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Gamma-glutamyltransferase is a strong predictor of secondary sclerosing cholangitis after lung transplantation for COVID-19 ARDS

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KEYWORDS:
Covid-19;
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BACKGROUND: Lung transplantation (LTx) can be considered for selected patients suffering from COVID-19 acute respiratory distress syndrome (ARDS). Secondary sclerosing cholangitis in critically ill (SSC-CIP) patients has been described as a late complication in COVID-19 ARDS survivors, however, rates of SSC-CIP after LTx and factors predicting this detrimental sequela are unknown.

METHODS: This retrospective analysis included all LTx performed for post-COVID ARDS at 8 European LTx centers between May 2020 and January 2022. Clinical risk factors for SSC-CIP were analyzed over time. Prediction of SSC-CIP was assessed by ROC-analysis.

Abbreviations: ALAT, Alanine aminotransferase; ALP, Alkaline phosphatase; ARDS, Acute respiratory distress syndrome; ASAT, Aspartate aminotransferase; AUC, Area under the curve; COVID-19, Coronavirus disease 2019; ECLS, Extracorporeal live support; ECMO, Extracorporeal membrane oxygenation; GGT, Gamma-glutamyltransferase; ICU, Intensive care unit; IQR, Inter-quartile range; LAS, Lung allocation score; LTx, Lung transplantation; mg/dL, Milligrams per deciliter; PEEP, Positive end-expiratory pressure; PGD, Primary graft dysfunction; POD, Post-operative day; ROC, Receiver operating characteristic; SSC-CIP, Secondary sclerosing cholangitis in critically ill patients; TBi, Total bilirubin; U/L, Units per liter

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RESULTS: A total of 40 patients were included in the analysis. Fifteen patients (37.5%) developed SSC-CIP. GGT at the time of listing was significantly higher in patients who developed SSC-CIP (median 661 (IQR 324-871) vs 186 (109-346); $p = 0.001$). Moreover, higher peak values for GGT (585 vs 128.4; $p < 0.001$) and ALP (325 vs 160.2; $p = 0.015$) were found in the 'SSC' group during the waiting period. Both, GGT at the time of listing and peak GGT during the waiting time, could predict SSC-CIP with an AUC of 0.797 (95% CI: 0.647-0.947) and 0.851 (95% CI: 0.707-0.995). Survival of 'SSC' patients was severely impaired compared to 'no SSC' patients (1-year: 46.7% vs 90.2%, log-rank $p = 0.004$).

CONCLUSIONS: SSC-CIP is a severe late complication after LTx for COVID-19 ARDS leading to significant morbidity and mortality. GGT appears to be a sensitive parameter able to predict SSC-CIP even at the time of listing.

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Lung transplantation (LTx) is an established treatment option for end stage chronic lung diseases. In addition, LTx has recently been established as a last resort in patients suffering from acute respiratory distress syndrome (ARDS) who do not recover despite several weeks of extra-corporeal membrane oxygenation (ECMO).[1] The detrimental coronavirus disease 2019 (COVID-19) pandemic has resulted in an increase of ventilator- and ECLS-dependent ARDS cases worldwide. As a result, ARDS has become an important indication for LTx. In recent reports, it represented 7-29% of the transplant volume in institutions offering transplantation for ARDS.[2-4]

Secondary sclerosing cholangitis in critically ill patients (SSC-CIP) is a rare complication following ARDS and entails severe morbidity and mortality. It is marked by increased total bilirubin (TBi), gamma-glutamyltransferase (GGT) and alkaline phosphatase (ALP) reflecting cholestatic liver injury.[5] SSC-CIP has been recognized as an underdiagnosed and underreported disease entity associated with intensive care treatment in different settings such as sepsis, shock, trauma and burns.[6,7] It can affect the intra- and extrahepatic biliary tree and usually evolves rapidly to biliary cirrhosis. The prognosis of SSC-CIP is poor.[8] In few cases, the disease can be stabilized by medical and endoscopic treatment, however the most severe cases require liver transplantation, when possible, in the overall clinical context. Without liver transplant, survival is limited to 55% at 1 year and 14% at 6 years.[9] The median survival for SSC-CIP is only 13 months, and thereby significantly lower than median survival rates of other forms of SSC.[10] Several risk factors of SSC-CIP have been proposed, including sepsis/SIRS, ischemia/hypoxia, prolonged prone positioning and abdominal obesity, ECMO and potentially hepatotoxic medication.[6,11,12] In critically ill COVID-19 patients, liver injury has been a widely reported extrapulmonary manifestation with deranged liver parameters in 10-58% of hospitalized patients.[13,14] Severe cholestatic liver dysfunction resembling SSC-CIP has also been observed in COVID-19 patients without the need for ECMO treatment.[15-19] Several studies have shown a direct damage of SARS-COV-2 on the small bile ducts.[20,21]

LTx for post-COVID-19 ARDS has shown promising short- and mid-term survival.[2,22] Several centers have reported liver damage with features of SSC-CIP as a late complication in these patients, but comprehensive data are still lacking. In this multicenter-study, we therefore aimed to (i) analyze the incidence of SSC-CIP in patients after LTx for COVID-19 ARDS and to (ii) explore predictive factors which could aid in patient selection and therefore avoid this severe complication.

Patients and methods

We retrospectively analyzed double LTx performed for post-COVID-19 ARDS within the Eurotransplant (ET) region between May 2020 and January 2022. The ET area covers 138 million inhabitants across 8 countries and currently has an annual lung transplant activity of around 1200 LTx in 22 centers. Eight of 22 ET-LTx centers performed LTx for post-COVID-19 ARDS patients within the study period. All 8 agreed to participate in this study and contributed patient data for transplanted patients for this analysis. Ethics approval was granted by the institutional review board of the Medical University of Vienna (EK-Nr 1528/2021) and the participating institutions.

The cohort was divided into 2 groups. Group 'SSC' included patients with clinically suspected SSC or SSC proven by endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography.[23] All other patients formed group 'no SSC'.

Management of COVID-19 ARDS patients

In all centers, the primary ECMO configuration of choice was VV ECMO either in femoro-femoral or femoro-jugular configuration or a double-lumen cannula. The type of cannulation was dependent on the preference of the treating intensive care teams, patient characteristics (thrombosis, anatomical situation) or expectation to achieve awake bridging. VA or VVA configurations were employed in case of additional hemodynamic instability.

For anticoagulation during ECMO bridging, either subcutaneous low-molecular weight heparin twice daily with a target antiXa

at 4 hours of 0.4 to 0.7 or intravenous unfractionated heparin with a target aPTT of 60 to 80 sec was used. In case of heparin-induced thrombocytopenia, argatroban was used.

All centers used lung-protective low tidal volume ventilation strategy as their standard for COVID-19 ARDS patients. This involved volume-limited or pressure controlled ventilation mode aiming for tidal volumes of ≤ 6 ml/kg ideal body weight, driving pressure limited to 15 cm H2O, and a target peak pressure of ≤ 30 cm H2O. Prone positioning was employed by all centers.

All centers aimed to bridge patients to transplantation in an awake. Otherwise, sedative medications included a combination of propofol and remifentanil (mainly employed in the first 7 days), midazolam, sufentanil, esketamine or dexmedetomidine (mostly used after the first 7 days) according to patient requirements.

Donor data

Each center provided basic donor data retrieved from the Eurotransplant donor registry. In addition, the Oto score[24] was calculated for each donor. This scoring system includes donor age, smoking history, chest radiograph assessment, bronchial secretions observed in bronchoscopy and paO2/FiO2 ratio. Lower numbers correspond to favourable donor characteristics.

Recipient data

Recipient data included basic demographic parameters, details on the course of COVID-19 disease and specific treatment, details on mechanical ventilation and ECLS bridging, data of the transplant procedure as well as perioperative data. Liver serum biochemistry parameters at the time of listing and at the time of transplantation were collected. In addition, the maximum pre- as well as post-transplant values were assessed. Liver parameters included TBi in milligrams per deciliter (mg/dL), alanine aminotransferase (ALAT) in units per liter (U/L), aspartate aminotransferase (ASAT) in U/L, GGT in U/L and ALP in U/L.

Outcome parameters

Early recipient outcome analysis included primary graft dysfunction (PGD) grades at T0, T24, T48 and T72 hours, length of post-transplant intensive care unit (ICU) stay and length of total hospital stay. PGD was graded according to the current guidelines of the International Society for Heart and Lung Transplantation.[25] Patients with postoperatively prolonged ECMO support were graded as PGD 3 or PGD 'ungradable' depending on the chest X-ray. Total length of mechanical ventilation was defined as the time to successful extubation without early reintubation (<3 days). In case of tracheostomy, length of mechanical ventilation was defined as the time when the patient tolerated mere oxygen insufflation without any mechanical breathing assistance for more than six continuous hours. Furthermore, postoperative complications and in-hospital mortality were recorded.

Statistical analysis

Statistical analysis was performed in IBM SPSS 26 (*IBM Analytics, Armonk, NY*). P-values below 0.05 were considered statistically significant. Missing data (only single data points with a random pattern) were appropriately coded and missing cases were excluded from each respective sub analysis. Continuous variables were reported as means ± standard deviation or medians with interquartile ranges (IQR) of 25% to 75% and compared using *t*-tests or Mann-Whitney-U-test according to distribution. Chi-square test or Fisher's exact test used for categorical variables. Comparison of PGD rates as well as in-hospital mortality was performed with Pearson's chi-square test or Fisher's exact test where applicable. Long-term outcome was analyzed by Kaplan-Meier curves and log-rank tests. For parameters significantly different between the 'SSC' group and the 'no SSC' group at listing and for the peak value between listing transplantation, receiver-operating characteristics (ROC) curve analysis was performed and the area under the curve (AUC) calculated. Sensitivity and specificity were determined according to the ROC curve coordinates and optimal thresholds assessed using the Youden index. Figures were created using GraphPad Prism 8 (*GraphPad Software, La Jolla, CA*).

Results

Recipient parameters

The patient cohort consisted of cases from Austria (n = 23), Germany (n = 8), Slovenia (n = 5), Belgium (n = 3) and the Netherlands (n = 1). Recipient data of both groups are described in detail in Table 1. Basic demographic parameters were similar. Median age was 57 years (IQR 42-61) in the SSC group compared to 54 years (IQR 44.5-56) in patients without SSC (p = 0.376). Patients who developed SSC were intubated after a median of 11 days (IQR 9-18) after diagnosis of COVID-19 compared to 12 days (8-20) for 'no SSC' patients (p = 0.847). Bridging by veno-venous ECMO was employed in a sedated state in 14 (93.3%) patients in the 'SSC' group and 1 patient (6.7%) was bridged awake. In the 'no SSC' group, 20 patients (80%) were sedated during ECMO and 4 (16%) were awake, while 1 patient (4%) did not require ECLS bridging. The majority of patients were bridged by veno-venous ECMO, 1 in the 'SSC' group was switched to veno-arterial and 1 patient in each group was bridged with a veno-veno-arterial ECMO configuration. Median time between confirmed disease and start of ECLS bridging was 18 days (IQR 15-41) in 'SSC' and 25 days (16-29) in 'no SSC' patients (p = 0.649). At least one ECMO-related complication was reported in 17 cases (42.5%) overall. Five centers reported episodes of bleeding, 2 of hemothorax, 1 of ECMO circuit clotting and 2 of vein thrombosis. Three centers reported no complications associated with ECMO. COVID-19-specific treatment included corticosteroids for the majority of patients, 3 patients received convalescent plasma, 3 remdesivir and 3 tocilizumab without significant differences between groups. Patients were listed for LTx at a median of 49.5 (IQR 41-62) vs 57.5 (IQR 49-72) days after initial COVID-19 diagnosis. All but 4 patients qualified for a lung allocation score (LAS) above 50 points (high LAS), with a median of 88.8 (IQR 85-91) in 'SSC' and 86.1 (72-91) in 'no SSC' cases, respectively. At the time of listing, 2 patients (13%) in the 'SSC' group and 1 in the 'no SSC' had estimated GFR levels below 60 ml/min/1.73m^2.

Table 1 Recipient Demographics and Perioperative Parameters

	SSC *n* = 15	no SSC *n* = 25	*p*-value
Age (median; IQR)	57 (42 – 61)	54 (44.5 – 56)	0.376
Sex (m%/f%)	80%/20%	76%/24%	0.999
BMI (median; IQR)	25 (24 – 28)	25 (23 – 28)	0.956
Onset to ICU (median; IQR)	8 (7 – 15)	8 (6 – 12)	0.999
Onset to intubation (median; IQR)	11 (9 – 18)	12 (8 – 20)	0.847
Tracheostomy (*n*; %)	13 (87%)	20 (83%)	0.999
Septicaemia during ICU stay (*n*; %)	7 (47%)	10 (40%)	0.749
CRRT during ICU stay (*n*; %)	2 (13%)	2 (8%)	0.631
ECLS bridging (*n*; %)			
- None	0 (0%)	1 (4%)	
- Sedated	14 (93%)	20 (80%)	0.484
- Awake	1 (7%)	4 (16%)	
Onset to ECLS (*n*; %)	18 (15 – 41)	25 (16 – 29)	0.665
Onset to listing (median; IQR)	49.5 (41 – 62)	57.5 (49 – 72)	0.192
LAS (median; IQR)	88.8 (85 – 91)	86.1 (72 – 91)	0.255
Transfusions of pRBC pre-Tx (median; IQR)	4 (2 – 8)	6.5 (2 – 16)	0.491
eGFR <60 at time of listing (*n*; %)	2 (13%)	1 (33%)	0.545
CRP at time of listing (median; IQR)	7 (3 – 12)	11 (6 – 17)	0.069
Waiting time (median; IQR)	7.5 (6 – 12)	6.5 (3 – 16)	0.525
Type of transplant (*n*; %)			
- whole lungs	7 (47%)	19 (76%)	
- Size reduction	7 (47%)	2 (8%)	0.017*
- Lobar Tx	1 (6%)	4 (16%)	
Intraoperative type of ECLS (*n*; %)			
- Central VA	6 (40%)	13 (52%)	
- Central VA and peripheral VV	7 (46%)	5 (20%)	
- VVA	1 (7%)	1 (4%)	0.095
- Peripheral VV	0 (0%)	6 (24%)	
- Peripheral VA	1 (7%)	0 (0%)	
Duration of surgery (median; IQR)	378 (306 – 478)	378 (335 – 481)	0.399
Surgical acess (*n*; %)			
- Clamshell	13 (87%)	18 (72%)	0.440
- Bilateral thoracotomies	2 (13%)	7 (28%)	
Mean ischemic time (median; IQR)	410 (330 – 467)	406 (375 – 445)	0.907
Intraoperative pRBC (median; IQR)	8 (6 – 9)	8 (5 – 10)	0.934
Intraoeprative FFP (median; IQR)	9 (5 – 13)	8 (4 – 13)	0.942
PostOP ECMO prolongation (*n*; %)	4 (26.7%)	4 (16%)	0.444
Primary graft dysfunction at 72 hours (*n*; %)			
- PGD 0	14 (93%)	16 (64%)	
- PGD 1	1 (7%)	5 (20%)	
- PGD 2	0 (0%)	2 (8%)	0.205
- PGD 3	0 (0%)	0 (0%)	
- PGD ungradable	0 (0%)	2 (8%)	
PostOP LMV (median; IQR)	15 (10 – 41)	15 (7 – 27)	0.377
PostOP ICU stay (median; IQR)	34.5 (27 – 52)	36 (19 – 51)	0.704

(Abbreviations**:** BMI, Body-mass index; CRP, C-reactive protein; CRRT, Continuous renal replacement therapy; ECLS, Extracorporeal life support; ECMO, Extracorporeal membrane oxygenation; eGFR, Estimated glomerular filtration rate; FFP, Fresh frozen plasma; ICU, Intensive care unit; IQR, Inter-quartile range; LAS, Lung allocation score; LMV, Length of mechanical ventilation; PGD, Primary graft dysfunction; PostOP, Postoperative; pRBC, Packed red blood cells; SSC, Secondary sclerosing cholangitis; Tx, Transplantation; VA, Venoarterial; VV, Venovenous; VVA, Venovenoarterial)

Median waiting time was 7.5 (6-12) days in the 'SSC' group and 6.5 (3-16) days in the 'no SSC' group. Liver imaging was performed in all patients pre-transplant as a part of the evaluation examinations. Abdominal computed tomography was performed in 10 cases (25%), sonography in 10 cases (25%) and 20 patients (50%) received both. Abnormalities concerning liver and biliary system were found in 5 cases (33.3%) of the SSC group and 7 (28%) of the no SSC group. These findings were generally unspecific. In the 'SSC' group, 1 patient had liver steatosis with cholecystolithiasis, 1 liver steatosis without any sign of cholangiectasis and 2 had a slightly enlarged common hepatic duct with minor intrahepatic cholangiectasis, but no concrement. In the 'no SSC' group, 4 cases of biliary sludge without cholangiectasis, 2 cases of hepatomegaly and 1 case of contrast enhancement of the gall bladder

Table 2 Donor Parameters

	SSC *n* = 15	no SSC *n* = 25	*p*-value
Age (median; IQR)	50 (38 – 60)	51 (42 – 58)	0.659
Aspiration (*n*; %)	4 (33%)	5 (22%)	0.685
Trauma (*n*; %)	2 (13.3%)	4 (16%)	0.999
Oto score (median; IQR)	6 (3 – 8)	4 (2.5 – 5)	0.163
Time on ventilation (hours) (median; IQR)	96 (45 – 249)	77 (50 – 204)	0.761
Donation type (*n*; %)			
- DBD	13 (87%)	24 (96%)	0.545
- DCD	2 (13%)	1 (4%)	
Cause of death (*n*; %)			
- Cerebral ischemia	3 (20%)	2 (8%)	0.597
- Intracerebral bleeding	8 (53%)	16 (64%)	
- Traumatic head injury	4 (27%)	6 (24%)	
- Other	0 (0%)	1 (4%)	
PaO2 at 1.0 FiO2 (median; IQR)	405 (378 – 480)	433 (363 – 477.5)	0.740
PaCO2 at 1.0 FiO2 (median; IQR)	37.8 (34 – 42)	41.4 (38 – 45)	0.033*

(Abbreviations**:** DBD, Donation after brain death; DCD, Donation after circulatory death; FiO2, Fraction of inspired oxygen; IQR, Inter-Quartile Range; PaCO2, Arterial partial pressure of carbon dioxide; PaO2, Arterial partial pressure of oxygen; SSC, Secondary sclerosing cholangitis).

were found. Clamshell incision was used as surgical access in the majority of patients in both groups (87% and 72%, respectively). Median surgical time was 378 min in both groups ($p = 0.399$). Cold ischemic time as an average of left and right side was similar (SSC: median 410 min (IQR 330-467); no SSC: median 406 min (375-445); $p = 0.907$). Intraoperative requirement of packed red blood cells and fresh frozen plasma concentrates were comparable ($p = 0.934$ and $p = 0.942$, respectively). Four patients (26.7%) in the 'SSC' group and 4 patients (16%) in the 'no SSC' group required post-operative prolongation of ECMO. Early graft performance was good in both groups. Of the 15 'SSC' patients, 14 (93%) had PGD 0 or 1 (7%) in PGD 1 at 72 hours after transplantation. In the 'no SSC' group, 16 (64%) were graded as PGD 0, 5 (20%) as PGD 1, 2 (8%) as PGD 2 and 2 (8%) as ungradable. Post-transplant length of mechanical ventilation (median 15 vs 15 days; p=0.377) and length of ICU stay (median 37 vs 35 days; $p = 0.406$) were similar. A center-specific breakdown of recipient parameters is presented in Suppl. Table 1.

Donor parameters

Donor characteristics are shown in Table 2. Overall, there were no significant differences in important donor parameters between patients who developed SSC and those who did not. Donor age, cause of death, history of aspiration or trauma and duration of ventilation before explantation were similar in both groups. The SSC group included 2 donors after circulatory death (13.3%) while it was 1 (4%) in the 'no SSC' group ($p = 0.545$). Oto scores in donors of 'no SSC' recipients with a median of 4 (IQR 2.5-5) were comparable to the SSC group. (median 6 (IQR 3-8)) ($p = 0.545$). PaO2/FiO2 ratios were similar in both groups ($p = 0.740$). PaC02 was significantly lower in the SSC group (median 37.8; IQR: 34-42; $p = 0.033$).

Liver serum biochemistry

Levels of bilirubin and liver enzymes at various time points are shown in Figure 1. At the time of listing, TBi ($p = 0.244$), ASAT ($p = 0.201$), ALAT ($p = 0.319$) and ALP ($p = 0.133$) did not show significant differences between groups. In contrast, median GGT was elevated up to 661 U/L (IQR 324-871) in patients who later developed SSC, compared to 186 U/L (IQR 109-346) in those without cholestatic disease ($p = 0.001$). Peak levels of TBi ($p = 0.160$), ASAT ($p = 0.058$) and ALAT (0.294) between listing and transplantation were not associated with a later SSC development. However, higher peak values during the waiting period for GGT (median 585 vs 128.4; $p < 0.001$) and ALP (median 325 vs 160.2; $p = 0.015$) were found in the 'SSC' group compared to the 'no SSC' group. At time of transplantation, all liver parameters were significantly higher in patients who later developed SSC compared to those who did not. Median TBi was 2.32 mg/dL (IQR 0.8-4.38) in the 'SSC' group vs 0.53 mg/dL (IQR 0.34-0.93) in the 'no SSC' group ($p = 0.001$). Both median ASAT (85 U/L (IQR 65-107) vs 31 U/L (26-52); $p < 0.001$) and ALAT (74 U/L (IQR 44-147) vs 28 U/L (17-47); $p < 0.001$) were higher in 'SSC' patients. Furthermore, GGT (585 U/L (IQR 412-1034) vs 128.4 U/L (IQR 47-352); $p < 0.001$), as well as ALP (325 U/L (IQR 227-856) vs 160.2 U/L (IQR 95-237); $p < 0.001$) were significantly increased. Hepatology was consulted for elevated liver serum biochemistry in one case pre-transplant who developed SSC after transplantation. Since GGT at time of listing and peak GGT during the waiting period were significantly associated with the later diagnosis of a SSC, we determined their individual predictive value with ROC curve analysis (Figure 2). GGT at the time of listing predicted SSC with an AUC of 0.797 (95% CI: 0.647-0.947). The optimal threshold value was 320 U/L, with a sensitivity of 80% and a specificity of 72%, resulting in a Youden index of 0.52.

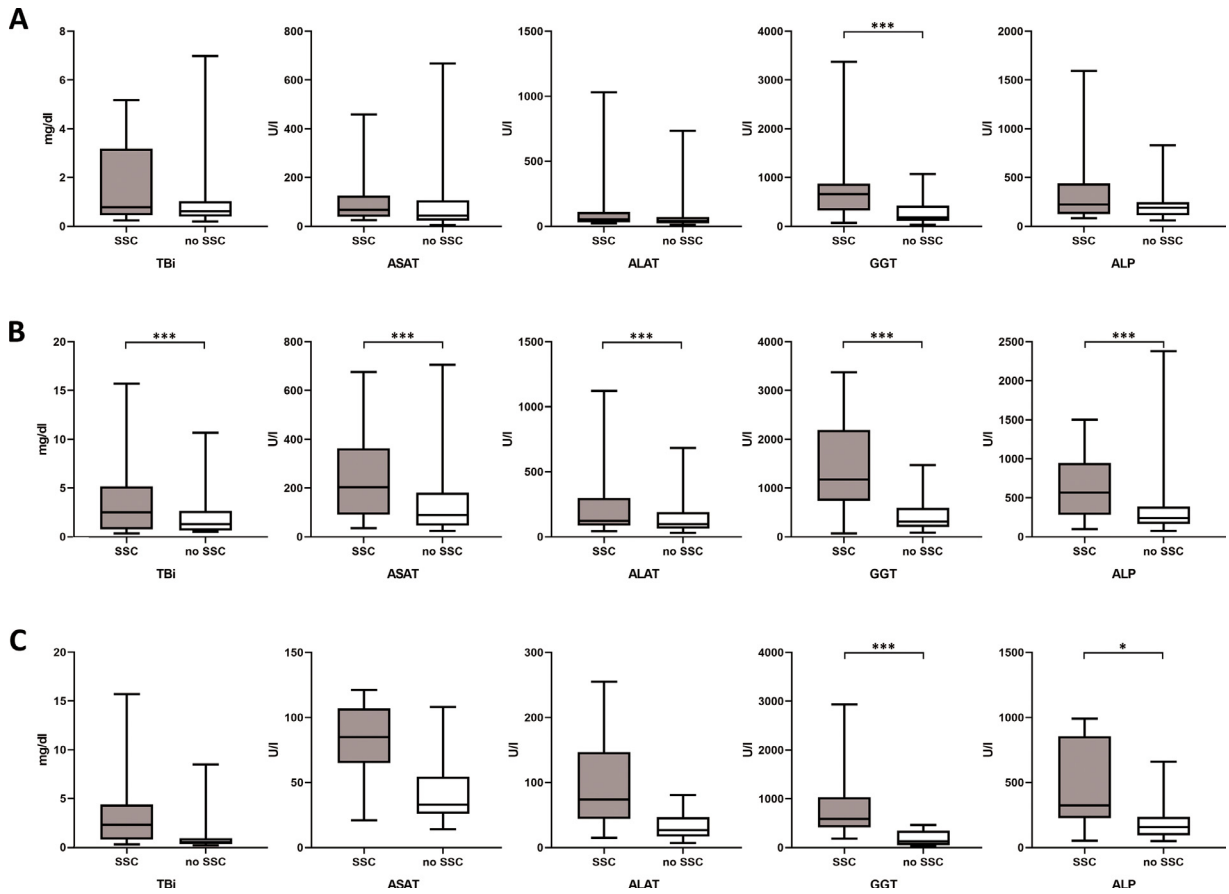

Figure 1 Liver serum biochemistry A – at time of listing B – highest value between onset and transplantation C – at time of transplantation * ($p \leq 0.05$); ** ($p \leq 0.01$); *** ($p \leq 0.001$); (Abbreviations: ALAT, Alanine aminotransferase; ALP, Alkaline phosphatase; ASAT, Aspartate aminotransferase; GGT, Gamma-glutamyltransferase; TBi, Total bilirubin; SSC, Secondary sclerosing cholangitis).

Peak GGT during the waiting period also provided an excellent prediction with an AUC of 0.851 (95% CI: 0.707-0.995). An optimal cut-off value was 633 U/L, where the sensitivity was 87%, the specificity was 81%, with a Youden index of 0.68. Various sensitivity and specificity values are given in Suppl. Table 2.

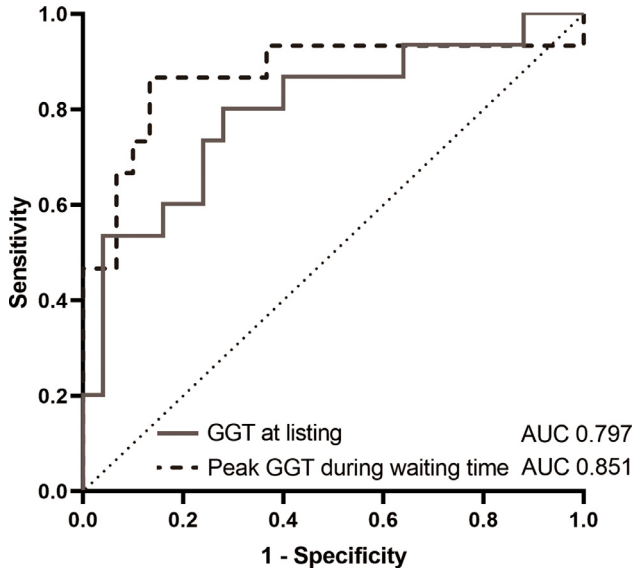

Figure 2 ROC curves for GGT at time of listing and for highest GGT between onset and transplantation (Abbreviations: AUC, Area under the curve; GGT, Gamma-glutamyltransferase).

Outcomes

Short-term outcomes were similar between groups (Table 1). At 72 hours after transplantation, most patients had PGD 0 and no PGD 3 was observed at this point. Two patients (8.0%) in the 'no SSC' group were ungradable due to prolonged ECMO while showing clear chest radiographs. Median post-operative length of mechanical ventilation was 15 days in both groups ($p = 0.377$). Median post-transplant ICU stay was also similar with 34.5 days (IQR 27-52) in surviving 'SSC' and 36 days (19-51) in 'no SSC' patients. The diagnosis of SSC was triggered by clinical suspicion due to jaundice and exceedingly elevated cholestasis parameters in 3 patients (20%) but confirmed by ECRP and/or MRCP in the majority of patients. The morbidity and mortality this diagnosis entailed was significant. Four patients (26.7%) recovered, while GGT and ALP still remained elevated. One patient (6.7%) is currently being evaluated for liver transplantation (POD 237), one did not reach transplantation and died on the waiting list (POD 157), one is currently on the liver waiting list while also requiring kidney dialysis due to cholemic nephropathy (POD 300) and one patient successfully received liver

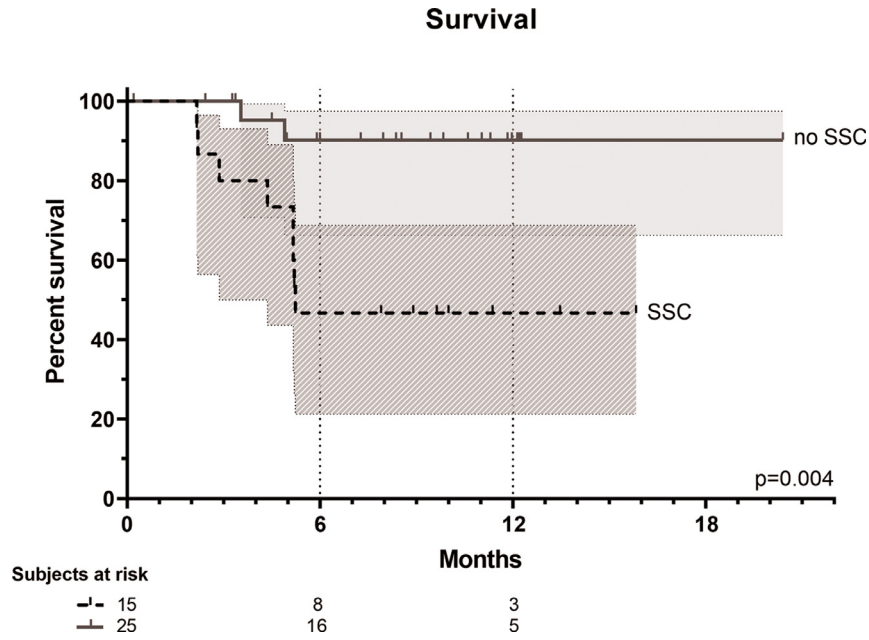

Figure 3 Kaplan-Meier survival with 95% confidence intervals (Abbreviations: AUC, Area under the curve; GGT, Gamma-glutamyltransferase; SSC, Secondary sclerosing cholangitis;).

transplantation 216 days after the initial LTx. In 5 of 8 patients who died after transplantation, the cause of death was SSC. Figure 3 shows the 1-year survival for patients in the 'SSC' group (47%) and the 'no SSC' patients (90%) (log-rank: $p = 0.004$).

Discussion

SSC-CIP is a known complication of COVID-19 ARDS patients surviving the ICU.[15-17] With increasing experience in salvage LTx for this patient group, a growing number of centers have also observed SSC-CIP. However, data is lacking, given the rarity of SSC-CIP in general and the limited number of COVID-19 ARDS patients transplanted worldwide to date. To the best of our knowledge, this multi-center study is the first to examine this entity in patients transplanted for ARDS. Our results suggest that i) the incidence of SSC-CIP in LTx for COVID-19 ARDS is substantial and entails significant non-graft-related morbidity and mortality and ii) GGT is a sensitive early biomarker that could be useful for patient selection.

While long-term outcomes remain to be assessed, the reported early- and mid-term outcomes reported for LTx in COVID-19 ARDS are encouraging.[22,26] The largest single-center experience to date has recently been published by Kurihara et al The authors showed a short-term survival of 100% with a median follow-up of 351 days in a cohort of 30 patients.[2] Roach et al reported the largest case series to date in a registry study of the UNOS database. Their cohort consisted of 140 COVID-19 ARDS patients and 74 COVID-19 associated pulmonary fibrosis patients and showed a 3-month survival of 95.6%.[3] Looking at early outcomes and graft performance, the results in our study cohort corroborate the concept of LTx for ARDS. Both study groups had initially successful courses with no perioperative mortality and survival was excellent in patients who did not develop SSC-CIP.

Patients transplanted due to COVID-19 are not a homogenous group and two phenotypes can be distinguished. Patients unweanable from ECLS with chronic fibrotic parenchymal changes seen after COVID-19 represent a less complex group. Excellent post-transplant outcome similar to idiopathic pulmonary fibrosis can be achieved. On the other hand, the setting of acute critical illness with necrotizing lungs, bacterial superinfection, pleural empyema and frequent episodes of bacteremia poses more surgical and peri-operative challenges. Our cohort predominantly represents this latter phenotype. Naturally, these complex patients are more prone for complications, among them hepatic problems. SSC-CIP has been previously described in non-COVID-19 ARDS[11,12,27] as well as COVID-19 associated critical illness.[15-17] Several risk factors have been proposed. Ischemic injury to the biliary system is thought to be an important underlying factor for SSC-CIP.[6] While ARDS itself constitutes a hypoxic situation to the organism, aggressive intensive care treatment can further contribute to these effects. Sustained high PEEP values are thought to impair splanchnic perfusion.[28] Of note, high PEEP levels are often applied as a part of lung-protective ventilation concepts with low tidal volumes and are very commonly used in COVID-19 ARDS patients. Similarly, prolonged prone positioning has been employed almost universally to improve oxygenation in these patients. However, prone positioning is thought to negatively impact blood supply to the liver and biliary system, especially in obese patients.[12] Ketamine has been widely used in COVID-19 patients as an additional sedative. It has been proposed as an important risk factor for SSC-CIP.[29] Especially high cumulative ketamine doses have been suspected to cause progressive cholangiopathy.[30] Interestingly, all 15 patients in the 'SSC'

cohort received ketamine pre-transplant, while only 50% of 'no SSC' patients did. Unfortunately, data on cumulative doses could not be collected due to the retrospective nature of this study. The severely destroyed lung parenchyma in ARDS is highly susceptible to recurring bacterial or fungal superinfections, potentially leading to septicaemia and potentially septic shock. Moreover, hepatotoxic antibiotics and antimycotics often cannot be avoided, which may add additional risk factors for the development of cholestatic liver injury.[5] In our cohort, all patients received various potentially hepatotoxic antibiotics or antimycotics over the course of the pre-transplant ICU treatment with no apparent differences between groups. In patients with severe elevations in serum liver parameters, these medications were avoided post-transplant as far as possible. While pre-transplant bridging with VV ECMO has become a routine in LTx,[31] the pandemic has led to very long bridging periods. Current guidelines for LTx in COVID-19 emphasize evaluating the potential for native lung recovery and suggest at least 4 weeks on optimal treatment including ECLS until considering listing.[32] As VV ECMO has been described as a risk factor for SSC-CIP, these prolonged ECMO-bridging durations could also contribute to its high incidence in LTx patients after COVID-19.[33] In our study, the vast majority of patients were bridged using VV ECMO in a femoro-jugular configuration. We found no association of ECMO mode and cannulation site with SSC.

In addition to the above mentioned risk factors, extrapulmonary viral effects of COVID-19 itself could be an important contributor to the development of SSC-CIP. Liver function impairment has been widely reported in critically ill COVID-19 patients.[13] In particular, cholestatic liver injury resembling SSC-CIP has been a known complication in these patients.[15-17] The ACE2-receptor as one of the main entry sites for the virus in the cell is also expressed in the bile ducts which have been shown to be infected by COVID-19.[14] This has been postulated as an explanation for the higher incidence of cholestatic problems in COVID-19 patients.[17,34]

Most patients in our overall cohort show a broad combination of the above mentioned risk factors accumulating over time (Table 3). As the majority of COVID-19 ARDS patients share these factors, the assessment of a patient's clinical risk for SSC is of limited value. In addition, routine liver imaging during pre-transplant evaluation performed within a short period before transplantation in these cases was non-prognostic for a later SSC in our study cohort. Sensitive laboratory values as proposed by our study could be more useful to determine the risk for SSC-CIP development.

Guidelines to approach evaluation of COVID-19 patients for LTx have been previously suggested.[32] LTx is only recommended in case of mono-organ failure of the lungs. However, the distinction between an extrapulmonary organ failure precluding LTx and a transient organ dysfunction is difficult. Our study illustrates these difficulties. Synthetic liver function can be preserved in SSC-CIP, misrepresenting the true condition of the liver.[11] Generally, the lack of clinical symptoms in the initial phase can delay or prevent the diagnosis of SSC-CIP and often, only persistent indicators of cholestasis parameters raises the suspicion over time.[35] Given the highly-urgent nature of LTx in the setting of ARDS, the window for diagnosis of SSC-CIP may be easily missed before transplantation. In addition, more transient factors such as sepsis-associated cholestasis or drug-induced liver injury are common differential diagnoses.[5,36] GGT is a non-specific and therefore frequently underestimated and highly sensitive marker for oxidative stress and bile duct injury.[37,38] Notably, in contrast to other cholangiopathies, the clinical value of ALP is increasingly controversially discussed in primary sclerosing cholangitis sharing several key features with SSC.[39] Increased GGT has been previously described as one of the earliest markers predicting SSC-CIP.[40] Our study corroborates these findings and suggests that persistent and excessive levels of GGT should raise a red flag and further diagnostic work-up should be recommended given the poor outcomes of SSC-CIP after LTx. Delisting should be considered. Our data further suggests that TBi and ALP should be closely observed on the waiting list.

Our study has several limitations. As a retrospective study, it is prone to missing or miscoded data. While the multi-center approach is invaluable to gain a substantial patient cohort, heterogenous recipient and donor selection, surgical standards and strategies in perioperative care may introduce bias. Patient numbers contributed by the different centers were not balanced and ranged between 1 and 23. The limited overall cohort size prevented us from

Table 3 Risk Factors for SSC-CIP

	SSC *n* = 15	no SSC *n* = 25	*p*-value
Ketamine (*n*; %)	11 (100%)	11 (50%)	0.001
Duration of ECLS bridging (median; IQR)	36 (25 − 46)	37.5 (28 − 49)	0.743
Hepatotoxic antibiotics (*n*; %)	14 (93%)	23 (92%)	0.999
Hepatotoxic antimycotics (*n*; %)	10 (67%)	15 (60%)	0.746
Pre-transplant ACE inhibitors (*n*; %)	0 (0%)	2 (9%)	0.519
Pre-transplant ARBs (*n*; %)	1 (8%)	0 (0%)	0.371
Hepatic comorbidities (*n*; %)	2 (13%)	1 (4%)	0.545

(Abbreviations: ACE, Angiotensin-converting enzyme; ARBs, Angiotensin receptor blockers; ECLS, Extracorporeal life support; SSC, Secondary sclerosing cholangitis).

meaningfully correcting our statistical analysis for volume of the individual centers. As the worldwide experience for LTx in COVID-19 patients is still limited and SSC-CIP is a rare disease, a limited cohort size is unavoidable. This may limit the generalizability of our findings. This fact also prevented us from meaningfully applying multivariate statistical calculations. Registry studies could provide larger cohorts but lack data granularity necessary to address the aims of our study. Lastly, while our study covers a relatively long follow-up period compared to other reports on LTx for COVID-19, it can still only assess a limited time frame and true long-term outcomes are beyond our scope.

In conclusion, our study shows that SSC-CIP is a severe complication after LTx for COVID-19 ARDS and entails significant morbidity and mortality in this cohort. While the risk-benefit ratio still should be considered favorable for LTx, this strongly underlines the importance of patient selection. GGT appears to be the most sensitive early parameter routinely available and predicts SSC-CIP even at the time of listing.

Author Contributions

SS and KH conceived and designed the study; CL, MH, TS, JVS, LJC, FI, JG, SK, MH, CA, NK, EAMV, JMS and PJ collected patient data; SS and KH performed the statistical analysis and interpreted the data; SS, MT and KH wrote the manuscript draft; ET, ES, PF, KM and PJ contributed important conceptual content; All authors helped revise the manuscript with significant intellectual contributions.

Disclosure statement

None of the authors have any relevant conflict of interest to declare.

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Supplementary materials

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