Feasibility of lung microdialysis to assess metabolism during clinical ex vivo lung perfusion

FEASIBILITY OF LUNG MICRODIALYSIS TO ASSESS METABOLISM DURING CLINICAL EX VIVO LUNG PERFUSION

Anna Teresa Mazzeo, MD,[a] Vito Fanelli, MD, PhD,[a] Massimo Boffini, MD,[b] Mariangela Medugno, MD,[a] Claudia Filippini,[a] Erika Simonato, MD,[b] Andrea Costamagna, MD,[a] Luisa Delsedime, MD,[c] Luca Brazzi, MD, PhD,[a] Mauro Rinaldi, MD,[b] V. Marco Ranieri, MD, PhD,[d] and Luciana Mascia, MD, PhD[e]

Affiliations

[a] University of Torino, Department of Surgical Sciences, Division of Anesthesia and Intensive Care, Torino, Italy. [b] University of Torino, Department of Surgical Sciences, Division of Cardio-Thoracic Surgery, Torino, Italy. [c] University of Torino, Department of Medical Sciences, Pathology Unit, Torino, Italy. [d] Alma Mater Studiorum University of Bologna, Department of Anesthesia and Intensive Care Medicine, Bologna, Italy. [e] Sapienza University of Rome, Department of Medical and Surgical Science and Biotechnologies, Rome Italy.

Corresponding Author:

Anna Teresa Mazzeo, MD

University of Torino. Department of Surgical Sciences. Division of Anesthesia and Intensive Care. Azienda Ospedaliera Universitaria Citta' della Salute e della Scienza di Torino. Presidio Molinette.

Corso Dogliotti 14

10126 Torino (Italy)

Email: anna.mazzeo@unito.it

Business Telephone: +39-011-6335502

Home Telephone: +39-011-6706555

Fax number: +39-011 6960448

Name of the Institution: University of Torino. Department of Surgical Sciences. Azienda Ospedaliera Universitaria Citta' della Salute e della Scienza di Torino. Presidio Molinette. 10123 Torino (Italy).

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Reprint request: Anna Teresa Mazzeo, University of Torino. Department of Surgical Sciences. Division of Anesthesia and Intensive Care. email address: anna.mazzeo@unito.it

Abstract

Background. Lung metabolism during ex vivo lung perfusion (EVLP) is increasingly studied. Microdialysis (MD) allows metabolic monitoring by sampling parenchymal interstitial fluid. The aim of this study was to investigate lung metabolism using MD during EVLP and to evaluate if microdialysate metabolites could improve selection and discriminate outcome of donor lungs.

Methods. MD monitoring was used during fourteen clinical EVLP procedures. Paired microdialysate and perfusate samples were analysed for glucose, lactate, pyruvate, glutamate, and lactate/pyruvate (L/P) ratio and values that best discriminated unfavorable outcome determined. Outcome was defined as unfavorable (lungs not transplanted or transplanted with primary graft dysfunction (PGD) at 72 hours >=2) or favorable (lungs transplanted with PGD<2).

Results. Microdialysisate markers and perfusate L/P ratio could discriminate unfavorable outcome with sensitivity and specificity of 0.85 and 0.81 for MD-glutamate >18.4micromol/L, 0.81 and 0.74 for MD-lactate >685micromol/L, 0.92 and 0.75 for MD-glucose >530 micromol and 0.85 and 0.65 for MD-pyruvate >25 micromol/L, 0.73 and 0.67 for perfusate L/P ratio >24.17. All microdialysate markers, perfusate and microdialysate L/P ratio, and perfusate lactate discriminated outcome when we limited analysis only to transplanted lungs.

Conclusions. We report the use of MD to evaluate lung metabolism during clinical EVLP, demonstrating that MD metabolites can contribute to selection of reconditioned lungs and discriminate early outcome after transplantation. Furthermore, glutamate as a marker of lung injury during EVLP is proposed and could hence be used as a potential target for future therapies.

Keywords: Ex vivo lung perfusion, lung metabolism, microdialysis, glutamate, lactate, glucose, lactate pyruvate ratio.

Introduction

Lung transplantation is a lifesaving therapy for patients with end-stage lung disease, but donor shortage still represents a significant problem in the clinical practice. With the use of normothermic ex vivo lung perfusion (EVLP), the retrieved donor lungs are ventilated and perfused in an ex vivo circuit, allowing repair and extended assessment of marginal donor lungs before transplantation.[1] Actual criteria used to evaluate lung suitability for transplantation during EVLP are lung function tests, radiographic and bronchoscopic findings and macroscopic assessment.

The study of lung metabolism during EVLP could add further insights in the clinical evaluation of lung viability. Indeed, an increase in lactate has been recently shown by Koike et al. in the perfusate taken from the reservoir of EVLP even if no correlation with post-transplant outcomes could be demonstrated.[2] Furthermore, Valenza et al demonstrated that glucose consumption correlated with lung edema in experimental models of EVLP[3] and, more recently, Iskender et al. reported that cytokine filtration during EVLP was associated with reduced glucose consumption, lactate production and lactate/pyruvate ratio in the perfusate of a large animal model.[4] Furthermore, using metabolomics approach, Hsin et al demonstrated that perfusate palmitoyl-sphingomyelin (a cell membrane component), 5-aminovalerate (a urea cycle metabolite), and decanoylcarnitene (a metabolite in carnitine metabolism) highly correlated with early lung transplantation outcomes potentially contributing to the clinician's decision to proceed to transplantation.[5] As cellular metabolism and respiratory function restart during EVLP, the same authors suggested that metabolomic markers could improve donor lung selection and lung transplant outcome.[5]

Directly sampling tissue interstitial fluid by microdialysis (MD) monitoring could be a more effective mean to study lung metabolism than sampling perfusate in the circuit of EVLP.

MD is a minimally invasive monitoring tool in which a thin probe, with a semipermeable dialysis membrane at its tip, is inserted into a tissue for continuous sampling of the interstitial fluid. Chemical substances from the interstitial fluid diffuse across the membrane into the perfusion fluid inside the catheter and can be collected and analyzed. [6-11]

In this study, we propose the use of lung MD during EVLP procedures.

We hypothesized that the assessment of lung metabolism using MD during EVLP could contribute to identify organs which did not match adequate criteria for transplantation or which developed severe primary

graft dysfunction (PGD) after transplant. Aim of our study was to investigate lung metabolism during EVLP with the use of MD and to evaluate if metabolic analytes (glucose, lactate, pyruvate) and marker of lung injury (glutamate) measured with MD could discriminate outcome.

Methods

The study was approved by the Ethic Committee of Azienda Ospedaliera Universitaria Citta' della salute e della scienza di Torino (2CEI/178). Consent was given by the recipient patients candidate to receive the reconditioned donor lungs. The study was conducted in compliance with the International Society for Heart and Lung Transplantation Statement on Transplant Ethics (April 2007).

Donor lungs that were considered suitable for EVLP procedure by cardiac surgeons, were consecutively enrolled in the study, starting on February 2015, for 30 months.

Clinical protocol of ex vivo lung perfusion

EVLP was performed according to methods previously described by Cypel and colleagues. [1] Briefly, lungs were perfused in the XVIVO™ (XVIVO Perfusion) chamber with 2 liters of buffered dextran containing extracellular-type solution (Steen Solution™), with addition of 500 mg of methylprednisolone, 500 mg of imipenem-cilastatin and 3000 UI of heparin. An amount equal to 250 mL of Steen Solution was replaced every hour, as by protocol.[1] Lung perfusion flow was progressively increased up to the value corresponding to 40% of the estimated donor cardiac output. Lungs were ventilated with a tidal volume of 7 ml/kg of donor predicted body weight, a respiratory rate of 7 breaths per minute, a positive end expiratory pressure of 5 cmH2O and an inspiratory fraction of oxygen of 0.21 once temperature reached 32°C.

Radiography and flexible bronchoscopy of lungs were performed at 1 and 3 hours during EVLP. At the end of the procedure, the evaluation of lungs reaching adequate criteria for transplantation was performed by the EVLP team using lung function tests, radiographic and bronchoscopic findings, and macroscopic assessment.

Study protocol.

Lung microdialysis technique.

At the beginning of EVLP procedure, after vessel cannulation and endotracheal tube placement, a sterile MD probe with a 10 mm-length flexible membrane, cut-off 20KDa, (CMA70, CMA/Microdialysis, Sweden) and an external diameter of 0.6 mm, was inserted in the left lung lingula by the surgeon. The probe was inserted for few (3-4) centimeters into the lung parenchyma through a splitable introducer, and then fixed with suture.

The probe was perfused at 2 microL/min, using sterile normal saline, through a precision MD pump (CMA 107, CMA/Microdialysis, Sweden), and MD monitoring was continued till the end of EVLP procedure (Online Figure 1). After allowing for one hour of stabilization of the system, the MD vials were collected every 40-60 min and immediately frozen. At the same time points paired perfusate samples from the reservoir of the EVLP circuit were collected and immediately frozen. The research team maintained a detailed procedure event log to identify important events and to record times of vial sampling. At the end of EVLP procedure the MD probe was removed by gently pulling it out of the parenchyma and the site of probe placement was used for lung biopsies, as per clinical protocol. A larger bioptic sample including the in situ MD probe was taken and fixed for histological analysis in a case of lungs that not reached adequate criteria for transplantation after EVLP, with the aim to exclude any damage by the MD catheter and to illustrate histology of the surrounding area which was object of interstitial metabolic study.

The lung microdialysate and perfusate samples were analyzed for glucose, lactate, pyruvate, and glutamate using CMA 600 Microdialysis Analyzer (CMA/Microdialysis, Sweden). The analyzer was calibrated before and after each batch, using standards within the ranges of human concentrations supplied by CMA. All analyses were carried out in duplicate.

Outcome measure

PGD was evaluated at 72 hours after lung transplantation according to International Society for Heart and Lung Transplantation guidelines.[12]

For the purpose of this study, in the **primary outcome** we identified two groups: favorable outcome (lungs transplanted with PGD at 72 hours <2) and unfavorable outcome (lungs not transplanted or transplanted with PGD at 72 hours $\geq$2). PGD2 was scored as PaO2/FiO2 ratio between 200 and 300 and diffuse allograft infiltrates on chest radiograph.[12]

In **secondary outcome** we only included transplanted lungs and identified two groups: PGD 0-1 and PGD 2-3.

Statistical analysis

Continuous data are presented as mean and standard deviation (SD), or median and interquartile range (IQR) depending on data distribution, while categorical data are presented as rate and proportion.

Comparison of continuous data between groups was performed using the unpaired Student's t-test or Wilcoxon Mann-Whitney as appropriate.

Temporal profile of each studied variables in microdialysate and perfusate in favorable and unfavorable outcome, was tested with general linear model for repeated measure.

Values of microdialysate and perfusate analytes that best discriminated unfavorable from favorable outcome were determined by assessing the receiver-operating characteristics curve (ROC) using all available microdialysate and perfusate values. Results are given as area under the curve (AUC) and confidence interval (CI) at 95% level. The selected threshold values were those that maximized sensitivity (minimizing false negative classifications) with a specificity value not lower than 0.65. A true positive result was defined as occurring when an analyte detected failure and this occurred. A true negative result occurs when an analyte detected success and transplantation had favorable outcome. False negative result occurs when an analyte detected success but transplantation had unfavorable outcome. A false positive result was defined when analyte detected failure but transplantation had favorable outcome. The level of statistical significance was set at 0.05. All statistical analyses were performed using SAS ® 9.4 software (SAS Institute Inc. Cary N.C. USA).

RESULTS

Clinical characteristics and outcome

Donor lung characteristics and physiological variables at the end of EVLP are reported in Table 1. We applied lung MD in fourteen consecutive EVLP procedures (Figure 1). One potential recipient patient, at the end of EVLP procedure, did not give consent to receive reconditioned organs, and this case was excluded from the analysis for missing outcome and withdrawn consent. Nine out of 13 EVLP (69%) resulted in organs matching criteria for transplantation (6 bilateral). Outcome was favorable (transplanted with PGD<2) in seven out of thirteen EVLP procedures (54%), unfavorable in 6 (46%). Recipients age was 46.5 ± 18.7 years, reason for transplantation were chronic obstructive pulmonary disease (n=3), cystic fibrosis (n=1), idiopathic pulmonary fibrosis (n=2), previous graft failure in cystic fibrosis (n=1), and severe ARDS (n=1). Of the nine transplanted patients, seven exhibited a PGD of 0-1 at 72 hours and two a PGD of 3. Median (days) and IQR of hospital length of stay (LOS) was 39 (24;56) versus 41.5 (20;63) (p>0.999), of ICU LOS was 4 (2;10) versus 41.5 (20;63) (p=0.0570) and duration of mechanical ventilation was 1.5 (1;2) versus 41.5 (20;63) (p=0.0519), respectively in PDG of 0-1 versus PGD 2-3. All patients with PGD of 0-1 were alive at 90 days, while the two PGD of 3 died in ICU while ventilated. Time course of physiological variables during EVLP in PGD 0-1 and PGD 2-3 patients is shown in Figure 2.

Microdialysis and perfusate analysis

The temporal profile of microdialysate and perfusate analytes according to outcome is presented in Figure 3, for the first 4 hours of monitoring, for which more data were available.

EVLP procedures with unfavorable outcome showed increased levels of glutamate and lactate than favorable outcome, in microdialysate as well as in perfusate; differences were significant in microdialysate. There was a significant decrease of glutamate over time in both microdialysate and perfusate, and a significant increase of lactate in the perfusate over time (Figure 3). MD levels of lactate were significantly different between favorable and unfavorable outcomes already at 1 hour and there was no further increase over time, as seen in perfusate.

The area under the ROC curve for lactate, glutamate, glucose and pyruvate in microdialysate and perfusate was calculated and CIs reported (Table 2). ROC curves of lactate, glutamate, glucose, pyruvate and L/P ratio in both microdialysate and perfusate are presented in Figure 4.

Sensitivity and specificity of microdyalisate glutamate >18.4 micromol/L to discriminate unfavorable outcome were 0.85 and 0.81, respectively (Table 2). Sensitivity and specificity of microdialysate lactate >685 micromol/L to discriminate unfavorable outcome were 0.81 and 0.74, respectively (Table 2).

EVLP procedures with unfavorable outcome showed significantly higher levels of microdialysate glucose and pyruvate, which was significant over time for pyruvate (Figure 3). Sensitivity and specificity of microdyalisate glucose >530 micromol/L to discriminate unfavorable outcome were 0.92 and 0.75, respectively. Sensitivity and specificity of microdyalisate pyruvate >25 micromol/L to discriminate unfavorable outcome were 0.85 and 0.65, respectively (Table 2).

Microdialysate and perfusate L/P ratio were increased in EVLP procedures with unfavorable outcome, but difference among outcome groups was not significant (Figure 3). Sensitivity and specificity of perfusate L/P ratio >24.17 to discriminate unfavorable outcome were 0.73 and 0.67, respectively (Table 2).

To evaluate if microdialysate markers can increase the ability of standard clinical criteria to identify unfavorable outcome, we compared[13] the area under the ROC curve for last Delta PaO2/FiO2 with AUC of a selected microdialysate analyte and AUC of the combination of both. For this purpose we selected glutamate and lactate which were the microdialysate markers with the stronger pathophysiological rationale.

Microdialysate glutamate combined with last Delta PaO2/FiO2 (AUC, 0.98 [0.89;0.99]) was significantly more accurate in discriminating unfavorable outcome than microdialystate glutamate alone (AUC, 0.89 [0.77; 0.96]; p=0.0488), and last Delta PaO2/FiO2 alone (AUC, 0.92 [0.81; 0.97]; p=0.0474) (Figure 5).

Microdialysate lactate combined with last Delta PaO2/FiO2 (AUC, 0.93 [95%CI 0.82;0.98]) was significantly more accurate in discriminating unfavorable outcome than microdialysate lactate alone (AUC, 0.81 [95%CI 0.67; 0.91]; p=0.0442), but not than last Delta PaO2/FiO2 alone (AUC, 0.90 [95%CI 0.79; 0.97]; p=0.3966), (Figure 5).

For the secondary outcome, when we limited analysis only to transplanted lungs, all microdialysate markers including L/P ratio, perfusate lactate and perfusate L/P ratio showed significant AUC to discriminate PGD 2-3: microdialysate glutamate (AUC = 1.00 [0.90; 1.00]; p=<0.0001), microdialysate lactate (AUC, 0.90

[0.74; 0.98]; p=<0.0001), microdialysate glucose (AUC, 0.95 [0.81; 0.99]; p=<0.0001), microdialysate pyruvate (AUC, 0.74 [0.56; 0.88]; p=0.0139), microdialysate L/P ratio (AUC, 0.75 [0.56; 0.88]; p=0.0139), perfusate lactate (AUC, 0.76 [0.62; 0.87]; p=0.0079), perfusate L/P ratio (AUC, 0.75 [0.61; 0.87]; p=0.0015).

Technical aspects. We were able to recover microdialysate samples from all the EVLP procedures and to measure the studied analytes in each sample, with very few values under detection limit. In four EVLP procedures microdialysis monitoring was interrupted before the end of the procedure due to displacement of the probe, mainly during recruitment maneuvers; in these cases, perfusate samples were collected till the end of the EVLP procedures. In two other cases MD probe was replaced in adjacent area after displacement. No complication related to MD monitoring during EVLP was observed, as well as no adverse effect in the recipients, nor in terms of air leak nor in terms of any radiological change in the area of MD catheter placement.

Figure 6 illustrates histologic features of a pulmonary biopsy in a case of lungs that did not match criteria for transplantation after EVLP. In Figure 6A is visible the pulmonary parenchyma surrounding MD probe site after its removal, without evidence of local phenomena of hemorrhage or tissue damage related to catheter insertion. Figure 6 B-D shows oedema and diffuse inflammatory infiltration at the end of an unsuccessful EVLP procedure.

Online Figure 2 shows localization of microdialysis probe at XR imaging of reconditioned donor lung.

Discussion

We reported the use of MD to evaluate lung metabolism during clinical EVLP demonstrating that MD markers of metabolism could contribute to donor lung selection and discriminate early outcome after transplantation. To the best of our knowledge, no previous demonstration of glutamate, as a marker of lung injury and a potential target for future therapies, at lung interstitial level and in the perfusate of donor lungs undergoing EVLP has ever been reported in literature.

The metabolic properties of lungs and the presence of transpulmonary lactate and pyruvate kinetics have been demonstrated in studies on isolated perfused lung preparations suggesting a possible metabolic role of lung.[14-16] The importance to assess lung metabolism during EVLP in humans has been increasingly proposed in literature. [2-5] In our study we were able to measure markers of energetic metabolism at lung interstitial level during clinical EVLP using MD.

Since the first clinical studies in the 90's,[6,17,18] MD has been increasingly used for sampling the chemistry of different tissues, especially the brain in neurocritically ill patients, so that cerebral MD is nowadays an important clinical and research tool. During last decade lung MD has been used to measure the extracellular concentrations of antibiotics in the lung, in order to assess antimicrobial exposure of the pathogen.[19] Several studies[19-24] have indeed demonstrated feasibility and safety of lung MD technique in patients undergoing elective thoracic surgery. No adverse effect related to lung MD in the clinical setting has ever been described.[19]

In our study we demonstrated, with the use of lung MD, that levels of microdialysate lactate and glutamate were higher in EVLP procedures with unfavorable outcome and could discriminate outcome.

Increased levels of lactate, as marker of anaerobic metabolism, could be related to pulmonary edema and inflammation affecting lung metabolism and altering substrate availability and end-product removal. Nevertheless, also mitochondrial dysfunction could play a role in high lactate levels, due to inability of dysfunctional mitochondria to metabolize lactate, as already documented in brain injury literature.[25] In our study microdialysate lactate >685 micromol/L was able to discriminate unfavorable outcome with a sensitivity of 0.82 and a specificity of 0.74.

The other interesting finding of our study is that we were able to measure glutamate both at lung interstitial level and in the perfusate, during clinical EVLP procedures. Sensitivity and specificity of microdialysate

glutamate >18.4 micromol/L to discriminate unfavorable outcome were 0.85 and 0.81, respectively. Combining glutamate with last Delta PaO_2/FiO_2 further increased the performance of the AUC in discriminating unfavorable outcome. The first report of glutamate excitoxicity in the lung is by Said et al. who demonstrated in perfused ventilated lung rats that N-methyl-D-aspartate (NMDA) receptor activation causes acute edematous lung injury that was prevented by competitive NMDA receptor antagonists.[26] Later, Tang et al. demonstrated that, by activation of the NMDA receptors, hyperoxia elevated NF-KB expression and increased ROS production, while hyperoxia-induced lung injury was attenuated by NMDA receptor antagonist MK-801.[27] More recently, it was shown that memantine, a NMDA receptor channel blocker, significantly mitigated bleomycin-induced lung injury in mice.[28] The role of glutamate as a marker of excitotoxicity not only in the brain but also in the lung and in other peripheral organs has been recently reviewed in a comprehensive review.[29] We propose a role for glutamate in the acute lung injury of donor lung undergoing EVLP, thus suggesting a potential therapeutic role for NMDA receptor antagonists in attenuating excitotoxic lung injury during reconditioning procedure.

Histological study of the lung parenchyma surrounding the brain showed no specific lesions due to the probe placement. Lung inflammation and edema visible at anatomo-pathological level in our bioptic sample including the MD probe in a lung which did not reach adequate criteria for transplantation (Figure 6) were compatible with metabolic derangements measured by MD at interstitial level.

Furthermore, in our study we also observed that levels of microdialysate pyruvate and glucose in EVLP procedures characterized by unfavorable outcome were higher and could discriminate outcome. High levels of pyruvate together with high levels of lactate could be the expression of mitochondrial dysfunction as published for brain parenchyma.[8] In our study microdialysate glucose levels > 530 micromol/L discriminated unfavorable outcome with a sensitivity of 0.92 and a specificity of 0.75. Valenza et al[3] in their experimental porcine EVLP model had previously showed that glucose consumption correlated with lung edema, measured as wet/dry ratio. In our series of human EVLP there was a significant reduction of perfusate glucose over time, but difference between outcome groups was not significant. Nevertheless, in our study microdialysate glucose was able to discriminate unfavorable outcome. Human versus experimental setting and different selected outcomes might partially explain differences between the two studies.

For future studies in this setting, the use of MD probe of 100 KDa allowing detection of larger size molecules, such as inflammatory cytokines or other possible mediator of injury should be considered.[32]

Another tool that nowadays can evaluate organ metabolism is positron emission tomography (PET), recently proposed to assess the magnitude and regional distribution of inflammatory metabolic activity in the lungs of patients with ARDS.[33] Even if the potential application of PET in the setting of EVLP is unknown, PET gives a snapshot picture of cellular metabolism, while MD allows monitoring of metabolic activity in near real time over the period of interest.

Conclusion

We report the use of lung MD to evaluate metabolism during clinical EVLP, demonstrating that microdialysisate lactate, glutamate, glucose and pyruvate and perfusate L/P ratio can discriminate unfavorable outcome. When we focused only on transplanted lungs, also microdialysate L/P ratio and perfusate lactate added a significant contribute to identify severe PGD. We also demonstrated that microdialysate glutamate is an important marker of lung injury during EVLP and a potential target for future therapies.

Accordingly, we do consider lung MD as a safe, feasible and useful adjunct to actual criteria for the evaluation of lung suitability for transplantation during EVLP. Further larger studies are required to validate these results.

Conflict of interest

The authors declare that they have no competing interests.

Aknowledgement

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Figure legend

Figure 1. Flow-chart illustrating primary and secondary outcomes according to disposition of donor lungs.

Figure 2. Time course of physiological variables during EVLP in patients with Primary graft dysfunction (PGD) 0-1 (n=7) versus PGD 2-3 (n=2). Data did not differ significantly between the groups and over time, for Delta PaO2/FiO2 ratio (panel A, p=0.0734 and p=0.9288), peak inspiratory pressure (panel B, p=0.1445 and p=0.5394), dynamic compliance (panel C, p=0.3059 and p=0.6161) and pulmonary vascular resistances (PVR) (panel D, p=0.7234 and p=0.4886), respectively. Data are expressed as mean and standard deviation.

Figure 3. Temporal profile of microdialysate and perfusate lactate (panel A), glutamate (panel B), glucose (panel C), pyruvate (panel D), and lactate/pyruvate (L/P) ratio (panel E), according to unfavorable and favorable outcome is presented at 1, 2, 3 and 4 hours after starting the EVLP procedure. Number of available determinations at each time for microdialysate (MD) and perfusate (PERF) analytes is reported. Data are expressed as mean and standard deviation. * $p<0.05$ over time, in microdialysate; ≠ $p<0.05$ over time, in perfusate; # $p<0.05$ between outcome groups, in microdialysate.

Figure 4. ROC curves of lactate (panel A), glutamate (panel B), glucose (panel C), pyruvate (panel D), and lactate/pyruvate (L/P) ratio (panel E) in microdialysate and perfusate. Microdialysisate lactate, glutamate, glucose and pyruvate and perfusate L/P ratio can discriminate unfavorable versus favorable outcome after transplantation.

Figure 5. Figure illustrates, in panel A, the Receiver-operating characteristic (ROC) curves of microdialysate glutamate, last Delta PaO2/FiO2, and microdialysate glutamate combined with last Delta PaO2/FiO2 to discriminate unfavorable outcome and, in panel B, the ROC curves of microdialysate lactate, last Delta PaO2/FiO2, and microdialysate lactate combined with last Delta PaO2/FiO2. Comparison of the AUC demonstrated that combining microdialysate glutamate with last Delta PaO2/FiO2 further increased the

performance of the AUC and was the most accurate to discriminate unfavorable outcome (AUC, 0.98 [0.89;0.99]; p=0.0474).

Figure 6. At histological examination the intraparenchymal microdialysis probe site after removal is observed (A); diffuse inflammatory infiltration of bronchial walls and alveoli, with relative architectural preservation (B). At higher power (C, D) diffuse airspace filling by oedema and inflammatory cells with predominance of neutrophil polymorphonucleates is observed. (Hematoxylin-eosin, original magnification x25(A), x40(B), x200 (C) and x400 (D).

Online Figure 1. Lung microdialysis procedure during ex vivo lung perfusion. After insertion of a splitable introducer into the lung parenchyma (A), the tip of the microdialysis probe is inserted through the introducer into the parenchyma (B), the introducer is retracted and peeled off (C), and the probe is fixed to the parenchyma to avoid displacement (white arrow) (D). After insertion, the probe (shown in panel E) is perfused with sterile normal saline through a microdialysis precision pump (blu arrow) and microdialysate is collected into microvials (red arrow)(F).

Online Figure 2. Representative lung X-ray showing microdialysis monitoring during ex vivo lung perfusion. The arrow identifies the microdialysis circuit outside the parenchyma, the circle identifies the area of lung parenchyma surrounding the microdialysis probe; the particular highlights the golden tip of the probe.

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Name of the Institution: University of Torino. Department of Surgical Sciences. Azienda Ospedaliera Universitaria Citta' della Salute e della Scienza di Torino. Presidio Molinette. 10123 Torino (Italy).

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Reprint request: Anna Teresa Mazzeo, University of Torino. Department of Surgical Sciences. Division of Anesthesia and Intensive Care. email address: anna.mazzeo@unito.it

Conflict of interest

The authors declare that they have no competing interests.

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Figure 1

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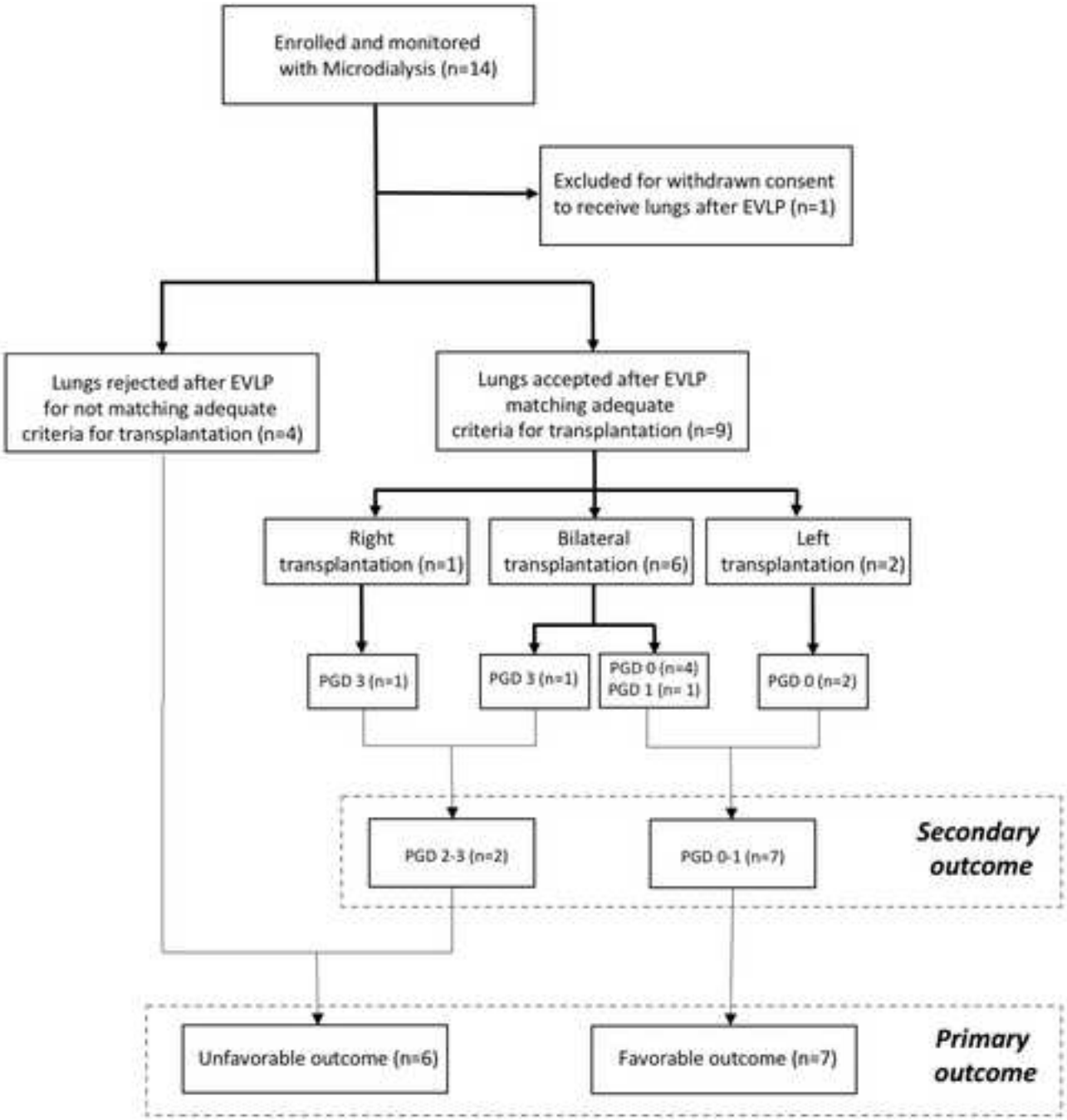

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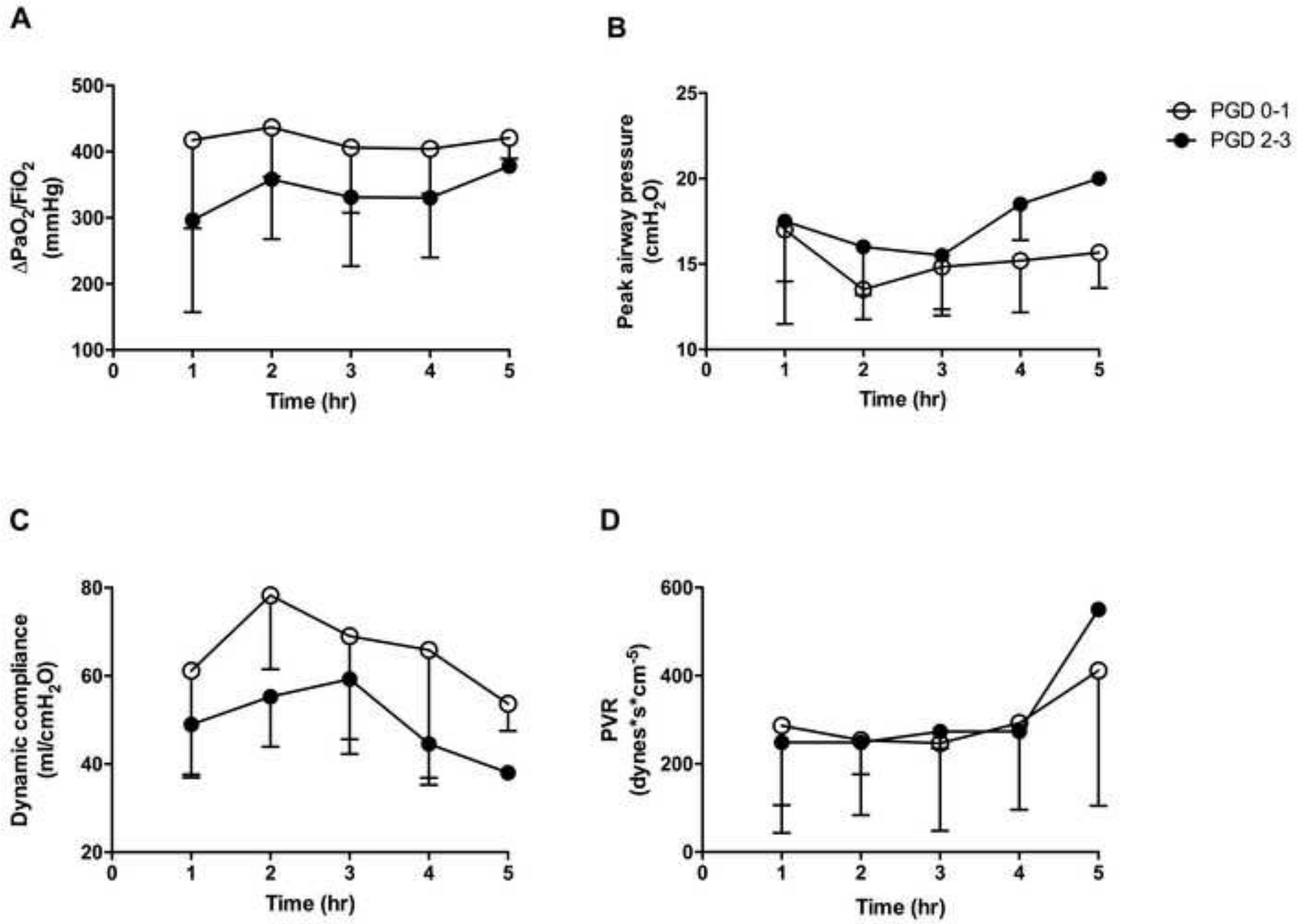

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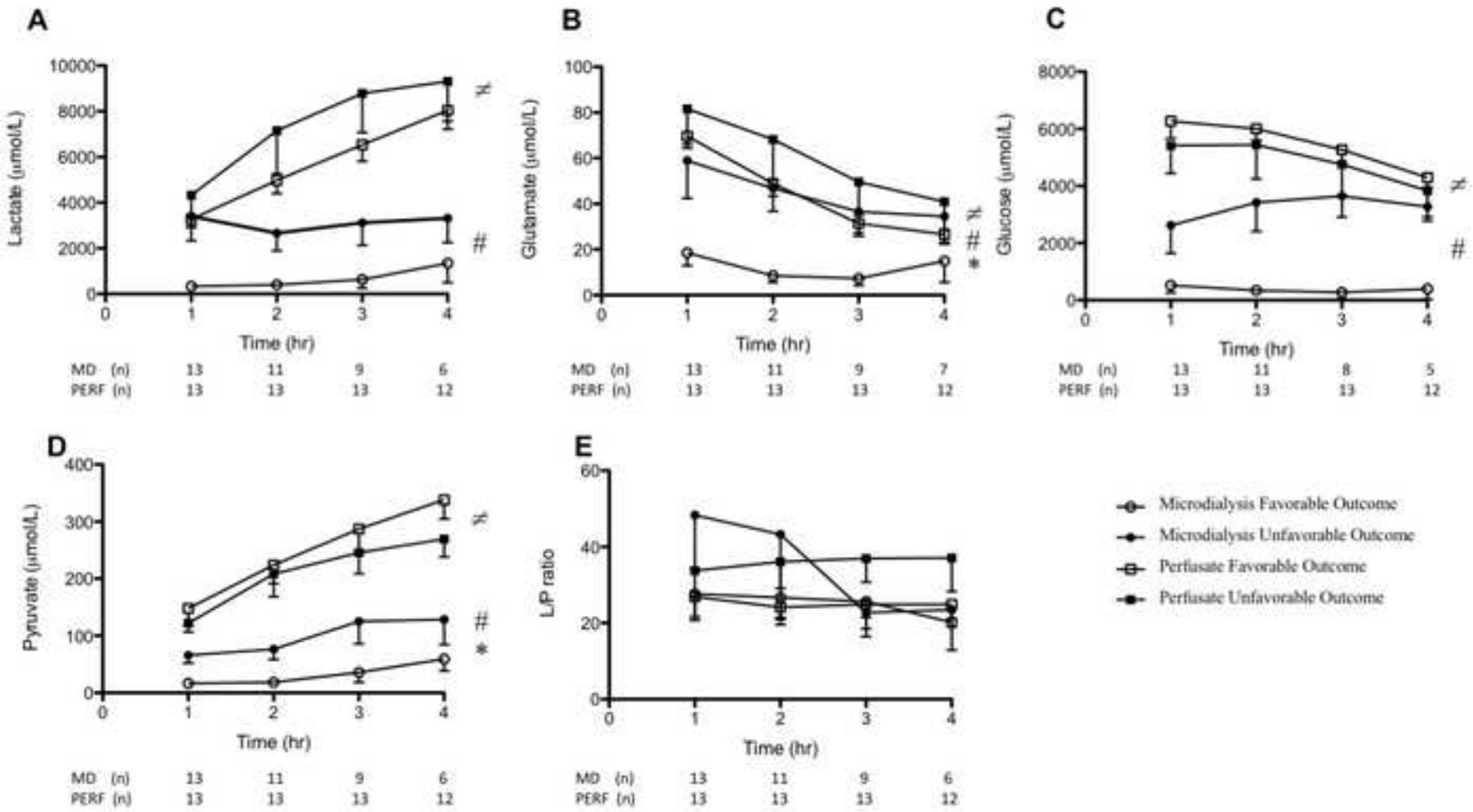

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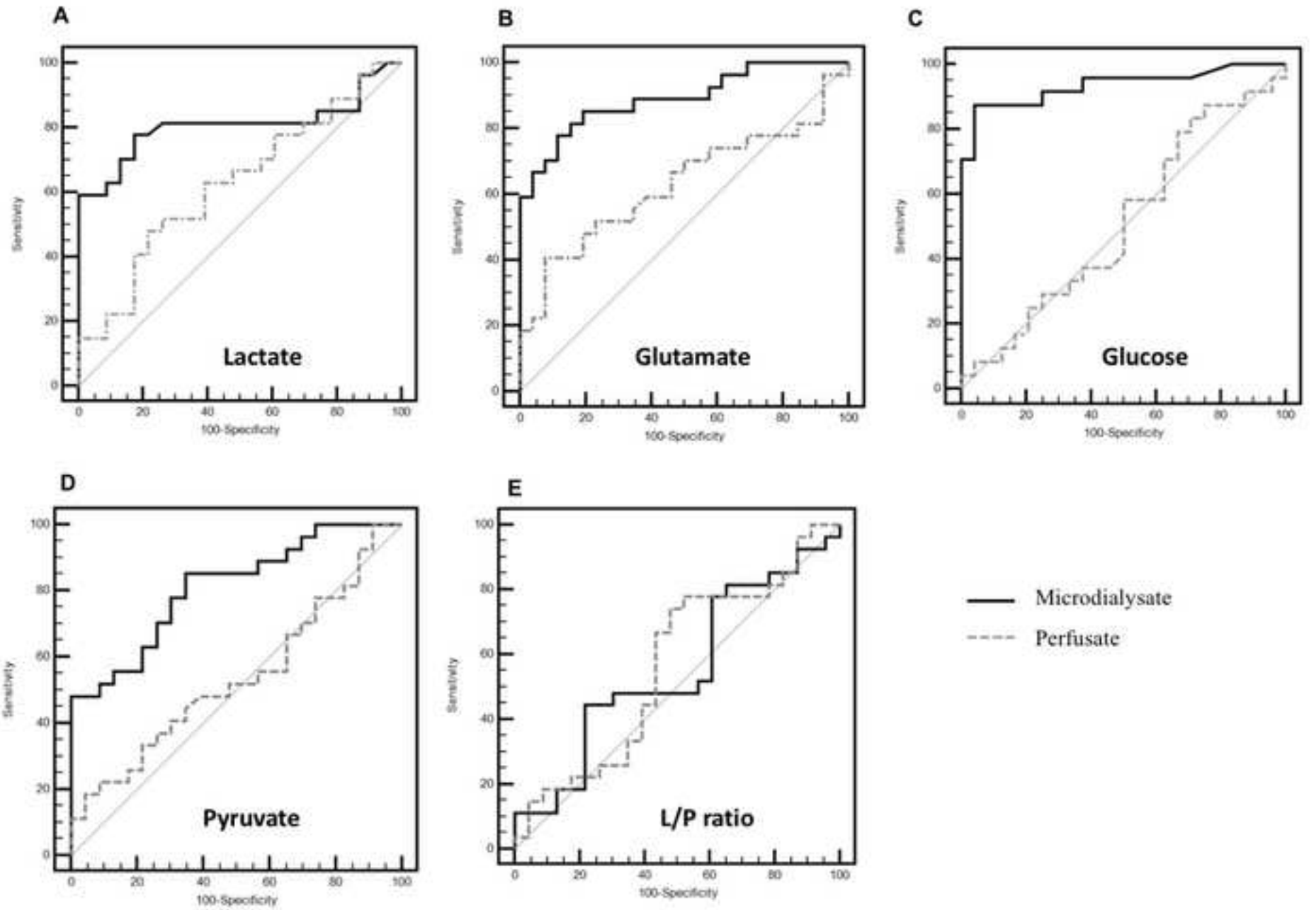

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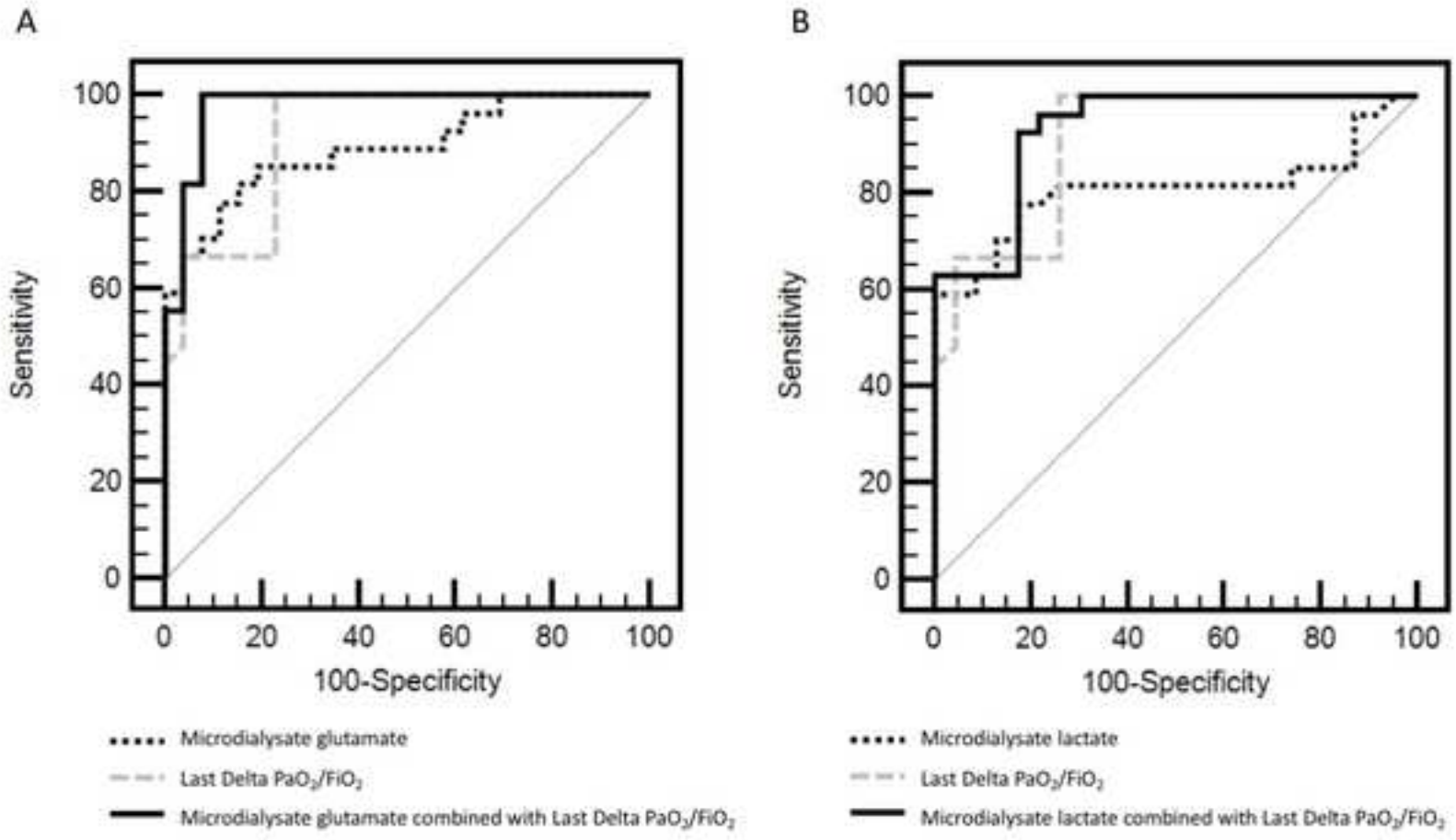

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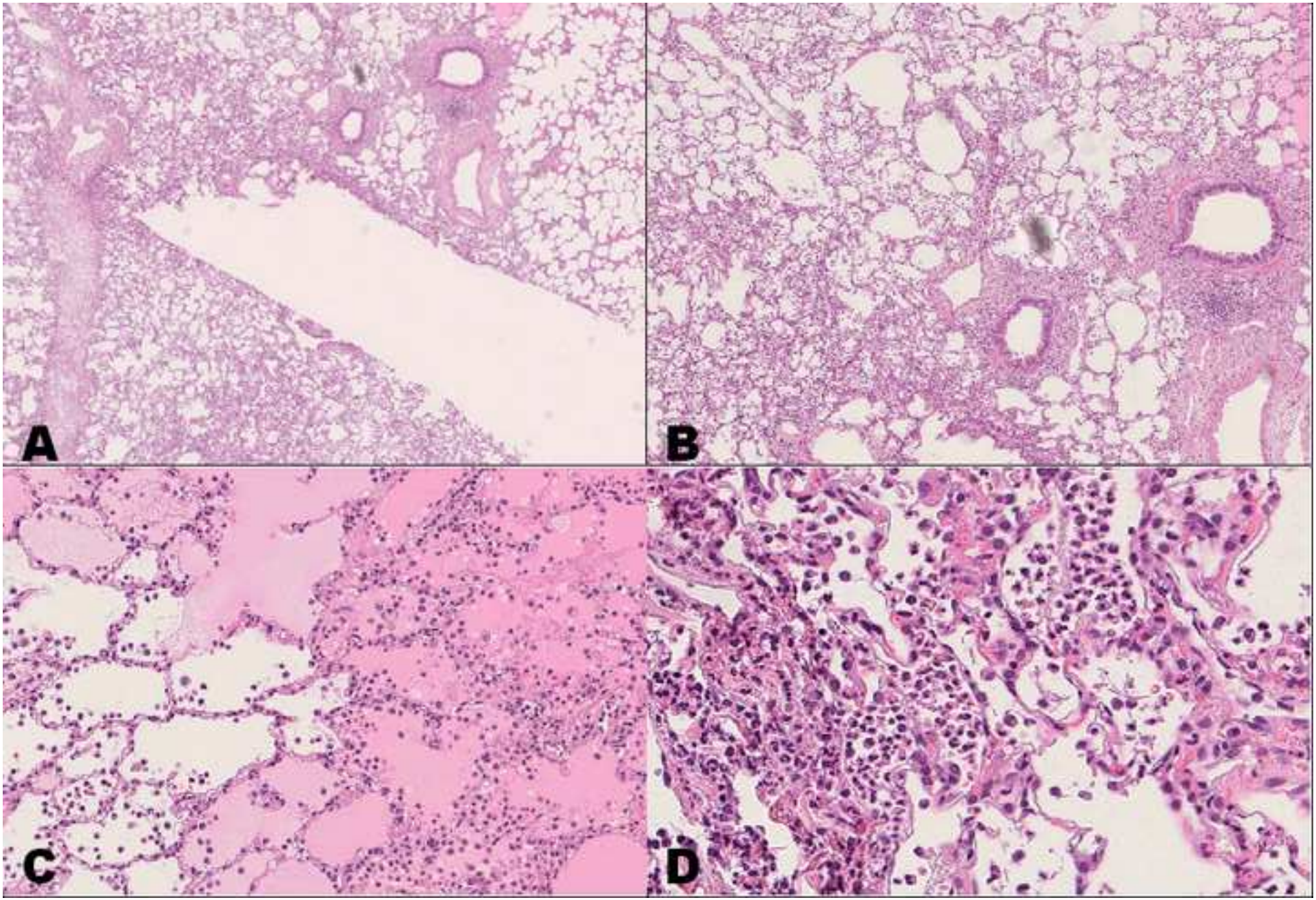

Table 1. Donor lung characteristics and EVLP data according to primary outcome

Variables	Favorable outcome[a] n = 7	Unfavorable outcome[b] n = 6	p-value
DONOR CHARACTERISTICS			
Age, years	39.3±13.2	35.8±16.8	0.6865
Cause of death, n (%)			
Post-anoxic encephalopaty	0	4 (67)	
Traumatic brain injury	2 (29)	0	
Subarachnoid hemorrhage	3 (43)	2 (33)	
Ischemic stroke	1 (14)	0	
Pulmonary embolism	1 (14)	0	
Last PaO_2/FiO_2 before organ retrieval, mmHg	251.7±160.2	295.2±142.6	0.6186
Donor Score	5 (3-8)	8.5 (6.5-9)	0.1578
Days of mechanical ventilation	2 (1-2)	3 (3-4)	0.0444
EVLP PROCEDURES			
Cold ischemic time, min	250.3±68.4	261±79	0.8067
EVLP duration, hours	5 (4-6)	5 (4-6)	0.8807
Last delta PaO_2/FiO_2, mmHg	379±62.9	271.3±80.2	0.197
Last dynamic compliance, ml/cmH_2O	65.6±25.5	39.1±16.5	0.0579
Last peak airway pressure, cmH_2O	15.5±2.7	23.7±11.4	0.1453
Last PVR, dynes • s • cm^{-5}	308.9±229.4	285±147.9	0.6889

Categoric data are given as numbers and percentage and continuous data as mean ± SD or median and IQR. [a]Favorable outcome= lungs transplanted with PGD at 72 hours <2. [b] Unfavorable outcome=lungs not transplanted or transplanted with PGD at 72 hours >2. PaO_2, fraction of inspired oxygen; FiO_2, partial pressure of arterial oxygen; EVLP, ex vivo lung perfusion; PVR, pulmonary vascular resistance.

Table 2 Receiver-operating characteristics curve analysis

	AUC (CI)	p-value	Sensitivity (CI)	Specificity (CI)	Cutoff	NPV	PPV	+LR	-LR
Microdialysate Glutamate	0.89 (0.77; 0.96)	<0.0001	0.85 (0.66;0.96)	0.81 (0.61;0.93)	>18.4	84.00	82.14	4.43	0.18
Microdialysate Lactate	0.81 (0.67; 0.90)	<0.0001	0.81 (0.62;0.94)	0.74 (0.52;0.90)	>685	77.27	78.57	3.12	0.25
Microdialysate Glucose	0.93 (0.82; 0.99)	<0.0001	0.92 (0.73;0.99)	0.75 (0.53;0.90)	>530	90.00	78.57	3.67	0.11
Microdialysate Pyruvate	0.81 (0.67; 0.91)	<0.0001	0.85 (0.66;0.96)	0.65 (0.43;0.84)	>25	78.95	74.19	2.45	0.23
Perfusate L/P ratio	0.69 (0.57; 0.79)	0.0031	0.73 (0.56;0.87)	0.67 (0.49;0.82)	>24.17	72.73	67.57	2.21	0.40

L/P ratio, lactate/pyruvate ratio. AUC, area under the curve; CI, Confidence interval.
NPV = negative predictive value; PPV = positive predictive value; +LR=positive likelihood ratio; -LR negative likelihood ratio
Microdialysis cutoff are expressed in micromol/L; microdialysis perfusion flow rate at 2 microL/min.