 38/15 h 41	07 h 00

BAT, bilateral anterolateral thoracotomy; BLTx, bilateral lung transplantation; CHS, controlled hypothermic storage; ECMO, extracorporeal membrane oxygenation; LTx, lung transplantation; MV, median value; NA, not applicable; UAT, unilateral anterolateral thoracotomy.

^a94-year-old male donor.

an ice cooler for 03 h 30 [interquartile range (IQR), 02 h 18–04 h 09], and after arrival in the recipient center, they were preserved in a 10°C temperature-controlled incubator for 07 h 48 (IQR, 05 h 46–09 h 37) until implantation. Preservation time (including lung implantation in this study) was 12 h 28 (IQR, 10 h 14–14 h 12) and 14 h 09 (IQR, 12 h 03–15 h 45) for the first and second implanted lungs, respectively. PGD3 incidence at 72 h was 5.7% vs. 9.3%, and 1-year patient survival was 94% vs. 87%, for the CHS vs. SIS groups, respectively. Minor differences were observed in ICU stay (5 vs. 5 days), hospital stay (25 vs. 30 days), and need for postoperative ECMO (5.7% vs. 9.3%).

In February 2022, the commercially portable CHS device LUNGguard was first implemented in North America. Specifically for this device, the clinical non-randomized post-market registry study “Global Utilization And Registry Database for Improved preservAtion of doNor LUNGS’ (GUARDIAN-LUNG) (NCT04930289) was started, with the objective of comparing the outcomes after LTx by CHS vs. SIS (24, 25). Preliminary registry data about the North American experience was presented at ISHLT 2023, enrolling 86 LUNGguard and 90 SIS patients. The median preservation temperature was 4.9°C, total ischemic time was 7 h 26 ± 1 h 51, and PGD3 incidence at 72 h was 8.1% (7/86).

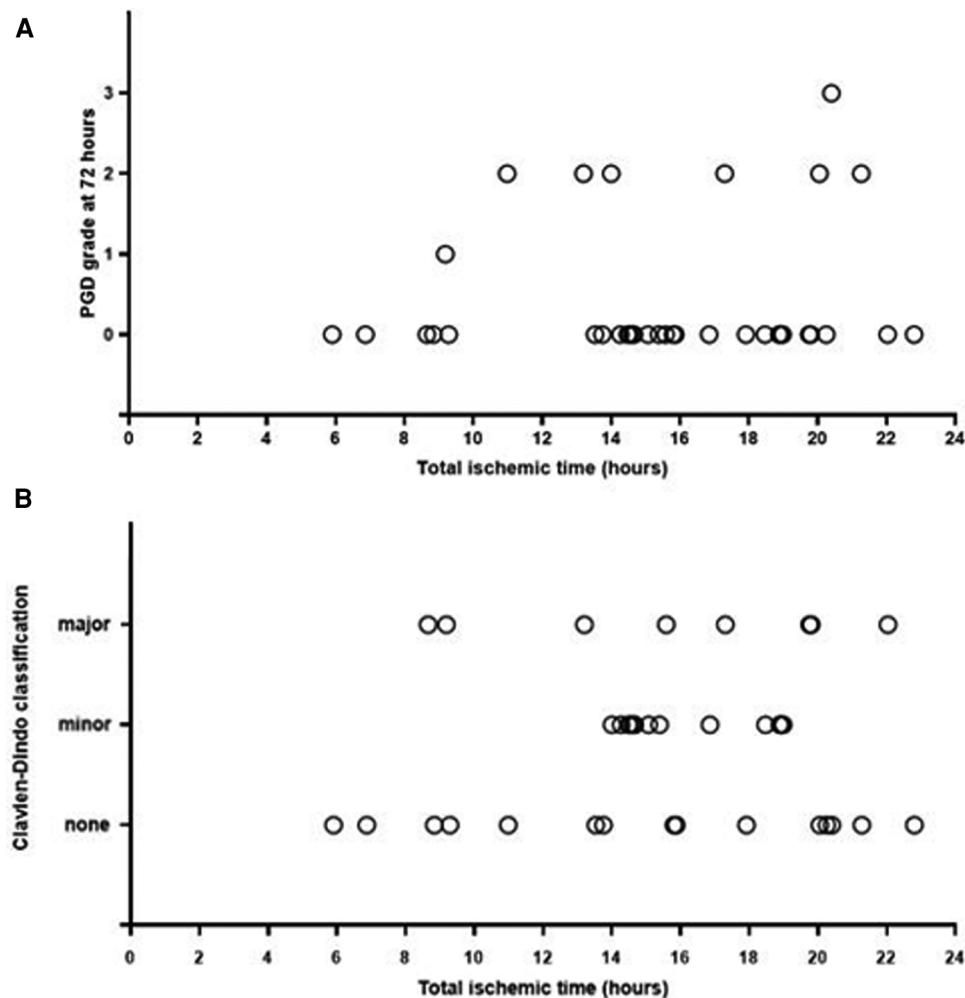


FIGURE 1

(A) Longest total ischemic time (h) and PGD grade (0–1–2–3) at 72 h. (B) Longest total ischemic time (hours) and Clavien–Dindo classification (none: grade 1–2, minor: grade 3a–3b, major: grade 4a–4b–5).

The CHS cohort had a clinically relevant 54% reduction in PGD3 incidence at 72 h ($p=0.058$) compared with SIS, and was also associated with significantly improved 1-year estimated patient survival [CHS 92.7% vs. SIS 82.2% ($p=0.02$)] (26).

Our manuscript describes the implementation of this CHS device over a 14-month period in two European centers. It is worth mentioning that the preservation temperature in our cohort was higher compared with the GUARDIAN-LUNG (6.5°C vs. 4.9°C). Both Leuven and Prague attempt to reach higher temperatures, based on the favorable outcome of lungs preserved at 10°C (4, 8, 9). Therefore, we adopted a strategy in which we use maximum 250 g of ice on the bench table, and target a donor lung surface temperature between 8°C and 12°C prior to storage in the CHS device. We observed that the starting surface temperature of the donor lung directs the average preservation temperature afterward: starting temperatures >10°C vs. 8–10°C vs. <8°C were associated with average preservation temperatures above 8°C vs. 6–8°C vs. 4–6°C, respectively. The duration of

preservation also influences the temperature curve, which changed during storage, finding equilibrium around 6°C.

Another major difference from the GUARDIAN-LUNG analysis concerns total ischemic time. Compared with the registry with total ischemic time of 7 h 26 ± 1 h 51, we report considerably longer total ischemic times with 15 h 41 (05 h 54–22 h 48) for the second implanted lung. In 52.8% ($n=19$) of the patients, total ischemic times exceeded 15 h, with a maximum of 22 h 48. Nevertheless, PGD3 at 72 h was only 2.8% in our series. The promising findings of this first European experience with extended preservation and total ischemic times have encouraged Leuven and Prague to implement and standardize overnight bridging ($n=26$; 72.2%) allowing a shift toward transplantation during the daytime. The literature suggests that nocturnal transplantation might be associated with worse outcomes because of limited resources, shortage of personnel, and lesser technical expertise (27–32). Moreover, fatigue and sleep deprivation of the LTx team might have a negative repercussion on the cognitive

TABLE 3 Postoperative outcomes.

	Postoperative ECMO	Time on ventilator (h)	PGD at 0/24/48/72 h	ICU stay (days)	Hospital stay (days)	Clavien–Dindo score
1	No	37	0/0/0/0	5	19	2
2	No	44	3/2/0/0	14	26	3a
3	No	42	0/0/0/0	6	31	3a
4	No	NA	3/3/3/2	62	87	4a
5	No	48 ^a	0/0/0/0	9	30	3b
6	Yes	108	UG/UG/UG/0	16	32	4a
7	No	16	0/0/2/1	4	18	4a
8	Yes	NA	3/3/3/2	7 ^b	7 ^b	5
9	No	29	3/2/0/0	8	16	3b
10	No	42	0/2/2/2	7	21	2
11	No	19	1/0/0/0	7	83	4a
12	No	15	0/0/0/0	4	31	2
13	Yes	86	3/3/3/0	15	45	3a
14	No	22	0/0/1/0	5	30	3a
15	Yes	64	UG/UG/UG/0	14	34	4a
16	No	20	3/2/2/2	8	28	2
17	No	19	0/0/0/0	7	40	2
18	No	49	0/0/3/0	12	27	4a
19	No	57	3/0/1/0	9	24	2
20	No	17	2/2/3/3	5	20	2
21	No	18	0/0/0/0	7	19	2
22	Yes	39	2/3/2/0	8	Ongoing	2
23	No	57	1/3/0/0	Ongoing	Ongoing	3b
24	No	19	2/1/3/2	Ongoing	Ongoing	2
25	No	6	0/0/0/0	11	20	3a
26	No	7	0/0/0/0	11	34	3b
27	No	10	0/0/0/0	7	13	2
28	No	17	0/0/0/0	7	20	2
29	No	31	0/0/0/0	11	19	2
30	No	21	0/0/0/0	8	19	2
31	No	41	0/0/0/0	46	52	3b
32	No	8	0/0/0/0	7	20	2
33	No	526 ^a	0/0/2/0	44	51	4a
34	No	12	0/0/0/0	9	34	3a
35	No	47	2/1/1/2	14	26	3a
36	No	6	0/0/0/0	20	32	3b
MV	—	25.5	—	8	28	—

ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; PGD, primary graft dysfunction; MV, median value; UG, ungradable.

^aTracheostomy due to failure from weaning.

^bDeath on day 7 postoperatively.

and psychomotor skills. Accordingly, the LTx policies of Leuven and Prague have considerably changed, with focus on flexibility and overnight bridging when donor cross-clamp time is planned after 6:00 PM. After procurement, the lungs are stored in the CHS device unattended in the surgical theater of the recipient center. Patient induction occurs the next day at 7:30 AM, and the LTx is performed during the daytime in optimal conditions with a well-rested team and maximal medical expertise. In addition, extended preservation and ischemic times allow expansion of the donor pool through long-distance lung procurement, rescue allocation, facilitation of immunological crossmatch test, and acceptance of a second pair of donor lungs in case of simultaneous or overlapping LTx.

Last but not least, we reported a higher number of DCD procedures ($n = 8$; 22.2%) compared with the registry [CHS $n = 15/85$ (17.6%) and SIS $n = 6/90$ (6.7%)] (26). These findings can

be attributed to the rapidly growing experiences of Leuven with DCD procedures (Leuven $n = 7$ vs. Prague $n = 1$). In fact, based on the favorable long-term survival in DCD-III and DBD lung donor recipients, as reported by ISHLT in 2019, Leuven increasingly performs DCD procurements to expand the donor pool (33).

Several questions on CHS remain, concerning the ideal temperature, extended vs. short preservation, long-term outcome, and potential benefits for extended-criteria donors. Randomized controlled trials (RCTs) and a propensity-matched study from a large GUARDIAN-LUNG cohort ($n = 500$) are awaited (24, 25). Furthermore, a multicenter RCT is currently being conducted by Toronto (X^oPort Lung Transport Device, Traferox Technologies Inc.): “Safety of 10°C Lung Preservation Versus Standard of Care: A Multi-Center Prospective Non-Inferiority Trial” (NCT05898776), comparing 160 CHS vs. 160 SIS (24, 34) cases.

5 Conclusion

CHS by LUNGguard seems feasible and safe, despite the relatively high-risk recipient and donor profiles (DCD 22.2%) and extended preservation periods. PGD3 at 72 h of 2.8% was observed in this series. The CHS technology potentially allows overnight bridging and shifting toward daytime transplantation, optimizing working conditions. However, several questions remain and multicenter randomized trials are awaited.

Data availability statement

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

Ethics statement

The study was approved by the research Ethics Committees of Leuven (S67697) and Prague (EK387/23). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) and minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

A-LP: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. RN: Writing – original draft, Writing – review & editing, Conceptualization, Investigation, Methodology, Software, Data curation. DVR: Writing – review & editing, Conceptualization. JVS: Writing – review & editing, Conceptualization, Data curation, Investigation, Methodology, Software. EP: Writing – review & editing. CMV: Writing – review & editing. AB: Writing – review & editing. XJ: Writing – review & editing. KD: Writing – review & editing, Data curation, Software. PDL: Writing – review & editing. HVV: Writing – review & editing. LD: Writing – review & editing. YJ: Writing – review & editing. JaP: Writing – review & editing. AN: Writing – review & editing. SB: Writing – review & editing. CI: Writing – review & editing. BJ: Writing – review & editing. LG: Writing – review & editing. LDS: Writing – review & editing. RV: Writing – review & editing. MS: Writing – review & editing. JaV: Writing – review & editing. JK: Writing – review & editing. JT: Writing – review & editing. JH: Writing – review & editing. ZOS: Writing – review & editing. JiP: Writing – review & editing. JS: Writing – review & editing. JiV: Writing – review & editing. RL: Writing – review & editing. LJC: Writing – original draft, Conceptualization, Data curation, Formal Analysis, Investigation,

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Conflict of interest

In relation to this manuscript, we disclose that Paragonix granted five LUNGguardTM devices to the authors to test their feasibility. No financial compensation was granted.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcvm.2024.1370543/full#supplementary-material>

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4.3. Anesteziologická rozvaha o intraoperační ECMO antikoagulaci při transplantaci plic: unicentrická, retrospektivní, observační studie

Vajter J, Holubova G, Novysedlak R, Svorcova M, Vachtenheim J Jr, Vymazal T, Lischke R. Anaesthesiologic Considerations for Intraoperative ECMO Anticoagulation During Lung Transplantation: A Single-Centre, Retrospective, Observational Study. Transpl Int. 2024 Mar 20;37:12752. doi: 10.3389/ti.2024.12752. PMID: 38585623; PMCID: PMC10996050.

V této retrospektivní studii jsme analyzovali výsledky 141 pacientů, kteří podstoupili transplantaci plic v období let 2020–2022. Do analýzy bylo zahrnuto 109 pacientů, u nichž byla transplantace provedena s podporou VA ECMO s centrální kanylací a ukončením ECMO podpory na konci výkonu. U všech pacientů jsme sledovali dávku nefrakcionovaného heparinu (UFH) podávaného během chirurgického výkonu, množství krevních ztrát a spotřebu krevních derivátů intraoperačně a do 48 hodin po přijetí na JIP. Hodnocena byla také nutnost chirurgické revize kvůli pooperačnímu krvácení, trombotické komplikace, mortalita a dlouhodobé přežití.

Výsledkem této studie bylo zjištění, že nižší dávky UFH podávány jako intraoperační antikoagulace na ECMO přispívají k signifikantnímu snížení spotřeby krevních derivátů a krevních ztrát. Kromě toho byly nižší dávky UFH asociovány s nižším počtem nutných chirurgických revizí pro hemothorax. Ve skupině s nižší dávkou UFH nebyl v prvních 72 hodinách po transplantaci zaznamenán výskyt PGD 3. stupně.



Anaesthesiologic Considerations for Intraoperative ECMO Anticoagulation During Lung Transplantation: A Single-Centre, Retrospective, Observational Study

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Background: Extracorporeal membrane oxygenation (ECMO) is frequently used during lung transplantation. Unfractionated heparin (UFH) is mainly used as part of ECMO support for anticoagulation. One of the most common perioperative complications is bleeding, which high-dose UFH can aggravate.

Methods: We retrospectively analyzed ($n = 141$) patients who underwent lung transplantation between 2020 and 2022. All subjects ($n = 109$) underwent central cannulated VA ECMO with successful intraoperative ECMO weaning. Patients on ECMO bridge, postoperative ECMO, heart-lung transplants and transplants without ECMO were excluded. The dose of UFH for the entire surgical procedure, blood loss and consumption of blood derivatives intraoperatively and 48 h after ICU admission were recorded. Surgical revision for postoperative bleeding were analyzed. Thrombotic complications, mortality and long-term survival were evaluated.

Results: Lower doses of UFH administered for intraoperative ECMO anticoagulation contribute to a reduction in intraoperative blood derivatives consumption and blood loss with no thrombotic complications related to the patient or the ECMO circuit. Lower doses of UFH may lead to a decreased incidence of surgical revision for hemothorax.

Conclusion: Lower doses of UFH as part of intraoperative ECMO anticoagulation might reduce the incidence of complications and lead to better postoperative outcomes.

Keywords: ECMO, UFH, anticoagulation, lung transplantation, anesthesiology

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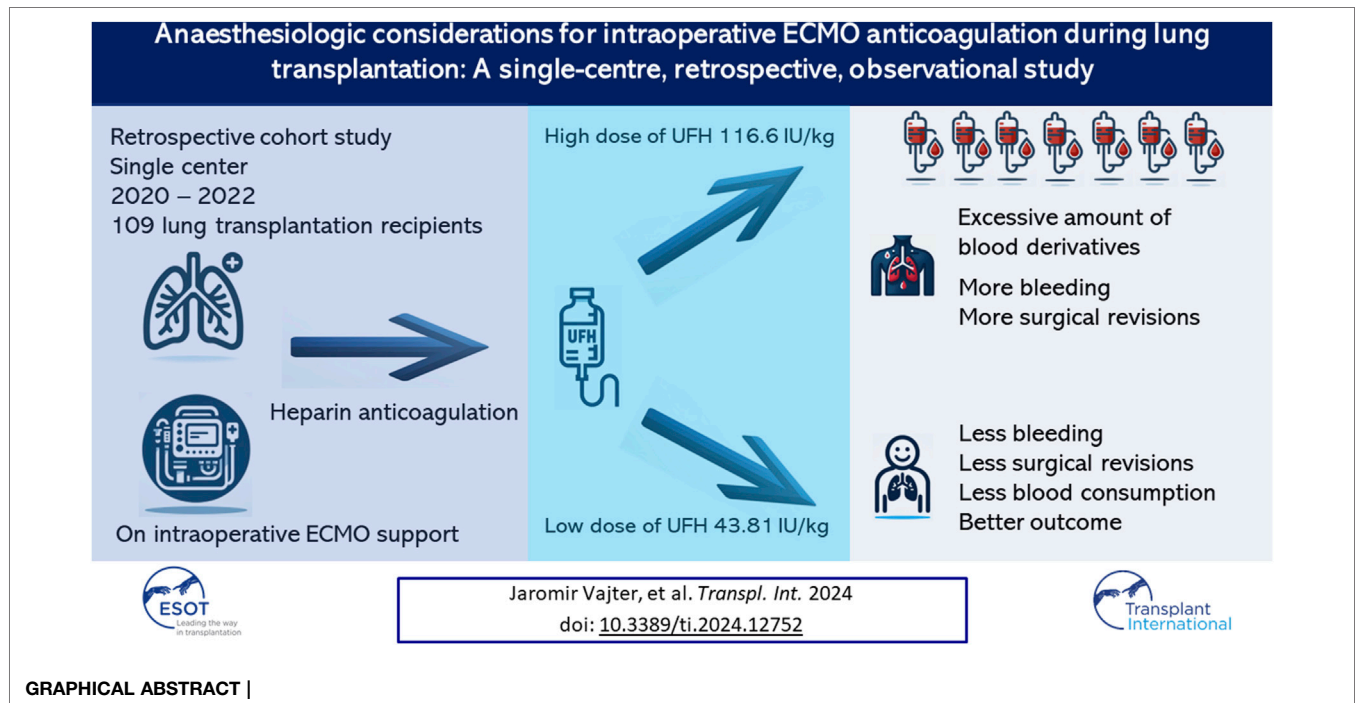
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Abbreviations: ACT, activated clotting time; APTT, activated partial thrombin time; BMI, body mass index; CONSORT, Consolidated Standards for Reporting Trials; CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation; FFP, fresh frozen plasma; HLTx, heart-lung transplant; ICU, intensive care unit; LUTx, lung transplant; MCS, mechanical circulatory support; NSAID, non-steroidal anti-inflammatory drugs; PEEP, positive end-expiratory pressure; PFA, platelet function assay; RBCs, red blood cells; ROTEM, rotational thromboelastometry; Tx, transplantation; UFH, unfractionated heparin.



INTRODUCTION

Intraoperative extracorporeal membrane oxygenation (ECMO) is routinely used during lung transplant surgery to provide the patient with temporary respiratory and circulatory support [1–3]. Over the past two decades, many centres have switched from intraoperative support with cardiopulmonary bypass (CPB) to ECMO [1, 2, 4, 5]. The intraoperative ECMO approach allows surgeons to perform the procedure with greater precision and efficiency while minimizing the risk of lung injury [2, 4]. Most centres that use intraoperative ECMO, to a greater extent, support the idea that ECMO helps to control lung graft reperfusion [4, 6, 7]. Another advantage of intraoperative ECMO support is the possibility of transplanting more patients with comorbidities who would not be able to undergo surgery on selective unilateral ventilation without extracorporeal support. For example, patients with severe pulmonary hypertension have a high risk of left-sided heart failure, pulmonary fibrosis, and very low pulmonary compliance [8, 9]. ECMO has a much lower pro-inflammatory potential than CPB [1]. However, ECMO also carries risks of bleeding, infection, and thrombotic complications [10–13]. The entire team should decide to use intraoperative ECMO on a case-by-case basis, considering the patient's medical history and condition [14, 15]. Our study aims to highlight that lower doses of anticoagulants administered during intraoperative ECMO support do not lead to increased risks and may be beneficial for the patient.

Intraoperative ECMO Anticoagulation During Lung Transplants

A certain amount of anticoagulation is crucial to prevent thrombotic complications during lung transplantation with

intraoperative ECMO support [1, 2, 11]. Thrombotic events, such as clot formation, can cause significant harm to the patient and negatively impact the transplant's success. However, the use of intraoperative anticoagulation is associated with a risk of bleeding, which can be problematic during surgery. Full heparinization with high activated clotting time (e.g., ACT >400 s) values are no longer necessary from CPB to ECMO support transition (also, thanks to heparin-coated ECMO cannulas and circuits). In contrast, the more knowledge we have about ECMO issues in the context of understanding coagulopathy, the more we strive for significantly lower doses of anticoagulation [4]. According to the current guidelines, the recommended procedure for ECMO cannulation is to administer a certain UFH bolus, usually 2000–5000 IU or 25–100 IU/kg, and then control the anticoagulation level using ACT [1, 11]. The ACT should be maintained in the range of 180–220 s [1, 11]. Other options (increasingly used over ACT) for anticoagulation monitoring are activated partial thrombin time (APTT) in the range of 60–90 s [16] and APTT ratio of 1.5–2.5 [16]. The anti-Xa assay is also a possible method for monitoring anticoagulation. The target values of the anti-Xa assay are 0.3–0.7 IU/mL [16]. According to the literature, it is also possible to perform anticoagulation monitoring using viscoelastic methods such as rotational thromboelastometry (ROTEM), explicitly using the CT INTEM/HEPTTEM ratio [17].

MATERIALS AND METHODS

This study was approved by the Local Ethics Committee (reference number EK-786/23) and was registered in the clinical trial database at ClinicalTrials.gov (identifier number

TABLE 1 | Standard, essential post-transplant ICU care.

Mechanical ventilation
• Maximum effort to achieve early extubation • Upon admission to the Intensive Care Unit • Pressure control mode mechanical ventilation (initially PEEP 8–10 cmH₂O) • Pressure support mode mechanical ventilation (PEEP 5 cmH₂O) • extubation
Vasopressor support
• First choice—Norepinephrine • Additional—Vasopressin
Immunosuppressives medication
• Tacrolimus • Mycophenolate-mofetil • Methylprednisolone • Basiliximab
Antibiotics and antivirals
• Piperacilline-Tazobactam i.v. • Amphotericin inh. • Ganciclovir i.v.
Analgesia
combination, according to a visual analogue scale and patient needs
• Bilateral ESPB • Sufentanil • Paracetamol • NSAID • Dexmedetomidine • LMWH (Enoxaparine) s.c. based on antiXa assay • Ketamin
Thrombosis prevention
• LMWH (Enoxaparine) s.c. based on antiXa assay
Specific examination and procedures
• Chest X-ray every 24 h • Physiotherapy 4 times/day or based on the needs of the patient • Microbiology findings every 24 h • Pulmonary artery pressure continual monitoring • Continual hemodynamic monitoring • Early enteral feeding

Abbreviations: PEEP, Positive end-expiratory pressure; cmH₂O centimeter of water; i.v. intravenous; inh. inhalation; NSAID, non-steroidal anti-inflammatory drugs; LMWH, low molecular weight heparin, s.c. subcutaneous.

NCT06054997). This study was designed as a single-centre, retrospective, observational study that included all lung transplants performed between January 2020 and December 2022 within the Prague Lung Transplant Program Motol University Hospital. A total of 141 patients underwent transplantation during the study period. The exclusion criteria were lung transplantation without intraoperative ECMO support, block heart-lung transplantation, ECMO bridge-to-lung transplant, and planned postoperative ECMO. The inclusion criteria were lung transplantation performed with intraoperative ECMO support, central ECMO cannulation, and successful ECMO support termination at the end of surgery. According to the study inclusion criteria, only lung transplants performed under intraoperative central ECMO cannulation with successful ECMO weaning were included. In total, 109 patients fulfilled the inclusion criteria. Thirty-two patients were excluded based on the following criteria: heart-lung transplant (HLT_x), $n = 4$; ECMO bridge, $n = 4$;

transplantation (Tx) without ECMO, $n = 8$; and planned prolonged ECMO, $n = 16$. UFH was used for ECMO anticoagulation in all the patients. The subjects were divided into two groups for further analysis according to the UFH dose administered during the entire surgical procedure. In the first group, the UFH dose was ≤ 60 IU/kg/surgery. In the second group, the UFH dose was greater than 61 IU/kg/surgery. A cutoff value of 60 IU/kg was determined based on the available literature review. Values ≤ 60 IU/kg/surgery were considered relatively lower doses, and values > 61 IU/kg/surgery were considered higher doses of UFH [2, 4, 10–13, 16–19]. The UFH effect was monitored using activated clotting time (ACT) values. The intraoperative haemoglobin level target for red blood cell (RBC) substitution was 100 g/L. Coagulopathy was managed according to the clinical experience of the anesthesiologist and viscoelastic Point of care methods (ROTEM, PFA). The parameters followed up intraoperatively in both groups were total blood loss in milliliters and related to the patient's weight during the surgery (assessed by the amount of blood in a calibrated suction device); the total amount of UFH administered to the patient during surgery in the international unit (IU) and related to patient weight; and the consumption of blood derivatives during the surgical procedure, such as RBC, fresh frozen plasma (FFP), and platelets (PLT). In both groups, ACT values were monitored after the administration of UFH before ECMO cannulation and then every 60 min. In both groups, protamine was administered at the end of the surgical procedure until physiological ACT values below 120 s were achieved (if needed). No type of biological glue to seal the anastomosis was used. In both groups, intraoperative VA ECMO was implanted electively, and no patient underwent urgent ECMO cannulation due to cardiac or pulmonary reasons. The Maquet Rotaflow RF-32 centrifugal pump provided Intraoperative VA ECMO support. Heparin-coated cannulas and a heparin-coated tubing system were used for cannulation. According to internal guidelines, the ECMO flow was maintained at 1/2 to 2/3 of the calculated cardiac output. In the postoperative period, we followed up on the development of hemothorax requiring surgical revision. We considered surgical revision for hemothorax to be a significant bleeding complication. We also evaluated blood product consumption in the period 48 h after ICU admission, PGD grade three in 72 h after LUT_x, 30-day and 90-day mortality, long-term survival and ECMO circuit-related and patient-related thrombotic complications. The basic, standard points of care in the Intensive care unit (ICU) are displayed in **Table 1**.

Recipient and Donor Characteristics

Both groups of recipients were relatively homogenous, even though the number of subjects was not the same in both groups (lower dose UFH/kg group, $n = 44$; higher dose UFH/kg group, $n = 65$). The p -values for sex, age, height and mean pulmonary artery pressure (mPAP) were above the significance level of 0.05. The lower dose UFH/kg group recipients had a slightly higher weight ($p = 0.048$) and a higher BMI ($p = 0.0093$). The distribution of diagnoses for which the recipients were transplanted was also homogeneous

TABLE 2 | Recipient and donor characteristics.

Recipient-characteristic variable	≤60 IU/kg UFH group (n = 44)	>61 IU/kg UFH group (n = 65)	p-value
Male sex, n (%)	26 (59.09%)	44 (67.69%)	0.36
Age (years; mean ± SD)	54.14 ± 11.53	52.09 ± 12.47	0.59
Weight (kg; mean ± SD)	79.91 ± 15.47	73.93 ± 15.26	0.048
Height (cm; mean ± SD)	172.1 ± 7.39	173.4 ± 7.72	0.39
Body mass index (mean ± SD)	26.9 ± 4.43	24.6 ± 4.57	0.0093
mPAP (torr; mean ± SD)	26.3 ± 9.6	27.8 ± 13.4	0.528
thoracotomy prior LUTx (sum)	1	1	0.78
Transplant indication			
COPD, n (%)	15 (34%)	26 (40%)	0.59
Pulmonary fibrosis, n (%)	18 (40.9%)	23 (35.38%)	0.50
Cystic fibrosis, n (%)	3 (6.8%)	5 (7.69%)	0.89
Others, n (%)	8 (18.18%)	11 (16.92%)	0.82
Donor-characteristic variable	≤60 IU/kg UFH group (n = 44)	>61 IU/kg UFH group (n = 65)	p-value
Male sex, n (%)	21 (41.72%)	37 (56.92%)	0.34
Age (years; mean ± SD)	47.41 ± 16.05	44.78 ± 16.32	0.41
Weight (kg; mean ± SD)	70.9 ± 13.62	76.7 ± 17.12	0.059
Height (cm; mean ± SD)	170.3 ± 10.12	172.6 ± 10.95	0.26
Body mass index (mean ± SD)	24.1 ± 3.64	25.6 ± 4.7	0.074
Cause of death			
Subarachnoid haemorrhage, n (%)	10 (22.7%)	14 (21.5%)	0.88
Intracerebral bleeding, n (%)	8 (18.2%)	14 (21.5%)	0.67
Traumatic brain injury, n (%)	15 (34.1%)	16 (24.6%)	0.28
Anoxic brain injury, n (%)	8 (18.2%)	13 (20.0%)	0.81
Others, n (%)	3 (6.8%)	8 (12.3%)	0.35

Abbreviations: IU, international unit; UFH, unfractionated heparin; SD, standard deviation; n, Number of Subjects; kg, Kilogram(s); COPD, chronic obstructive pulmonary disease; mPAP, mean pulmonary arterial pressure.

($p > 0.05$). These numbers are listed in **Table 2**. Donor characteristics were completely homogeneous in both groups. The p -values of sex, age, weight, height, BMI, and cause of death were above the significance level of 0.05 (**Table 2**).

Statistical Analysis

Statistical analyses were performed with version 8.0.1 (244) of GraphPad Prism statistical software. Statistical significance was set at $p < 0.05$. The unpaired t -test was used to statistically evaluate blood loss, UFH dose, consumption of blood derivatives, and ACT values. For the statistical evaluation of surgical revision for hemothorax and PGD, we chose chi-square test. Kaplan-Mayer curve and log-rank test have been performed for 30-day and 90-day” and long-term survival assessment (long-term survival time endpoint October/2023).

RESULTS

Patients were recruited between January 2020 and December 2022, and based on the exclusion criteria, a total of 32/141 patients were excluded from the study. A flow diagram based on the Consolidated Standards of Reporting Trials (CONSORT) is shown in **Figure 1**. The lower dose of UFH (≤60 IU/kg/surgical procedure) and higher dose of UFH (>61 IU/kg/surgical procedure) groups ultimately consisted of 44 and 65 patients, respectively. For most parameters, we obtained surprising results, clearly in favour of the administration of lower doses of UFH (≤60 IU/kg). Total

blood loss during surgery was significantly lower in the group treated with lower doses of UFH (≤60 IU/kg). The mean total intraoperative blood loss was 753 and 1,470 mL, respectively ($p < 0.0001$) (**Table 3**). Blood loss related to body weight was also significantly lower in the UFH group ≤60 IU/kg. The mean intraoperative blood loss/patient body weight was 9.628 mL/kg and 20.97 mL/kg, respectively ($p < 0.0001$) (**Table 3**). The total UFH dose was significantly lower in the UFH group (≤60 IU/kg). The mean total intraoperative UFH doses were 3491 IU and 8694 IU, respectively ($p < 0.0001$) (**Table 3**). The total UFH dose, based on body weight, was significantly lower in the UFH group (≤60 IU/kg). The mean dose of UFH/patient bodyweight intraoperatively was 43.81 IU/kg and 116.6 IU/kg, respectively ($p < 0.0001$) (**Table 3**). We also noticed a significant difference in favour of reducing the consumption of blood derivatives in the group with lower doses of UFH ≤60 IU/kg. The consumption of RBCs during the surgical procedure was 0.5581 and 1.908 units, respectively ($p = 0.0009$) (**Table 3**). The FFP consumption during surgery was 0.4186 and 1.862 units, respectively ($p = 0.0009$) (**Table 3**). The platelet consumption during surgery was 0.1628 and 0.4154 units, respectively ($p = 0.1461$) (**Table 3**). The mean ACT values before ECMO cannulation and 3 min after the administration of UFH were lower in the UFH group ≤60 IU/kg (156.3 and 209.1) ($p < 0.0001$) (**Table 3**). There was a significant reduction in bleeding complications in terms of surgical revision for hemothorax in the lower UFH dose group ≤60 IU/kg, with only one revision (2.27%) and nine revisions (13.85%), respectively ($p = 0.040$) (**Table 3**). In the postoperative period 48 h after admission to the ICU, we did not

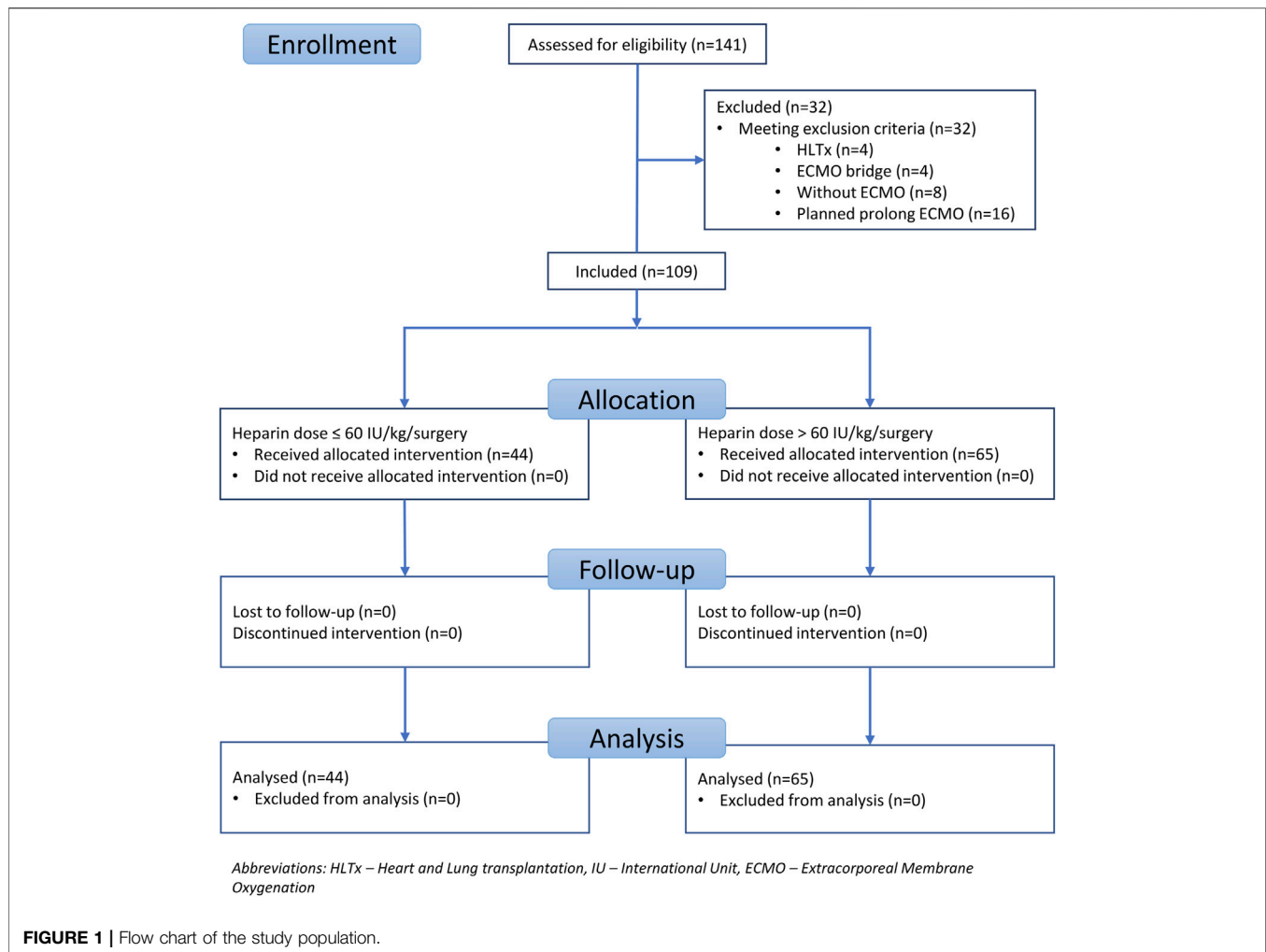


TABLE 3 | Variables blood loss, intraoperative blood product consumption, UFH dose, and surgical revision for haemothorax.

Variables	≤60 IU/kg UFH group (n = 44)	> 61 IU/kg UFH group (n = 65)	p-value
Total blood loss (ml; mean ± SD)	753.5 ± 522.5	1,470 ± 1,014	<0.0001
Blood loss per kg (ml/kg; mean ± SD)	9.63 ± 6.59	20.97 ± 15.64	<0.0001
Total dose of heparin (IU; mean ± SD)	3,491 ± 1,088	8,694 ± 3,790	<0.0001
Dose of heparin per kg (IU/kg; mean ± SD)	43.81 ± 12.08	116.6 ± 44.98	<0.0001
RBC consumption (unit)	0.5581 ± 1.119	1.908 ± 2.435	0.0009
FFP consumption (unit)	0.4186 ± 1.349	1.862 ± 2.543	0.0009
PLT consumption (unit)	0.1628 ± 0.5314	0.4154 ± 1.044	0.1461
Surgical revision due to haemothorax (% of revision)	2.27	13.85	0.040

Abbreviations: IU, international unit; UFH, unfractionated heparin, mL, millilitre; kg, kilogram; SD, standard deviation; RBC, red blood cells; FFP, fresh frozen plasma; PLT, platelets. Bold values are statistically significant.

observe a significant decrease in the consumption of blood derivatives (Table 4). However, there was a significantly lower incidence of third-degree PGD 72 h after LUTx in the group where a lower dose of UFH was administered ($p = 0.038$) (Table 4; Figure 2). The 30-day, 90-day, and long-term survivals were not different (Figure 3). The log-rank test was $p = 0.6879$ (Figure 3). Mortality rates were not different in either group. We did not record any thrombotic complications arising

from the ECMO circuit or patient-related complications in any group.

DISCUSSION

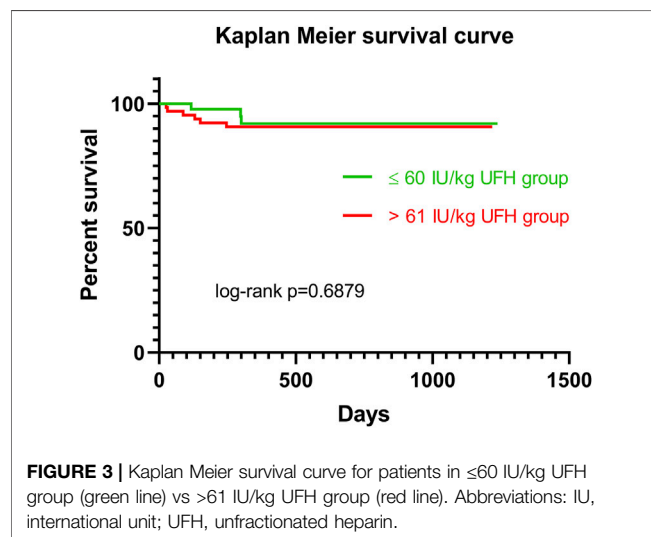
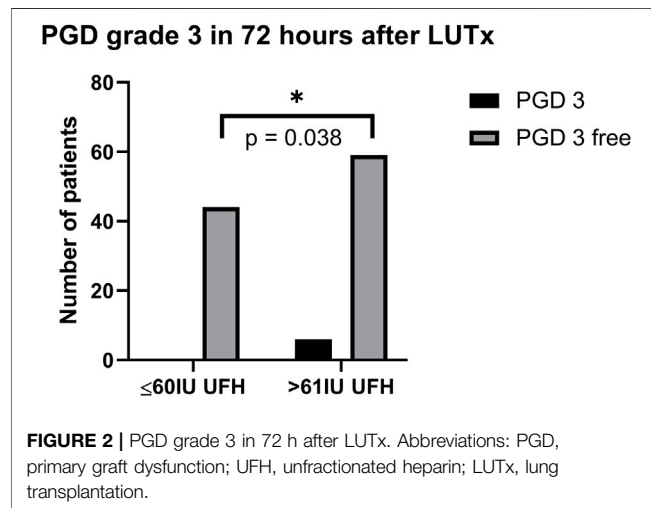
Intraoperative ECMO support during lung transplantation is a routine method frequently used to facilitate surgical procedures.

TABLE 4 | Variables post LUTx (PGD 3 in 72hours post LUTx), (FFP, RBC, PLT 48 h post LUTx).

Variables	≤60 IU/kg UFH group (n = 44)	> 61 IU/kg UFH group (n = 65)	p-value
PGD grade 3 (total, percentage)	0 (0%)	6 (9.23%)	0.038
RBCs consumption mean (unit)	1.43 ± 2.0	1.55 ± 2.4	0.7996
FFP consumption mean (unit)	0.41 ± 1.4	1.12 ± 3.0	0.1567
PLT consumption mean (unit)	0.23 ± 1.1	0.35 ± 1.1	0.5615

Abbreviations: IU, international unit; UFH, unfractionated heparin; LUTx, lung transplantation; PGD, primary graft dysfunction; FFP, fresh frozen plasma; RBCs, red blood cells; PLT, platelets; IU, international unit.

Bold values are statistically significant.



This enables the procedure to be performed in significantly more polymorbid patients who cannot handle selective ventilation. Another indisputable advantage of ECMO is the possibility of controlled reperfusion of lung grafts. During ECMO support, the blood is in contact with allogeneic materials such as cannulas, circuits, and oxygenators. All of these factors can cause potential complications, including bleeding and thrombosis. Therefore,

balancing the edge between anticoagulation and procoagulation is very important to minimize complications during intraoperative ECMO support. UFH remains the most widely used drug for ECMO anticoagulation. From the available recommendations of thoracosurgery societies, we know the recommendation for UFH dosing, most often between 25–100 IU/kg and effect control with ACT in the range of 180–220 s. However, the literature and the guidelines more frequently report the possibility of reducing the dose of UFH and, very importantly, without an increase in thrombotic complications. According to the recommendations of the thoracosurgery societies, in the case of bleeding complications or anticipated intraoperative bleeding (for example, significant intrapleural adhesions), it is recommended to minimize the dose of UFH or completely eliminate UFH and perform heparin-free ECMO. For example, Bernhardt et al. mentioned in The International Society for Heart and Lung Transplantation/Heart Failure Society of America Guidelines on Acute Mechanical Circulatory Support that “bleeding complications in acute mechanical circulatory support (MCS) are common and frequently necessitate withdrawal of anticoagulation” and stated that “in the settings of life-threatening bleeding, full discontinuation of all anticoagulation may be necessary” [20]. Hartwig et al. stated in The American Association for Thoracic Surgery guidelines that “low or no heparin regimes are recommended for patients with significant adhesions and impaired coagulation status” [1]. Additionally, Lorusso et al., in the EACTS/ELSO/STS/AATS expert consensus, mentioned that “anticoagulation is required during prolonged ECLS to prevent circuit thrombus formation with embolization and/or circuit failure. However, bleeding remains the most frequent complication associated with ECLS. UFH infusion is typically delayed until haemostasis is achieved, often within 24–48 h. Reports that suggest the safety of prolonged withdrawal of anticoagulation for as long as 3 days when faced with bleeding are important” [21]. This trend was also confirmed by our study, which suggests that a lower dose of UFH as part of intraoperative ECMO support may not pose a risk but, on the contrary, can have substantial benefits for the patient. Using lower doses of UFH below 60 IU/kg (43.81 IU/kg based on our study) can benefit the patient and the entire perioperative period. Nonetheless, these results must be interpreted with caution, and numerous limitations should be considered. The major limitation is that the dosage of the UFH used in the study (high or low dose) has been based on subjective criteria, i.e., the clinical experience of the

anesthesiologist guides its dosage. Another possible limitation of this study may be the division of subjects according to UFH into groups below and above 60 IU/kg since the available literature does not define the exact dose of UFH but only the range. Therefore, it was necessary to set a limit for dividing the patients into groups. However, the medians of the two groups were very far apart 43.81 IU/kg vs. 116.6 IU/kg; we presume this is more of a minor bias. Furthermore, having a more significant number of investigated subjects would be advisable, which would add even more weight to the entire study. Similarly, a particular bias may have been introduced into this study because the primary hemostasis disorder was not investigated; primary hemostasis can be disturbed as part of ECMO support during lung transplantation, thereby potentiating intraoperative bleeding [22]. The authors hypothesize that the significantly higher incidence of PGD grade 3 in 72 h postop in the group with higher doses of UFH may be caused by the need to administer a larger number of blood derivatives, which is one of the possible reason for the development of PGD. The anesthesiologist plays a crucial role in the decision-making process and is mostly responsible for anticoagulation management of intraoperative ECMO support [15, 23, 24]. Such management depends not only on their experience but also on their knowledge of the latest findings and recommended practices. The decision to withhold anticoagulation therapy or decrease its dosage involves balancing the competing risks between bleeding and clotting.

Conclusion

The results of this study generates a hypothesis that lower doses of UFH (mean dose of UFH: 43.81 IU/kg) administered for intraoperative ECMO anticoagulation may contribute to a reduction in intraoperative blood loss and decrease the incidence of surgical revisions for haemothorax. Furthermore, lower doses of UFH may reduce the intraoperative consumption of blood derivatives such as RBCs and FFP. Notably, the concept of lower doses of UFH did not have a negative effect on 30-day, 90-day and long-term survival. No thrombotic complications of the ECMO circuit or thrombotic complications related to the patient were observed. Further investigation in this area is needed to provide deeper insights into the potential use of lower doses of UFH during ECMO.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving humans were approved by the Local Ethics Committee (reference number EK-786/23). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

JaV designed the study; JaV and GH collected the study data; JaV analyzed the data and wrote the major part of the manuscript; GH, JiV, MS, RN, TV, and RL participated in the research performance and/or substantially contributed to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4.4. Dopady SARS-CoV-2 na obor transplantace plic: očkování a evaluace dárce

4.4.1. Hodnocení virové nálože SARS-CoV-2 při transplantaci plic

Novysedlak R, Vachtenheim J Jr, Stráž I, Viklický O, Lischke R, Strizova Z. SARS-CoV-2 viral load assessment in lung transplantation. Physiol Res. 2021 Dec 16;70(S2):S253-S258. doi: 10.33549/physiolres.934760. PMID: 34913356; PMCID: PMC8884369.

V níže prezentované práci jsme analyzovali dva případy potenciálních dárců plic s nejednoznačnými výsledky stran infekce COVID-19. První dárkyní byla 28letá žena, která zemřela na trombózu bazilární tepny. Její PCR test na SARS-CoV-2 byl původně pozitivní, ale následně v jiné instituci negativní, přičemž opakované PCR testy z BAL a sérologické vyšetření anti-SARS-CoV-2 protiálek nepotvrdily naázkzu virem, avšak vzhledem k nespecifickým infiltrátům na CT plic byly plíce k transplantaci zamítnuty, zatímco srdce, játra a ledviny byly transplantovány. Druhým dárce byl 58letý muž, který zemřel na kraniotrauma. Jeho PCR testy na SARS-CoV-2 vykazovaly hraniční hodnoty Ct (38 a 39.4). Dodatečná sérologická analýza prokázala přítomnost protilátek IgG i IgA proti viru, avšak vzhledem k pleurálnímu výpotku a přidruženým změnám v plicním parenchymu na CT nebyly jeho plíce k transplantaci akceptovány. Na základě těchto případů jsme formulovali doporučení, aby hodnota prahového cyklu (Ct) polymerázové řetězové reakce (PCR) byla při rozhodování o akceptaci dárce zohledněna, přičemž rozhodnutí o provedení transplantace by mělo být učiněno na základě komplexního zhodnocení více kritérií. Mezi ně patří výsledky PCR testů z nosohltanového stěru a bronchoalveolární laváže, hodnota Ct, radiologické nálezy a detailní anamnestické údaje. Jelikož samotná přítomnost virové RNA neimplikuje

infekčnost, bylo v době pandemie nezbytné urychleně objasnit, jaké hodnoty Ct jsou spojeny s viabilitou viru. Dále jsme formulovali názor, že detekce anti-SARS-CoV-2 IgA protilátek a kvantifikace nukleokapsidového antigenu SARS-CoV-2 v séru mohou být cennými nástroji pro podporu rozhodovacího procesu při akceptaci dárce. Chybné rozhodnutí při výběru dárce může vést k rozvoji těžké formy PGD a závažným komplikacím v pooperačním období, které mohou negativně ovlivnit celkový výsledek transplantace.

RAPID COMMUNICATION

SARS-CoV-2 Viral Load Assessment in Lung Transplantation

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Summary

In the era of COVID-19 pandemic, organ transplantation programs were facing serious challenges. The lung transplantation donor pool was extremely limited and SARS-CoV-2 viral load assessment has become a crucial part of selecting an optimal organ donor. Since COVID-19 is a respiratory disease, the viral load is thought to be more important in lung transplantations as compared to other solid organ transplantations. We present two challenging cases of potential lung donors with a questionable COVID-19 status. Based on these cases, we suggest that the cycle threshold (Ct) value should always be requested from the laboratory and the decision whether to proceed with transplantation should be made upon complex evaluation of diverse criteria, including the nasopharyngeal swab and bronchoalveolar lavage PCR results, the Ct value, imaging findings and the medical history. However, as the presence of viral RNA does not ensure infectivity, it is still to be clarified which Ct values are associated with the viral viability. Anti-SARS-CoV-2 IgA antibodies may support the diagnosis and moreover, novel methods, such as quantifying SARS-CoV-2 nucleocapsid antigen in serum may provide important answers in organ transplantations and donor selections.

Key words

COVID-19 • Lung transplantation • Organ donor • PCR • Cycle threshold

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Coronavirus disease 2019 (COVID-19) pandemic has become one of the greatest challenges in the recent history (Lacina *et al.* 2021, Hubacek *et al.* 2021, Paces *et al.* 2020). The disease outbreak has impacted the entire society with one of the most influenced area being the healthcare (Huang *et al.* 2021).

Due to an extremely high transmissibility and a high mortality of COVID-19, especially among immunocompromised patients, both diagnostic and therapeutic processes have significantly changed (Paces *et al.* 2020, Patt *et al.* 2020, Sica *et al.* 2021, Khairallah *et al.* 2021, Vasku 2020). In order to manage this unprecedented situation, intensive care units, as well as standard hospital wards and ambulatory care practices, were accordingly reshaped to provide the best protection against COVID-19 transmission and to allow gaining a control over the COVID-19 (Dzupova *et al.* 2021, Baccolini *et al.* 2021).

Nonetheless, while the management of the COVID-19 dominated the diagnostic and therapeutic landscape in most European countries, other medical areas were negatively altered (Patt *et al.* 2020, Sica *et al.*

2021, Khairallah *et al.* 2021, Dzupova *et al.* 2021, Allam *et al.* 2020).

In the era of COVID-19 pandemic, organ transplantation programs faced serious challenges (Azzi *et al.* 2021, Esagian *et al.* 2020, DeFilippis *et al.* 2020). The lung transplantation donor pool was extremely limited and novel guidelines for the management of organ donors and recipients had to be created (Azzi *et al.* 2021, Keller *et al.* 2020).

The COVID-19 mortality among transplant recipients was found to be substantially high and thus, a great effort is still being made to search for SARS-CoV-2 in both organ donors and recipients (Lieberman *et al.* 2020). Currently, all potential organ recipients on the transplant waiting list are advised to undergo vaccination against the COVID-19 and are screened for the COVID-19 infection prior to transplantation (Scharringa *et al.* 2021). Vaccination against the COVID-19 is also recommended in organ recipients after the transplantation despite the fact that

several studies already indicated the impairment of the anti-SARS-CoV-2 specific immunity in solid organ transplant recipients (Shostak *et al.* 2021, Havlin *et al.* 2021). In fact, mRNA vaccines were found to elicit only limited antibody responses in lung transplant recipients but contrarily, displayed a capacity to promote the induction of anti-SARS-CoV-2 reactive T cells (Shostak *et al.* 2021, Havlin *et al.* 2021).

Still, there are many issues that accompany the SARS-CoV-2 viral load assessment in organ donors. For instance, various studies already reported cases of unclear COVID-19 status in individuals that required a highly personalized and careful evaluation of their infectivity based on multiple criteria (Lieberman *et al.* 2020, Oran *et al.* 2021, Messika *et al.* 2021, Amor *et al.* 2021, Rao *et al.* 2020, Theodore *et al.* 2021, Miller *et al.* 2021, Fisher *et al.* 2021).

In this report, we present two challenging cases of potential lung donors with a questionable COVID-19 status, Figure 1.

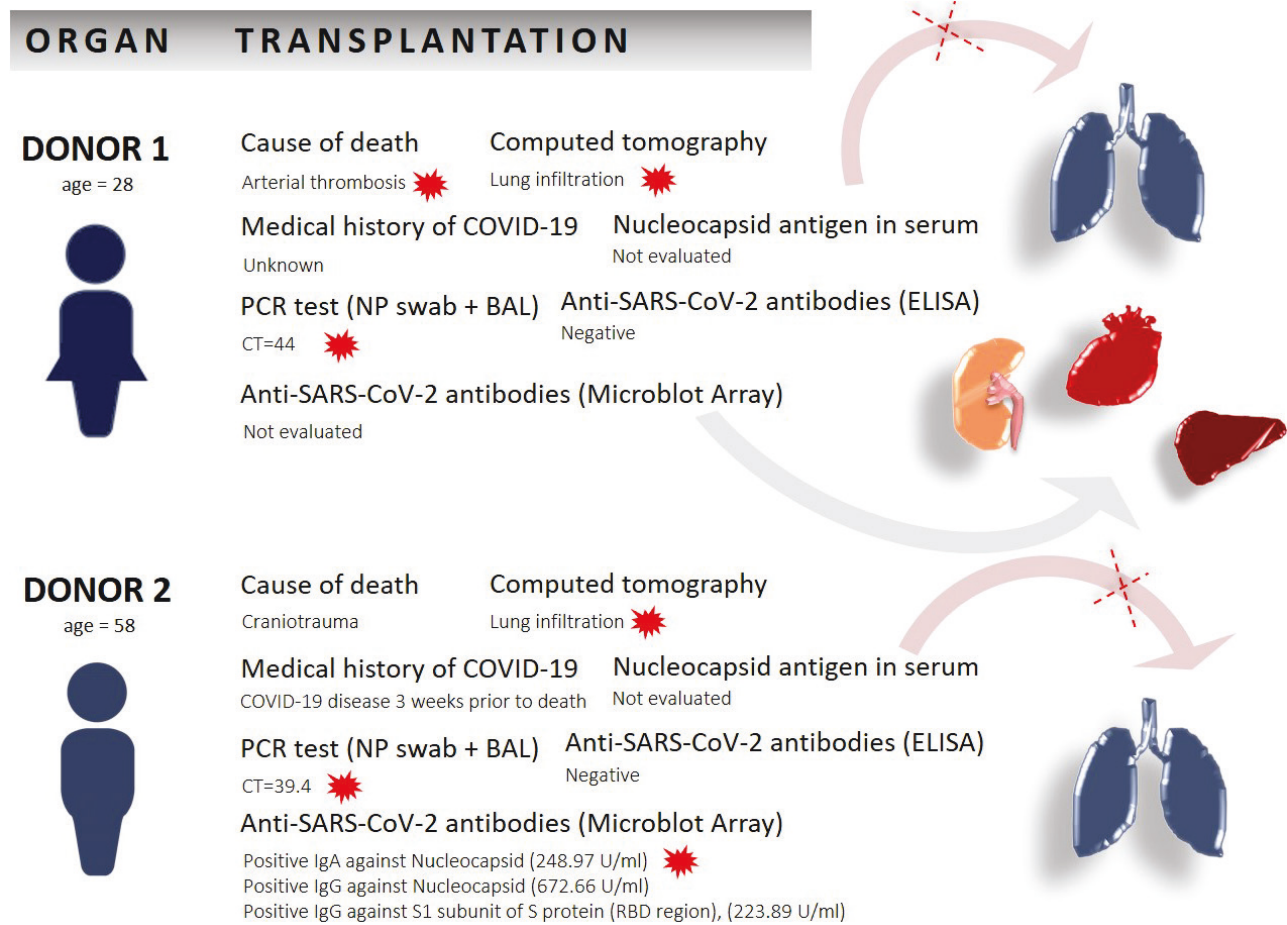


Fig. 1. Two donors and relevant factors associated with the clinical decision-making in relation to SARS-CoV-2 viral load and organ transplantation. Red marks label the factors that served as the exclusion criteria for lung transplantation.

The first donor was a young 28-years old female that died due to a thrombosis of basilar artery. This donor was presented as SARS-CoV-2 PCR positive at the primary Institution but came out PCR negative one day later at another Institution. Her organs were being considered for transplantation.

According to the generally accepted recommendations, bronchoalveolar lavage (BAL) should be examined in patients with suspected COVID-19 infection after a negative nasopharyngeal (NP) swab (Theodore *et al.* 2021, Gao *et al.* 2021). In our case, two consecutive PCR tests performed on BAL were negative, as well as the anti-SARS-CoV-2 antibodies that were retrospectively examined. To assess the level of positivity of the first PCR, we have requested the cycle threshold (Ct) count from the primary Institution. The Ct was 44 and, in most cases, Ct above 40 is considered negative (Falasca *et al.* 2020). Nevertheless, PCR assays are highly sensitive and allow a detection of an extremely low viral load (Oran *et al.* 2021, Messika *et al.* 2021). Therefore, in the process of decision-making whether to transplant, this became an issue. Also, the *computed tomography (CT)* revealed non-specific lung infiltration that could imply viral affection. Based on these findings, the lungs were not transplanted while the heart, the liver and the kidney transplantations were performed.

The second case was a 58-years-old male who died due to a craniotrauma that had a recent medical history of COVID-19 disease. This donor was tested SARS-CoV-2 PCR positive with the Ct 38 two days before the admission and the Ct 39.4 at the day of the planned transplantation. To obtain more data, we have retrospectively examined the antibodies by a microblot-array and found positive IgG against the *RBD region* and against the nucleocapsid, and positive IgA against the nucleocapsid (248.9 U/ml). CT revealed pleural effusion with associated lung parenchyma abnormalities and thus, the lung transplantation was not carried out.

SARS-CoV-2 viral load assessment is a crucial part of selecting an optimal organ donor (Lieberman *et al.* 2020). Since COVID-19 is a respiratory disease, the viral load may be more important in lung transplantations as compared to other solid organ transplantations (Messika *et al.* 2021). General criteria for accepting/declining lungs remain controversial and to date, there is still a significant proportion of possible donor lungs that are declined due to parenchyma abnormalities, such as pulmonary arterial thrombosis, pulmonary infarction, bronchopneumonia, and other findings (Verleden *et al.* 2017).

CT examination was proposed as a simple and valuable tool to decide whether to accept or decline donor lungs. Yet, there is no evidence that lungs with CT alterations could not be transplanted (Verleden *et al.* 2017).

In the era of COVID-19, accepting or declining donor organs for transplantations has become extremely challenging and especially the quality of lung allografts has raised serious concerns (Azzi *et al.* 2021, Keller *et al.* 2020).

While the COVID-19 pandemic has led to an increase of patients dying while on transplant waiting list, the risk of severe illness and death from COVID-19 in transplant recipients seems to outweigh the risk of donor organ shortage (Miller *et al.* 2021, Fisher *et al.* 2021).

PCR testing allow prompt and accurate detection of SARS-CoV-2, however, the information about the positivity/negativity alone may not be sufficiently sensitive to discriminate acceptable and nonacceptable lungs (Lanser *et al.* 2021).

Based on our previous experiences, we suggest that in unclear cases, the Ct value should always be requested and the decision should be made upon complex evaluation of diverse criteria, including the NP swab and BAL PCR results, the Ct value, imaging findings and medical history, Figure 2. To note, it is still to be clarified which Ct values are associated with the viral viability. Anti-SARS-CoV-2 IgA antibodies may support the diagnosis and moreover, novel methods, such as quantifying SARS-CoV-2 nucleocapsid antigen in the serum may provide important answers in organ transplantations and donor selections (Ogata *et al.* 2020).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

BAL – bronchoalveolar lavage, COVID-19 – coronavirus disease 2019, CT – computed tomography, Ct – cycle threshold, Ig – immunoglobulin, NP – nasopharyngeal, PCR – polymerase chain reaction, RBD – receptor binding domain, RNA – ribonucleic acid, SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2, SOT – solid organ transplant.

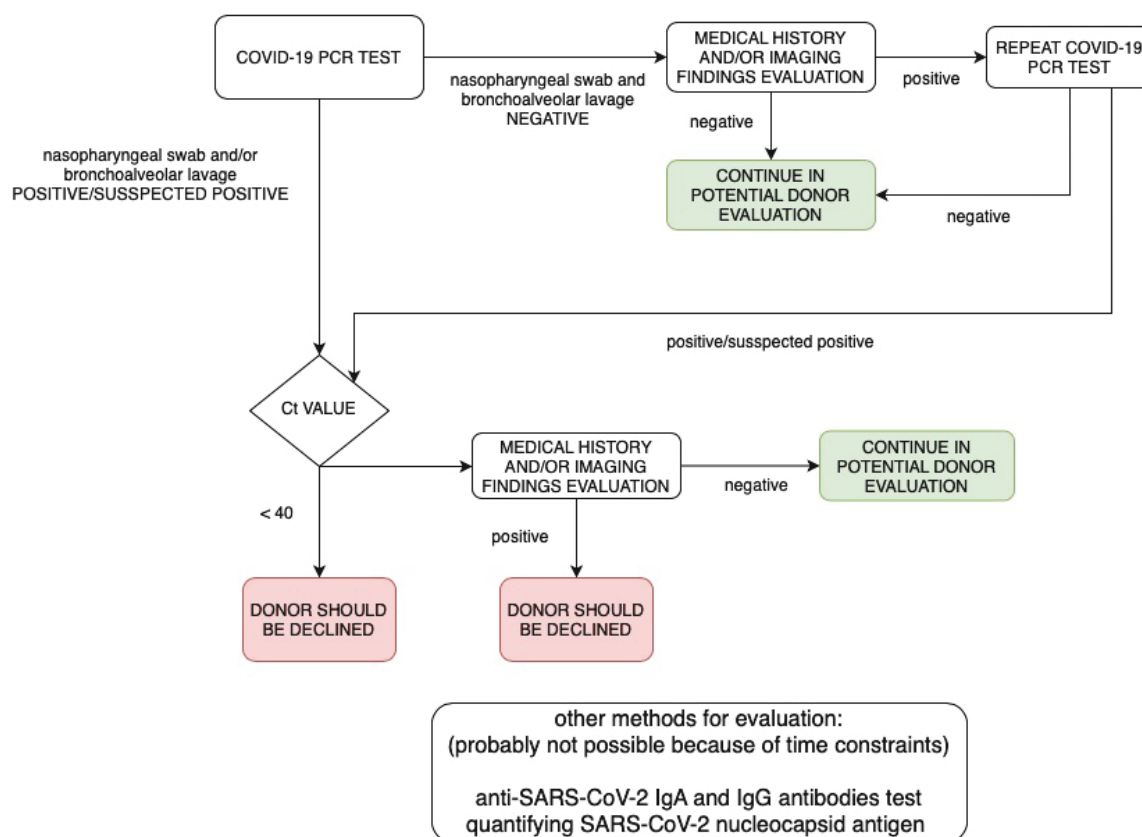


Fig. 2. Diagnostic algorithm for the evaluation of COVID-19 status in organ donor in lung transplantation.

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4.4.2. Očkování proti SARS-CoV-2 u příjemců transplantace plic:

Imunogenicita, účinnost a bezpečnost

Svorcova M, Novysedlak R, Lischke R, Vachtenheim J Jr, Strizova Z. Vaccination Against SARS-CoV-2 in Lung Transplant Recipients: Immunogenicity, Efficacy and Safety. Front Immunol. 2022 Jun 1;13:906225. doi: 10.3389/fimmu.2022.906225. PMID: 35720376; PMCID: PMC9198330.

Předmětem této práce je také problematika COVID-19. Vzhledem k tomu, že plíce představují primární orgán zasažený touto infekcí, považovali jsme za zásadní věnovat se této problematice v kontextu probíhající pandemie. Shruli jsme aktuální, byť omezené, poznatky o imunogenicitě, účinnosti a bezpečnosti vakcinace proti COVID-19 u transplantovaných pacientů, kteří představují vysoce rizikovou skupinu z hlediska rozvoje těžkého průběhu onemocnění COVID-19.

Velké klinické studie ukázaly, že vakcíny proti COVID-19 výrazně snižují závažnost onemocnění, avšak tyto studie nezahrnovaly pacienty užívající imunosupresiva. Dle některých studií převážná většina transplantovaných pacientů nevyvine po očkování ani humorální, ani buněčnou imunitní odpověď.

U transplantovaných pacientů proto vznikla potřeba zvážit podání dalších dávek vakcíny proti COVID-19, přičemž třetí posilovací dávka byla považována za potenciálně účinný nástroj prevence závažného průběhu infekce. Nicméně, zůstává otázka, zda silnější imunitní odpověď vyvolaná další dávkou vakcíny může zvýšit riziko vzniku akutní rejekce nebo jiných komplikací, což představuje významnou neznámou v péči o tuto skupinu pacientů.

V naší práci jsme se zaměřili na hlavní výzvy spojené s očkováním proti SARS-CoV-2 u příjemců plicních štěpů v době pandemie COVID-19. Shromáždili jsme dostupná data týkající se imunogenicity, účinnosti a bezpečnosti vakcín proti COVID-19 u této specifické skupiny pacientů a zdůraznili klíčové otázky, které je nutné zohlednit při vývoji personalizovaných očkovacích strategií, které minimalizují riziko komplikací, jako je reakce, a současně zajišťují optimální ochranu proti těžkým formám COVID-19.



Vaccination Against SARS-CoV-2 in Lung Transplant Recipients: Immunogenicity, Efficacy and Safety

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Lung transplant (LuTx) recipients are considered to be at higher risk of developing serious illness from COVID-19. COVID-19 vaccines were shown in randomized clinical trials to substantially reduce the severity of COVID-19, however, patients receiving immunosuppressants were excluded from these trials. Observational studies report a proportion of solid organ transplant (SOT) recipients being able to mount sufficient titers of SARS-CoV-2 specific IgG antibodies, however, other studies demonstrate that more than 90% of the SOT recipients elicit neither humoral nor cellular immune response after vaccination. Currently, the third booster dose of the COVID-19 vaccines was shown to elicit strong immune responses and may, thus, represent a potent tool in the prevention of severe COVID-19 infection in SOT recipients, including patients after lung transplantation. To address the main challenges of SARS-CoV-2 vaccination in LuTx recipients in the era of COVID-19, we have closely collected all available data on the immunogenicity, efficacy and safety of COVID-19 vaccines in LuTx recipients.

Keywords: COVID-19, mRNA vaccination, Pfizer, Moderna, transplant, immunosuppression

INTRODUCTION

Immunosuppressive therapy prevents both acute and chronic rejection in a significant proportion of solid organ transplant (SOT) recipients and to date, calcineurin inhibitors, cell cycle inhibitors, and corticosteroids belong to the most commonly-prescribed anti-rejection drugs for the treatment of lung transplant (LuTx) patients (1). Yet, bacterial, viral, and fungal infections make up the largest proportion of serious adverse events associated with anti-rejection therapy (2). For that reason, lung transplant (LuTx) recipients are considered to be at higher risk of developing serious illness from COVID-19 (3).

The field of organ transplantation has been severely affected in the era of COVID-19 (4). Most of the SOT centers have experienced a significant decline in transplant activity given a variety of factors, such as limited donor pools, extensive SARS-CoV-2 testing, and decreased intensive care unit (ICU) capacities (4, 5). Lung transplantations were associated with a number of donor-derived COVID-19 infections which raised the urge to set novel guidelines for the prevention and treatment of LuTx recipients (4, 5).

COVID-19 vaccines in SOT recipients could provide at least a certain level of protection and in randomized clinical trials, COVID-19 vaccines were shown to substantially reduce the severity of COVID-19 (6, 7). Vaccination against COVID-19 was strongly encouraged but initially, patients receiving immunosuppressants were excluded from the randomized clinical trials (8, 9). For that reason, the development of protective immunity after COVID-19 vaccination in SOT recipients has been investigated mostly throughout observational and/or real-life evidence studies (10). While several studies evaluated the serological responses in patients receiving post-transplant medication, little is still known about the mechanisms of protective immunity and the duration of such protection (11–14). The interpretation of the vaccination efficacy based on serological responses bears certain limitations, such as highly variable antibody titers and kinetics among individuals (15, 16). The strict cutoff values that are being applied by most antibody tests may not efficiently classify the seropositivity as negative control values may overlap true positive low-titer values (17). Furthermore, it is crucial to investigate virus-neutralizing antibody responses in vaccinated individuals, including those after lung transplantation. Virus neutralizing antibodies serve as a reliable marker of protection against COVID-19 and were previously well-established in other viral infections (18, 19). Previous experiences with influenza vaccination in LuTx recipients reported pre-transplant patients being able to mount a more vigorous immune response as compared to LuTx recipients (20). Similarly, COVID-19 vaccination is currently recommended primarily in waitlisted patients to achieve a stronger humoral and cellular immune response (21). Even though the re-exposure may sustain protective antibodies, it is yet to be clarified which factors have affect the adaptive immune responses (15).

The fear of suboptimal immune response is often accompanied by the fear of severe adverse events in SOT recipients (22). Although the benefit of COVID-19 vaccination outweighs the risk of adverse events, several acute rejections have been reported in SOT recipients which raises a number of safety concerns (23–25). On the other hand, most of the COVID-19 studies in SOT recipients show no association between the vaccination and the development of organ rejection (22).

In this review, we attempted to address the main challenges of SARS-CoV-2 vaccination in LuTx recipients in the era of COVID-19. We have closely collected all available data on the immunogenicity, efficacy and safety of COVID-19 vaccines in LuTx recipients to provide a deeper insight into this complex and challenging issue.

METHODS

We conducted a comprehensive review of the literature on the immunogenicity, efficacy and safety of COVID-19 vaccines in LuTx recipients. COVID-19, lung transplantation, SARS-CoV-2, vaccine, and vaccination, were used as the keywords in the search strategy. The following databases were used: Medline/PubMed, Scopus, and Web of Science. Only English-written and peer-

reviewed studies that were published in indexed international journals were reviewed.

ANTI-COVID-19 VACCINATION IN LUNG TRANSPLANT RECIPIENTS: FIRST AND SECOND DOSES

Randomized clinical trials have evaluated the immunogenicity of diverse anti-SARS-CoV-2 vaccines, however, the efficacy of anti-SARS-CoV-2 vaccines in immunosuppressed patients, including those receiving anti-rejection drugs, remains to be clarified (10).

It has been already demonstrated that patients that were vaccinated while on the transplant waiting list were able to mount much higher anti-SARS-CoV-2 immune responses as compared to patients that were vaccinated after the transplantation (5, 21).

In a study by Narasimhan et al., evaluating serological responses to two doses of COVID-19 mRNA vaccine in LuTx recipients, only 25% of LuTx recipients mounted SARS-CoV-2 specific IgG response with 36% of responders in the Moderna cohort and 19% of responders in the Pfizer cohort (11). Also, the antibody titers in the LuTx recipients were significantly lower as compared to non-transplanted COVID-19-naïve subjects (11). These data suggested that in the majority of LuTx patients, receiving two doses of the anti-COVID-19 mRNA vaccine did not guarantee protection against a COVID-19 infection. Furthermore, the Moderna vaccine was found to generate a trend towards a more robust humoral response than the Pfizer vaccine in LuTx patients (7).

A prospective observational cohort study by Shostak et al. evaluated the immunogenicity and safety of the BNT162b2 vaccine in a total of 168 LuTx recipients (8). This was one of the first studies to investigate the generation of the humoral immune response after COVID-19 vaccination in LuTx recipients. Interestingly, the SARS-CoV-2 specific IgG antibodies were detected in only 4% of the study participants after the first dose, and in 18% of the study participants after the second dose of the mRNA vaccine. The participants who were receiving either antimetabolites or mammalian target of rapamycin (mTOR) inhibitors were less likely to develop sufficient antibody titers. These findings were in accordance with a study by Boyarsky et al. demonstrating diminished humoral responses in SOT recipients receiving antimetabolites (26). Despite the fact that neither the virus-neutralizing antibodies nor the cellular immune response was evaluated in the study by Shostak et al., the safety of the BNT162b2 vaccine in LuTx recipients was carefully assessed and only minor adverse events, such as injection site reactions, were observed.

Another study addressing both the humoral and cellular immune response to the BNT162b2 vaccine in 50 transplant patients (42 heart Tx, 7 LuTx, and one heart-lung Tx) was conducted by Schramm et al. (27) In this study, 96% of the study participants displayed non-detectable anti-SARS-CoV-2 antibodies 21 days after the first dose. Moreover, 90% of the SOT recipients elicited neither humoral nor cellular immune

response three weeks after the two doses of the BNT162b2 vaccine (27). Interestingly, in a small subgroup of transplant recipients where humoral response was non-detectable, a slight IFN- γ release was observed. Although these findings could indicate a possible T-cell mediated protection in SOT recipients after COVID-19 vaccination, the authors also state a possibility of cross-reactivity with a former coronavirus infection.

In a study by Hallet et al., vaccine-elicited immune responses of 103 LuTx recipients and 134 heart transplant recipients were evaluated (12). In this study, all tested subjects had dramatically decreased humoral responses as compared to the general population. Of 103 LuTx recipients, 9% responded to the first dose, 27% responded to the second dose and 64% were classified as non-responders. Heart transplant recipients were more likely to mount detectable humoral responses (12). In this study, SOT recipients receiving anti-metabolite maintenance immunosuppression therapy were less likely to mount protective antibody titers after vaccination and these diminished antibody responses were in dire contrast with clinical trials in the general population. Severe adverse events, such as acute rejection or anaphylaxis, were not reported throughout the study (8).

The immunogenicity of the BNT162b2 mRNA COVID-19 vaccine was also evaluated in 48 LuTx recipients where none of the study participants had detectable anti-SARS-CoV-2 specific IgG antibodies after the first, nor after the second vaccine dose (16). Interestingly, these results were in contrast with the LuTx recipients infected with COVID-19 where humoral responses were observed in 85% of the patients within 90 days following the COVID-19 diagnosis (28). However, only four out of twelve patients exhibited SARS-CoV-2 specific CD4⁺ and CD8⁺ T cells (28). The analysis of the safety outcomes has not been assessed in this study, however, one patient did not receive a second vaccine dose due to acute rejection.

The authors Yanis et al. correlated the humoral and cellular immune responses after two doses of COVID-19 mRNA vaccine in 56 SOT patients and 26 healthy individuals (29). In this study, four LuTx recipients were included, however, the majority of SOT types were kidney (41%) and liver (37%). The authors presented only 21.6% of the SOT patients eliciting comparable antibody responses to healthy individuals.

A single-center retrospective study by Bolinelli et al. was designed to evaluate the clinical effectiveness of COVID-19 vaccines in LuTx patients by assessing the respiratory symptoms, oxygen saturations, imaging findings and other clinical variables, in SARS-CoV-2 positive LuTx patients with/without COVID-19 vaccination (30). The study has shown lower levels of inflammatory markers during the acute phase of COVID-19 and lower risk of COVID-19 complications, such as respiratory failure or ventilation need, in vaccinated individuals.

ANTI-COVID-19 VACCINATION IN LUNG TRANSPLANT RECIPIENTS: THIRD DOSE

Since the two doses of COVID-19 vaccine did not elicit strong immune responses in SOT recipients, the administration of the

third vaccine dose was initiated. Subsequently, several observational studies reported the immunogenicity and safety of the third dose in LuTx recipients (28).

In a study by Kamar et al., 101 consecutive SOT recipients were given a third dose of the BNT162b2 mRNA vaccine. In this study, eight LuTx recipients were included and the administration of a third dose significantly promoted the increase in antibody titers and was proven as a reliable booster of the vaccine immunogenicity (31). Patients with only limited humoral response were mostly older and had a higher degree of immunosuppression. The authors, however, highlight that although the vaccine immunogenicity was significantly improved with the third dose, the clinical efficacy in SOT recipients is yet to be clarified. Therefore, SOT recipients should still avoid COVID-19 exposure and encourage their relatives to receive an anti-COVID-19 vaccination (19).

The authors Hall et al. conducted a randomized clinical trial in 120 SOT recipients to evaluate the immunogenicity of the third dose of the vaccine compared to a placebo. In this study, the third-dose booster substantially improved the immunogenicity of the mRNA-1273 vaccine (32). In addition, the median percent virus neutralization was 71% in patients receiving the Moderna vaccine as compared to 13% in patients receiving the placebo. The third dose administration was safe and well-tolerated in all SOT study participants. The authors reported neither cases of acute organ rejection nor serious adverse events in the SOT recipients within the study period (20).

Since there are currently only limited data on the efficacy, immunogenicity and safety of the third dose of anti-COVID-19 vaccine in LuTx recipients, promising results demonstrated by Peled et al. may further promote attempts to evaluate these outcomes in individuals after lung transplantation (21). In this study, the humoral response increased from 23% to 67% after the third dose of the BNT162b2 vaccine in heart transplant recipients (33). Moreover, the vaccination with a third dose was not associated with any severe side effects, nor with organ rejection (33).

Safety concerns regarding the third dose application were raised in a review by Ison et al. (34) The study by Ou et al. demonstrated an increased risk of organ rejection in a cohort receiving three doses of COVID-19 vaccine as compared to the two-dos cohort. However, the conclusions were based on one case of rejection per cohort (23).

DISCUSSION

Vaccination against COVID-19 is an essential step in the prevention of severe COVID-19 infection in LuTx recipients (28). Currently, LuTx candidates are recommended to undergo vaccination while on the transplant waiting list (5, 21). These recommendations stem from the fact that the COVID-19 vaccine immunogenicity, efficacy and safety were shown to be greater in the pre-transplant patients as compared to the SOT recipients (5, 8, 35). Similar findings were previously reported in LuTx patients receiving influenza vaccination prior transplantation (20, 36).

The immunogenicity of anti-SARS-CoV-2 vaccines in patients receiving anti-rejection drugs varies (10). Several

studies report a proportion of SOT recipients being able to mount sufficient titers of SARS-CoV-2 specific IgG antibodies, while other studies show that more than 90% of the SOT recipients elicit neither humoral nor cellular immune response after vaccination (27). Furthermore, SOT recipients receiving antimetabolites were shown to have rather limited humoral responses to COVID-19 vaccination (8, 26).

LuTx recipients were less likely to develop humoral immune response as compared to healthy individuals, but also as compared to heart transplant recipients (12). However, SARS-CoV-2 reactive T cells were observed in a small number of LuTx recipients (28).

Interestingly, the third booster dose of the COVID-19 vaccines was shown so far to elicit strong immune responses and may, thus, represent a potent tool in the prevention of severe COVID-19 infection in SOT recipients (33). The safety concerns regarding COVID-19 vaccines in LuTx recipients seem to be outweighed by the benefits of vaccination, and rare cases of organ rejection in SOT recipients do not provide a rationale for withholding the vaccination (22).

The clinical efficacy of COVID-19 vaccines in LuTx recipients is yet to be elucidated. To date, large cohort studies attempted to provide real-life evidence of COVID-19 vaccines efficacy in

diverse SOT recipients. These studies show almost 80% reduction of symptomatic COVID-19 illness in vaccinated individuals (37). However, in lung transplantation, more data are needed to understand the extent of immune protection.

AUTHOR CONTRIBUTIONS

MS, RN, JV and ZS contributed to the conceptualization, data curation, and writing of the original draft. MS, RN, RL, JV and ZS contributed to the review and editing of the final draft. All authors contributed to the article and approved the submitted version.

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5. Závěr

Včasná diagnostika, predikce na základě rizikových faktorů a zejména prevence PGD, ACR a CLAD jsou klíčové pro dosažení optimálních výsledků přežití a kvality života pacientů po transplantaci plic.

Náš výzkum přináší nové perspektivy v diagnostice ACR s využitím imunohistochemie. Zároveň může v budoucnu sloužit jako jeden z klíčových projektů pro další explorační roli checkpoint inhibitorů v patofyziologii ACR.

V klinické části práce jsme poukázali na přínosy spojené s uchováním dárcovských plic v optimálních teplotních podmínkách a na to, že ani dlouhá studená ischemie (nad 15 hodin) při optimální teplotě nemusí být asociována s vyšším rizikem rozvoje těžké PGD.

Naše data navíc podpořila trend snižování dávek antikoagulace při ECMO podpoře. Prokázali jsme, že vhodně zvolenou antikoagulační strategií při použití ECMO během transplantace lze minimalizovat i riziko PGD.

Poukázali jsme také na nutnost detailnějšího zkoumání viability viru při akceptaci dárcovských orgánů. Dále jsme zhodnotili dostupné studie ohledně vakcinace proti onemocnění COVID-19 u transplantovaných pacientů plic s cílem předejít možným rejekčním komplikacím.

6. Vlastní publikace

Níže je uveden přehled publikací autora aktuální k datu odevzdání disertační práce.

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