# THE UNIVERSITY OF QUEENSLAND

### AUSTRALIA

**Sick Patients, Starved Circuits and Sticky Drugs: Understanding pharmacokinetics during extracorporeal membrane oxygenation to optimise drug dosing and improve patient outcomes**

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M.B.B.S.

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## Abstract

Extracorporeal membrane oxygenation (ECMO) is the final option for patients with acute severe cardiac and/or respiratory failure that is unresponsive to conventional management. ECMO plays a supportive role and its success relies on optimal drug therapy to reverse the underlying disease process and to prevent or treat complications. Vital drugs may be sequestered and/or degraded in the ECMO circuit resulting in altered pharmacokinetics (PK). This may be further complicated by the PK changes that occur in the presence of critical illness and may lead to therapeutic failure and/or drug toxicity.

However, much of the currently available PK data is from neonates receiving ECMO and may not be extrapolated to adult patients given the developmental and physiologic differences. Equally, such PK alterations are complex and are challenging to investigate in a critically ill patient on ECMO. The aim of this research was to investigate each of the circuit, drug and critical illness factors affecting PK during ECMO in adult patients. A combination of linear and non-linear mixed effects modelling, compartmental and population methods and dosing simulations was utilised to characterise antibiotic, sedative and analgesic PK. The incremental research plan comprised of;

(i)     *ex vivo* experiments for drug stability testing in human blood;

(ii)    *ex vivo* drug disposition studies in ECMO circuits primed with fresh whole human blood;

(iii)   PK studies in healthy and critically ill ovine models of ECMO with appropriate non-ECMO controls and;

(iv)    an international multi-centre clinical population PK study in critically ill adults on ECMO.

The introductory chapter (Chapter 1) outlines the origins of this research based on clinical observations, hypothesis generating preliminary data (Chapter 1.1), summarises the available extracorporeal life support therapies (Chapter 1.2) and provides a clinical context to PK alterations induced by ECMO (Chapter 1.3). This is followed by a literature review on PK alterations during ECMO (Chapter 2). Thus, an understanding of the clinical problem, available extracorporeal therapies, and identifying the gaps in literature (Chapter 3) facilitated the development of a comprehensive research plan (Chapter 4.1). Chapter 4.2 describes the methodology for the development of *ex vivo* and ovine models of ECMO. The protocol for the international multi-centre population PK study that aims to develop dosing guidelines for 18 key antibiotic, sedative and analgesic drugs is presented in Chapter 4.3. Ex vivo studies identified drug stability, lipophilicity (Chapter 5.1) and protein binding (Chapter 5.2) as the key drug factors determining their sequestration and /or degradation in

ECMO circuits. An increase in peripheral volume of distribution (Vd) of the highly protein-bound, lipophilic drug midazolam was identified in both healthy and critically ill sheep on ECMO (Chapter 6.1) providing further *in vivo* evidence of circuit drug sequestration. PK of eight antibiotic drugs that exhibited a wide range of lipophilicity and protein binding was characterised in healthy sheep controls and healthy and critically ill sheep on ECMO (Chapter 6.2). This experiment identified that ECMO may variably influence Vd and decrease clearance (CL) in the presence of critical illness, based on lipophilicity and protein binding characteristics and these properties may be used to predict their PK during ECMO. Population PK modelling of meropenem in critically ill patients on ECMO (with or without renal replacement therapy) was performed and compared with data from non ECMO controls in a matched-cohort study (Chapter 7). This study provided preliminary evidence that in the absence of significant circuit sequestration for hydrophilic and less protein-bound drugs such as meropenem, an increased Vd (critical illness and ECMO factors) may be to an extent countered by a decreased CL (from ECMO and kidney injury) minimising the net influence ECMO has on PK. The closing chapter (Chapter 8) discusses key findings of this research and lays a roadmap for future work.

The overarching finding of this research is that drug factors lipophilicity and protein binding play a significant role in altering PK during ECMO and drug factors may be used to predict PK of antibiotic and sedative drugs. The combined effect of critical illness and ECMO may be limited for hydrophilic and less protein-bound drugs compared with lipophilic and more protein-bound drugs. The ongoing clinical population PK study will provide robust dosing guidelines for prescription of commonly used antibiotics, sedative and analgesic drugs during ECMO.

Future research should focus on optimising the interactions between drug, device and the disease. While it may be challenging to alter drug physicochemistry, whilst maintaining safety and efficacy, further refinements to ECMO circuitry may be considered. Equally, the impact of ECMO on pathophysiology, especially on hepatic and renal organ systems which are responsible for metabolism and excretion of most drugs need to be better understood. Building pharmacodynamic (PD) models based on animal and clinical studies will add another dimension of PK/PD during ECMO as the PD alterations induced by ECMO, if at all, are yet to be investigated.

**Declaration by author**

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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## Publications during candidature

*Peer-reviewed papers*

1. **Shekar K**, Roberts JA, McDonald CI, Fisquet S, , Ghassabian S, Anstey C, Wallis SC, Mullany DV, Fung YL, Smith MT, Fraser JF. Protein-bound drugs are prone to sequestration in the extracorporeal membrane oxygenation circuit: results from an *ex vivo* study. Crit Care. 2015 Apr 14;19(1):164.

2. Shruti Rateesh, **Kiran Shekar**, Rishendran Naidoo, Dolly Mithal , Balu Bhaskar. Use of Extracorporeal Membrane Oxygenation for mechanical circulatory support in a patient with 5-fluorouracil -induced Acute Heart Failure. Circ Heart Fail. 2015 Mar;8(2):381-3C

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1. Passmore M, Fung YL, Dunster KR, Diab S, **Shekar K**, Fraser JF. Lung inflammatory changes in an ovine model of VV ECMO. Am J Respir Crit Care Med 189;2014:A3064 American Thoracic Society 2014

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7. D. Platts, S. Diab, C. MacDonald, M. Tunbridge, S. Chemonges, K. Dunster, **K. Shekar,** D. Burstow, D. Mullany, J. Fraser. .The Impact of Continuous Flow From Venovenous Extracorporeal Membrane Oxygenation Cannulae on Tricuspid Valve Geometry and Function. Heart Lung & Circulation 01/2013; 22, Supplement 1:S199

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**Publications included in this thesis**

**Kiran Shekar**, Daniel V Mullany, Bruce Thomson, Marc Ziegenfuss, David G Platts, John F Fraser. Extracorporeal life support devices and strategies for management of acute cardiorespiratory failure in adult patients: a comprehensive review. Crit Care. 2014 May 9;18(3):219. Incorporated as Chapter 1.2.

| Contributor | Statement of contribution |
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| Kiran Shekar (Candidate) | Conceptualised paper (80%), Wrote paper (75%) |
| Daniel V. Mullany | Conceptualised paper (20%), Wrote paper (20%), Edited paper (40%) |
| Bruce Thomson | Wrote paper (5%), Edited paper (20%) |
| Marc Ziegenfuss | Edited paper (5%) |
| David G. Platts | Edited paper (5%) |
| John F. Fraser | Edited paper (30 %) |

**Shekar K**, Roberts JA, Mullany DV, Corley A, Fisquet S, Bull TN, Barnett AG, Roberts JA, Fraser JF. Increased sedation requirements in patients receiving extracorporeal membrane oxygenation for respiratory and cardio-respiratory failure. Anaesth Intensive Care 2012; 40:648-655. Incorporated as Chapter 1.3.

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| Shekar K.  (Candidate) | Conceptualised paper (100%), Wrote paper (80%), Collected data (20%), Analysed (50%), interpreted data (80%) |
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| Corley A. | Collected data 20% |
| Fisquet S. | Collected data 20% |
| Bull T.N. | Collected data 20% |
| Barnett A.G. | Analysed data (50%) |
| Roberts J.A. | Wrote paper (10%), Edited paper (30%) |
| Fraser J.F. | Edited paper (30%) |

**Shekar K**, Fraser JF, Smith MT, Roberts JA. Pharmacokinetic changes in patients receiving extracorporeal membrane oxygenation. J Crit Care. 2012 Dec; 27(6):741.e9-18. Incorporated as Chapter 2.

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| Roberts J.A. | Conceptualised paper (10%), Wrote paper (20%), Edited paper (50%) |

**Shekar K**, Roberts JA, Smith MT, Fraser JF. The ECMO PK Project: an incremental research approach to advance understanding of the pharmacokinetic alterations and improve patient outcomes during extracorporeal membrane oxygenation. BMC Anesthesiology 2013, 13:7. Incorporated as Chapter 4.1.

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**Shekar K,** Fung YL, Diab S, Mullany DV, McDonald CI, Dunster KR, Fisquet S, Platts DG, Stewart D, Wallis SC, Smith MT, Roberts JA, Fraser JF. Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit-host interactions. Crit Care Resusc (2012) June; 14(2):105 – 111. Incorporated as Chapter 4.2.

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46. **Shekar K**, Roberts JA, Welch S, Buscher H, Rudham S, Burrows F, Ghassabian S, Wallis SC, Levkowich B. Pellegriono V, McGuinness S, Parke R, Barnett AG, Walsham J, Mullany DV, Smith MT, Fraser JF. ASAP ECMO: Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation: A multi-centre study to optimise drug therapy during ECMO. 2012. BMC Anesthesiol. 2012 Nov 28; 12(1):29. Incorporated as Chapter 4.3.

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| Shekar K. (Candidate) | Conceptualised and designed study 80% |
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| | Site coordination St Vincent's Hospital 5% |
| Burrows F. | Study design 1% and co-ordination 1% |
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| Levkovich B. | Site coordination ,The Alfred 60% |
| Pellegrino V. | Study design 2%, Edited paper 5% |
| | Site coordination,The Alfred 40% |
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| Parke R. | Co-ordination, Auckland City Hospital 30% |
| Gilder E. | Co-ordination, Auckland City Hospital 30% |
| Barnett A.G. | Statistical plan 100% |
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| Mullany D.V. | Edited paper 10% |
| Fung Y.L. | Edited paper 10% |
| Smith M.T. | Sedative drug assays 80%, Edited paper 10% |
| Fraser J.F. | Conceptualised and designed study 5% |

Edited paper 10%

**Shekar K**, Roberts JA, McDonald CI, Fisquet S, Barnett AG, Mullany DV, Ghassabian S, Wallis SC, Fung YL, Smith MT, Fraser JF. Sequestration of drugs in the circuit may lead to therapeutic failure during extracorporeal membrane oxygenation. Crit Care. 2012 Oct 15; 16(5):R194. Incorporated as Chapter 5.1.

| Contributor | Statement of contribution |
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| Shekar K. (Candidate) | Design experiment 80% |
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| | Wrote paper 25%, edited paper 45% |
| McDonald C.I. | Experimental design 10%, Performed experiment 30%, Edited paper 10% |
| Fisquet S. | Study drug sourcing and dispensing 100% |
| Barnett A.G. | Statistical analysis 80% |
| Mullany D.V. | Edited paper 10% |
| Ghassabian S. | Sedative drug assays 80% |
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| Wallis S.C. | Antibiotic drug assays 100% |
| | Assisted with experiment 5% |
| Fung Y.L. | Design experiment 5%, Edited paper 10% |
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| Fraser J.F. | Experimental design 5 %, Edited paper 20% |

**Shekar K**, Roberts JA, McDonald CI, Fisquet S, , Ghassabian S, Anstey C, Wallis SC, Mullany DV, Fung YL, Smith MT, Fraser JF. Protein-bound drugs are prone to sequestration in the extracorporeal membrane oxygenation circuit: results from an *ex vivo* study. Crit Care. 2015 Apr 14;19(1):164. Incorporated as Chapter 5.2.

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| Shekar K. (Candidate) | Design of experiment 80%, Performed experiment 60%, Analysed data and wrote paper 80% |
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| Mullany D.V. | Edited paper 10% |
| Fung Y.L. | Design experiment 5%, Edited paper 10% |
| Fraser J.F. | Design experiment 5%, Edited paper 20% |

**Shekar K,** Roberts JA, Ghassabian S, Diab S, Fung YL, Smith MT, Fraser JF. Pharmacokinetics of midazolam and its metabolites in healthy and critically ill ovine models of extracorporeal membrane oxygenation. Manuscript under review. Critical Care and Resuscitation. Incorporated as Chapter 6.1.

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| Shekar K. (Candidate) | Design and conduct of PK experiment 80% |
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| | Edited the paper20% |
| Smith M.T. | Sedative drug assays 20% |
| Fraser J.F. | Design ovine ECMO experiment 45% |
| | Assisted with experiments 5% |
| | Edited paper 10% |

**Shekar K,** Roberts JA, Barnett AG, Diab S, Dunster KR,Wallis SC, Fraser JF. Can physicochemical properties predict pharmacokinetics of antibiotic drugs during extracorporeal membrane oxygenation: illustrative data from an ovine model. Manuscript under review. Critical Care. Incorporated as Chapter 6.2.

| | |
|---|---|
| Shekar K. (Candidate) | Design and conduct of PK experiment 80% |
| | Assisted with experiments 15% |
| | Analysed data 50%, Interpreted data 90% |
| | Wrote paper 90% |
| Roberts J.A. | PK experiment design 20%, Interpreted data 10%, |
| | Wrote paper 10%, Edited paper 40% |
| Barnett A.G. | Analysed data 50%, Edited paper 20% |
| Diab S. | Assisted with experiments 80% |
| | Design of ovine experiment 5% |
| Dunster K.R. | Technical and medical equipment  100% |
| Wallis S.C. | Antibiotic drug assays 100%, Edited paper 10% |
| Fung Y.L. | Design of ovine experiment 45% |
| | Edited the paper 20% |
| Fraser J.F. | Design of ovine ECMO experiment 50% |
| | Assisted with experiments 5% |
| | Edited the paper 10% |

**Shekar K**, Fraser JF, Taccone FS, Welch S, Wallis SC, Mullany DV, Lipman J, Roberts JA. The combined effects of extracorporeal membrane oxygenation and renal replacement therapy on meropenem pharmacokinetics: a matched cohort study. Crit Care. 2014 Dec 12; 18(6):565. Incorporated as Chapter 7.1.

| Contributor | Statement of contribution |
|---|---|
| Shekar K. (Candidate) | Design and conduct of study 70% |
| | Analysed data 40%, Interpreted data 80% |
| | Wrote the paper 90% |
| Fraser J.F. | Study design 10% |
| | Edited the paper 20% |
| Taccone F.S. | Interpreted data 10% |
| | Edited the paper 20% |
| Welch S. | Study design and site coordination 10% |
| Wallis S.C. | Developed antibiotic drug assays 100% |
| Mullany D.V. | Edited paper 10% |
| Lipman J. | Edited paper 10% |
| Roberts J.A. | Study design 20%, Analysed data 60% |
| | Interpreted data 10%, Wrote paper 10% |
| | Edited paper 40% |

## Contributions by others to the thesis

**Professor John Fraser**: I am eternally grateful to John for being such an inspiration for my research journey. John's knowledge, experience, hard work, enthusiasm and humanistic qualities have allowed him to break all silos and build a unique multidisciplinary research group that provides an excellent research infrastructure to a RHD student. His advice, support, guidance and encouragement throughout my project were invaluable. John provided me with a free reign to follow my own ideas and provided sound advice as required.  This allowed me not only to complete this thesis but also to get a deeper understanding of the research process. If it wasn't for John, my research career would have probably ended well before it began.

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**<u>Statement of parts of the thesis submitted to qualify for the award of another degree</u>**

None.

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# LIST OF ABBREVIATIONS USED IN THIS THESIS

| | |
|---|---|
| CI | confidence interval |
| CL | clearance |
| ECMO | extracorporeal membrane oxygenation |
| VV | venovenous |
| VA | venoarterial |
| MIC | minimum inhibitory concentration |
| PD | pharmacodynamics |
| PK | pharmacokinetics |
| TPCH | The Prince Charles Hospital |
| Vd | volume of distribution |
| BIS | bispectral index |
| TDM | Therapeutic drug monitoring |

# CHAPTER 1

# INTRODUCTION

## 1.1 ORIGINS OF THIS RESEARCH

The Adult Intensive Care Unit at TPCH in Brisbane, Queensland, Australia officially commenced its extracorporeal membrane oxygenation (ECMO) service in 2009 at the height of the H1N1 pandemic. The service was modelled on The Alfred, a high volume, experienced ECMO centre in Melbourne, Australia. Under the strong leadership of Dr Dan Mullany, TPCH has now evolved to become an ECMO centre with world class outcomes.

In the early days of ECMO at TPCH, clinicians and nurses at the bedside made some observations in ECMO patients that were not typical of a critically ill patient not supported with ECMO. One such observation that triggered this research was the unusually high sedation requirement in ECMO patients. "It doesn't seem to touch them ", remarked a senior clinician after his desperate attempts to sedate his ECMO patient with high doses of multiple sedative agents. Another nurse tired of giving sedative boluses to keep her patient safe in bed noted "looks like all the drugs we give are sucked up by this hungry machine". Puzzled by the unusual sedative requirements, another clinician wondered "what was happening to all the antibiotics we are giving these ECMO patients, at least with sedation we know when it's not working". Based on such clinical observations, Professor Fraser suggested that "drugs and ECMO" would be very relevant topic for my PhD studies and thus the "ECMO Pharmacokinetic (PK) Project" was born.

In 2010, under the leadership of Professor Fraser and Associate Professor Lin Fung, the Critical Care Research Group at the Prince Charles Hospital secured a 3 year National Health and Medical Research Council Project Grant to examine inflammation and coagulation effects of ECMO in ovine models. A team of experienced clinicians, scientists, perfusionists, cardiologists, cardiac surgeons and research assistants was assembled for the ovine ECMO study. Although not originally intended, the ovine experiments provided a perfect opportunity to mechanistically investigate PK of drugs during ECMO.

Encouraged by the NHMRC grant success, an ECMO Special Interest Group Meeting which was attended by clinicians and researchers across Australasia was held at TPCH in December 2010. This meeting facilitated collaboration with Professor Jason Roberts, a well-known PK expert, clinicians and pharmacists from other ECMO centres to form the multi-centre clinical PK study collaborative that helped the translation side of this research. The PK work was well supported by the two state of the art analytical laboratories at the University of Queensland in Brisbane. The Burns Trauma and Critical Care Research Centre developed and performed PK assays for antibiotic study drugs and the Centre for

Integrated preclinical Drug Development assisted with sedative and analgesic drug assays.

This research represents an attempt to further the field of ECMO, a life saving technique. Since the 2009 H1N1 pandemic, there has been a significant surge in the use of ECMO globally. This has occurred despite the lack of definitive evidence for the use of ECMO. Undoubtedly, ECMO is an invasive therapy with risks. Encouraged by excellent results reported especially in centres with higher case volumes (even when used as a salvage therapy) many clinicians believe that significant refinements in ECMO technology, its clinical application and service delivery are possible and may shift the risk: benefit ratio in favour of ECMO. Thus the H1N1 pandemic was the perfect trigger not only for increased ECMO up take in the clinical arena, but also for research aimed at improving all aspects of ECMO. To witness many young patients survive a potentially 'unsurvivable" illness with "salvage ECMO" provided necessary motivation and impetus for this translational research.

The remaining sections in this chapter provide a context for this research and also present some preliminary hypothesis generating work that was performed prior to embarking on a comprehensive research plan which was both labour and resource intensive. This pilot work was approved by the Institutional Human Ethics and Research Committee and informed consent was obtained from patients and/or surrogate decision makers as applicable.

### 1.1.1. ECMO: a life saving therapy

ECMO is a final treatment option for patients who are at a high risk of death from acute cardiorespiratory failure that is unresponsive to maximal conventional therapy[1,2]. Conventional modern intensive care management, although extra-ordinary, is usually limited to mechanical ventilation with adjuncts[3], high dose pharmacological cardiovascular support, renal dialysis and other supportive measures. This is partly due to lack of viable, less invasive mechanical options to acutely support cardiopulmonary function[4]. With refinements in technology, ECMO has emerged to fill this void. ECMO is among the most rapidly increasing life support techniques used in intensive care. Its use in the United States has increased more than 433% in a space of only 5 years[5]. ECMO is highly versatile and not only provides temporary cardiorespiratory support in the acute setting, but it also effectively complements cardiac surgical and interventional cardiology procedures, timely implantation of long-term cardiac assist devices, heart and lung transplantation programmes and cardiopulmonary resuscitation[4,6].

### 1.1.2 ECMO circuits are not passive conduits for blood

ECMO is considered a supportive therapy whilst drug therapy is used to treat the underlying disease and minimise complications [8]. In a manner analogous to the lung it attempts to mimic, ECMO is critically dependent upon the large surface area of the oxygenator and associated tubing to ensure adequate blood flows through the circuit to facilitate gas transfer. This bio-synthetic interface results in significant sequestration of the administered drugs making it difficult to provide optimal drug therapy to reverse the underlying disease[7]. For many important drugs, available data from neonates illustrates an interaction between the drug and the artificial surfaces of the ECMO device[8-15]. This results in significantly altered drug concentrations in the most severely ill patients who already have profound PK changes. Given the morbidity and mortality associated with suboptimal antibiotic and sedative therapy in ICU patients, altered PK of these drugs [8-12] is of high clinical relevance. Published data from neonates demonstrate that ECMO dramatically affects PK in the most severely ill patients who already have significant PK changes[9,11,16,17]. However, there is a significant paucity of PK data in adult patients on ECMO.

In critically ill patients not receiving ECMO, it has been shown PK changes can result in highly significant changes to drug exposure through interactions between the patient, pathology and the drug[18-21]. The ECMO system introduces additional variables - the circuit itself, and the effects of systemic inflammation due to the prolonged use of an extracorporeal circuit. Sequestration of drugs in the circuit, increased Vd and CL are the major PK changes associated with ECMO[17], although the extent of these changes remains poorly characterised. Previous studies in the neonatal circuit highlight the influence that drug properties such as molecular size, degree of ionization at physiological pH, lipophilicity and plasma protein binding have on drug disposition during ECMO[22,23].

Drug sequestration may be further influenced by the age of circuit components, type of the pump, oxygenator and tubing as well as the variety of fluids that can be used for circuit priming[24-27]. Patient factors such as systemic inflammation, haemodilution, bleeding and transfusion, organ dysfunction and renal replacement therapy all add to the clinical challenges of drug dosing during ECMO[17].


### 1.1.3 Defining the burden of altered PK during ECMO

Given that most neonatal PK data originates from older generation of ECMO circuits and the paucity of PK data from adult patients on ECMO, preliminary data was obtained from

retrospective data base analysis and prospective PK sampling in adult patients receiving ECMO to further inform hypothesis development.

*1.1.3.1 Risks of suboptimal antimicrobial therapy:*

Plasma meropenem concentrations were measured in patients receiving the drug in standard doses (1g/8h) based on standard regimes. Standard dosing resulted in suboptimal plasma meropenem concentrations raising concerns of therapeutic failure in these complex patients (Figure 1). Administering higher doses of antibiotics to account for the PK alterations is not viable solution during ECMO as this may lead to potential toxicity from some drugs and their active metabolites[28] (e.g. oseltamivir and oseltamivir carboxylate).
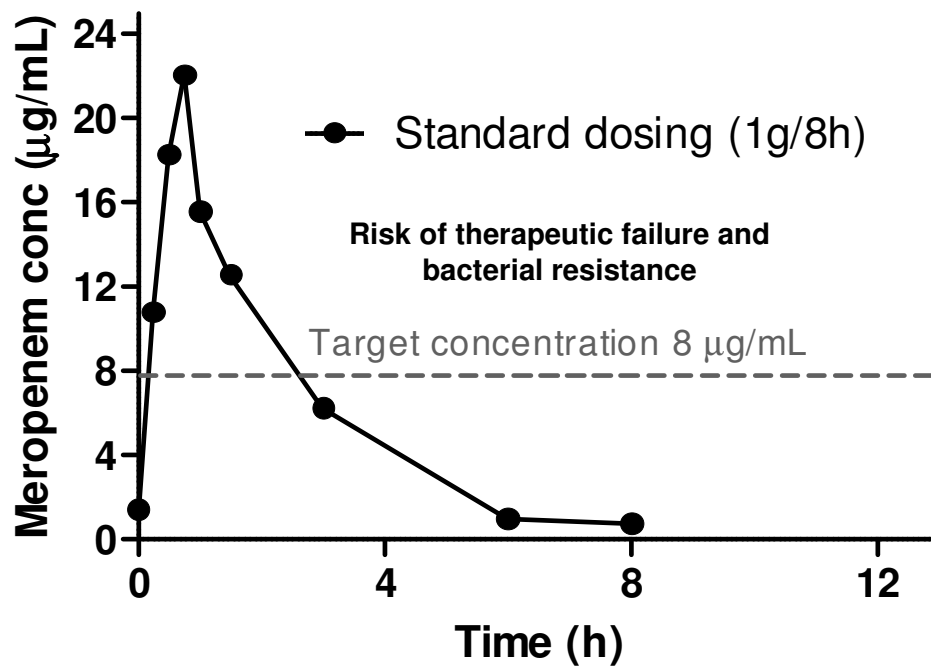


**Figure 1. Plasma meropenem concentrations over time with conventional meropenem dosing (1g/8h)**. In this patient who developed enterobacter sepsis on venovenous ECMO, whilst a minimum target of 40% time that the meropenem concentration was maintained above the MIC (40% T>MIC) was achieved with standard dosing the more aggressive target of 4 x MIC used locally, 8 µg/mL, was only achieved for 30% of the dosing interval[8].

*1.1.3.2 Escalating sedation requirements during ECMO:*

A retrospective review of sedation requirements in 30 consecutive ECMO patients[11] was

performed to evaluate sedation practices on ECMO. Sedation in these patients was titrated by a senior bedside nurse. The average 24-hourly dose increased by 18mg per day for midazolam (95% CI: 8, 29mg, p=0.001) and 29mg per day for morphine (95% CI: 4, 53 mg, p=0.021) from the first day of ECMO. The venovenous group required a daily midazolam dose that was 157 mg higher on average than the venoarterial group (95% CI: 53, 261 mg, p=0.005). Patients often received up to 1500mg of morphine and midazolam per day despite supplemental sedation with propofol, dexmedetomidine and thiopentone. Extubation or tracheotomy was not possible in all patients and this heightened sedation requirement raised concerns of increased morbidity due to excessive sedation in the ICU[29].

### 1.1.3.3 Is escalating sedation requirement associated with a decrement in sedative drug concentration?

To further investigate this clinical problem, opportunistic PK sampling was performed in a patient prior to and after commencement of ECMO. Sedation prior to after commencement of ECMO was titrated to a Richmond Agitation Sedation Scale of -3 to -4 and a bispectral index (BIS) of 40-45. The patient also received neuromuscular paralysis with vecuronium (0-5mg/h, titrated to 1-2 twitches on train-of-four monitoring) to optimise ECMO flows and ventilation. Pre- ECMO sedation comprised of propofol (100mg/h), morphine and midazolam (20mg/h of each). In the first 3 hours after commencement of ECMO, the propofol infusion regimen was increased to 200mg/h (p=0.4) and the morphine and midazolam infusion regimens were each increased to 50mg/h (p< 0.001) to achieve pre-ECMO sedation levels (Fig 2a). In addition, propofol was also administered as repeated boluses (30-50mg, up to total of 300mg in the first hour).

The escalation in morphine and midazolam doses correlated with a decrement in plasma concentrations of these drugs and their active metabolites. There was a significant reduction in the plasma morphine (20%), midazolam (11%), 1-hydroxy midazolam (17%), morphine-3-glucuronide (36%) and morphine-6-glucuronide (35%) concentrations upon commencement of ECMO as compared to pre-ECMO levels, which then increased consistent with the marked increase in administered drug doses (Fig 2b, 2c). The increased requirement for sedation persisted for the entire duration of ECMO (19 days). Tracheotomy performed on day 7 did not lead to a significant reduction in sedative doses*.*
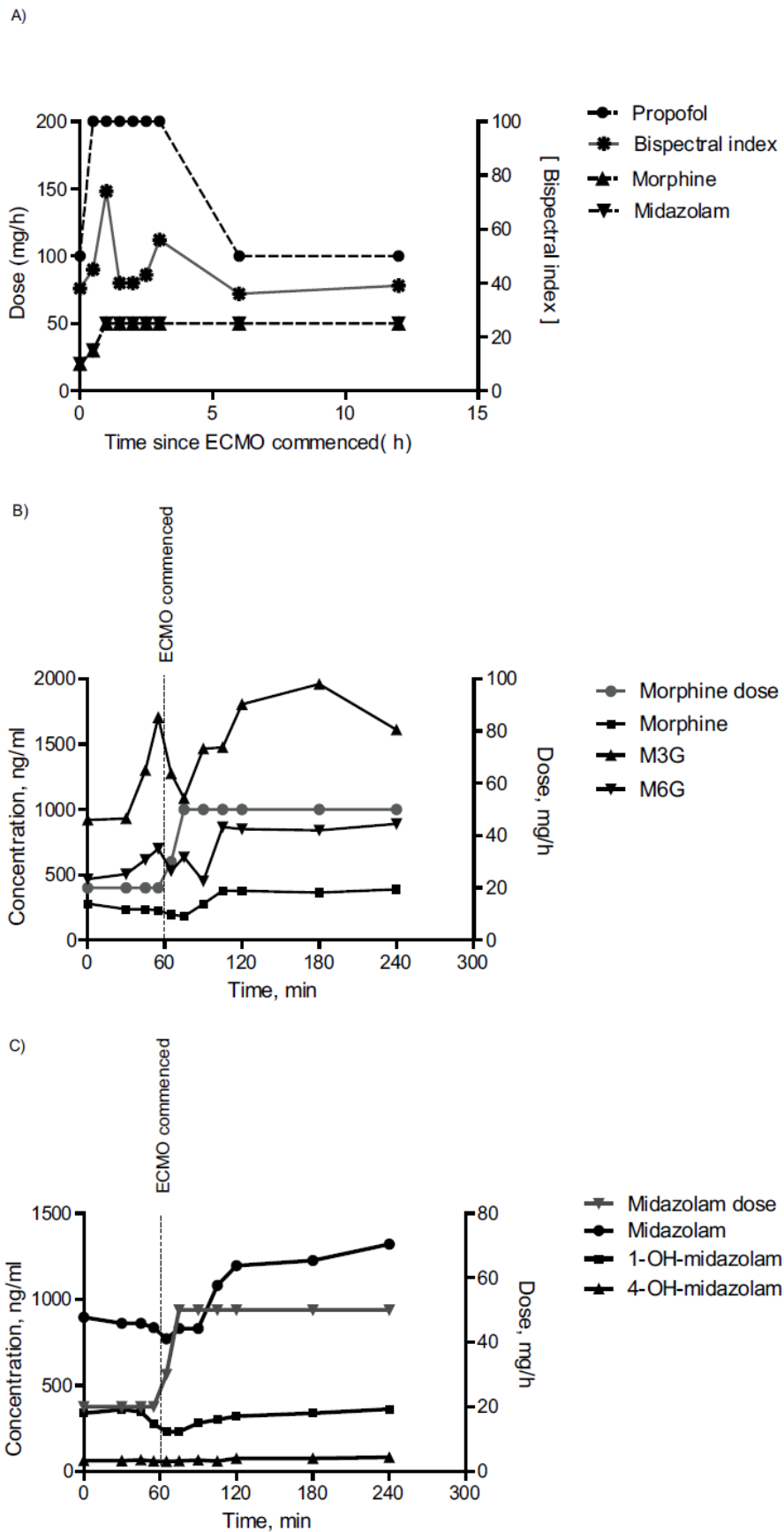
**Figure 2. Increased sedation during ECMO.** Escalating sedative drug doses (a) upon commencement of ECMO correlated with a decrement in plasma concentrations (b, c) of these drugs and their active metabolites[9].

### 1.1.4 Morbidity from altered PK during ECMO

There is increasing awareness of the implications of altered PK and its impact during critical illness[30-32]. The success of ECMO, especially in critically ill patients with severe pneumonia or a pandemic viral respiratory illness relies heavily on the success of antibiotic therapy. Optimal antibiotic therapy in these patients is a balance between potency and exposure[21,33-36]. A recent review of Extracorporeal Life Support Organization (ELSO) data[37] revealed a total of 2,418 infections during 20,741 (12%) ECMO cases. Infections increased the duration of ECMO, post-ECMO ventilator support and were associated with an increased risk of death. Neonatal studies have reported severe PK variations, however limited data is available to guide antibiotic therapy in adults[22,25,38-42]. Sub-optimal prescription of antibiotics in patients on ECMO can worsen the problem by selecting for resistant microorganisms[34].

By default, clinicians prescribe standard doses to these complicated patients, thereby risking therapeutic failure or toxicity. Neonatal studies also consistently demonstrate the need to escalate sedative doses during ECMO[22,43-45]. By acting as a reservoir, ECMO may also prolong the pharmacological effect of sedatives even after drugs have been ceased. Sedation practices in the ICU are changing and emerging data supports its judicious use[46,47]. This is concerning as it is now well established that excessive sedation in critically ill patients is associated with increased mortality and morbidity[29]. However, unlike sedation, there are no real-time PD end-points for antibiotic therapy and therapeutic drug monitoring [48] is available for only a handful of antibiotics.

### 1.1.5 Why study PK during ECMO in adult patients?

There are no other reliable techniques currently available to provide flexible and tailored extracorporeal support to adult patients with acute cardiorespiratory failure that is unresponsive to maximal conventional treatment[49,50]. ECMO certainly has shown promise and future research is vital not just in area of PK but many other areas of ECMO therapy to improve patient outcomes.

**i)      *Generate new knowledge of the interaction between ECMO and drugs:***

Population PK modelling is crucial to ensure drug safety and efficacy. The success of ECMO is dependent on optimal disease modifying drug delivery. This research will not only identify the drugs that are more suitable for use during ECMO but the findings may also inform the development of strategies for drug dosing using PK/ PD principles in critically ill

patients receiving ECMO. Such PK modelling may likely be able to translate the physicochemical / PK correlations from adults to children.

### ii) Developing adequate antimicrobial drug dosing regimens:

Optimal antimicrobial prescription has significant implications not only for the patients on ECMO but also for other ICU patients and the community in general. Optimal dosing of an antimicrobial agent will not only lead to improved microbiological and clinical cure rates in an individual patient, but also will reduce the emergence of resistant organisms.

### iii) Improving sedation practices during ECMO:

Using sedative agents at an appropriate dose may minimise ICU morbidity[47] related to risk of infections, duration of mechanical ventilation, inotrope and vasopressor requirements, drug withdrawal and post traumatic stress and length of hospital stay. This not only has resource implications but significantly affects patient outcomes. This research also evaluates existing ICU sedation protocols as compared with BIS monitoring.

### iv) Recognising potential and refining ECMO:

It is foreseeable that with further refinements in technology, ECMO will not only establish itself as a viable option for management of severe acute cardiorespiratory failure but also its role is likely to expand further to benefit a wide range of critically unwell patients [2,5]. Technological improvements need to be complimented by research directed at its judicious clinical application and this research is one critical step in this direction.

As highlighted in the literature review in subsequent chapters, drugs are variably affected by the presence of ECMO and meaningful recommendations for antibiotic, sedative and analgesic drug dosing can only be developed after systematic investigation of this complex problem. Thus, the above preliminary data underpins the importance of a comprehensive research plan that addresses the key determinants of altered PK during ECMO and development of dosing guidelines for key drugs used during ECMO.

# 1.2 EXTRACORPOREAL LIFE SUPPORT DEVICES AND STRATEGIES FOR MANAGEMENT OF ACUTE CARDIORESPIRATORY FAILURE IN ADULT PATIENTS: A COMPREHENSIVE REVIEW

### *1.2.1. Introduction to publication*

This chapter provides an overview of available extracorporeal life support therapies and strategies for management of acute cardiorespiratory failure in adult patients. Extracorporeal membrane oxygenation (ECMO) is one of the most common applications of extracorporeal life support and is used to support heart and/or lungs in patients who fail conventional treatment. Equally, the ECMO circuit may be used in many different configurations (e.g. use without the artificial oxygenator and with a pump just to provide circulatory support).This chapter emphasises that the success of extracorporeal therapies relies not only on the individually tailored application of the devices but also on the optimisation of pharmacotherapy directed at underlying disease and at minimising complications. Deeper understanding of extracorporeal life support therapy being applied is critical to choose an optimal phamacotherapeutic strategy in these complex patients.

**CRITICAL CARE**

## REVIEW

# Extracorporeal life support devices and strategies for management of acute cardiorespiratory failure in adult patients: a comprehensive review

Kiran Shekar[1*], Daniel V Mullany[1], Bruce Thomson[1,2], Marc Ziegenfuss[1], David G Platts[1,3] and John F Fraser[1]

## Abstract

Evolution of extracorporeal life support (ECLS) technology has added a new dimension to the intensive care management of acute cardiac and/or respiratory failure in adult patients who fail conventional treatment. ECLS also complements cardiac surgical and cardiology procedures, implantation of long-term mechanical cardiac assist devices, heart and lung transplantation and cardiopulmonary resuscitation. Available ECLS therapies provide a range of options to the multidisciplinary teams who are involved in the time-critical care of these complex patients. While venovenous extracorporeal membrane oxygenation (ECMO) can provide complete respiratory support, extracorporeal carbon dioxide removal facilitates protective lung ventilation and provides only partial respiratory support. Mechanical circulatory support with venoarterial (VA) ECMO employed in a traditional central/peripheral fashion or in a temporary ventricular assist device configuration may stabilise patients with decompensated cardiac failure who have evidence of end-organ dysfunction, allowing time for recovery, decision-making, and bridging to implantation of a long-term mechanical circulatory support device and occasionally heart transplantation. In highly selected patients with combined severe cardiac and respiratory failure, advanced ECLS can be provided with central VA ECMO, peripheral VA ECMO with timely transition to venovenous ECMO or VA-venous ECMO upon myocardial recovery to avoid upper body hypoxia or by addition of an oxygenator to the temporary ventricular assist device circuit. This article summarises the available ECLS options and provides insights into the principles and practice of these techniques. One should emphasise that, as is common with many emerging therapies, their optimal use is currently not backed by quality evidence. This deficiency needs to be addressed to ensure that the full potential of ECLS can be achieved.

## Review

### Introduction

Extracorporeal life support (ECLS) is a therapeutic option increasingly used in the management of patients with cardiorespiratory failure that is refractory to maximal conventional treatment [1,2]. This support may facilitate therapeutic intervention, bridge to recovery, bridge to a long-term support device, heart or lung transplantation, or bridge to palliation. Despite the renewed interest in ECLS technology following the 2009 H1N1 influenza pandemic [3], there is a lack of definitive evidence regarding its routine application. Current ECLS equipment has evolved to allow a plethora of perfusion strategies enabling tailored temporary support for patients and the ability to transition between configurations. A number of factors limit more frequent utilisation. These factors include challenges in patient selection, choice of an appropriate strategy, technical aspects of initiation and maintenance, and minimising complications [4].

This review provides a summary of the available ECLS options and cannulation techniques for short-term support of adult patients with cardiorespiratory failure that is refractory to conventional treatment.

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## Extracorporeal respiratory support for respiratory failure

### Extracorporeal membrane oxygenation

Details about the technology, principles and practice of extracorporeal membrane oxygenation (ECMO) can be found elsewhere [5,6]. Venovenous (VV) ECMO is predominantly used as a rescue therapy for selected patients with acute respiratory distress syndrome (ARDS) and refractory hypoxia [1,7,8]. Patients with refractory hypoxia due to ARDS continue to have a high mortality, in the order of 70 to 90%. Whether VV ECMO further improves survival in this group of patients when compared with lung-protective ventilation (LPV) and adjuncts such as neuromuscular blockade [9] and prone ventilation [10] is yet to be established. Recent studies demonstrating harm or no benefit with the use of high-frequency oscillation in patients with moderate to severe ARDS exclude this technique as a routine rescue option for refractory hypoxia and may further expand the scope of VV ECMO [11,12]. Despite unfavourable results in the early studies, the CESAR trial showed improved disability-free survival at 6 months in 90 patients who were randomised to receive ECMO (37% vs. 53% on LPV, $P = 0.03$) [13]. However, 22 of these patients did not receive ECMO and a majority of them improved with LPV. The study was criticised for lack of standardisation of LPV in the control group. This may not be seen as definitive evidence supporting the use of VV ECMO. However, the CESAR trial and the UK data from patients with H1N1-related ARDS do confirm that referral to an ECMO centre may lower hospital mortality compared with matched non-ECMO-referred patients [14].

Analysis of 2009 H1N1 pandemic data [3,15-18] demonstrates that while VV ECMO may be in equipoise with LPV, it may play a vital role in younger patients with critical oxygenation who have fewer failed organ systems and fail LPV [8]. Although VV ECMO is relatively easy to institute technically, the complexities relate more to the availability of the service, practicalities of transfer to an ECLS centre, timing and patient selection, care of the patient on ECMO and minimising complications [4]. Patients often need to be retrieved whilst supported by ECMO, and data suggest that this can be undertaken safely in trained hands with an appropriate system [19]. VV ECMO and the more portable proprietary extracorporeal respiratory support devices such as Cardiohelp™ (Maquet Cardiopulmonary AG, Hirrlingen, Germany) can be utilised for transport [20].

A number of configurations of VV ECMO can be applied based on individual patient requirements (Table 1). Although there are many other factors, patient arterial oxygenation is critically dependent on the fraction of total cardiac output passing through the oxygenator while adequate carbon dioxide ($CO_2$) clearance can still occur with lower blood flows [21]. The bicaval dual-lumen Avalon™ cannula (Avalon Laboratory, Los Angeles, CA, USA) inserted through the internal jugular vein allows single-site cannulation for VV ECMO [22], but flows are unlikely to be as high compared with the use of two large venous drainage cannulae positioned in the venae cavae via internal jugular and femoral veins. Additionally, meticulous positioning is required, usually with transoesophageal echocardiography. Use of a dual-lumen cannula in the neck may facilitate mobilisation in bed, extubation and rehabilitation in patients who receive prolonged ECMO support [22].

### Extracorporeal carbon dioxide removal

Hypercapnia and respiratory acidosis, although usually well tolerated, is a barrier to implementing ultra-

**Table 1 Available extracorporeal respiratory support devices and strategies**

| ECLS strategy | Principle indication(s) |
| --- | --- |
| VV ECMO standard (femoral vein–femoral vein) | Default strategy for complete extracorporeal respiratory support |
| VV ECMO (dual-lumen cannula) | Complete or partial respiratory support predominantly |
| | Bridge to lung transplant |
| VV ECMO high flow (SVC and IVC access) | Complete respiratory support for larger patients; for example, male weight >90 kg |
| VV ECMO high flow with two oxygenators in parallel | Complete respiratory support for very large patients; for example, male weight >120 kg |
| Femoral VV with pump (iLA™Activve; Novalung GmbH, Hechingen, Germany) | Complete or partial respiratory support |
| Pulmonary artery–left atrium pumpless with oxygenator (iLA™; Novalung GmbH) | Bridge to lung transplant |
| | Salvage for refractory hypoxia during complete respiratory support on VV ECMO |
| | Salvage for severe pulmonary hypertension with normal left heart |
| Femoral arterio-venous pumpless (iLA™; Novalung GmbH) | Partial respiratory support only in a very haemodynamically stable patient |
| VV ECCOR (Hemolung™; Alung Technologies, Pittsburgh, PA, USA) | Partial respiratory support |

Complete respiratory support, oxygenation and carbon dioxide removal; partial respiratory support, carbon dioxide removal and some or no oxygenation. ECCOR, extracorporeal carbon dioxide removal; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; IVC, inferior vena cava, iLA, interventional lung assist; SVC, superior vena cava; VV, venovenous.

protective ventilation [21]. This barrier has renewed interest in extracorporeal technologies that facilitate extracorporeal $CO_2$ removal (ECCOR). Refinements in technology [21,23] have resulted in fewer complications when used as adjuncts to LPV [24], but definitive evidence is lacking [25].

ECCOR requires an arterial or venous access cannula, a pump to drain blood during venous access, a membrane lung and a return venous cannula. Heparin-coated wire-reinforced cannulae may be placed percutaneously in a femoral–femoral or a femoral–jugular orientation. Alternatively, a wire-reinforced double-lumen catheter may be inserted under ultrasound guidance via the right internal jugular vein with the drainage port positioned in the intra-hepatic inferior vena cava and the return port in the right atrium [21,26]. A flow of fresh gas containing little or no $CO_2$ is utilised to create a diffusion gradient across the membrane and allows $CO_2$ removal. While ECMO necessitates high blood flow rates (5 to 7 l/minute) to ensure optimal oxygenation, ECCOR allows $CO_2$ removal at much lower blood flow rates (<1 l/minute) due to significant differences in $CO_2$ and oxygen kinetics [21,23]. Although lower blood flows can be achieved with smaller cannulae with greater ease, vascular complications may still occur especially with arterial cannulation.

Various novel ECCOR devices are currently available to facilitate LPV and are reviewed in detail elsewhere [21,23]. The available and emerging devices are summarised in Table 1. The pumpless interventional lung assist iLA™ membrane ventilator marketed by Novalung GmbH (Hechingen, Germany) is a low-gradient device (Figure 1) that can be employed peripherally (femoral artery access and femoral vein return) and allows complete $CO_2$ removal in patients with adequate oxygenation and robust haemodynamics. There have been reports of its successful use in patients with ARDS [28] and severe asthma [29] and as a bridge to lung transplantation [30]. However, the risks of arterial access have to be carefully considered in these patients. The pulmonary artery–left atrial configuration of the same device has been used as a bridge to lung transplantation particularly in those who have significant pulmonary hypertension [31]. A VV configuration of the membrane oxygenator with a pump (iLA™ Activve; Novalung GmbH) is also available for partial or complete respiratory support [32].

Devices such as the Decap™ system (Hemodec, Salerno, Italy) that serve the dual purpose of renal replacement therapy and ECCOR are also available [33]. By combining the membrane lung and the pump into a single unit, the Hemolung™ (Alung Technologies, Pittsburgh, PA, USA) achieves efficient $CO_2$ removal at flows between 400 and 600 ml/minute [21] using dual-lumen catheters similar to those used for renal replacement



**Figure 1 Interventional lung assist device (iLA™; NovaLung GmbH, Talheim, Germany) for pumpless arterio-venous carbon dioxide removal.** Reproduced with permission from [27].

therapy. These low-flow systems provide partial $CO_2$ removal only and do not provide any oxygenation benefit. Even though modern low-flow VV ECCOR devices reportedly require a lower degree of anticoagulation, concerns remain over risks of circuit thrombosis. Other emerging technologies such as intravascular gas exchange and respiratory dialysis [21] are beyond the scope of this article. Similarly, bridging the acutely ill patients to lung transplantation [34,35] with ECLS is a highly specialised service beyond the scope of this review.

Extracorporeal respiratory support can thus be provided with ECCOR or ECMO depending on the lung pathology, pulmonary compliance and oxygenation and decarboxylation requirements of an individual patient. ECMO and ECCOR can also bridge highly selected patients to lung transplantation.

## Extracorporeal life support in acute cardiac failure
Providing temporary mechanical circulatory support (MCS) support to patients with acute refractory cardiac failure using ECLS techniques is a rapidly evolving area; intervention may be time critical and mortality is higher than ECLS for isolated respiratory failure [36,37]. The use of ECLS in the setting of cardiopulmonary resuscitation is discussed elsewhere [38,39]. Patient outcomes with the use of long-term ventricular assist devices (VADs) in cardiogenic shock (INTERMACS class 1) are poor [40,41]. Recently published International Society for Heart and Lung Transplantation Guidelines for MCS
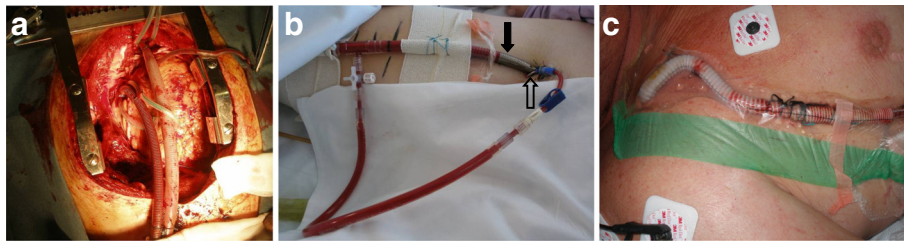
**Figure 2 Venoarterial extracorporeal membrane oxygenation.** Venoarterial extracorporeal membrane oxygenation can be instituted: **(a)** centrally by cannulating the right atrium/inferior venacava and the aorta; **(b)** peripherally using the femoral vein and femoral artery (solid arrow, arterial return cannula; hollow arrow, back-flow cannula for distal limb perfusion); or **(c)** peripherally using the axillary/subclavian artery. The choice is often guided by the clinical setting, the expected duration of support and pulmonary function.

provide recommendations for long-term MCS options for patients with cardiac failure [42], and these are not discussed in this article. These guidelines strongly recommend consideration of the use of temporary MCS in patients with multiorgan failure, with sepsis or on mechanical ventilation to allow successful optimisation of their clinical status and neurologic assessment prior to placement of a long-term MCS device [42].

The severity of noncardiac organ system failures can be defined using scoring systems such as the Sequential Organ Failure Assessment score. Severe multiorgan failure (for example, Sequential Organ Failure Assessment score >15) has been considered a contraindication to VV ECMO [43] and similar criteria may be applicable for venoarterial (VA) ECMO or for the use of an ECMO circuit as a temporary VAD. Factors considered in the initial cannulation strategy include: the underlying cause of cardiac dysfunction and projected time course of recovery; the severity of

pulmonary dysfunction and projected time course of recovery; the functional reserve of each ventricle; the presence and severity of valvular pathology; risk of arterial access and size of vessels; the severity of coagulopathy and risk of sternotomy; and planned future surgery, such as long-term VAD or transplant.

For patients with predominant cardiac failure with preserved pulmonary function, the available MCS devices provide several options (Table 2). Central VA ECMO has been traditionally applied as a bridge to recovery in patients who fail to wean from cardiopulmonary bypass after cardiac surgery (Figure 2). Central VA ECMO outside this setting in adults is uncommon. Femoral VA ECMO (Figure 2) is more commonly used in adults requiring urgent cardiac support because it can be achieved rapidly and a sternotomy is avoided. One of the major limitations of peripheral femoro-femoral VA ECMO is left ventricular (LV) afterload mismatch and

**Table 2 Extracorporeal life support strategies for mechanical circulatory support in isolated cardiac failure**

| ECLS strategy | Principle indication(s) |
|---|---|
| VA ECMO (return femoral artery) | Default strategy for potentially reversible cardiogenic shock of any cause |
| Central VA ECMO (return aorta) | Failure to wean from cardiopulmonary bypass where recovery expected within 7 days |
| | Salvage for small patients with cardiogenic shock where femoral arterial access inadequate |
| VA ECMO (return axillary artery) | Reversible cardiogenic shock where high flows not required |
| | Reversible cardiogenic shock with lower-limb vascular disease |
| Centrimag™ (Levitronix LLC, Waltham, MA, USA) LVAD (access left atrium/left ventricle, return aorta) | Isolated LV support where recovery is expected in 8 weeks |
| Centrimag™ (Levitronix LLC) RVAD (access right atrium, return pulmonary artery) | Isolated RV support where recovery is expected in 8 weeks |
| Centrimag™ (Levitronix LLC) BiVAD | Biventricular support where recovery is expected in 8 weeks |
| TandemHeart (CardiacAssist, Inc., Pittsburgh, PA, USA) percutaneous LVAD (access left atrium via femoral vein, return femoral artery | Isolated LV support |
| Impella™ (Abiomed, Aachen, Germany) percutaneous LVAD (access femoral artery) | Isolated LV support |
| Peripheral VA ECMO + Impella™ (Abiomed) percutaneous LVAD | Isolated LV support with better LV decompression |
| Implantable LVAD + temporary RVAD (±oxygenator) | Met criteria for LVAD but unexpected reversible RV dysfunction occurred |

BiVAD, biventricular assist device; ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; LV, left ventricular; LVAD, left ventricular assist device; RV, right ventricular; RVAD, right ventricular assist device; VA, venoarterial.

inadequate LV decompression/venting. This limitation is particularly so in patients with very low native cardiac output states and severe mitral valve regurgitation, and may result in severe hydrostatic pulmonary oedema in some patients. Although some centres use an intra-aortic balloon pump in conjunction with peripheral VA ECMO to reduce LV afterload and pulmonary congestion, no definitive data exist to support routine use.

LV and aortic root stasis from lack of cardiac ejection and failure of aortic valve opening may result in catastrophic intracardiac and aortic root thrombosis. Increased anticoagulation to minimise this risk may heighten the risk of significant bleeding. Minimally invasive strategies such as percutaneous transseptal left atrial decompression [44] and subxiphoid surgical approaches to drain the left ventricle [45] have been described to reduce LV distension. The residual atrial defect may require correction once the patient has been weaned from mechanical support. Use of a percutaneously inserted VAD (Impella™; Abiomed, Aachen, Germany) to decompress the left ventricle has also been reported in this setting [46], alleviating the need for a high-risk septostomy or surgical venting.

Femoral VA ECMO is also limited by femoral arterial size, and thus cannula size and the requirement for distal limb perfusion. Given its less invasive nature (compared with thoracic access), peripheral VA ECMO – with attention to optimal LV afterload, minimising LV distension with optimal fluid and inotrope therapy, anticoagulation and pulmonary management – is a viable first-line option for patients with isolated acute cardiac failure refractory to conventional management.

The limitations of peripheral VA ECMO have prompted the use of ECMO devices [47] to facilitate ventricular unloading by changing to a temporary left ventricular assist device (LVAD) or a biventricular assist device configuration (Figure 3). Any perfusion strategy that creates a right to left shunt requires an oxygenator in the circuit. Oxygenators may additionally provide temperature control. This strategy effectively provides biventricular support and gas exchange through a single pump configuration with the ability to cease right ventricular (RV) support when not required. However, this configuration requires sternotomy and cannulation of the left ventricle (or left atrium) and aorta. A reoperation (sternotomy or thoracotomy) is then required for explantation of the cannulae upon cardiac recovery or for implantation of a long-term mechanical assist device. Less invasive techniques for temporary cardiorespiratory support including a transition strategy to an intermediate-term support configuration [48] allowing mobilisation have been described (Figure 4). Although



**Figure 3 Biventricular assist device along with respiratory support provided by the oxygenator in the circuit.** The hybrid Centrimag™ (Levitronix LLC, Waltham, MA, USA) extracorporeal membrane oxygenation system can be used as a biventricular assist device along with respiratory support provided by the oxygenator in the circuit. Reproduced with permission from [47]. LVAD, left ventricular assist device; RA, right atrium.

**Figure 4 From venoarterial extracorporeal membrane oxygenation (ECMO) to use of ECMO as a temporary ventricular assist device.**
**(A)** Emergent femoro-femoral venoarterial extracorporeal membrane oxygenation. **(B)** Left ventricular apical cannulation and decompression. **(C)** Right ventricular recovery and isolated temporary left ventricular support. **(D)** Axillary artery cannulation to facilitate mobilisation. Reproduced with permission from [48].

this configuration requires a left thoracotomy, sternotomy is avoided, potentially reducing risk for subsequent surgery in the absence of cardiac recovery (long-term VAD implantation as a bridge to destination or heart transplantation).

Temporary RV support can be provided with the Centrimag™ ECMO system (Levitronix LLC, Waltham, MA, USA) through percutaneous femoral venous access to the right atrium and return to the pulmonary artery via a cannulated exteriorised Dacron graft. This strategy is described for temporary support of the RV with insertion of a long-term LVAD but is applicable to other causes of severe isolated RV dysfunction. An oxygenator can be included in the circuit to ensure adequate oxygenation, $CO_2$ removal and temperature regulation. Upon RV recovery, the graft can be ligated and buried upon decannulation without re-sternotomy.

Percutaneously inserted LVADs such as TandemHeart™ (CardiacAssist, Inc., Pittsburgh, PA, USA) and Impella™ (Abiomed) [49] are potential options for MCS in the acute setting. However, there is a paucity of supportive evidence for their use and the complications with arterial access such as bleeding and limb ischemia cannot be understated. TandemHeart™ utilises a centrifugal pump to drain the left atrial blood from a catheter placed transseptally via the femoral vein and returns it to the

femoral artery. The Impella™ device uses an axial pump that is inserted retrogradely across the aortic valve via the femoral artery. These devices provide LV support and lack the ability to provide extracorporeal respiratory support if required. However, there are case reports pertaining to their successful use as RV assist devices and/or biventricular assist devices [50,51].

Even though the third-generation, implantable LVADs designed for long-term MCS are a significant improvement on earlier devices [52], their use in a deteriorating patient with multiorgan dysfunction is associated with poor outcomes and is not currently recommended.

## Advanced extracorporeal life support in severe cardiorespiratory failure

The number of patients with severe combined cardiac and respiratory failure who fail conventional treatment is very small and ECLS in this group is controversial, being considered either heroic or futile. In the setting of pneumonia or sepsis and severe cardiac dysfunction, where feasible, VV ECMO with inotropic support should be the initial perfusion strategy [53]. Septic myocardial depression may improve with management of the sepsis, improved oxygenation and normalisation of respiratory acidosis [54]. Peripheral femoral VA ECMO (Figure 2) may be considered a rescue option for these patients if

myocardial depression is profound [55] or if the diagnosis is uncertain and conditions such as myocarditis are considered likely. Use of this strategy in septic patients with multiple organ failure who may have severe coagulation and hepatic dysfunction may be futile. However, heroic measures can result in good outcomes [56].

Upper body hypoxia can occur if myocardial recovery occurs and lung function remains poor. This may be overcome by transition to VV ECMO if myocardial recovery is satisfactory or with the use of VA–venous ECMO, which allows return of oxygenated blood to both arterial and venous sides of the circulation, thereby minimising the risk of upper-body hypoxia. Although peripheral arterial cannulation for VA–venous ECMO is less invasive and is an attractive option, balancing the oxygenation and perfusion needs of an individual patient by regulating the return of oxygenated blood to the underperfused coronary and cerebral circulation may be challenging, and a central configuration may be preferred in this setting. Returning the oxygenated blood in the ascending aorta by cannulating the axillary [57,58] or subclavian artery cannulation has also been described (Figure 2) in this setting. However, axillary artery side graft cannulation may be complicated by ipsilateral upper-limb hyperperfusion and bleeding from the arterial graft [59].

A more invasive, high-risk option in this setting includes use of the Centrimag™ ECMO system (Levitronix LLC) as a temporary LVAD/biventricular assist device with an oxygenator in the circuit. The device can be employed in several configurations (Table 3) to support both the left and/or right ventricles and the oxygenator can be removed from the circuit when pulmonary function stabilises. This strategy can support patients for a longer period of time, allowing more time to recover, and minimises the risks of LV distension and thrombosis. This is ideally suited to patients with suspected acute myocarditis in whom myocardial recovery is possible but prolonged support may be required. Alternatively, central VA ECMO may be used in a patient *in extremis* [60,61] when femoral cannulation is expected to be difficult. Regardless of the initial strategy used, transition to VV ECMO (Figure 5) should be considered as soon as adequate cardiac function returns and is pragmatically possible. Continued vigilance as well as prospective risk management of the potential for LV and/or aortic root thrombosis must be considered when exploring specific potential configurations, and must be assessed prior to implantation.

By providing a range of support options based on the degree of cardiac and respiratory failure (Table 3), ECLS thus redefines the contemporary management of this condition.

## Experimental extracorporeal life support therapies

Several other ECLS cannulation strategies merit consideration and further investigation. These strategies are necessitated by inherent limitations of ECLS therapies

**Table 3 Advanced extracorporeal life support strategies for cardiac and respiratory support: bridging to intermediate or long-term support may be required**

| ECLS strategy | Principle indication(s) |
|---|---|
| VA ECMO (return femoral artery) | Default strategy for potentially reversible cardiogenic shock of any cause |
| VA ECMO (return axillary artery) | Reversible cardiogenic shock where high flows are not required |
| | Reversible cardiogenic shock with lower-limb vascular disease |
| | Reversible cardiogenic shock with poor gas exchange |
| VA ECMO (return ascending aorta) | Failure to wean from cardiopulmonary bypass where recovery expected within 7 days |
| | Salvage for small patients with cardiogenic shock where femoral arterial access inadequate |
| | Salvage for severe combined cardiac and respiratory failure |
| VA–venous ECMO | Patients developing circulatory instability on venovenous ECMO |
| | Salvage for severe combined cardiac and respiratory failure |
| Venous–pulmonary artery ECMO | Reversible RV dysfunction expected duration up to 2 weeks |
| Centrimag™ (Levitronix LLC, Waltham, MA, USA) RVAD (femoral access + oxygenator) | Reversible RV dysfunction expected duration up to 2 weeks |
| Centrimag™ (Levitronix LLC) RVAD (right atrium access + oxygenator) | Reversible isolated RV dysfunction expected duration up to 8 weeks with plan to remove oxygenator and convert to RVAD |
| Centrimag™ (Levitronix LLC) hybrid (requires oxygenator) | Severe LV after load mismatch on VA ECMO |
| | Severe combined cardiac and respiratory failure where early RV recovery is expected before intermediate term LV recovery |
| Implantable LVAD + temporary RVAD (±oxygenator) | Met criteria for LVAD but unexpected reversible RV dysfunction occurred |

ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; LV, left ventricular; LVAD, left ventricular assist device; RV, right ventricular; RVAD, right ventricular assist device; VA, venoarterial.
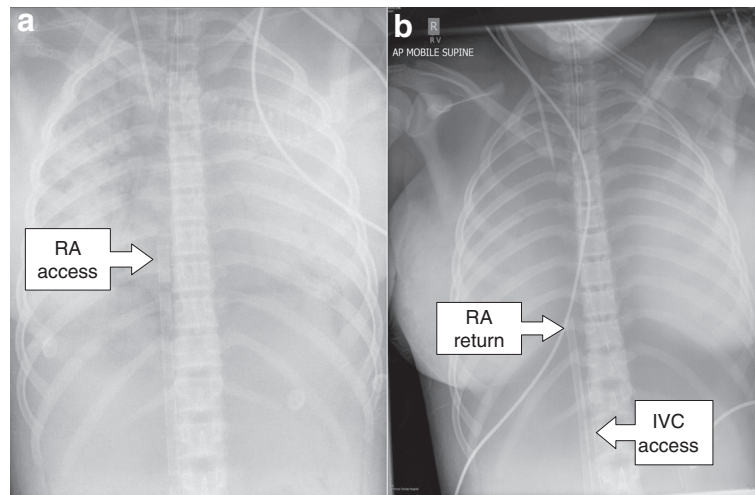
**Figure 5 Patient *in extremis* initially receiving femoro-femoral venoarterial extracorporeal membrane oxygenation (ECMO) with transition to venovenous ECMO.** Patient *in extremis* initially received femoro-femoral venoarterial (VA) ECMO for severe cardiorespiratory failure with transition to venovenous (VV) ECMO on day 4 following satisfactory cardiac recovery. **(a)** Chest X-ray scan shows multistage access cannula in the right atrium (RA) during VA ECMO, **(b)** which was later withdrawn into the inferior vena cava (IVC) during VV ECMO. **(b)** A venous return cannula can also be seen in the right atrium.

such as VV ECMO and VA ECMO and also to make some of the support options less invasive. A summary of these therapies is presented in Table 4.

Refractory hypoxia and severe pulmonary hypertension may be encountered on VV ECMO and is often terminal. Percutaneous transeptal cannulation of the left atrium to return the oxygenated blood has been proposed as an option to avoid a sternotomy, which needs to be further investigated. Similarly, percutaneous transeptal cannulation of the left atrium for access along with percutaneous right atrial cannulation may assist in venting the left ventricle during VA ECMO. Other emerging less invasive ECLS options include hybrid systems that can provide renal replacement therapy and $CO_2$ removal.

## Limitations of extracorporeal life support therapies

ECLS therapies are high-risk invasive interventions undertaken in a few specialised centres. Despite the advancements in ECLS technology, the associated complications such as bleeding, thrombosis and infections cannot be underestimated. Apart from the risk profile, the success of

ECLS relies heavily on its clinical application. Careful selection of both patients and the ECLS perfusion strategy is the key and not all centres may have the experience or the resources to provide the full complement of ECLS therapies discussed in this paper.

The lack of robust evidence is a significant limitation. The complexities in delivering ECLS include resources and cost-effectiveness, staff training, governance and availability of funding for other programmes such as cardiothoracic surgery, long-term mechanical assist devices, and heart and lung transplantation. Such undertaking may be feasible in resource-rich settings, but significant innovation and refinement is required prior to its widespread use. With minimal improvement in outcomes in ARDS over the years, widespread use of VV ECMO and ECCOR may be a reality in years to come. VA ECMO and its use as a temporary VAD for MCS is a complex undertaking and its use will probably be limited to specialised centres.

## Conclusion

ECLS therapies hold promise and further research is indicated to explore their full potential. Given the small

**Table 4 Experimental extracorporeal life support options**

| ECLS strategy | Possible indication(s) |
|---|---|
| VV ECMO + atrial septostomy | Refractory hypoxia and/or pulmonary hypertension on VV ECMO avoiding sternotomy |
| VV ECMO + transeptal return to left atrium | Refractory hypoxia and/or pulmonary hypertension on VV ECMO avoiding sternotomy |
| Venoarterial ECMO + transeptal access from left atrium and right atrium | Refractory left ventricular distension on venoarterial ECMO |

ECLS, extracorporeal life support; ECMO, extracorporeal membrane oxygenation; VV, venovenous.

number of patients who receive ECLS for cardiac and/or respiratory support globally, it may not be feasible to generate evidence-based guidelines for all available therapies. However, ongoing refinements in technology, development of minimally invasive techniques, better understanding of the physiological impact of the ECLS circuit (for example, altered pharmacokinetics of vital drugs [62]) and improved clinical delivery may improve patient outcomes. ECLS therapies will probably play a vital future role in the management of adult patients with acute cardiorespiratory failure. Collaboration between global ECLS centres is the key in designing and conducting high-quality clinical trials that will hopefully provide more clarity in patient selection, choice of ECLS device and the appropriate perfusion strategy to be used.

### Abbreviations

ARDS: Acute respiratory distress syndrome; $CO_2$: Carbon dioxide; ECCOR: Extracorporeal carbon dioxide removal; ECLS: Extracorporeal life support; ECMO: Extracorporeal membrane oxygenation; LPV: Lung-protective ventilation; LV: Left ventricular; LVAD: Left ventricular assist device; MCS: Mechanical circulatory support; RV: Right ventricular; VA: Venoarterial; VAD: Ventricular assist device; VV: Venovenous.

### Competing interests

The authors declare that they have no competing interests.

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## 1.3 INCREASED SEDATION REQUIREMENTS IN PATIENTS RECEIVING EXTRACORPOREAL MEMBRANE OXYGENATION FOR RESPIRATORY AND CARDIORESPIRATORY FAILURE.

### 1.3.1. Introduction to this publication

This analysis of sedation data from patients receiving ECMO was the first report of altered sedative PK in adult patients. Patients on ECMO required much more sedation (often greater than 4 fold) when compared with critically ill patients not on ECMO. Sedative drugs have clinically titrable endpoints where as this is not the case for antibiotic drugs for many of which even therapeutic drug monitoring is not readily available. Thus this paper serves not only as a hypothesis generator but makes the case for further systematic research.

# Increased sedation requirements in patients receiving extracorporeal membrane oxygenation for respiratory and cardio-respiratory failure

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## SUMMARY

Critically ill patients receiving extracorporeal membrane oxygenation (ECMO) are often noted to have increased sedation requirements. However, data related to sedation in this complex group of patients is limited. The aim of our study was to characterise the sedation requirements in adult patients receiving ECMO for cardio-respiratory failure. A retrospective chart review was used to collect sedation data for 30 consecutive patients who received venovenous or venoarterial ECMO between April 2009 and March 2011. To test for a difference in doses over time we used a regression model. The dose of midazolam received on ECMO support increased by an average of 18 mg per day (95% confidence interval 8, 29 mg, $P=0.001$), while the dose of morphine increased by 29 mg per day (95% confidence interval 4, 53 mg, $P=0.021$) The venovenous group received a daily midazolam dose that was 157 mg higher than the venoarterial group (95% confidence interval 53, 261 mg, $P=0.005$). We did not observe any significant increase in fentanyl doses over time (95% confidence interval 1269, 4337 μg, $P=0.94$). There is a significant increase in dose requirement for morphine and midazolam during ECMO. Patients on venovenous ECMO received higher sedative doses as compared to patients on venoarterial ECMO. Future research should focus on mechanisms behind these changes and also identify drugs that are most suitable for sedation during ECMO.

*Key Words: extracorporeal membrane oxygenation, sedation and analgesia, drug toxicity, cardio-respiratory failure, pharmacokinetics*

Extracorporeal membrane oxygenation (ECMO) temporarily supports patients with severe cardiac and/or respiratory failure that is not responsive to maximal conventional treatment[1]. It is usually a bridge to organ recovery, but can also be used as a bridge to long-term mechanical assist devices or transplantation[2,3]. Its role extends even further as an adjunct to cardiopulmonary resuscitation (CPR)[4], as well as high risk percutaneous cardiac interventions[5]. Improvements in equipment, patient selection and a better understanding of extracorporeal circuit technology have resulted in some centres reporting significantly improved outcomes[6-8]. The Conventional Ventilation or ECMO for Severe Adult Respiratory Failure (CESAR) trial[9], and the Australian and New Zealand Intensive Care Society ECMO investigator study into Influenza A (H1N1)[10], support its role in the advanced management of respiratory failure unresponsive to maximal conventional therapy[11-14]. Data from the Extracorporeal Life Support Organization registry[15] suggest an increasing use of ECMO in adults with refractory respiratory and/or cardiac failure, with a reported survival of 63% for venovenous (VV) ECMO and 53% for venoarterial (VA) ECMO.

ECMO is a highly invasive treatment in a group of patients who have failed conventional treatment and optimal sedation for this complex group of patients is not clearly defined. Sedative and analgesic medications promote patient

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comfort and eliminate pain, anxiety, agitation, delirium and other forms of distress induced by the intensive care unit (ICU) environment[16]. However, excessive sedation may lead to adverse outcomes. Use of objective scoring systems, sedation protocols[17] allowing lighter sedation with daily interruptions[17-19] and minimising administration of paralytic agents have all been shown to reduce morbidity in ICU. Although evidence-based, these practices may not always be applicable to a critically ill patient on ECMO. Sedation during ECMO minimises the risk of catheter malposition or dislodgement; as well as coughing, which induces 'suck down' or chatter, resulting in haemolysis. Deep sedation and paralysis may be required, especially early in the course of VV ECMO to achieve better circuit flows, minimise oxygen consumption and optimise ventilation. These factors may lead to a degree of 'permissiveness' when sedating patients during ECMO. To overcome this problem, some centres perform an early tracheostomy[20] but the risks of this procedure in a patient on extracorporeal life support (ECLS) cannot be underestimated. Although neonatal studies have highlighted the increased sedation requirements during ECMO[21-24], no such data is available for adult patients.

The aim of the study was to retrospectively review the sedative and analgesic drug requirements in adult patients receiving ECMO in our intensive care unit.

## METHODS

### Study design and participants

A retrospective chart review was performed after obtaining local ethics approval (HREC/11/QPCH/3). All patients undergoing ECMO since the commencement of the program at the Prince Charles Hospital, Brisbane, from April 2009 to February 2011 were included. One patient was excluded as death occurred in the operating room before meaningful data was charted.

### Details of sedation, ventilation and ECLS

Our local protocol requires patients to be heavily sedated (equivalent to a Riker Sedation-Agitation Scale of 1 to 2) during the first few days of ECMO. This is particularly relevant for patients receiving VV ECMO to minimise oxygen consumption, optimise ECMO flows and ventilation. The senior nurses at the bedside titrate sedation and analgesia to patient comfort, degree of agitation, delirium and distress while preventing inadvertent disconnection of the circuit. Sedation is lightened where possible to assess neurology on a regular basis. Midazolam is used for sedation

in all patients. Morphine is used for analgesia in patients with conserved renal function and fentanyl is used in patients with renal dysfunction or those requiring renal replacement therapy (RRT). Patients requiring additional sedation are given propofol, dexmedetomidine and thiopentone. Where necessary patients also may be prescribed enteral diazepam and/or neuroleptic agents if clinically indicated. Sedation is rapidly de-escalated after discontinuation of ECMO, particularly where a tracheostomy is performed.

The patients in the VV group receive a tidal volume of less than 6 ml/kg lean body weight with a positive end expiratory pressure of 10 to 15 $cmH_2O$, limiting plateau pressures and fractional inspired oxygen ($FiO_2$) to less than 30 $cmH_2O$ and 0.5 respectively. Ventilator settings in the VA group were chosen to offer adequate lung aeration, prevention of atelectasis, avoidance of overdistension and prevention of coronary and cerebral hypoxemia (tidal volume 6 to 8 ml/kg lean body weight, rate 6 to 12 breaths/minute, $FiO_2$ ≤0.5, positive end expiratory pressure >5 $cmH_2O$, end tidal carbon dioxide -20 to 30 mmHg).

The standard ECMO circuitry comprised poly vinyl chloride tubing, centrifugal pump (Jostra Rotaflow™, Maquet, Germany) and a hollow fibre oxygenator (Quadrox D™, Maquet, Germany). RRT included both continuous (Aquarius™, Baxter International Inc., USA), using a percutaneous vascular catheter, and extended daily diafiltration (ARrT Plus™, Fresenius USA Inc., USA) directly via the ECMO circuit.

### Data collection

Data collection from the medical record was performed using a detailed data collection instrument. Hourly total doses of midazolam, fentanyl and morphine including infusion and boluses were recorded from the time of ECMO commencement to the end of the day when ECMO was ceased. Drug dosing is described as total daily dose and was the study variable unless otherwise specified. Demographic and severity of illness data were obtained from the local ECLS database. Sequential Organ Failure Assessment (SOFA) scores were recorded on days 1, 3 and 5 of the ICU stay, and Acute Physiology and Chronic Health Evaluation (APACHE) III scores within the first 24 hours of ICU stay.

### Statistical analysis

To provide a visual display of drug dosing over time we plotted the daily drug dose for each patient. We also plotted the average drug dose per day to

show the average dosing over time. To test for differences in drug dosing over time we used a regression model. To allow each patient to have a different profile over time we used a mixed effects model and fitted a random intercept and slope for each patient. We examined the residuals of the final model to check for normality. We also included variables for RRT and VA/VV ECMO to examine differences in these patients both on average and over time. To examine the association between APACHE III and SOFA scores and midazolam dose, we used scatter plots and linear regression. These analyses used each patient's mean midazolam dose over their entire ECMO run. Results are presented as means and 95% confidence intervals. We used a two-sided statistical significance level of 0.05. Analyses were performed using R version 2.12.2 (www.r-project.org).

RESULTS

Of the 29 patients analysed, 13 received VV and 16 received VA ECMO. In total, 342 days of ECMO support was cumulatively received by all patients; VV, median 356 hours (range 52 to 645 hours); VA, median 160 hours (range 0 to 514 hours). The patient characteristics and severity of illness data are presented in Table 1.

Patients received between 0 and 1440 mg per day of midazolam. The median daily midazolam dose was 175 mg (range 24 to 1092 mg). Figure 1

and Table 2 shows the summary results for midazolam, morphine and fentanyl. On average, the daily dose of midazolam increased by 18 mg per day (95% confidence interval 8, 29 mg, $P=0.001$) after commencement of ECMO, representing an increased dose of 10.2%. The VV group had a daily midazolam dose 157 mg higher on average than the VA group (95% confidence interval 53, 261 mg, $P=0.005$). There was no statistically significant difference in average dose of midazolam for the RRT group ($P=0.16$). On average, the daily dose of morphine increased by 29 mg per day (95% confidence interval 4, 53 mg, $P=0.02$). There was no statistically significant increase in fentanyl doses over time ($P=0.94$). Fifteen of the 29 patients required propofol (100 to 300 mg/hour) as a rescue agent at various times and there was an increased use between days 3 and 8. Nine of these 15 patients received VV ECMO. Dexmedetomidine and thiopentone were used in two patients. All patients on VV ECMO received boluses of a neuromuscular blocker (vecuronium, median daily dose 20 mg, range 20 to 40 mg/day) early in their course of ECMO.

In Figure 2 the predicted daily doses of midazolam, fentanyl and morphine are graphed according to whether RRT was used. There was no significant influence of RRT on average doses over time (one to 21 days) for midazolam, morphine and fentanyl in both VA and VV groups. In Figure 3, measures of sickness severity, SOFA

TABLE 1
*Demographic and severity of illness data*

|  | VV ECMO | VA ECMO |
|---|---|---|
| Age, median, y | 35 (18-55) | 54 (16-77) |
| Gender, male (%) | 6 (46) | 12 (71) |
| Admission weight, kg | 71.5 (47-106) | 79 (44-112) |
| Duration of ECMO, h | 356 (52-645) | 160 (1-514) |
| APACHE II score | 20 (16-35) | 21 (9-39) |
| APACHE III score | 80 (51-123) | 79 (48-128) |
| APACHE III ROD estimate | 0.39 (0.13-0.73) | 0.36 (0.04-0.91) |
| SOFA score day 1 | 10 (6-16) | 10 (6-16) |
| SOFA score day 3 | 10 (5-17) | 10 (4-17) |
| SOFA score day 5 | 11(4-14) | 10 (5-11) |
| Pre-ECMO creatinine, $\mu$mol/l | 166 (35-422) | 127 (65-383) |
| Pre-ECMO bilirubin, $\mu$mol/l | 20 (4-189) | 35 (6-101) |
| PRBC transfusion, units | 11(4-53) | 31(8-191) |

VV=venovenous, ECMO=extracorporeal membrane oxygenation, VA=venoarterial, APACHE=Acute Physiology and Chronic Health Evaluation, ROD=risk of death, SOFA=Sequential Organ Failure Assessment, PRBC=packed red blood cells.

and APACHE III scores are compared with daily midazolam dose. Higher SOFA scores were associated with lower doses, although this association was of borderline statistical significance ($P=0.053$). Higher APACHE III scores were non-significantly associated with lower midazolam doses ($P=0.14$).

DISCUSSION

This study highlights the challenges in maintaining optimal sedation in patients receiving ECMO. The data presented are unique and advance our understanding of sedative drug use during ECMO, which is at present very limited. In general, most
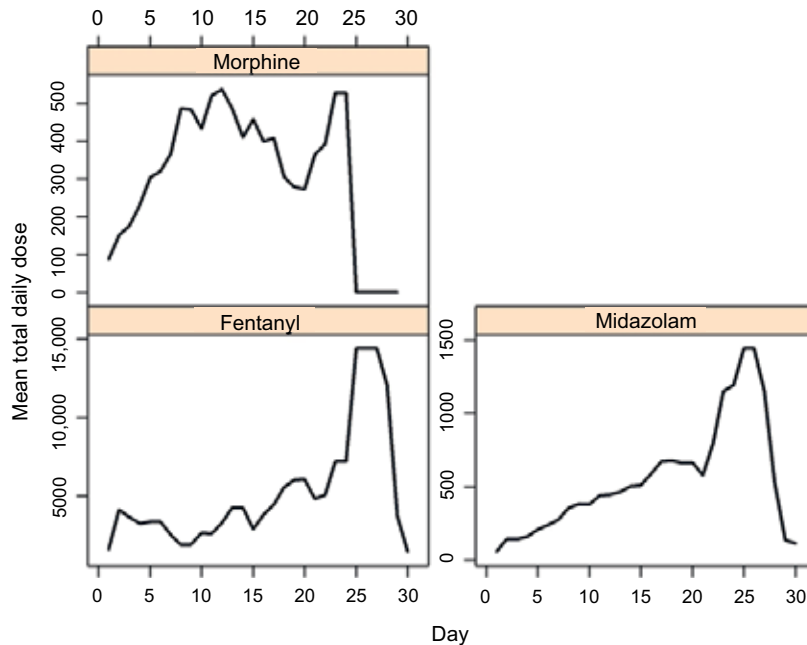


FIGURE 1: Average dose requirements for morphine, midazolam and fentanyl appear to increase with the duration of ECMO. In some patients, this occurred despite the concurrent use of other sedative agents (propofol, dexmedetomidine and thiopentone). Tracheostomy on liberation from ECMO led to a significant decrease in sedative and analgesic drug requirements seen between days 25 to 30. This data is particularly influenced by patients on venovenous ECMO.

TABLE 2
*Mixed model results for midazolam, morphine and fentanyl*

| Drug | Variable | Mean | 95% CI | P value |
|------|----------|------|--------|---------|
| Midazolam | Day | 18 | 8, 29 | 0.001 |
| | RRT=Y | -72 | -175, 32 | 0.164 |
| | VA/VV=VV | 157 | 53, 261 | 0.005 |
| Morphine | Day | 29 | 4, 53 | 0.021 |
| | RRT=Y | -23 | -247, 202 | 0.83 |
| | VA/VV=VV | 129 | -103, 361 | 0.24 |
| Fentanyl | Day | -7 | -198, 184 | 0.94 |
| | RRT=Y | 419 | -1426, 2264 | 0.64 |
| | VA/VV=VV | 166 | -1698, 2030 | 0.86 |

To allow each patient to have a different profile over time we used a mixed effects model and fitted a random intercept and slope for each patient to examine changes in the daily doses of midazolam, morphine and fentanyl over time with or without RRT, and with or without VV or VA ECMO. On average the 24-hour dose increased by 18 mg for midazolam and 29 mg for morphine from the day of commencement of ECMO. There was no significant increase in fentanyl doses over time. CI=confidence interval, RRT=renal replacement therapy, Y=yes, VA=venoarterial, VV=venovenous.

patients received increasing doses of midazolam and morphine with time. This occurred despite the concurrent use of multiple sedative agents like propofol, dexmedetomidine, thiopentone and neuroleptic drugs in some patients. Interestingly, in this small group of patients RRT did not significantly influence the drug doses used. The difference in the severity of illness between patients receiving VA and VV ECMO was not significant enough to explain the higher dose requirement in the VV group. Patients
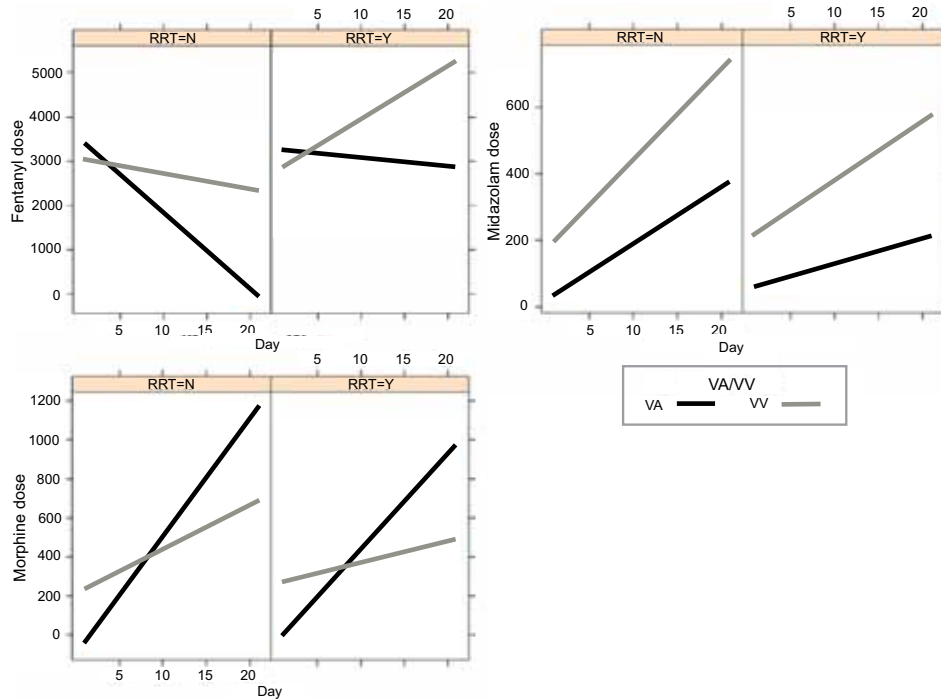


FIGURE 2: Average doses for all patients over time (one to 21 days) for midazolam, morphine and fentanyl. Data is presented as to whether the patient was receiving renal replacement therapy (RRT) with the left panels representing doses without RRT and the panels on the right describing data in the presence of RRT. The grey line represents data from patients receiving venovenous ECMO and the black line represents data from patients receiving venoarterial ECMO. N=no, Y=yes, VA=venoartertial, VV=venovenous.
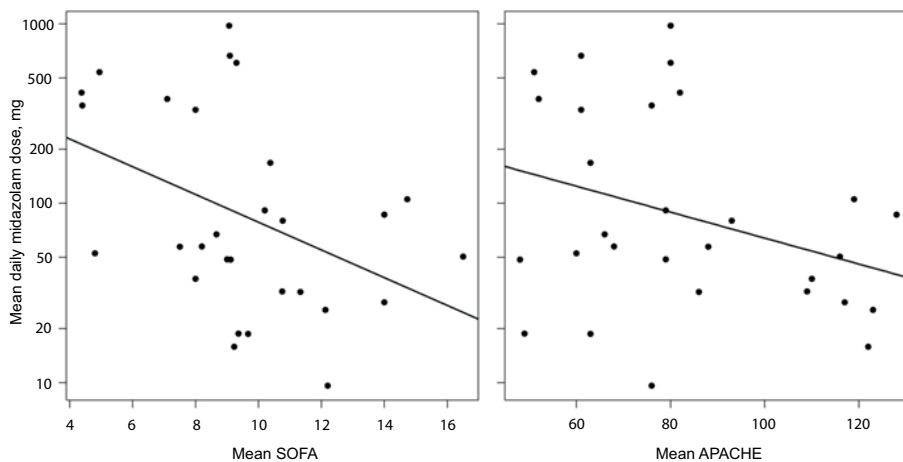


FIGURE 3: Severity of illness and midazolam requirement during ECMO. Mean 24-hourly midazolam dose for all patients plotted against the average Sequential Organ Failure Assessment (SOFA) score (left panel) and Acute Physiology and Chronic Health Evaluation (APACHE) II score (right panel). A log scale was used on the Y axis to account for two patients who received very high doses. The fitted line is from a regression with log-transformed dose as the dependent variable and the SOFA or APACHE score as the independent variable. The R-squared for SOFA and midazolam is 42% and the $P$ value is 0.024. The R-squared for APACHE and midazolam is 33% and the $P$ value is 0.083.

in the VV group are more critically balanced from an oxygenation point of view and are very dependent on optimal circuit flows, especially early in their course of ECMO. While this may explain the increased sedation requirements early in the course of VV ECMO, the mechanisms behind the escalating sedation needs (later in the course of VV ECMO in patients with resolving lung injury) are poorly understood.

It is unclear if the very high sedation requirement seen in our patients is entirely attributable to circuit factors alone. Mechanical ventilation, bleeding, delaying tracheostomy until liberation from ECMO, possible alterations in pharma-codynamics, development of tolerance and recovering organ function with time may all have contributed to the increase in sedation requirements in this group of patients. However, it is possible that ECMO may influence pharmacokinetics (PK) significantly and this effect may be more pronounced for certain drugs depending on their physicochemical properties. The common mechanisms are likely to include increased volume of distribution, decreased clearance and adsorption of the drugs in the circuit[25]. ECMO probably constitutes another compartment for drug distribution from a PK point of view. The pharmacodynamic effects of some of the sedative drugs may be further complicated by the presence of their active metabolites.

The ability of ECMO to alter the PK of sedative drugs has been highlighted in studies involving neonates and simulated ECMO circuits. Neonates receiving ECMO demonstrated an escalation of fentanyl and morphine doses with time[26,27]. Development of tolerance, organ maturation[28] and resolution of organ failure may all account for increased drug clearance in the neonatal population in addition to circuit factors. Clearance of morphine and its metabolites was reduced in several studies and correlated with severity of illness[29-31]. Variable data exists regarding the PK of morphine and fentanyl in neonates receiving ECMO[32,33].

Midazolam and its metabolites exhibited similar properties during ECMO in neonates. There were substantial increments in doses over time despite 1-hydroxy midazolam glucuronide accumulation during ECMO[28,34,35]. These effects have also been observed with lorazepam[29,36]. There is limited data on other sedative agents used during ECMO. A requirement for higher doses of phenobarbital has been reported[22] during ECMO in neonates. The escalating sedation requirement with time in adult patients on ECMO appears to be similar to that in neonates despite the differences in development, physiology, PK and pharmacodynamics.

Simulated circuit studies using neonatal circuits and pumps confirm the potential role of the extracorporeal circuit in altering the PK of administered drugs[29,38]. Though limited by variability in the technology used, these studies have clearly demonstrated the unpredictable behaviour of sedative drugs in the ECMO circuit[21]. Type of oxygenator[36], pump[39] and age of the membrane[40] have all been shown to have a variable influence on PK.

Limitations of currently available studies include the fact that some data are from ex vivo and in vitro models that do not account for the altered physiology common to critical illness. Other data from neonates may not be applicable to adults, due to the developmental and age-related physiological differences. The studies that do exist have used different equipment, such as polymethylpentene vs silicone membrane oxygenators, or uncoated tubing, which is different from presently available equipment. The mean duration of ECMO support has increased with the changing demographics of ECMO[41] leading to the possibility of saturation of circuit binding sites over time[42].

Critical illness and ECMO may have profound effects on the pharmacokinetics[21,25,36,42-45] of drugs. Pathophysiological factors such as sickness severity, haemodilution[42], altered serum protein concentrations[43], the inflammatory response induced by the extracorporeal circuit[46], bleeding and massive transfusion, changes in the extracellular fluid volume and total body water may all lead to an increase in the volume of distribution of administered drugs. Drug adsorption and/or sequestration in the extracorporeal circuit tubing and the oxygenator may further increase the volume of distribution[39]. Organ dysfunction, changes in gastrointestinal[43], renal, hepatic and pulmonary blood flow[47] as a result of a non-pulsatile circulation[48,49] may result in decreased drug clearance. Renal replacement therapy can further complicate the PK of drugs by adding another parallel extracorporeal circuit that can potentially filter drugs[50].

There are clear limitations to this study which include the absence of an ECMO-specific sedation scale and use of Bispectral index monitoring[51], the small sample size, absence of a control group and limited generalisability to other settings. Long-stay patients have more influence on the results over time due to their longer stays. The dose requirements for morphine, fentanyl and midazolam may have been much higher in the absence of other rescue drugs like propofol, thiopentone and dexmedetomidine, which

were used in our patients. More detailed evaluation of organ function, total body water, extracellular fluid volume and the effects of RRT and its type are also required[25]. However, what is clear is that ECMO does dramatically increase sedative requirement and that this initial observational data supports the call for further prospective studies to improve safety and efficacy of ECMO.

The degree of variability seen in our study and limited existing data at present make it difficult to draw any meaningful conclusions on the use of sedative agents for patients on ECMO. However, the morbidity associated with excessive sedation calls for further research. Future studies should focus on development of sedation protocols that will allow optimal usage of available sedative drugs to meet the increased sedation requirements in this important subgroup of patients. It may be prudent to slowly taper opioid and benzodiazepine dosages after decannulation to prevent withdrawal. Our patients received regular enteral benzodiazepines and opioid replacement after decannulation to minimise risks of withdrawal.

CONCLUSIONS

This preliminary study highlights the sedation-related issues in patients receiving ECMO. Unfortunately, a lack of knowledge of the underlying mechanisms presents challenges for clinicians. Future research should aim at characterising the effect of the circuit, drug and patient factors on the PK of sedatives and analgesics during ECMO. Identifying the sedative agents that are least affected by the PK alterations during ECMO, development of ECMO specific sedation protocols and further refinements in ECMO circuitry may minimise morbidity associated with excessive sedation in this most unwell cohort of intensive care patients.

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# CHAPTER 2
# LITERATURE REVIEW

## 2.1 PHARMACOKINETIC CHANGES IN PATIENTS RECEIVING EXTRACORPOREAL MEMBRANE OXYGENATION.

### 2.1.1 Introduction to this publication

This chapter represents the next logical step towards formulation of a research plan to address the complex issue of altered PK during ECMO. Most available PK data was available from neonates not surprisingly given that ECMO had its beginnings in this patient population. This review confirms significantly altered PK during ECMO in neonates and also highlights the limitations of translating neonatal data to adult patients. The review identifies an absolute paucity of data on sedative and antibiotic drug dosing in adult patients on ECMO and emphasises how suboptimal drug therapy may lead to adverse clinical outcomes based on adapt from critically ill patients not on ECMO. It also identifies research gaps and outlines potential approaches for systematic investigation of this complex problem.

# Pharmacokinetic changes in patients receiving extracorporeal membrane oxygenation ☆

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**Abstract** Extracorporeal membrane oxygenation (ECMO) is a form of prolonged cardiopulmonary bypass used to temporarily sustain cardiac and/or respiratory function in critically ill patients. Extracorporeal membrane oxygenation further complicates the management of critically ill patients who already have profound physiologic derangements with consequent altered pharmacokinetics. The purpose of this study is to identify and critically review the published literature describing pharmacokinetics in the presence of ECMO. This review revealed a dearth of data describing pharmacokinetics during ECMO in critically ill adults, with most of the available data originating in neonates. Of concern, the present data indicate substantial variability and a lack of predictability in drug behavior in the presence of ECMO. The most common mechanisms by which ECMO affects pharmacokinetics are sequestration in the circuit, increased volume of distribution, and decreased drug elimination. While lipophilic drugs and highly protein-bound drugs (eg, voriconazole and fentanyl) are significantly sequestered in the circuit, hydrophilic drugs (eg, $\beta$-lactam antibiotics, glycopeptides) are significantly affected by hemodilution and other pathophysiologic changes that occur during ECMO. Although the published literature is insufficient to make any meaningful recommendations for adjusting therapy for drug dosing, this review systematically describes the available data enabling clinicians to make conclusions based on available data. Furthermore, this review serves to highlight the need for well-designed and conducted clinical and laboratory-based studies to provide the data from which robust dosing guidance can be developed to improve clinical outcomes in this most unwell cohort of patients.
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## 1. Introduction

Extracorporeal membrane oxygenation (ECMO) is a temporary life support system that can provide partial or complete support for patients with severe cardiorespiratory failure [1,2]. It is usually used as a bridge to recovery but can also be used as a bridge to long-term mechanical assist devices or heart and/or lung transplantation [3-6]. Clinically, ECMO is a temporizing measure that can facilitate planning

of definitive treatment, optimization of patient physiology, and organ perfusion while the underlying pathology is being assessed and treated [1].

Two main forms of ECMO are currently practiced. Venovenous (VV) ECMO supports the lungs only, whereas venoarterial (VA) ECMO provides support for both the heart and the lungs. During VV ECMO, venous blood from the patient is accessed from the large central veins and returned to the venous system near the right atrium after it has passed through the oxygenator. In VA ECMO, deoxygenated blood is drawn from the right atrium, and oxygenated blood is returned to the distal descending aorta. Regardless of whether ECMO is used as VV or VA, a typical circuit will consist of polyvinyl chloride (PVC) tubing, a hollow fiber (poly-methylpentene) oxygenator, and a heat exchanger (Fig. 1). More detailed reviews on the principles and practice of ECMO can be found elsewhere [1,7-9].

Extracorporeal membrane oxygenation has established itself as an effective tool in the treatment of neonates and children with severe respiratory failure when compared with contemporary conventional care [10-14].

Venoarterial ECMO is being increasingly used in adult patients [15] with cardiac failure and has a reported survival of up to 53%. The first successful use of ECMO in adult respiratory failure was reported in 1972 [16]. Initial adult data failed to demonstrate significant benefit [17,18], but more recent studies have reported a survival rate to discharge of up to 78% in patients with severe respiratory failure [19-21]. Extracorporeal membrane oxygenation continues to broaden its scope [22,23] and holds great promise, but it is not without risks. Issues surrounding bleeding and anticoagulation have taken center stage in the management of patients on ECMO. However, alterations in

drug pharmacokinetics (PK) in the presence of ECMO are infrequently addressed despite significant preliminary data from neonatal studies. It should be emphasized that PK data from neonates cannot be extrapolated to older adults because physiologic processes that influence absorption, distribution, metabolism, and excretion are immature [24] in a neonate and because their maturation while on ECMO may significantly influence the derived PK data. In clinical circles, effective dosing of analgesics, sedatives, and antibiotics, in particular, are considered a significant challenge. The aim of this structured review is to describe changes in the PK of drugs commonly used during ECMO and the implications for drug therapy for this complex cohort of patients.

## 2. Methods

Data for this review were obtained by searches of the PubMed (1965 to August 2011), EMBASE (1965 to August 2011), and the Cochrane-Controlled Trial Register database and references from those searched articles. Search terms included "pharmacokinetics," "ECMO," "critical illness," "sedatives," and "antibiotics." Studies in languages other than English were excluded. All relevant studies describing the pharmacokinetic alterations during ECMO in in vitro and ex vivo experiments, animals, neonates, and adults were included in the review. The literature search identified 64 relevant articles, which included 35 PK studies in neonates, infants, and children; 11 in vitro and ex vivo studies; 1 animal study; 5 case reports in adults; and 12 review articles. These search results highlight the paucity of PK data in adult patients receiving ECMO.
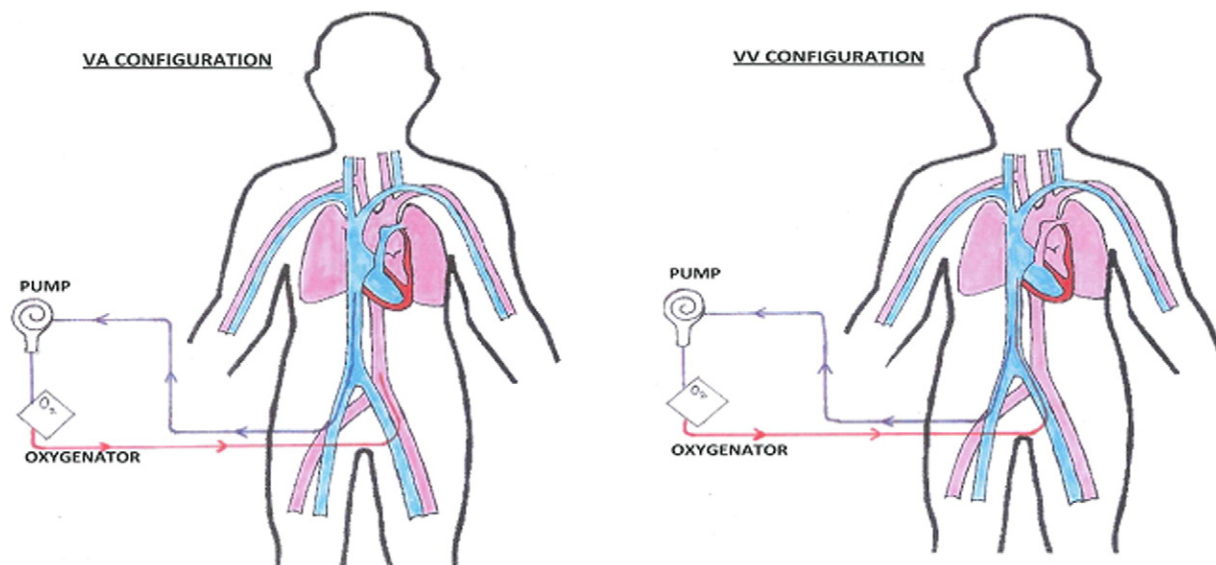


**Fig. 1**    Schematic representation of ECMO. In both modalities, blood is drained from the venous system (blue). In VA ECMO, it is returned (red) to the arterial system, and in VV ECMO, it is returned to the venous system. The direction of blood flow in the ECMO circuit is indicated by arrows.

## 3. Pharmacokinetic changes during ECMO

Describing PK in individual patient populations is essential to understand whether special dosing approaches may be required. In critically ill patients, PK changes can be highly significant and are dictated by the patient, pathology, and the drug [25]. Systemic inflammatory responses, organ dysfunction, drug interactions, and organ support are all known to affect PK. Pharmacokinetic changes in critically ill patients not receiving ECMO have been reviewed extensively and can be found elsewhere [25-31]. The available but limited data suggest that PK changes seen during critical illness are more pronounced in the presence of ECMO. Generally, ECMO can alter PK by increasing the volume of distribution (Vd), decreasing drug elimination, and sequestering drugs in the ECMO circuit [32]. Other factors affecting drug PK in the presence of ECMO are summarized in Fig. 2. The following sections will address the PK changes during ECMO in greater detail.

### 3.1. Extracorporeal membrane oxygenation and drug sequestration

Drug sequestration in ECMO circuits is a well-known but poorly characterized phenomenon. Drug properties such as molecular size, degree of ionization, lipophilicity, and plasma protein binding may all influence the degree of binding to the circuit components [32,33]. Given the large surface area of the tubing and membranes, significant amounts of drugs used in patients on ECMO may be sequestered over a period of time, resulting in a significant increase in Vd (Fig. 3). Conversely, the circuit may continue to release the sequestrated molecules once a particular drug infusion is ceased. This phenomenon is unpredictable and

may prolong the pharmacologic effect in a manner that is undesirable [33].

The type and age of a circuit also appear to be significant factors in the level of sequestration [34,35]. In a single-dose in vitro experiment using a membrane oxygenator and roller pump circuits, up to 50% of morphine and 40% of lorazepam were sequestered at 24 hours [36], with older circuits recording higher losses. This may partly explain the need to escalate sedative drug doses overtime as seen in neonatal studies [36,37], but the mechanisms behind this are unclear. A recent circuit study using neonatal and pediatric circuits compared roller with centrifugal pumps and silicone membrane with hollow fiber oxygenators [38]. Maximal drug recovery was observed in centrifugal pump circuits with polypropylene hollow fiber membrane oxygenators as opposed to silicon membrane with roller pumps (midazolam 63.00% vs 0.62%, fentanyl 33.00% vs 0.35%). Preston et al [39] studied losses of fentanyl and morphine in circuits with and without the oxygenator. Eighty percent of fentanyl was lost in circuits without oxygenators, 86% in the circuits containing the polypropylene Quadrox D oxygenator, and 83% in the circuits with the membrane oxygenator. Morphine losses were up to 40% in all circuits and were not influenced by the presence of the oxygenator. These contrasting results for morphine and fentanyl probably stem from the relative differences in their lipophilicity and are highly relevant for a patient on ECMO. Despite the large surface area of the oxygenator (1.8 m$^2$), most of the drug losses occurred in the uncoated PVC tubing. However, the newer modified surface-coated PVC tubing have also been shown to sequester a significant amount of fentanyl (30%-40%) and morphine (35-58%) in an in vitro study [40].

Another ex vivo study under physiologic conditions estimated the amount of drug sequestered over time using
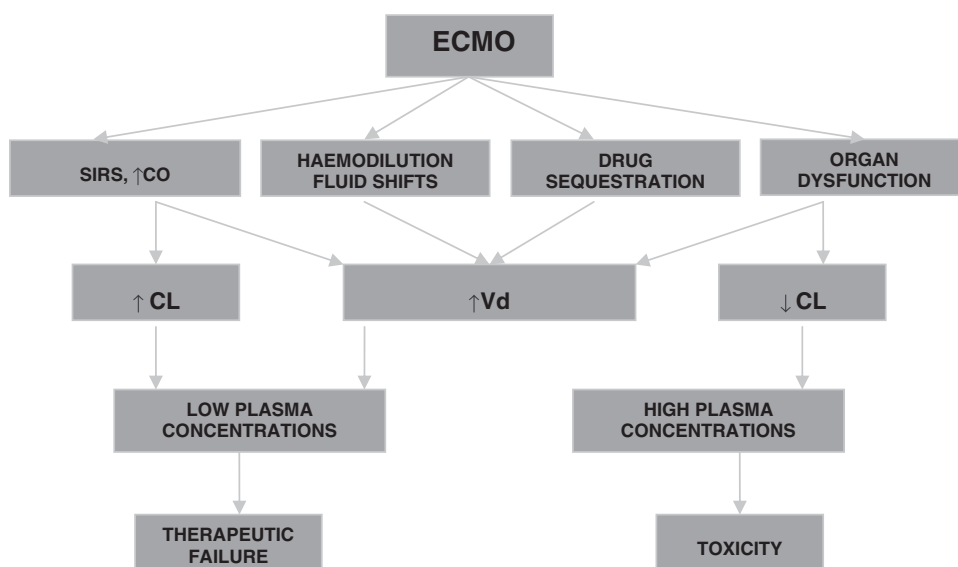


**Fig. 2** Impact of critical illness, inflammation, and ECMO on drug PK. SIRS indicates systemic inflammatory syndrome; CO, cardiac output.
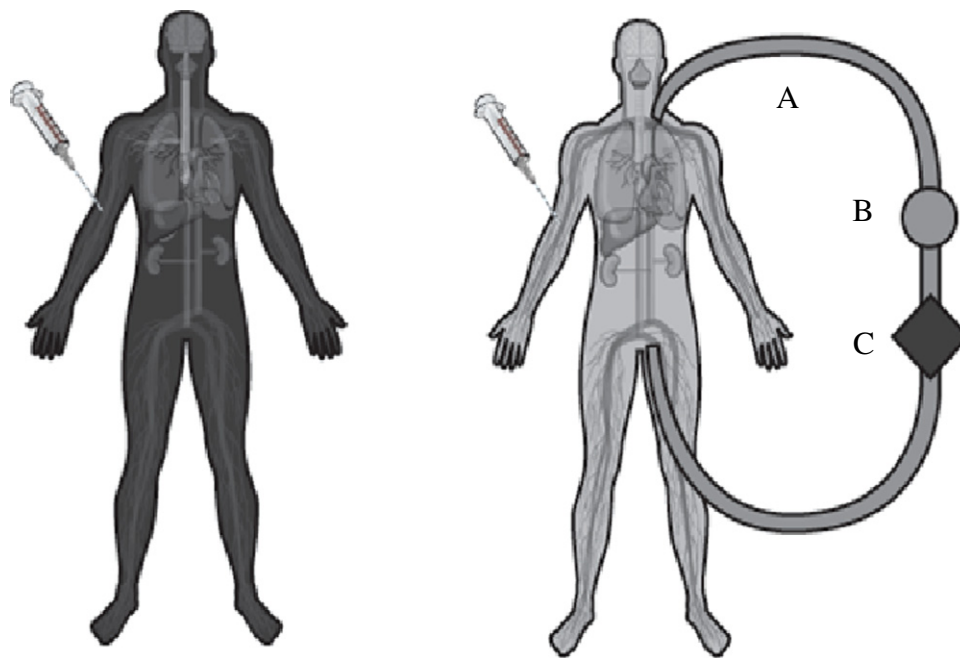
**Fig. 3** Significant sequestration of drugs in the ECMO circuit increases their volumes of distribution, leading to suboptimal drug concentrations in the body. A mere increase in administered dose for all drugs during ECMO may not suffice because the less sequestered drugs may reach toxic levels. A, PVC tubing. B, Pump head. C, Oxygenator.

neonatal circuits and silicon membrane oxygenators. A comparison of crystalloid and blood-primed circuits showed a loss of 71% and 15% of ampicillin, 17% and 31% of fosphenytoin, 33% and 53% of heparin, and 87% and 100% of fentanyl, respectively, at 24 hours. There was a statistically significant decrease in overall drug concentrations from 30 minutes to 24 hours, which could also be considered clinically significant, for both crystalloid ($P$ = .023) and blood-primed circuits ($P$ = .04). Interestingly, priming with blood increased the losses of fosphenytoin, fentanyl, and heparin [41].

However, it is unclear how priming of a circuit with various fluids differentially affects drug sequestration. The circuits are often primed with crystalloids, colloids, blood, and added electrolytes to adjust for pH and are maintained at normal body temperature. All of these factors could affect the protein binding of injected drugs as well as adsorption onto the PVC tubing and the oxygenator, although this has been poorly characterized [36]. There may be a saturation point for drug sequestration in the circuit; however, current understanding of this is limited. In vitro simulated circuit studies that duplicate physiologic conditions as closely as possible using standardized adult ECMO circuits have the potential to provide insights into circuit drug interactions and help further refine ECMO circuitry. Extracorporeal membrane oxygenation is a technology in evolution [42], and further studies are required to quantify drug losses in miniaturized circuits that use polymethylpentene oxygenators, magnetically levitated centrifugal pumps, coated tubing, and cannulae.

### 3.2. Extracorporeal membrane oxygenation and Vd

By definition, the apparent Vd of a drug is the theoretical volume of fluid into which the total drug administered will have to be diluted to produce a concentration equal to that observed in plasma. Extracorporeal membrane oxygenation can independently alter the Vd of drugs by several mechanisms as summarized in Table 2 and Fig. 2. However, most of these data originate from neonates. Newborn infants have a high proportion of total body water and a low proportion of adipose tissue that results in higher Vd for hydrophilic drugs (eg, $\beta$-lactams and aminoglycosides) and lower Vd for lipophilic drugs (eg, fluoroquinolones and macrolides) relative to adults. Similarly, decreased plasma protein binding in neonates increases the unbound drug concentrations resulting in increased Vd [24], especially for highly protein-bound drugs (eg, ceftriaxone and teicoplanin). These physiologic alterations limit the use of neonatal PK data to guide pharmacotherapy in adults.

Extracorporeal membrane oxygenation circuits probably represent another PK compartment because they are thought to sequester significant amounts of drugs, thereby increasing Vd [41,43]. Hemodilution from priming solutions on commencement of ECMO, ongoing blood product transfusions, and administration of volume to maintain circuit flows may all increase Vd, particularly for hydrophilic drugs. Although hemodilution can often enhance the pharmacologic effect of highly protein-bound drugs by increasing the unbound fraction of the drugs, redistribution of the free drug into tissues may result in lower serum concentrations.

Patients receiving ECMO often have significant alterations in pH, which may lead to further alterations in protein binding and drug distribution [33]. As with critically ill patients not receiving ECMO, inflammation, fluid shifts, renal dysfunction, and fluid retention can all lead to an increased Vd. Up-regulation of the renin-angiotensin system during a nonpulsatile circulation as in VA ECMO may significantly alter renal handling of fluid and electrolytes, resulting in increased circulating blood volume and, thereby, Vd [44].

### 3.3. Extracorporeal membrane oxygenation and drug clearance

Extracorporeal membrane oxygenation is associated with reduced drug elimination and is thought to occur by several mechanisms, in addition to those related to critical illness. The incidence of renal dysfunction (serum creatinine concentration >1.5 mg/dL) in adults has been reported as 32% and 47% for VV and VA ECMO, respectively [15]. Patients subjected to ECMO often have a preceding hypoxia/hypoperfusion-related insult to their kidneys. Pulseless perfusion of the kidneys during VA ECMO may be associated with a decrease in glomerular filtration [45]. However, available studies show similar elimination half-lives for gentamicin in infants receiving VA and VV ECMO [46], questioning the relevance of a pulsatile circulation in the development of a kidney injury and altered PK. Decreased clearance (CL) of drugs (eg, aminoglycosides and $\beta$-lactams) during ECMO as demonstrated in neonatal studies should, once again, be interpreted in the light of immature glomerular and tubular functions [24]. It should be noted that the hepatic metabolism for drugs in neonates differs significantly from that in adults because the enzymatic pathways are still in the process of maturation, resulting in reduced CL rates of those drugs (eg, macrolides and linezolid), for which metabolism is a significant mechanism for elimination [47]. There may be alterations in regional liver blood flows, which can also affect CL of drugs, especially those with a high extraction ratio [33]. Decreased pulmonary blood flows [48], especially during VA ECMO, may affect sequestration and metabolism of many sedative and analgesic drugs by the lungs [49]. Drug removal by the ECMO circuit may reduce the bioavailability of the first dose of a drug and also affect the overall CL of a drug [36]. Decreased elimination predisposes patients to drug toxicities, especially for drugs that have a narrow therapeutic index.

### 3.4. Extracorporeal membrane oxygenation and renal replacement therapy

The Extracorporeal Life Support Organisation recently reported that up to 50% of patients on VV ECMO and 41% on VA ECMO may require some form of renal replacement therapy (RRT) during the ECMO run [15]. Characterizing altered PK in patients receiving RRT while on ECMO can be complex. Variability in the techniques used for RRT and ECMO is a significant limitation to PK modeling. Although in vitro circuit studies may provide insights into interactions between 2 independent ECCs, population PK studies in patients receiving RRT on ECMO may guide clinicians until such data become available. However, given the paucity of data in this regard, most clinical approaches are theoretical rather than evidence based. Available studies have shown a significant variability that highlights the importance of therapeutic drug monitoring [50]. Surprisingly, some drugs in the setting of ECMO and RRT in infants and young children, such as ribavirin and ticarcillin, are not recommended to have dose adjustments, which may predispose to clinical failure [50,51]. More research is needed to guide effective dosing in the presence of this common cotreatment of ECMO in critically ill patients.

## 4. Pharmacokinetics of drugs commonly prescribed during ECMO

As discussed previously, ECMO has the potential to alter the PK of many drugs, although existing data are almost exclusively available only for sedatives, analgesics, and antibiotics.

### 4.1. Sedatives and analgesics

Extracorporeal life support (ECLS) is a highly invasive treatment for patients who have failed conventional treatment, and optimal sedation for this complex group of patients is not clearly defined. Evidence-based practices such as use of sedation protocols [52], allowing lighter sedation with daily interruptions [52-54] and minimizing administration of paralytic agents, have all been shown to reduce morbidity in critically ill patients. However, these practices may not always be practical in a critically ill patient on ECLS. Apart from the usual benefits [55], sedation during ECLS minimizes the risk of catheter malposition or accidental dislodgement and coughing, which induces "suck down" resulting in hemolysis within the circuit. Deep sedation and paralysis may be required, especially early in the course of VV ECLS, to optimize circuit flows and ventilation and to minimize oxygen consumption.

In clinical practice, it is observed that patients receiving ECMO often have substantially higher sedative drug dosing requirements than do corresponding patients not on ECMO. There are no data on sedation practices in adults during ECMO, and it is unclear if this increased sedative dosing requirement is also present in neonates [36,37,56]. Certainly, in neonates, PK variability can be caused by non-ECMO factors including organ maturation

and development of tolerance. Knowledge of the available PK data can guide the clinician in rational drug dosing in the presence of ECMO.

Neonatal studies consistently demonstrate a need to escalate fentanyl doses with time. The reported fentanyl dose range in these studies (9-71 $\mu$g kg$^{-1}$ h$^{-1}$) highlights the variability involved [32]. Morphine is also a problem, with one study reporting morphine CL as 0.57±0.3 L kg$^{-1}$ h$^{-1}$ during ECMO, which nearly doubled to 1.05± 0.72 L kg$^{-1}$ h$^{-1}$ after its cessation [57]. The mean serum morphine concentration during and after ECMO was 87±58 and 35±17 $\mu$g/L, respectively. Metabolism to morphine-3-glucuronide (the primary metabolite) and morphine-6-glucuronide was found to be reduced in another study [58]. The high serum levels, in part, reflect the higher doses of morphine required clinically during ECMO. The nonstandardized sedation practices during ECMO and the presence of active metabolites add to the challenges in interpreting these results. Variable data exist regarding the degree of sequestration and CL of the 2 most commonly used opioids, morphine and fentanyl, in neonates receiving ECMO [59,60]. It appears that morphine may be superior to fentanyl even after accounting for organ failures and the presence of active metabolites because it has been reported to provide superior analgesia compared with fentanyl, while reducing drug withdrawal and length of hospital stay significantly [61].

A population PK model for midazolam and its major metabolites in 20 neonates during VA ECMO was developed by Ahsman and colleagues [62]. The dose requirement increased substantially after 5 to 7 days despite accumulation of the metabolite 1-hydroxy midazolam glucuronide during ECMO, up to 34% after 7 days. The Vd for midazolam increased from 4.2 to 14.0 L/kg on the commencement of ECMO. A similar study found a significant increase in Vd and plasma half-life for midazolam and its metabolite 1-hydroxyl midazolam [63]. Dose concentration relationships of midazolam in 20 neonates undergoing VA ECMO demonstrated an increased midazolam requirement in first 24 hours of ECMO [64]. Similar effects have been observed with lorazepam [36,43]. The results from these studies should be interpreted in the light of altered PK in the neonatal population but do suggest that larger drug doses may be required with ongoing ECMO prescription.

There are limited data on other sedative agents used in the intensive care unit (ICU). Propofol, which is lipophilic and highly protein bound, is significantly sequestered in the circuit. In vitro studies have shown recoveries of 65% and 25% of predicted concentrations at 5 and 120 minutes after the addition of propofol into the circuit [65]. The degree of variability and limited PK data at present make it difficult to develop any evidence-based guidelines for the use of sedative agents for patients on ECMO, although, it may be prudent to slowly taper opioid and benzodiazepine dosages after decannulation to prevent withdrawal.

## 4.2. Antibiotics

Antibiotics are commonly prescribed drugs in ICU, and often, the success of ECMO may rely on the success of antibiotic therapy. Unfortunately, PK data in adult patients on ECMO are sparse. Neonatal and animal studies are available but have shown significant variability and unpredictability of antibiotic PK during ECMO [32,35,46,50,66-79] (Table 1). However, no dosing guidelines exist for this group of patients. Vancomycin and gentamicin have been subject to a number of neonatal PK studies [46,66,74,75]; however, changes in ECMO technology over the last decade make them less relevant to current practice.

The PK of cefotaxime and its active metabolite, desacetylcefotaxime, was described in 37 neonates receiving ECMO [80]. The standard cefotaxime dose regimen (50 mg/kg of body weight twice a day [postnatal age, or PNA], <1 week), 50 mg/kg 3 times a day (PNA, 1-4 weeks), or 37.5 mg/kg 4 times a day (PNA, >4 weeks) provided sufficiently long periods of supra-minimum inhibitory concentration to provide adequate treatment of infants on ECMO. Neonatal PK studies of gentamicin have demonstrated an increase in the Vd for gentamicin (0.43-0.66 L/kg), a lower CL (41.0-101.4 mL kg$^{-1}$ h$^{-1}$), and a prolonged elimination half-life (5.2-12.9 hours) [32,35,46,66-69]. Clinically, it is recommended to monitor gentamicin levels and extend the dosing intervals where necessary for patients on ECMO.

Neonatal studies have uniformly shown an increase in Vd for vancomycin (0.27-1.6 L/kg). A population PK model of 45 patients including term neonates, older children, and adults confirmed that the CL of vancomycin was decreased and its Vd increased in patients receiving ECMO [75].

Interestingly, age- and weight-matched neonates on ECMO and controls did not have any significant differences in Vd and CL, although an increased elimination half-life was present [74]. Other studies on neonates support a lower CL (0.78±0.19; range, 0.49-1.07 mL min$^{-1}$ kg$^{-1}$) and, consequently, a longer vancomycin half-life [73,74]. Loading doses are suggested (20-30 mg/kg) to account for the larger Vd, with therapeutic drug monitoring (TDM) recommended to guide maintenance

**Table 1**    Studies demonstrating the variability in Vd of antibiotics during ECMO

| Drug | Vd during ECMO(L/kg) | Reference |
| --- | --- | --- |
| Gentamicin [a] | 0.51-0.748 | 0.47-0.49 |
| Vancomycin [a] | 0.56–2.1 | 0.48-0.69 |
| Ticarcillin [a] | 0.26-0.27 | 0.26-0.27 |
| Caspofungin [b] | 0.137 | 0.13-0.16 |
| Voriconazole [b] | 1.38 | 1.39-4.6 |
| Ceftriaxone [a] | 0.73-3.02 | 0.39-0.45 |

The reference population comprises the critically ill neonates and adults not on ECMO.
  [a] Neonatal and pediatric studies.
  [b] Adults [31,34,44,45,60-73].

dosing in patients receiving ECMO until further PK data become available.

In clinical studies, adequate caspofungin peak (11.9 $\mu$g/mL) and trough levels (3.7 $\mu$g/mL) were maintained during ECMO [72]. However, being more lipophilic, voriconazole was significantly sequestered in the circuit, necessitating higher doses of the drug to maintain adequate trough levels. Mehta et al [41] reporting a 71% loss of voriconazole in the circuit, which is highly relevant because of the association between voriconazole trough blood levels and outcome. A problem of this circuit loss may be the circuit saturation that occurs with time, meaning that the initial high doses should be later decreased to avoid drug toxicity [70,72,81]. TDM of voriconazole is suggested to guide this dosing.

Oseltamivir PK was not significantly influenced by ECMO in influenza A–infected children (0-18 years). There was an increase in oseltamivir carboxylate plasma concentrations consistent with a higher dose of oseltamivir used to counter expected decreased plasma drug concentrations during ECMO (<15 kg: 60 mg/d every 12 hours, 15-23 kg: 90 mg/d every 12 hours, 23-40 kg: 120 mg/d every 12 hours, and >40 kg: 150 mg/d every 12 hours) [82]. Intravenous ribavirin (20 mg/kg per day) was administered to a neonate with disseminated adenovirus infection requiring ECMO and hemofiltration. Despite plasma concentrations at steady state being low (4.81 to 8.47 $\mu$g/mL),

results of viral cultures were negative within 48 hours of initiation of ribavirin therapy [51]. Although these are only small case studies and case series, the data may be useful for the clinical use of these drugs.

The variations in antibiotic PK in these patients are concerning. Although therapeutic failure and toxicity add to the morbidity and mortality of an individual patient in ICU, emergence of resistant microorganisms [83,84] may have much wider implications, including those who never require ECMO.

### 4.3. Other drugs

There are limited data available regarding the PK of other drugs. The estimated CL for theophylline in term neonates supported by ECMO was significantly lower, and Vd higher, as compared with patients of similar age not receiving ECMO [85]. TDM is widely available for theophylline and should be used in the presence of ECMO.

Significant loss of frusemide in the circuit components has been reported in in vitro studies [86], which may explain why frusemide infusions were shown to be effective in a neonatal study [87]. This is significant because patients on VV ECMO often receive diuretics to minimize their extra vascular lung water and improve pulmonary compliance. Given that that this drug is dosed to clinical end points, prescribers should be aware that larger-than-usual doses may be required in the

**Table 2**   Summary of PK changes during ECMO and their clinical implications

|  | Changes in PK | Therapeutic implication | Drugs affected |
|---|---|---|---|
| **Priming/transfusion** | | | |
| Hemodilution | ↑ Vd <br> ↓ Cmax | ↑ Loading dose | Hydrophilic drugs, eg, $\beta$-lactams and aminoglycosides |
|  | ↑ Free drug concentrations | ↑ Loading dose <br> ↑ Frequency | Highly protein-bound drugs, eg, teicoplanin and ceftriaxone |
| **Circuit-related factors** | | | |
| Drug sequestration | ↑ Vd <br> ↓ Cmax | ↑ Loading dose | Lipophilic drugs, eg, fluoroquinolones fentanyl, and midazolam |
| Drug inactivation | ↑ CL <br> ↓ Bioavailability | ↑ Frequency regarding dose with circuit changes | |
| **Patient factors** | | | |
| Systemic inflammation/sepsis | ↑ Vd <br> ↓ Cmax <br> ↑ CL | ↑ Loading dose | Hydrophilic drugs, eg, $\beta$-lactams and glycopeptides |
| Organ failures | ↓ CL <br> ↑ Vd | ↓ Frequency | Renally or hepatically excreted drugs |
| **Drug factors** | | | |
| Hydrophilicity | ↑ Vd <br> ↓ Cmax <br> ↑↓ CL (dependent on renal function) | ↑ Loading dose <br> ↑↓ Frequency | For example, $\beta$-lactams aminoglycosides |
| Lipophilicity | Vd largely unchanged <br> ↑ Circuit sequestration <br> ↓↑ CL (dependent on hepatic function) | ↑ Loading dose <br> ↑ Frequency | For example, fluoroquinolones, macrolides, and propofol |

Cmax indicates peak concentrations.

presence of ECMO. Limited data indicate that no dose adjustments are required for neonates on ECMO [88].

A study on 13 term neonates with stable renal and hepatic function who were treated with ECMO concluded that standard ranitidine doses do not need to be administered more frequently than every 12 hours [89].

Heparin PK was studied in 5 infants during ECMO and demonstrated that more than one half of the administered heparin is eliminated by the extracorporeal circuit itself or by blood components in the circuit [90]. This may have significant implications because anticoagulation on ECMO still remains a challenge and is, perhaps, one of the reasons that make ECMO a high-risk therapy. It is unclear if newer heparin-bonded circuits exhibit any heparin adsorption. Despite of this, standard activated partial thromboplastin time monitoring is essential for heparin in these patients and more frequent monitoring may even be appropriate while individualized patient doses are being identified.

Other data suggest that ECMO may also be associated with loss of vital trace elements, vitamins, micronutrients and macronutrients, and hormones [91,92]. A summary of the key PK changes during ECMO and their clinical implications can be found in Table 2.

## 5. Future directions

There is an urgent need to more fully elucidate the behavior of drugs during ECMO. This review is valuable because it summarizes the data presently available while highlighting the areas in need of further research. Relative quantification of the contributions of the drug, device, and disease to the altered PK in a patient on ECMO is challenging. Systematic research using in vitro circuit models, animal models of ECMO, and clinical population PK studies may be required to optimize drug therapy during ECMO. These studies should focus on identifying drugs, developing dosing regimes that are most suitable for use during ECMO, and developing circuitry that has the least influence on PK. In the interim, PK modeling of routinely used drugs may help procure drug dosing strategies that will increase the likelihood of achieving optimal pharmacologic effects. It is also important to standardize ECMO circuitry for the PK data to be widely applicable. Clearly, more research is needed to make ECMO a standard of care rather than a therapy of high risks.

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# CHAPTER 3

# KNOWLEDGE GAP, HYPOTHESIS AND AIMS

## 3.1 KNOWLEDGE GAPS

Following review of the literature, the following major knowledge gaps were identified;

1. Current antimicrobial and sedative drug dosing on ECMO is arbitrary.
2. There is a paucity of PK data in adult ECMO.
3. PK alterations in neonatal and premature infants may represent developmental issues.
4. Available *in vitro* and *ex vivo* mechanistic data uses out-dated technology and is not relevant.
5. There is minimal *in vivo* mechanistic data to describe the interplay between drug, device and disease factors.
6. No dosing recommendations exist for antibiotic and sedative drug dosing in this complex group of patients receiving ECMO.

## 3.2 HYPOTHESIS

ECMO represents a supportive therapy, thus effective drug therapy to reverse the underlying disease is critical for a successful outcome. The **hypothesis of this thesis** is:

*ECMO alters the PK of sedative, analgesic and antimicrobial drugs and their metabolites independently of patient and pathological factors, thereby contributing to elevated risk of therapeutic failure, drug toxicity and/or an emergence of antibiotic resistance in critically ill adult patients on ECMO.*

## 3.3 AIMS

The overall aim was to investigate the complex array of factors affecting PK during ECMO using an incremental approach[51] to delineate the interrelationships between the Drug, Device and the Disease and develop evidence-based dosing guidelines for use in adult patients on ECMO.

## 3.4 OBJECTIVES

This research will characterise the PK of 18 important drugs (Chapter 4) used during ECMO utilising validated *ex vivo* and large animal models of ECMO[52,53] ,efficient drug and metabolite assays[54] and a multi-national population PK collaboration[55]. This mechanistic data can be translated into clinical practice rapidly, facilitating optimal pharmacotherapy, minimizing the risk of therapeutic failure and the rise of antibiotic resistance from sub-therapeutic concentrations.

# CHAPTER 4

# MATERIALS AND METHODS

## 4.1 THE ECMO PK PROJECT: AN INCREMENTAL RESEARCH APPROACH TO ADVANCE UNDERSTANDING OF THE PHARMACOKINETIC ALTERATIONS AND IMPROVE PATIENT OUTCOMES DURING EXTRACORPOREAL MEMBRANE OXYGENATION.

### *4.1.1 Introduction to this publication*

This chapter describes a holistic approach to a challenging clinical problem. Critically ill patients have significantly altered PK Isolating the independent effects of ECMO on PK in such patients is nearly impossible. ECMO creates a bio-synthetic interface between the circuit and the patient and the dynamic interactions between drug, device and the disease process may result in profound PK alterations. All these factors need to be investigated in isolation and then in the clinical setting in totality to improve mechanistic understanding and to develop population PK models that will inform the development of dosing guidelines for adult patients receiving ECMO. This chapter provides a detailed outline of research methods and also cites other methodology papers that are not included in this thesis.

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## STUDY PROTOCOL

**Open Access**

# The ECMO PK Project: an incremental research approach to advance understanding of the pharmacokinetic alterations and improve patient outcomes during extracorporeal membrane oxygenation

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## Abstract

**Background:** Extracorporeal membrane oxygenation (ECMO) is a supportive therapy and its success depends on optimal drug therapy along with other supportive care. Emerging evidence suggests significant interactions between the drug and the device resulting in altered pharmacokinetics (PK) of vital drugs which may be further complicated by the PK changes that occur in the context of critical illness. Such PK alterations are complex and challenging to investigate in critically ill patients on ECMO and necessitate mechanistic research. The aim of this project is to investigate each of circuit, drug and critical illness factors that affect drug PK during ECMO.

**Methods/design:** An incremental research plan that encompasses *ex vivo* experiments for drug stability testing in fresh human and ovine whole blood, *ex vivo* drug disposition studies in standard and modified adult ECMO circuits primed with fresh human or ovine whole blood, PK studies in healthy and critically ill ovine models of ECMO with appropriate non ECMO controls and an international mutli-centre clinical population PK study will be utilised to comprehensively define the PK alterations that occur in the presence of ECMO. Novel drug assays that will allow quantification of multiple drugs in small volumes of plasma will also be developed. Mixed-effects regression models will be used to estimate the drug loss over time in *ex vivo* studies. Data from animal and clinical studies will be analysed using non-linear mixed-effects models. This will lead to generation of PK data that enables the development evidence based guidelines for antibiotic, sedative and analgesic drug therapy during ECMO.

**Discussion:** Systematic research that integrates both mechanistic and clinical research is desirable when investigating the complex area of pharmacokinetic alterations during ECMO. The above research approach will provide an advanced mechanistic understanding of PK during ECMO. The clinical study when complete will result in development robust guidelines for prescription of 18 commonly used antibiotic, sedative and analgesic drugs used in ECMO patients. This research may also pave the way for further refinements in circuitry, drug chemistry and drug prescriptions during ECMO.

**Trial registration:** ACTRN12612000559819.

**Keywords:** Pharmacokinetics, Extracorporeal membrane oxygenation, Pharmacodynamics, Therapeutic failure, Toxicity

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## Background

Extracorporeal membrane oxygenation (ECMO) temporarily supports patients with severe cardio-respiratory failure that is not responsive to maximal conventional treatment [1-4]. Following the 2009 H1N1 pandemic, ECMO has re-emerged as a versatile device that not only provides cardio-respiratory support when medical therapy fails but also compliments existing mechanical cardiopulmonary assist devices, heart /lung transplantation, cardiology and hospital based cardiopulmonary resuscitation services effectively. As ECMO is a supportive therapy, effective drug therapy directed at reversing the underlying disease is critical to ensure successful liberation from ECMO. Indeed, the clinicians applying ECMO recognise that contemporary use of this therapy is far from perfect with patients suffering ongoing morbidity because the clinicians are no longer able to confidently achieve the desired effects from pharmacotherapies. Published data demonstrates that ECMO dramatically affects pharmacokinetics (PK) in the most severely ill patients who already have significant PK changes [5-8].

It is essential that each of the drug, device and disease factors affecting PK during ECMO (Figure 1) is studied to improve treatment and outcomes of patients. We hypothesise that ECMO negatively alters the PK of sedative, analgesic and antibiotic drugs and their metabolites independent of patient and pathological factors, thereby contributing to elevated risk of therapeutic failure, drug toxicity and/or an emergence of microbial resistance in critically ill patients receiving ECMO. Our aim is to use an incremental research approach that include studies investigating drug, circuit and critical illness factors in isolation and combined to arrive at meaningful conclusions.
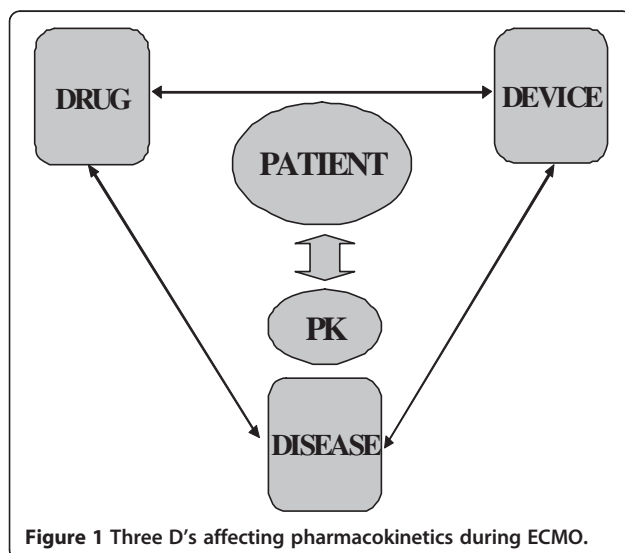


**Figure 1 Three D's affecting pharmacokinetics during ECMO.**

## ECMO circuits are not passive conduits for blood

In critically ill patients not receiving ECMO, it has been shown PK changes can result in highly significant changes to drug exposure through interactions between the patient, pathology and the drug [9-12]. The ECMO system introduces additional variables, which are the circuit itself, and the effects of systemic inflammation due to the prolonged use of an extracorporeal circuit. Sequestration of drugs in the circuit, increased volume of distribution (Vd) and decreased clearance (CL) are the major PK changes associated with ECMO [8], although the extent of change remains poorly characterised. Published data from neonatal circuit studies highlight the influence that drug properties such as molecular size, degree of ionization at physiological pH, lipophilicity and plasma protein binding have on drug disposition during ECMO [13,14]. In a manner analogous to the lung it mimics, ECMO is critically dependent upon the large surface area of the oxygenator and associated tubing to ensure adequate blood flows through the circuit and facilitate gas transfer. This bio-synthetic interface results in significant sequestration of the administered drugs resulting in a compartment effect on PK (Figure 2). The type and age of circuit components including type of the pump, oxygenator and tubing as well as circuit priming may influence the level of drug sequestration [15-18]. Patient factors such as systemic inflammation, haemodilution, bleeding and transfusion, organ dysfunction and renal replacement therapy all add to the clinical challenges of drug dosing during ECMO [8].

## The burden of altered pharmacokinetics during ECMO

There is increasing awareness of the implications of altered PK during critical illness in adult patients [19-21]. The PK changes during critical illness appear to be magnified during ECMO. This can affect any drug, however given the scientific and clinical PK gap, robust PK data for sedative, analgesic and antibiotic drugs are urgently required.

### Excessive sedation use and related morbidity

Sedation practices in the ICU are changing and emerging data supports its judicious use [22,23]. Neonatal studies consistently demonstrate a need to escalate sedative doses during ECMO [13,24-26]. In a retrospective review of 30 patients [7], the average 24-hourly dose increased by 18 mg per day for midazolam (95% CI: 8, 29 mg, p=0.001) and 29 mg per day for morphine (95% CI: 4, 53 mg, p=0.021) from the first day of ECMO. The VV group required a daily midazolam dose that was 157 mg higher on average than the VA group (95% CI: 53, 261 mg, p=0.005). Patients often received up to 1500 mg of morphine and midazolam per day despite supplemental sedation with propofol, dexmedetomidine and thiopentone. By acting as a reservoir, ECMO may also prolong the pharmacological
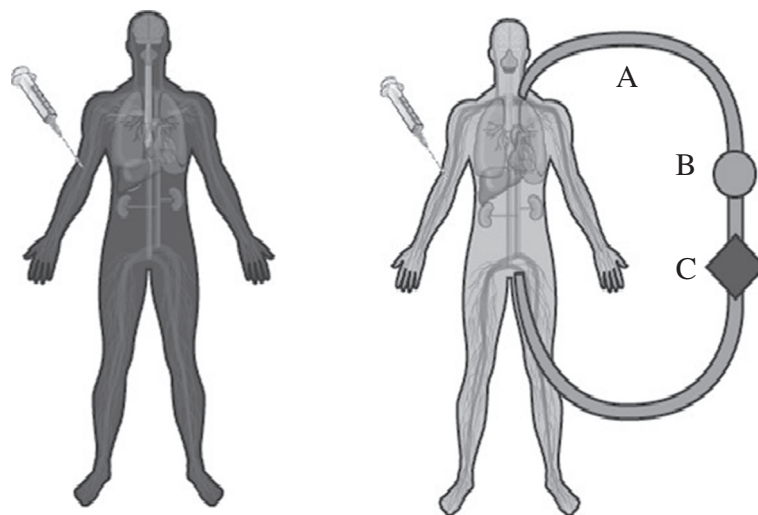
**Figure 2 Significant sequestration of drugs in the ECMO circuit increases their volumes of distribution leading to suboptimal drug concentrations in the body.** A mere increase in administered dose for all drugs during ECMO may not suffice, as the less sequestered drugs may reach toxic levels. **A** - PVC tubing, **B**- pump, **C**- oxygenator (Reproduced with Permission, Shekar et al Journal of Crit Care 2012).

effect of sedatives even after drugs have been ceased. This is concerning as it is now well established that excessive sedation in critically ill patients is associated with increased mortality and morbidity [27].

### Infection, antibiotic failure, drug toxicities and emergence of microbial resistance

ECMO is a supportive therapy and not a disease modifying treatment in itself. The success of ECMO, especially in patients with severe pneumonia or a pandemic viral respiratory illness relies heavily on the success of antiviral/antibiotic therapy. Optimal antibiotic therapy in these patients is a balance between potency and exposure [12,28-31]. A recent review of Extracorporeal Life Support Organization (ELSO) data [32] revealed a total of 2,418 infections during 20,741 (12%) ECMO cases. Infections increased the duration of ECMO, post-ECMO ventilator support and were associated with an increased risk of death. Neonatal studies have reported severe PK variations, however limited data is available to guide antibiotic therapy in adults [13,16,33-37]. Sub-optimal prescription of antibiotics in patients on ECMO can worsen the problem by selecting for resistant microorganisms [29].

## Methods/design
### A rational approach to understand the pharmacokinetic changes
Although data from clinical studies of the impact of ECMO on altering the PK of drugs used in patients on

ECMO will have great applicability for optimisation of pharmacotherapy, mechanistic research is required to identify the specific factors contributing to these PK changes. To gain insight into these factors, research using simulated circuits and large animal models are required so that individual variables can be altered in a systematic manner enabling the impact of each change to be quantified in an accurate and cost-effective manner. Additionally, this will define the interplay between critical illness and the extracorporeal circuit that result in altered PK during ECMO. The new knowledge to be generated has major implications for improving patient outcomes during ECMO therapy and extracorporeal technology in general. A proposed research plan that is being currently being implemented uses an incremental approach as shown schematically in and Figures 3 and 4. The study drugs are tabulated in Table 1.

### Drug factors
*Ex vivo* controls to examine baseline stability of drugs at 37°C is an important consideration in interpreting the PK alterations during ECMO. Stability testing in fresh human and sheep whole blood will be performed for all study drugs (Table 1). This is critical as drug losses in the circuit can only be meaningfully interpreted after establishing stability. Preliminary results highlight this as drugs such as meropenem [38] are highly unstable at 37°C.

| | *Ex vivo* drug stability at 37°C | Studies in *ex vivo* ECMO circuits (5 circuits per variable) | | Studies in healthy sheep (single/repeat dose PK) | | Studies in critically ill sheep (single/repeat dose PK) | | Clinical PK studies |
|---|---|---|---|---|---|---|---|---|
| | | Human blood (24 hrs) | Sheep blood (24 hrs) | 12 hr run (single dose) | 24 hr run (re-dose) | 12 hr run (single dose) | 24 hr run (re-dose) | |
| **Studies** | **Stability testing in fresh human (n=5) and sheep (n=5) whole blood** | • Physiologic<br>• Re dose PK<br>• Flow rates 2.5 vs. 5.0 L/min<br>• Circuit without oxygenator | • Physiologic<br>• Hyperoxia<br>• Hypothermia | Ventilated | Ventilated ECMO | Ventilated S-ALI | Ventilated S-ALI ECMO | PK studies in patients on ECMO |
| **Sample size (n)** | n=10 | n=25 | n=15 | n=12 Own controls | | n=12 Own controls | | n=12 |

**Figure 3 Proposed approach to quantify the PK changes and identify factors underlying these changes to inform the development of guidelines for antibiotic and sedative drug therapy during ECMO.** S-SLI-smoke inhalation acute lung injury.

## Circuit factors

These studies will identify the PK changes attributable to the circuit and drugs and will be used to describe single and repeat dose kinetics in standard circuits.

### Disposition of drugs in standard ECMO circuit

A validated *ex vivo* model of ECMO has been previously published [38,39]. Briefly, Maquet PLS ECMO circuits will be used (Maquet Cardiopulmonary AG, Hechinger Straße, Germany). A reservoir bladder (Medtronic R38) will allow sampling from the closed circuit (Figure 5). The circuit will be primed with Plasmalyte, 4% albumin followed by fresh whole blood to obtain a post oxygenator pressure of 230–250 mmHg. The final estimated volume of the pressurised circuit is 668 mL. A centrifugal pump maintained a circuit flow rate of 4–5 L /min. Oxygen tension and circuit temperature and pH will be maintained at 100–150 mm Hg and 37°C. Carbon dioxide gas or sodium bicarbonate solution will be added to

the circuit to maintain the pH of the circulating blood in the range 7.25–7.55. Study drugs (Table 1) will be injected post oxygenator to achieve clinically relevant concentrations in the circuit. Serial samples will be obtained post oxygenator over 24 hours. For re-dose PK studies, study drugs will be reinjected at 6, 8 or 12 hours (as per clinical dosing guidelines). This will further investigate potential saturation of the circuit with time and its affect on drug disposition during ECMO.

### Disposition of drugs in modified ECMO circuit
#### Circuit primed with fresh whole human Blood

**Circuit without oxygenator** These studies will examine the role of the oxygenator in sequestering drugs in the ECMO circuit. By comparing the data from standard ECMO circuit experiments, the relative contribution of the oxygenator may be quantified. It has been established in neonatal circuit studies that the
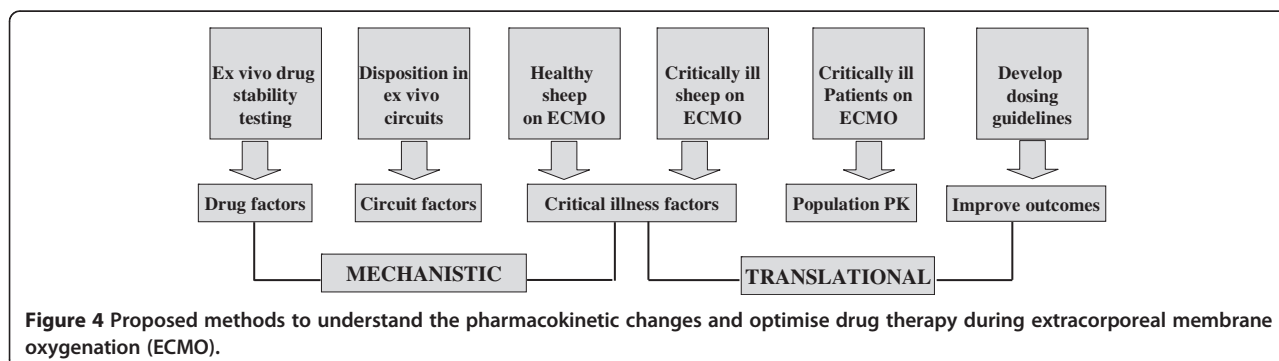
**Figure 4 Proposed methods to understand the pharmacokinetic changes and optimise drug therapy during extracorporeal membrane oxygenation (ECMO).**

**Table 1 Study drugs for which mechanistic and clinical pharmacokinetic (PK) data will be generated**

| Studies | Sedatives & analgesics | Antivirals /antifungals | Antibacterials |
|---|---|---|---|
| Ex vivo stability | Morphine | Fluconazole | Ceftriaxone |
| Ex vivo circuits | Morphine -3 -glucuronide | Caspofungin | Meropenem |
| Ovine ECMO | Morphine -6 –glucuronide | | Vancomycin |
| Population PK | Fentanyl, nor-fentanyl | | Ciprofloxacin |
| | Midazolam | | Gentamicin* |
| | 1 & 4 hydroxy midazolam | | |
| Ex vivo stability | Propofol ,Thiopentone | Oseltamivir | Piperacillin/tazobactum Ticarcillin/clavulunate |
| Population PK | Dexmedetomidine | Voriconazole | Cefepime, Linezolid |

OC –oseltamivir carboxylate. * Gentamicin will not included in the *ex vivo* circuit studies due to its incompatibility with other study drugs.

type of the pump and the oxygenator can influence drug PK [15].

### Circuit at varying flow rates (2.5 and 5.0 L/min)
Flow rates are thought to influence PK [40], however this has not been adequately tested. Higher ECMO flows usually reflect greater severity of illness and identifying the PK alterations is essential to maximise the chances of survival in this very unwell subgroup of patients. This experiment will also provide insight into whether or not flow rate adjusted standardisation of drug therapy is required.

### Circuits primed with fresh whole sheep blood
### Circuit under hyperoxic conditions (PaO2 300–400 mm Hg)
Hyperoxia is not uncommon in patients receiving ECMO [41]. Hyperoxic conditions may affect PK by changes in the catalytic activity of drug metabolising enzymes and changes in membrane permeability, affecting drug distribution [42]. Carbon dioxide gas or sodium bicarbonate solution was added to the circuit to maintain the pH of the circulating blood in the range 7.25–7.55.

### Circuit under hypothermic conditions (32–34°C)
Hypothermia can affect PK significantly [43,44] however there is limited published data. Circuits will be primed with sheep blood as it is relatively easy to replicate an *in vivo* experiment if required in sheep. The cooling device (Jostra™ Heater-Cooler Unit HCU 30 A) will be added to the ECMO circuit to induce hypothermia. This is relevant as patients on ECMO following CPR often receive therapeutic hypothermia as part of their post resuscitation care. Hypothermia may sometimes be induced to minimise oxygen consumption during VV ECMO. Patients on cardiopulmonary bypass are routinely exposed to hypothermia. Understanding the effect of hypothermia on PK is an important aspect for optimisation of drug dosing during ECMO.

### Host factors
### Healthy and critically ill controls
Baseline PK samples will be obtained from healthy sheep and sheep with smoke inhalation acute lung injury (S-ALI) over a 12 hour period prior to commencement of ECMO. In an appropriately equipped theatre, a central venous line will be placed in the right internal jugular vein (IJV) under local anaesthesia. Alfaxalone, ketamine and midazolam was used for induction and maintenance of anaesthesia. Buprenorphine 0.01 mg/kg
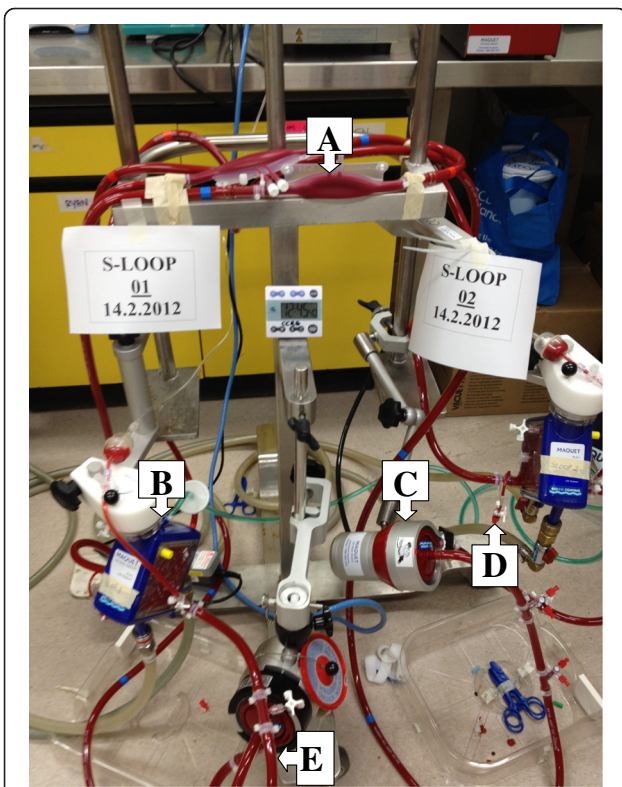


**Figure 5 Ex vivo ECMO circuit model. A** -reservoir bladder, **B**- oxygenator, **C**- centrifugal pump, **D**-drug injection and sampling port, **E**- circuit tubing.

will be used for supplemental analgesia. The sheep will be intubated and ventilated with a Hamilton Galileo ventilator (Hamilton Medical AG). The facial artery will be cannulated for invasive arterial blood pressure monitoring. A pulmonary artery catheter will provide continuous measurements of the central venous pressure, mixed venous saturation and cardiac output (CO). Additional sheaths will be placed in both IJV to facilitate ECMO cannulation and intra-cardiac echo (ICE). Sedative study drug infusions will be titrated to clinical effect. Antibiotics will be infused over 30 mins and serial blood samples will be obtained for drug assays using validated LC-MS/MS methods, and subsequent PK analysis.

For critically ill control sheep, S-ALI will be induced using a validated, reproducible technique that has been published [45]. Briefly, a stainless steel plate will be heated to 750°C and placed on top of 8 g of cotton in a cup. The smoke resulting from combustion will be delivered to the sheep by manual compression of the bellows (tidal volume VT, 10–12 mL/kg) to achieve a carboxyhaemoglobin content of 45–50% is achieved. The sheep will be ventilated using ARDSNet criteria (VT 4–6 ml/kg, PEEP 10–15 cm $H_2O$) for lung protective ventilation [46]. Sedative study drug infusions will be titrated to clinical effect. Antibiotics will be infused over 30 mins and serial blood samples will be obtained for drugs assays using validated LC-MS/MS methods, and subsequent PK analysis. Such an approach will provide insights into the effects of critical illness on sedative and antibiotic drug PK.

### Healthy sheep on ECMO

Following 12 hours of ventilation and PK sampling the healthy control sheep will be maintained on ECMO for 24 h. We have recently published a detailed description of our ovine model (Figure 6) of ECMO [39]. Cannulation will be performed in the supine position by rewiring the previously placed IJV venous sheaths. A 21Fr (50 cm) femoral Carmeda Bioactive Surface coated (CBAS®) venous cannula (Medtronic Inc, Minneapolis, MN, USA) will be inserted into the right IJV using a Seldinger technique and positioned using intra cardiac echocardiography (ICE) [47] in the proximal inferior vena cava (IVC). A 19Fr (50 cm) Carmeda coated femoral venous cannula will be used for return blood and also inserted in the right IJV and positioned at the mid right atrium using ICE. Pump speeds will be titrated to target flows at least 2/3$^{rd}$ of pre-ECMO CO (or 60–80 mL/kg). Sedative study drug infusions will be titrated to clinical effect. Antibiotics will be infused over 30 mins upon commencement of ECMO and at 8 and 12 h (for re-dose PK) to obtain serial blood samples for drug assays using validated LC-MS/MS methods, and subsequent PK analysis.

### Critically ill sheep on ECMO

After 12 h of lung protective ventilation, the control S-ALI sheep will be maintained on ECMO for 24 h. Cannulation, ECMO set up and initiation of ECMO have been described in earlier sections. Sedative study drug infusions will be titrated to clinical effect. Antibiotics will be infused over 30 mins upon commencement of ECMO and at 8 and 12 h (for re-dose PK) to obtain serial blood samples for drugs assays using validated LC-MS/MS methods, and subsequent PK analysis. Upon completion of these studies, PK data from critically ill sheep on ECMO will be compared with data from controls and healthy sheep on ECMO to obtain crucial PK data that will inform our understanding of the factors underpinning the PK changes induced by ECMO that is distinct from the impact of critical illness itself.

The physiologic data collection for the sheep experiments will include; weight, advanced haemodynamic and respiratory monitoring data, ECMO flow rates, urine output, fluid balance, inotrope and vasopressor use and blood loss if any. Eight hour urinary creatinine clearance, serum creatinine, serum total protein, serum albumin, *alpha1-acid glycoprotein*, serum bilirubin, alanine aminotransferase (ALT) measurements will be performed prior to (controls) and during ECMO. Pharmacokinetic studies in critically ill patients on ECMO.

An international multi-centre, clinical PK study [48] will enrol critically ill patients admitted to the intensive care units in Australia and New Zealand. The study centres include; The Prince Charles Hospital, Brisbane, St Vincent's Hospital, Sydney; The Alfred, Melbourne; Auckland City Hospital, Auckland and Princess Alexandra Hospital, Brisbane. Informed consent will be obtained from the patients or from their next of kin as appropriate. A total of 10–12 patients will be enrolled for each study drug (Table 1) for this descriptive study. Sedative drugs will be titrated to clinical sedation scores and bispectral index. Antibiotic drug selection and dosing is at the discretion of the treating clinicians. In some patients, blood samples relating to only antibiotics may be collected, whereas in other patients, samples for analysis of analgesics and sedatives may also be collected. Patient selection will be based on the below criteria;

### Inclusion criteria

- Age > 18 years and < 90 years
- Currently undergoing ECMO for respiratory and/or cardiac dysfunction
- Clinical indication for the antibiotics listed in Figure 3
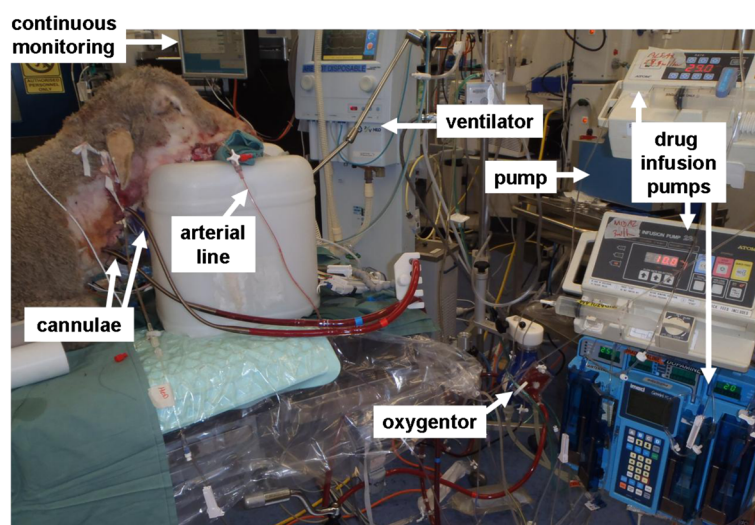- Clinical indication for the sedatives and analgesics listed in Figure 3

**Figure 6 Validated ovine ECMO model.** Reproduced with permission, Fung et al, ISBT Science Series 2012.

### Exclusion criteria

- No consent
- Known allergy to study drug
- Pregnancy
- Serum bilurubin > 150 μmol/L
- Ongoing massive blood transfusion requirement (> 50% blood volume transfused in the previous 8 hours)
- Therapeutic plasma exchange in the preceding 24 hours

Our clinical service model of ECMO has been recently published [49]. Feasibility studies are now completed and the study protocol has now been validated and published [48]. Eight hour urine creatinine or effluent creatinine (in patients on renal replacement therapy [RRT]) will provide estimates for renal clearance. Plasma assays and PK modelling will be undertaken for the study drugs (Table 1) using techniques described below.

### PK sample analysis

To reduce the sample burden per patient, validated bioanalytical methods are required to quantify multiple drugs and their metabolites selectively and sensitively in small volumes of plasma. A validated bioanalytical method that uses a fully automated on-line solid phase extraction (SPE) system (Symbiosis, SPARK Holland) combined with liquid chromatography-mass spectrometry (LC-MS/MS –API 5000) to simultaneously quantify morphine, morphine 3-β-D-glucuronide, morphine 6-β-D-glucuronide, midazolam, 1- hydroxymidazolam, 4-hydroxymidazolam, fentanyl and nor-fentanyl in samples of human plasma has been developed [50]. The technique

will also be expanded to analyse propofol, thiopentone and dexmedetomidine. This approach enables simultaneous measurement of the plasma concentrations of these molecules of interest with high accuracy and precision in a single specimen. Previously developed and validated antibiotic assays (HPLC and LC-MS/MS) will be used in these studies.

### PK modelling and statistical analysis

The sample size calculations used 10 circuits/subjects, with 10 observations over time per circuit/subject, and an 80% power with a 2-sided 5% significance level. The detectable differences over time are on a standardised scale (Cohen's d). The within correlations are from previous data. We have the power to detect relatively small changes with our small sample sizes because of the multiple observations per circuit/subject.

### Circuit studies

Data will be plotted over time and analysed for statistically significant temporal losses. Mixed-effects regression models with random slopes will be used to estimate the loss over time. Octanol-water partition coefficients (log $P$) for the individual drugs are available from the University of Alberta Drug bank website. We will examine the relation between the partition coefficients and the extent of drug loss in the circuit using simple linear regression. Correlation between log $P$ values and drug loss will be calculated by using two-sided Spearman test.

### Animal and clinical studies

Data from these studies will be analysed using non-linear mixed-effects models. This allows the estimation of typical population PK parameters and their inter- and

intra-individual variability, plus the estimation of residual random variability. We will fit random intercepts and slopes to allow for between patient differences in their average response and changes over time. This modelling allows us to visualise the average patient and individual patients. It also allows PK to be described in the absence of fixed protocol times, making it ideally suited to calculate PK parameters from drug concentration data collected at with varying times during routine care. Differential equations will be used to describe the population PK of study drugs and their metabolites expressed as PK parameters. Where relevant, results will be normalised to a median patient bodyweight of 70 kg, using allometry.

### Ethical considerations

Appropriate ethics approval has been obtained for all the phases of the ECMO PK project,

- *Ex vivo* circuit experiments using human blood (HREC/12/QPCH/90)
- *In vivo* ovine studies and *ex vivo* circuit experiments that utilise sheep blood (approval no. 1100000053)
- Multi-site ethics approval for the clinical studies in Australia (HREC/11/QPCH/121)
- Single-site ethics approval for the clinical study in New Zealand (LRS/12/06/020)

### Collaborating organisations

This project is co-ordinated by The Critical Care Research Group at The Prince Charles Hospital in Brisbane, Australia. This group will collaborate closely with The Burns Trauma and Critical Care Research Centre, and The Centre for Integrated Preclinical Drug Development, The University of Queensland in Brisbane for antibiotic and sedative drug assays. The Critical Care Research Group will also collaborate closely with all clinical sites involved in the multi-centre population PK study.

### Discussion

This research will not only identify the drugs that are most suitable for use during ECMO but our findings will also inform the development of strategies for drug administration using PK/PD principles in critically ill patients receiving ECMO. These patients receive a variety of pharmacological and other extracorporeal therapies such as RRT and these modalities have a potential to interact with each other (Figure 7). A lack of understanding of the impact of ECMO on drug Vd and CL predisposes to an increased likelihood of therapeutic failure or drug toxicity. PK modelling is crucial to drug safety. The ECMO PK Project seeks to provide the key information for development of evidence-based dosing schedules and sedation protocols for use by clinicians looking after patients receiving ECMO.
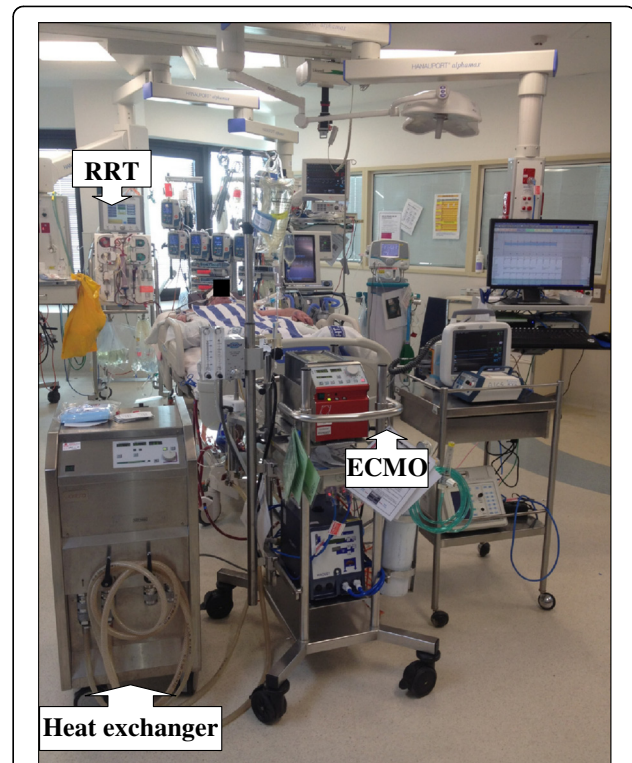


**Figure 7 Challenges in drug dosing during extracorporeal membrane oxygenation (ECMO).** This critically ill patient received concomitant venovenous ECMO, renal replacement therapy (RRT) and induced hypothermia, all of which can significantly alter pharmacokinetics of vital drugs.

Using the right sedative agent at an appropriate dose may minimise ICU morbidity related to risk of infections, duration of mechanical ventilation and length of hospital stay, inotrope and vasopressor requirement, drug withdrawal, post traumatic stress etc. This not only has resource implications but significantly affects patient outcomes [27]. The clinical study will also evaluate the adequacy of existing ICU sedation protocols as compared to bispectral index monitoring and provide data to inform recommendations for improving sedation practices during ECMO.

There is widespread consensus that in-hospital antibiotic resistance influences patient outcome and the allocation of resources. Optimal antibiotic prescription has significant implications not only for the patient on ECMO but also for other ICU patients and the community in general. Antibiotic PK studies in patients receiving ECMO will help the development of dosing regimes that are effective against the microorganism, but not harmful to the patient. The right dose of the right antibiotic will not only lead to improved microbiological and clinical cure rates in an individual patient, but also will reduce the emergence of multi-resistant organisms.

## Conclusions

Systematic research that integrates both mechanistic and clinical research is necessary when investigating the complex area of pharmacokinetic alterations during ECMO. The methods described in this paper will result in an advanced understanding of drug, circuit and critical illness factors that influence PK during ECMO. This will allow meaningful interpretation of clinical population PK data so that rational and robust guidelines may be generated to guide clinicians in optimising antibiotic, sedative and analgesic drug therapy during ECMO. The research methods described here are resource intensive and rely on extensive collaborations. Hopefully such an effort can be extended to comprehensively investigate many of the other complex issues in intensive care practice.

### Abbreviations

ECMO: Extracorporeal membrane oxygenation; ICU: Intensive care unit; PK: Pharmacokinetics; Vd: Volume of distribution; CL: Clearance; RASS: Richmond agitation sedation scale; HPLC: High performance liquid chromatography; LC-MS/MS: Liquid chromatography tandem mass spectrometry; RRT: Renal replacement therapy.

### Competing interests

The authors declared that they have no competing interest.

### Author's contributions

KS designed the project and wrote the initial protocol. JAR, MTS, YLF and JFF provided further advice and input into the study design and the protocol. All authors read and approved the final manuscript.

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## 4.2 DEVELOPMENT OF SIMULATED AND OVINE MODELS OF EXTRACORPOREAL LIFE SUPPORT TO IMPROVE UNDERSTANDING OF CIRCUIT - HOST INTERACTIONS.

### *4.2.1 Introduction to this publication*

This chapter describes the development of ex vivo and ovine models of VV ECMO that were crucial to an advanced mechanistic understanding of the PK changes. At that time of publication, this was the first ever report of a large animal model of VV ECMO. Prior to this, Zwischenberger et al [76] had published pathophysoiology of smoke inhalation in an ovine model of VA ECMO. It should be noted that VA ECMO is quite a contrasting extracorporeal life support technique used to support patients with predominant cardiac failure. VV ECMO is predominantly used for respiratory support[2] and has significantly different pathophysiological consequences on the host when compared with VA ECMO. Equally, the ECMO circuit components used in our model are far more refined than those reported by Zwischenberger et al 20 years ago. Lung injury was induced by smoke inhalation in both these models. This allowed us compare the effects of smoke inhalation and VV ECMO on ovine pathophysiology with the effects of smoke inhalation and VA ECMO reported by Zwischenberger et al. Detailed pathophysiological comparisons between these models are outside the scope of this thesis and will be reported elsewhere.

# Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit–host interactions

Kiran Shekar, Yoke L Fung, Sara Diab, Daniel V Mullany, Charles I McDonald, Kimble R Dunster, Stephanie Fisquet, David G Platts, David Stewart, Steven C Wallis, Maree T Smith, Jason A Roberts and John F Fraser

Use of extracorporeal life support (ECLS) in cardiorespiratory failure refractory to maximal medical therapy is now well described, and use in adults is increasing.[1,2] Patients requiring ECLS are a critically ill, although otherwise heterogeneous, cohort with varying diagnoses, age, body size and degrees end-organ dysfunction. ECLS itself can induce myriad additional pathophysiological changes. Clinical research in this group of patients is challenging, given the multitude variables that are difficult to control in any given study. Simulated extracorporeal circuits and in-vivo animal models provide valuable means to undertake detailed, systematic research into complex clinical scenarios such as ECLS, and to identify strategies for optimising clinical interventions.

An ideal animal model should mimic the severity of patient illness, reproduce key haemodynamic and immunological aberrations, serve as its own control, mimic histological findings in relevant organs, and provide a means to gain insight into factors contributing to interpatient variability.[3] There are many well established ovine models used to study clinically relevant interventions and pathological states, such as cardiopulmonary bypass, myocardial reperfusion, burn injury, sepsis and acute lung injury (ALI).[4-16] These

**Abbreviations**

| | |
|---|---|
| ABP | Arterial blood pressure |
| ACT | Activated clotting time |
| ALI | Acute lung injury |
| CCO | Continuous cardiac output |
| CVP | Central venous pressure |
| ECG | Electrocardiogram |
| ECLS | Extracorporeal life support |
| ECMO | Extracorporeal membrane oxygenation |
| ICE | Intracardiac echocardiography |
| IJV | Internal jugular vein |
| IVC | Inferior vena cava |
| SvO$_2$ | Mixed venous saturation |

## ABSTRACT

**Background:** Extracorporeal life support (ECLS) is a life-saving technology that is being increasingly used in patients with severe cardiorespiratory failure. However, ECLS is not without risks. The biosynthetic interface between the patient and the circuit can significantly alter inflammation, coagulation, pharmacokinetics and disposition of trace elements. The relative contributions of the pump, disease and patient in propagating these alterations are difficult to quantify in critically ill patients with multiple organ failure.

**Objective:** To design a model where the relevance of individual components could be assessed, in isolation and in combination.

**Design and subjects:** Four ECLS models were developed and tested — an in-vitro simulated ECLS circuit; and ECLS in healthy sheep, sheep with acute lung injury (ALI), and sheep with ALI together with transfusion of old or new blood.

**Main outcome measures:** Successful design of in-vitro and in-vivo models.

**Results:** We successfully conducted multiple experiments in the simulated circuits and ECLS runs in healthy and ALI sheep. We obtained preliminary data on inflammation, coagulation, histology, pharmacokinetics and trace element disposition during ECLS.

**Conclusions:** The establishment of in-vitro and in-vivo models provides a powerful means for enhancing knowledge of the pathophysiology associated with ECLS and identification of key factors likely to influence patient outcomes. A clearer description of the contribution of disease and therapeutic interventions may allow improved design of equipment, membranes, medicines and physiological goals for improved patient care.

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models highlight the appropriateness of the use of sheep in the study of human cardiopulmonary disease.

To assess the relative contribution of circuit factors to ECLS ovine model outcomes, we have also established a validated

in-vitro model of simulated ECLS using contemporary circuitry. Simulated circuits provide conditions that allow control of one variable at a time, which is difficult to achieve in animal and clinical studies. Hence, the aim of this study was to develop and validate more extensive and informative descriptors of ECLS using in-vitro and in-vivo models.

## Methods

### Establishment of a simulated model of extracorporeal life support

We used PLS ECLS circuits (Maquet Cardiopulmonary, Hirrlingen, Germany). This circuit comprises Bioline tubing, a PLS Quadrox D oxygenator and Rotaflow pump head. An



**Figure 1. Simulated extracorporeal life support circuit model; circuits primed with fresh whole human blood**



**Figure 2. Overview of experimental model**

ECMO = extracorporeal membrane oxygenation. PRBC = packed red blood cells.
SALI = smoke-inhalation acute lung injury.

R38 reservoir bladder (Medtronic, Minneapolis, Minn, USA) was added to allow fluid sampling from the closed circuit. The circuits were primed with 900 mL Plasma-lyte 148 (Baxter, Sydney, NSW, Australia), then exchanged for 500 mL Albumex 4 (human albumin, 40 g/L; CSL Bioplasma, Melbourne, Vic, Australia). Porcine mucous heparin (5000 U; Pfizer Australia, Sydney, NSW, Australia) was added to the circuits. Fresh whole human blood (mean volume, 420 mL [SD, 52 mL]) was used for the final prime to obtain postoxygenator pressures of 230–250 mmHg. The final circuit configuration is shown in Figure 1.

The final volume of the pressurised circuit was 668 mL (SD, 2 mL) with a mean haemoglobin concentration of 64 g/L (SD, 13 g/L). Activated clotting time (ACT) was maintained between 220 and 250 seconds. A Jostra Rotaflow centrifugal pump (Maquet Cardiopulmonary) was used to maintain a circuit flow rate of 4–5 L/min. Oxygen tension was maintained between 150 and 200 mmHg. Circuit temperature was maintained at 37°C. Carbon dioxide gas or sodium bicarbonate solution was added to the circuit to maintain the pH of the circulating blood between 7.25 and 7.55.

This simulated circuit model system was used to study the effect of circuit factors on drug concentrations, coagulation, inflammation and platelet cell functions under standard physiological conditions. In future, this model will incorporate variables that are of high relevance for a patient on ECLS, such as hypoxia, hyperoxia and hypothermia.

### Establishment of an ovine model of extracorporeal life support

Establishment and validation of the ovine model of ECLS was conducted with approval of the Queensland University of Technology Animal Ethics Committee (approval no. 1100000053). An overview of the ECLS ovine model is presented in Figure 2. A summary of the key steps (Figure 3) and rationale is outlined below.

#### Theatre set-up

The theatre was set up to incorporate all equipment required to manage and monitor animal health and wellbeing. The operating table was equipped with a warming blanket to maintain normothermia during chronic instrumentation. A cradle was used to safely maintain the sheep's sternum in the recumbent position, and a "head box" was used to immobilise the head and neck in a natural position.
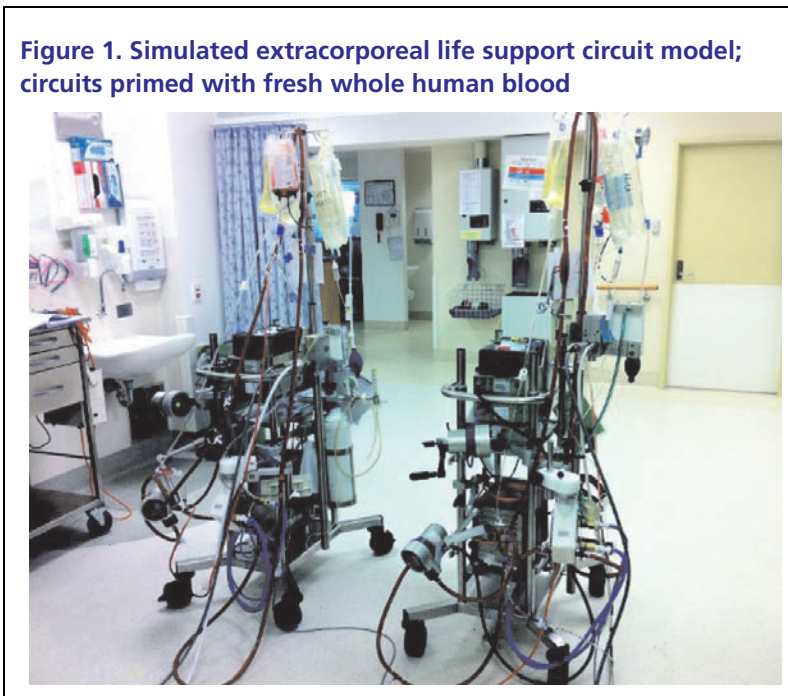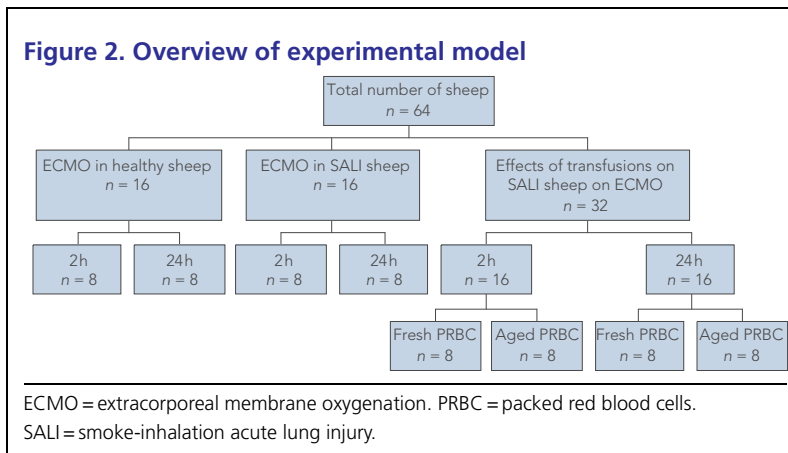
**Figure 3. Steps in development of an ovine model of extracorporeal life support**

Preparation: ethics, staffing, animal selection, theatre

↓

Access, anaesthesia, airway control, monitoring

↓

Smoke inhalation, lung-protective ventilation

↓

ECMO set-up, cannulation, intracardiac echocardiography

↓

Maintenance of ECMO
• Anticoagulation
• Haemodynamic management
• Fluid and electrolyte management
• General supportive care

↓

Euthanasia and postmortem handling

ECMO = extracorporeal membrane oxygenation.



**Figure 4. Operating room equipped for monitoring and organ support**
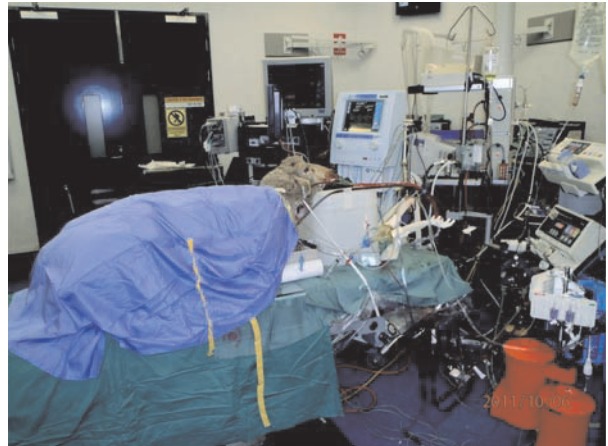
Electrocardiograms (ECGs), physiological and pump pressures, saturation and end-tidal carbon dioxide were measured with a Marquette Solar 8000 monitor (GE Healthcare, Little Chalfont, UK) and recorded at 5 s intervals using custom software. Ventilation data were recorded on a breath-by-breath basis using software provided by the manufacturer (Hamilton Medical, Reno, Nev, USA), and ECLS pump data were recorded at 5 s intervals using custom software. Mixed venous saturation ($SvO_2$) and continuous cardiac output (CCO) were measured with a Vigilance II Monitor (Edwards Lifesciences, Irvine, Calif, USA) and recorded at 5 s intervals using software provided by the manufacturer. Syringe drivers and large- and small-volume infusion pumps were used for anaesthesia maintenance and drug/fluid administration. A fibre-optic bronchoscope was used to obtain bronchoalveolar lavage samples. The operating theatre was set up as shown in Figure 4.

*Venous access, induction of anaesthesia and airway management*

The sheep were 18-month-old ewes and weighed 40–45 kg. After fasting overnight, the animal was brought into the preparation room in a sling with face, neck and chest shaved. A multilumen central venous catheter and 8 Fr sheath were placed in the left internal jugular vein (IJV) under local anaesthesia. Baseline blood samples were taken. Anaesthesia was induced with intravenous

midazolam (0.5 mg/kg) and alfaxalone (3 mg/kg). The animal was intubated with an orotracheal tube, brought into the operating room and positioned in the left lateral position; mechanical ventilation was commenced with expired air capnography. The sheep was ventilated with a Galileo ventilator (Hamilton Medical) using the Acute Respiratory Distress Syndrome Network criteria for lung-protective ventilation.[17] No muscle relaxants were used. Anaesthesia was maintained with infusions of alfaxalone (4–6 mg/kg/h), midazolam (0.25–0.5 mg/kg/h), and ketamine (3–5 mg/kg/h). Doses were titrated to physiological and clinical parameters such as arterial blood pressure (ABP), heart rate, respiratory rate, central venous pressure (CVP), jaw movements, loss of the eyelash reflex and limb withdrawal response to ensure a constant surgical plane and optimal depth of anaesthesia and analgesia. A bolus dose of intravenous buprenorphine 0.01 mg/kg was given for analgesia and subsequently every 6 hours. Three venous sheaths (10 Fr) were introduced in the right IJV. These would later be used for intracardiac echocardiography (ICE) and extracorporeal membrane oxygenation (ECMO) cannulation. A tracheostomy was performed using a size 10 Portex (Smiths Medical, London, UK) tracheostomy tube and the endotracheal tube removed. The length of the sheep's neck otherwise would prevent adequate bronchoscopy.

*Establishment of monitoring*

Routine monitoring consisted of pulse oximetry, ECG, ABP, CVP, pulmonary artery pressure and CCO. A pulmonary artery catheter was inserted via the left IJV sheath to measure CCO and $SvO_2$. A urinary catheter and orogastric tube were inserted. The facial artery was exposed and cannulated under direct vision for ABP monitoring.

## Figure 5. Schematic diagram of sheep on extracorporeal life support



Sheep have capacious jugular veins, which make multiple cannulations in the vein possible.
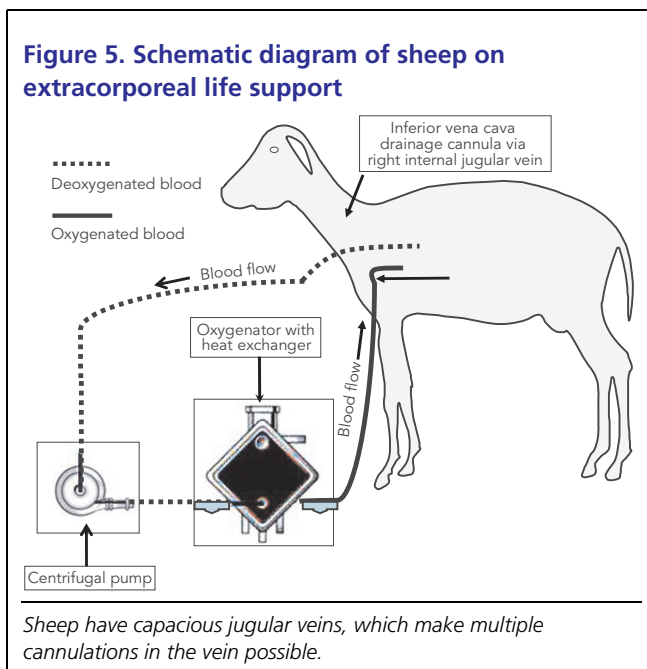
*Inducing acute lung injury*

Our validated reproducible smoke-inhalation ALI method was used and has been described in detail elsewhere.[18] Briefly, a stainless steel plate is heated to 750°C and placed on top of 8 g of cotton in a cup. A bellows with transparent walls and a tidal volume of 400 mL is placed on top. The smoke from the combustion then passively fills the bellows. The bellows are then compressed by hand, exhaling the smoke through the base to a one-way valve, acting as one tidal volume breath (10–12 mL/kg) to the sheep. Exhalation is via a one-way valve, thus minimising rebreathing. Twelve breaths are delivered with first load of cotton. After the first cycle, new fuel is placed in the cup, and the process is repeated with eight breaths per cycle. Blood gas analysis provides both oxygenation details and carboxyhaemoglobin content (target 45%–50%), which demonstrates the reproducibility of the smoke "dose" between sheep.

*Extracorporeal life support circuit set-up and cannulation*

The circuits were primed with 900 mL Plasma-lyte 148 (Baxter) and then exchanged for 500 mL Albumex 4 (human albumin, 40 g/L; CSL Bioplasma). The oxygenator was connected to a water heater (Cincinnati Sub-zero, Cincinnati, Ohio, USA) to maintain ovine normothermia (39°C). A PLS ECMO circuit was used (Maquet Cardiopulmonary AG). This circuit comprised Bioline tubing, a PLS Quadrox D oxygenator and Rotaflow pumphead. The circuit was primed with 900 mL Plasma-lyte 148 (Baxter), then exchanged for 500 mL Albumex 4 (CSL Bioplasma). Porcine mucous heparin (1000 U; Pfizer Australia) was then added to the circuit. The pump driver was a Bio-Medicus 550 Bio-

Console pump speed controller (Medtronic) with external drive head. All data from the Bio-Medicus device were captured using the serial data port. Precise mixing of air and oxygen was achieved using a Sechrist air–oxygen mixer (Sechrist Industries, Anaheim, Calif, USA).

Cannulation was performed in the supine position by rewiring the previously placed venous sheaths. A 21 Fr (50 cm) femoral Carmeda BioActive Surface coated venous cannula (Medtronic) was first inserted into the right IJV using the Seldinger technique and positioned using ICE in the proximal inferior vena cava (IVC). After insertion of an access cannula, 30 U/kg of unfractionated porcine heparin (Pfizer Australia) was administered intravenously. A 19 Fr (50 cm) Carmeda BioActive Surface coated femoral venous cannula was used for return blood and also inserted in the right IJV using the Seldinger technique and positioned at the mid right atrium using ICE. Sheep femoral vessels are too small for ECLS cannulation, whereas the jugular veins are much more capacious. Arterial cannulae (25 cm) were too short for this model.

On confirmation of correct cannula positioning, each cannula was locked with unfractionated heparin (10 U/mL) and saline, and anchored around the neck with white endotracheal tube tape. Standard cyanoacrylate adhesive was found to be very effective in fixation of the cannula, but it limited any subsequent manipulation. The sheep was then repositioned from the supine to the sternal recumbent position. The clamped ECLS circuit was connected to the access and return cannulae. The pump speed was dialled to 1000 rpm before releasing the clamps. Pump speed was further increased to target flows at least two-thirds of pre-ECLS cardiac output (or 60–80 mL/kg). Gas flow was set to 80% of pump flows and inspired oxygen concentrations set at 100%. Ventilator settings were then set to "rest" settings (respiratory rate, 6; FiO$_2$, 0.21; tidal volume, 4–6 mL/kg, positive end-expiratory pressure, 10 cmH$_2$O). PaCO$_2$ was adjusted by altering sweep gas flows with ventilator settings held constant. A schematic model of the sheep ECMO model is shown in Figure 5.

*Intracardiac echocardiography and confirmation of cannula position*

Echocardiography plays a fundamental role in initiating and maintaining ECLS.[19,20] Conventionally, transoesophageal echocardiography is used to determine cannula positioning in the clinical setting.[21] However, sheep have a particularly capacious oesophagus, preventing good transducer contact and hence poor images are produced. Transthoracic echocardiography is possible in sheep, but there are limited acoustic windows available. ICE has the dual benefits of excellent spatial resolution combined with multiple accessible acoustic windows.[22]

**Figure 6. Intracardiac echocardiography to confirm cannula position**
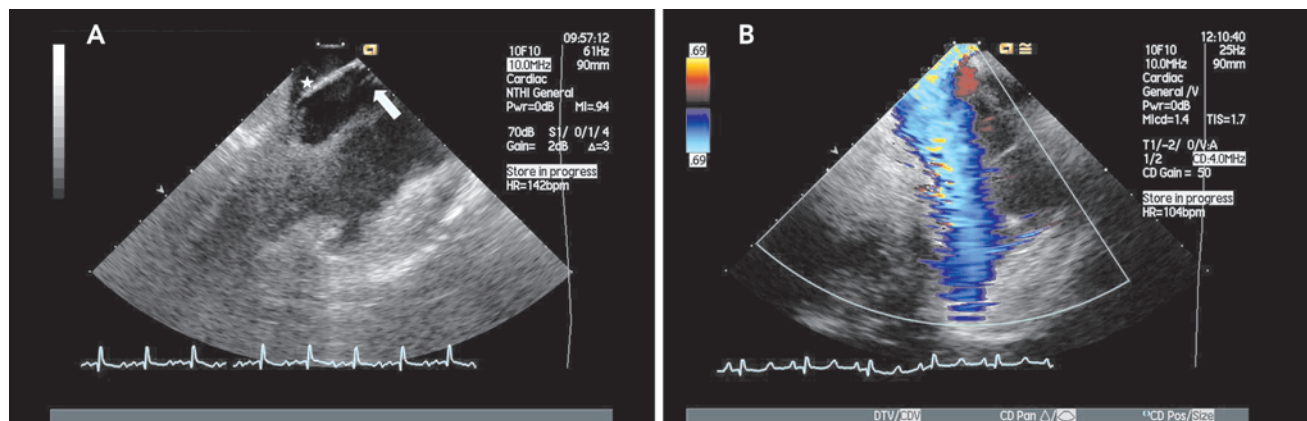


A. *Arrow points to the tip of the venovenous return cannula. Asterisk denotes Swan–Ganz catheter.* B. *Venovenous outflow in diastole with tricuspid valve open.*

An 11 Fr venous access sheath was placed in the right IJV. A 10 Fr ICE catheter was connected to an ACUSON Sequoia C512 echocardiography machine (Siemens, Munich, Germany) via a Swiftlink catheter connector (Siemens). The ICE catheter was passed down the sheath, through the right heart and into the IVC before cannulation. The guide wire for the access cannula was then passed through the heart and into the IVC under ICE guidance. The access cannula was inserted over this guide wire, and the tip could be visualised on the ICE images as it passed into the IVC and positioned as appropriate. A similar process was followed for the return cannula, but the imaging was performed more proximally. The ICE catheter was withdrawn into the mid right atrium. Appropriate passage of the return guide wire was confirmed with ICE. The return cannula was then passed over this wire and positioned in the mid right atrium (Figure 6).

*Anticoagulation*

The heparin infusion was commenced 4 U/kg/h for a target ACT of 200–300 s. The ACT checks were measured at 1 hour, 2 hours and every 2 hours subsequently.

*Haemodynamic management*

Invasive monitoring allowed for standardised management of haemodynamic variables using crystalloids (0.9% sodium chloride solution), colloids (human albumin, 40 g/L), vasopressor (vasopressin, noradrenaline) and inotrope (adrenaline) infusions as required.

*Fluid and electrolyte management*

Sheep were weighed on the day before and the day of surgery. Volume administration was determined by standard haemodynamic variables including elevated systemic vascular resistance, poor urine output and a low CVP. Maintenance fluids were run at 2 mL/kg/h throughout the study. Additional fluid boluses were allowed to maintain circuit flow and/or for resuscitation. Electrolyte levels were checked via regular blood gas analysis. Serum potassium levels were maintained > 3 mmol/L. If required, a potassium chloride infusion was commenced at a rate of 5–20 mmol/h, depending on serum potassium concentrations, urine output, and gastric losses. Any large-volume gastric content lost was returned to normalise acid balance.

*Temperature management*

Temperature control was facilitated by the heater unit connected to the oxygenator. The ambient room temperature could also be adjusted to maintain goal temperatures.

*Euthanasia and postmortem handling*

At completion of experimentation, animals were euthanased with sodium pentobarbitone (295 mg/mL, 0.5 mL/kg). Death was confirmed by loss of cardiac electrical activity, ABP and cardiac output. After euthanasia, organs were retrieved surgically. Histological analysis of organs, including the right lung (right lower lobe), left lung (left lower lobe), heart (left and right ventricles), liver, pancreas, kidneys, spleen, adrenals, stomach, intestine and brain, was performed. The remains of the animals were frozen and stored until disposed of via high temperature incineration.

**Results**

The simulated ECLS circuit experiments ($n = 4$) examining drug disposition in the extracorporeal circuit primed with fresh whole human blood have showed significant alterations in drug behaviour. Preliminary data show significant

sequestration of drugs in the circuit, which may have important clinical implications.

By commencing with a short-term ECLS experiment using healthy sheep, we conducted multiple experiments in the sheep comprising 2-hour ECLS runs in healthy ($n=6$) and ALI sheep ($n=6$). We were also successful with 24-hour runs in healthy ($n=4$) and ALI sheep ($n=1$). Significant preliminary data were obtained in relation to inflammation, coagulation, histology, pharmacokinetics, biomarkers and trace element disposition.

From a technical point of view, the cannulation, initiation and maintenance of ECLS were uneventful. We captured up to 70% of the cardiac output through our IVC access cannula with minimal recirculation, which is a significant improvement over previously reported ovine models,[23] and is the key to maintaining satisfactory oxygenation in animal models of severe ALI. We had no serious issues related to bleeding or clotting, unlike the clinical situation.[24] We have now optimised our anticoagulation, haemodynamic management and overall conduct of the experiment.

## Discussion

This article describes the use of standard circuitry that includes centrifugal pumps and polymethyl pentene oxygenators to establish simulated circuit and ovine models of ECLS. Previously reported models have used variable technology comprising roller pumps, membrane oxygenators and drainage reservoirs.[23,25-28] Some models have used venoarterial ECLS, which is not entirely comparable to the venovenous group. Changes in technology influence circuit–host interactions significantly and having a contemporary model adds to clinical relevance.

A major advantage of the sheep as an animal model of human disease is their large size. With mean body weight between 30 and 90 kg, depending on breed,[29] the size is comparable to adult humans (67–87 kg).[30] Haemodynamic and respiratory measurements can be made using the same clinical techniques and equipment that are used in clinical intensive care units. Also, large volumes of blood, plasma and bronchoalveolar lavage samples can be collected at serial time points during an experiment without compromising the animal's health. Apart from the size, there are immunological similarities, which are important when studying host–circuit interactions.[9,31-34] Previous successful use of other ovine models of human disease, especially ALI,[3] as well as closer similarities between sheep and human pulmonary anatomy and physiology, immunology and size, justify the choice of sheep as a relevant in-vivo large animal model of ECLS.

The simulated circuit and ovine models of ECLS described here allow an incremental and systematic approach to characterising the effects of ECLS on host physiology. Knowledge of how each ECLS component contributes to tissue injury will provide novel insight to inform new strategies to control, reduce or eliminate extracorporeal associated tissue injury, which will result in improved ECLS survival rates. The information gathered from this study will also benefit other extracorporeal therapies such as cardiopulmonary bypass. Knowledge gained using this ovine model of ECLS will improve our preparedness for future severe influenza outbreaks. Finally, the establishment and validation of this in-vivo ovine ECLS model provides a means for evaluating interventions (eg, drugs such as serine protease inhibitors) to reduce extracorporeal induced inflammation and tissue injury.

Future work beyond the scope of that described here will use simulated circuits together with healthy and critically ill sheep receiving ECLS to gain insight into the contributions of circuit factors relative to the influence of critical illness on changes in the pharmacokinetics of drugs such as antibiotics, sedatives and analgesics that are used in patients receiving ECLS in the clinical setting. These studies, together with studies in patients receiving ECLS, will provide important data for informing the development of antibiotic dosing guidelines as well as specific sedation protocols for patients receiving ECLS.

## Conclusions

We have successfully established reproducible in-vitro and ovine models of ECLS that will be used in future studies to provide novel insight on altered pathophysiology during ECLS. Our on-going work involves collaborative research between major ECLS centres across Australasia and our model systems described herein will enable clinical hypotheses to be tested in a systematic fashion in order to optimise patient outcomes and further refinements in the use of ECLS in the clinical setting.

## Competing interests

The funding sources acknowledged had no influence on study design.

# ORIGINAL ARTICLES

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# 4.3 ASAP ECMO: ANTIBIOTIC, SEDATIVE AND ANALGESIC PHARMACOKINETICS DURING EXTRACORPOREAL MEMBRANE OXYGENTION: A MULTI-CENTRE STUDY TO OPTIMISE DRUG THERAPY DURING ECMO.

### *4.3.1 Introduction to this publication*

Designing this international multi-centre population PK study was crucial to translation of the research findings. The ASAP ECMO study in many ways pioneered international multi-centre research in the field of ECMO. This effort represents collaboration between pharmacists, ECMO and PK experts with experience in guideline development. This study will generate vital PK data that would then lead to development of robust dosing guidelines for 18 key antibiotic, sedative and analgesic drugs used in ECMO patients.

BMC
Anesthesiology

**STUDY PROTOCOL**                                                    **Open Access**

# ASAP ECMO: Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation: a multi-centre study to optimise drug therapy during ECMO

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## Abstract

**Background:** Given the expanding scope of extracorporeal membrane oxygenation (ECMO) and its variable impact on drug pharmacokinetics as observed in neonatal studies, it is imperative that the effects of the device on the drugs commonly prescribed in the intensive care unit (ICU) are further investigated. Currently, there are no data to confirm the appropriateness of standard drug dosing in adult patients on ECMO. Ineffective drug regimens in these critically ill patients can seriously worsen patient outcomes. This study was designed to describe the pharmacokinetics of the commonly used antibiotic, analgesic and sedative drugs in adult patients receiving ECMO.

**Methods/Design:** This is a multi-centre, open-label, descriptive pharmacokinetic (PK) study. Eligible patients will be adults treated with ECMO for severe cardiac and/or respiratory failure at five Intensive Care Units in Australia and New Zealand. Patients will receive the study drugs as part of their routine management. Blood samples will be taken from indwelling catheters to investigate plasma concentrations of several antibiotics (ceftriaxone, meropenem, vancomycin, ciprofloxacin, gentamicin, piperacillin-tazobactum, ticarcillin-clavulunate, linezolid, fluconazole, voriconazole, caspofungin, oseltamivir), sedatives and analgesics (midazolam, morphine, fentanyl, propofol, dexmedetomidine, thiopentone). The PK of each drug will be characterised to determine the variability of PK in these patients and to develop dosing guidelines for prescription during ECMO.

**Discussion:** The evidence-based dosing algorithms generated from this analysis can be evaluated in later clinical studies. This knowledge is vitally important for optimising pharmacotherapy in these most severely ill patients to maximise the opportunity for therapeutic success and minimise the risk of therapeutic failure.

**Trial registration:** ACTRN12612000559819

**Keywords:** ECMO, Pharmacokinetics, Pharmacodynamics, Antibiotics, Sedatives, Analgesics, Therapeutic failure, Drug toxicity

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## Background

Extracorporeal membrane oxygenation (ECMO) is an invaluable tool for the management of acute severe cardiac and/or respiratory failure in patients failing maximal medical therapy [1]. ECMO is a temporary organ support system and is currently used only for a limited time as a "bridge to recovery", "bridge to bridge" or "bridge to decision" device [2,3]. Veno-venous (VV) ECMO supports the lungs only, whereas veno-arterial (VA) ECMO provides support for both the heart and lungs. The reported survival for VA ECMO in adult patients with cardiac failure is up to 53% [4]. VV ECMO has a survival rate of up to 71% in patients with severe respiratory failure [5]. The findings of the CESAR trial [6], and the Australian and New Zealand Intensive Care Society ECMO investigator study into H1N1 [5], partly support its role in the advanced management of respiratory failure [7]. With refinements in technology, expanding scope [3] and favourable outcomes in recent studies, it is likely that ECMO may emerge as an indispensible tool in management of critically ill adult patients with cardio-respiratory failure. However, as ECMO finds its niche in adult intensive care as an adjunct to medical therapy, it is important that its effects on the pharmacokinetics (PK) of commonly used intensive care drugs are fully understood to ensure optimal drug therapy and improve patient outcomes. Given that drugs are important for reversing the underlying disease process, unknown interactions between ECMO and pharmacotherapy may seriously impair patient recovery.

In critically ill patients not receiving ECMO, numerous PK studies have demonstrated highly significant changes to drug exposure through interactions between the patient, pathology and the drug [8-11]. The ECMO system introduces additional variables, which are the circuit itself, as well as the systemic inflammation that results from prolonged use of an extracorporeal circuit. Sequestration of drugs in the circuit, increased volume of distribution (Vd) and decreased clearance (CL) are the major PK changes associated with ECMO [12], although the extent of such changes remains poorly characterised. Neonatal studies have reported significant alterations in antibiotic, sedative and analgesic PK [13], but these results may not be generalisable to adult patients due to the developmental and physiologic differences [14]. There is emerging data on the altered dose requirements in adult patients on ECMO [15,16]. *In vitro* studies in the neonatal circuit studies highlight the influence that drug properties such as molecular size, degree of ionization at physiological pH, lipophilicity and plasma protein binding have on drug sequestration during ECMO [13,17]. Recently, significant antibiotic, sedative and analgesic drug sequestration has been demonstrated in ECMO circuits used for adult patients [18]. The type and age of circuit components including the type of the pump, oxygenator and tubing, as well as circuit priming, may influence the level of drug sequestration [19-22]. Patient factors such as systemic inflammation, haemodilution, bleeding and transfusion, organ dysfunction and renal replacement therapy (RRT) all add to the clinical challenges of drug dosing during ECMO [23-25].

## Methods/Design

The Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation (ASAP ECMO) study is a prospective, multi-centre, open-label, descriptive, PK study of 19 drugs commonly used during ECMO. This study will recruit over 200 patients from 5 ICUs across Australia and New Zealand over 3 years. The primary and secondary aims are:

### Primary aim

- To develop PK models for the antibiotic, sedative and analgesic study drugs and their relevant metabolites described in Table 1 in patients receiving ECMO.

### Secondary aims

- To assess the adequacy of current antibiotic dosing regimens in patients on ECMO.
- To develop guidelines for antibiotic drug dosing during ECMO.
- To develop ECMO-specific sedation protocols.

### Participants

Informed consent will be obtained from study participants or surrogate decision makers as applicable. Eligible patients will be the critically ill admitted to the ICUs at The Prince Charles Hospital, Brisbane, Australia; St Vincent's Hospital, Sydney, Australia; The Alfred, Melbourne; Auckland City Hospital, Auckland, New Zealand; The Princess Alexandra Hospital, Brisbane, Australia and who have a clinical indication for ECMO. These patients will be receiving sedation and analgesia as part of their routine care, as well as being prescribed a study antibiotic for a clinical indication. A target of 10–12 patients will be enrolled for each study drug. We will examine key antibiotics, analgesics and sedatives (Table 1), and will opportunistically collect blood samples. In some patients, blood samples relating to only antibiotics may be collected, whereas in other patients, samples for analysis of analgesics and sedatives may also be collected.

**Table 1 Study drugs and their relevant metabolites for which population PK models will be developed**

| Sedative and analgesics | Antiviral/antifungal | Antibacterial |
|---|---|---|
| Morphine | Fluconazole | Ceftriaxone |
| Morphine-3 & 6-glucuronide | Caspofungin | Meropenem |
| Fentanyl & nor-fentanyl | Voriconazole | Vancomycin |
| Midazolam | Oseltamivir | Ciprofloxacin |
| 1& 4 hydroxy midazolam | Oseltamivir carboxylate | Gentamicin |
| Propofol | | Piperacillin-tazobactum |
| Dexmedetomidine | | Ticarcillin-clavulunate |
| Thiopentone | | Linezolid |

### Inclusion criteria

- Age > 18 years and < 90 years.
- Currently undergoing ECMO for respiratory and/or cardiac dysfunction.
- Clinical indication for the antibiotics listed in Table 1.
- Clinical indication for the sedatives and analgesics listed in Table 1.

### Exclusion criteria

- No consent
- Known allergy to study drug
- Pregnancy
- Serum bilurubin > 150 μmol/L
- Ongoing massive blood transfusion requirement (> 50% blood volume transfused in the previous 8 hours)
- Therapeutic plasma exchange in the preceding 24 hours

### Drug administration
#### Antibiotics
Antibiotic selection and dosing will be at the discretion of the clinician, based on the clinical context and unit guidelines. Doses will be reconstituted in 10 ml of diluent and given as bolus intravenous (IV) infusion in 50 ml over 30 minutes (except ciprofloxacin, vancomycin and linezolid – 1 or 2 hour IV infusion), or as per local hospital protocols. Antifungals will be infused IV as per local hospital guidelines and oseltamivir will be administered via enteral feeding tube (contents of capsule mixed in 20mL water followed by further 20-50mL water flush).

#### Sedative and analgesic drugs
Sedative and analgesic drugs will be administered according to local policies at each ICU. As a guide, IV infusions and boluses of morphine (10–30 mg/hr) and midazolam (10–30 mg/hr) titrated to a Richmond Agitation Sedation Scale (RASS) [26] of –3 to –4 and a

bispectral index (BIS) [27] of 40–45. Patient ventilator interactions may also be used as a guide to titrate sedation especially in patients on venovenous ECMO. Therapeutic paralysis is at the discretion of the treating clinician and will be guided by neuromuscular monitoring.

Additional IV sedation if required may be provided with one of the following agents:

- Propofol IV (10–200 mg/hr)
- Dexmedetomidine IV (1 mcg/kg bolus and 0.1–1.5 mcg/kg/min)
- Fentanyl IV (50–300 mcg/hour) if morphine is discontinued for clinical reasons
- Thiopentone IV (100–200 mg/hour)[*]

[*] Note- Thiopentone is uncommonly used as an ultimate rescue sedative in some patients on ECMO.

### Sample collection
Blood samples will be drawn from an existing arterial line and collected in 2 ml tubes with a lithium heparin anticoagulant. Where possible, a closed loop Venous Arterial blood Management Protection system (VAMP[TM], Edward Life sciences, Canada Inc) will be used to minimise blood loss during sampling. The minimum sample volume is 2 mL per time point. Another 0.5 mL of blood will be drawn for each additional drug studied. It is considered unlikely for a patient to be receiving more than 4 study drugs at a given time during PK sampling. Labels for the storage tubes will be provided by the central laboratory and a site specific study number will be allocated to each patient.

#### Blood sampling: antibiotics
All patients will be sampled over a single dosing period during ECMO. Where two or more antibiotics of interest are prescribed for one patient, data on the timing of administration for both drugs will be collected (Figure 1) with sampling performed according to the antibiotic with the longer dosing interval. For example if

**Figure 1 Sampling schedule for antibiotic drugs.**

vancomycin 1g IV q12h and piperacillin 4.5g IV q6h were both prescribed, sampling would be performed according to the vancomycin 12-hourly dosing schedule.

- Six-hourly dosing schedule – Blood will be sampled from an existing arterial line at the following times: 0, 15, 30, 45, 60, 90, 120, 180 and 360 minutes.
- Eight or 12-hourly dosing schedule – Blood samples will be collected from an existing arterial line at the following times: 0, 15, 30, 45, 60, 90, 120, 180 and 480 minutes.

*Blood sampling: sedatives and analgesics*
Blood samples will be taken from an existing arterial line at 0, 15, 30, 45, 60, 120, 180 and 240 minutes on commencement or cessation of a new sedative drug infusion. The details of drugs, doses and rates of administration to be documented are on the data sheet (Figure 2).

*Urine specimens*
Urinary creatinine clearance collections will be performed for patients not receiving renal replacement therapy as an 8-hour urinary collection. Assay of the urine specimens will be performed by the local

pathology service as a surrogate for glomerular filtration rate. For patients receiving RRT the type and dose of the treatment will be documented on the data sheet.

**Data collection and management**
For each patient various de-identified clinical and demographic data will be collected by trained research staff at each participating centre and entered onto a data collection form (Figure 3). Each study site will maintain an electronic database for their subjects which will be subsequently consolidated into a single database. The data to be collected includes.

**Demographic data**

- Age
- Gender
- Weight
- Height

**Clinical data**

- Admission diagnosis, allergies
- Illness severity scores [Sequential Organ Failure Assessment (SOFA)on the day of PK

| Sedative and Analgesic Drugs<br><br>**ASAP – ECMO Study**<br><br>*Instructions: Document any boluses during the sampling period. If the sampling is outside the stipulated time points please document the actual time* | | Patient No. |
|---|---|---|
| **BIS** | | **RASS** |
| | | |
| **Context of PK sampling (please tick )** | | |
| **Infusing new drug/drugs?** ☐ | **Ceasing a current drug infusion?** ☐ | |
| **New Drug Name** | **Ceased Drug Name** | |
| | | |
| **Initial Bolus Dose** | **Initial Infusion Rate** | **Rate before ceasing Drug** |
| | | |
| **Boluses during the sampling period:** *(Specify Time)* | **Change in Rate of Infusion:** *(Specify Rate)* | |

| Sampling time points (minutes) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **0** | **15** | **30** | **45** | **0** | **15** | **30** | **45** |
| Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ |
| **60** | **120** | **180** | **240** | **60** | **120** | **180** | **240** |
| Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ | Actual Time:<br><br>__ __:__ __ |

**Figure 2 Sampling schedule for sedative and analgesic drugs.** BIS- bispectral index, RASS- Richmond Agitation Sedation Scale.

sampling & Acute Physiology and Chronic Health Evaluation (APACHE III) on admission]
- Use of renal replacement therapy
- Sedation scores (RASS, BIS)

**Organ function data**

- Serum bilurubin, total protein and albumin concentrations
- Serum creatinine concentrations
- 8-hour urinary creatinine clearance
- 24 h fluid balance and blood product requirements

**ECMO data**

- Days of ECMO
- ECMO flows during sampling period
- Type of oxygenator and pump

**Study drug dosing data**

- Dose, time and frequency
- Time of sampling
- Day of therapy

| | | | | | Patient No. |
|---|---|---|---|---|---|
| **Data collection form**<br><br>**ASAP – ECMO  Study** | | | | | |
| Age | Sex   M / F | Weight (kg) | | Height (cm) | |
| ICU Admission Diagnosis | | APACHE III | | SOFA* | |
| Days/hours on ECMO | | | | | |
| ECMO flow rate | | | | | |
| Type of ECMO | | ☐ VA          ☐ VV          ☐ Other | | | |
| Pump | | ☐ Jostra RotaFlow          ☐ Cardiohelp<br><br>☐ Levotronix Centrimag | | | |
| Oxygenator | | ☐ Quadrox<br><br>☐ Other (please specify) _____ | | | |
| **During pharmacokinetic sampling** | | | | | |
| Serum bilurubin (µmol/L): | | RRT:   Yes / No<br>**If YES,** please specify mode and flows below. | | | |
| Serum creatinine (µmol/L): | | CVVH | CVVHDF | | SCUF |
| Serum Albumin (g/L): | | EDD | IHD | | OTHER |
| Total proteins (g/L) | | Effluent flow rate (ml/h): | | | |
| | | Blood flow rate (ml/min): | | | |
| Blood urea (mmol/L) | | **If NO,** 8 hour creatinine clearance: | | | |
| Blood product transfusion details: | | 24 h fluid balance: | | | |

**Figure 3 Demographic and clinical data collection form.**

## Specimen processing, storage and distribution

Samples will be centrifuged at 3000 g for 10-minutes to separate plasma. Samples containing sedatives and analgesics will be stored locally at −20°C. Samples containing antibiotics will be stored locally at −80 °C. Labels and cryovials will be provided by the central laboratory to the participating sites. Dichlorvas (an inhibitor of plasma esterase activity) will be added to the tubes dedicated to oseltamivir and oseltamivir carboxylate assays. Fluoride oxalate tubes can be used as an alternative.

All samples will then be batched together for transport to the Burns Trauma and Critical Care Research Centre and the Centre for Integrated Preclinical Drug Development at The University of Queensland, Brisbane, Australia. The distribution of samples to the central laboratory will be handled by a commercial clinical trials courier company.

### Pharmacokinetic sample analysis

To reduce the sample burden per patient, validated bioanalytical methods have been developed to quantify multiple drugs and their metabolites selectively and sensitively in small volumes of plasma. A fully automated on-line solid phase extraction (SPE) system (Symbiosis, SPARK Holland) combined with liquid chromatography-mass spectrometry (LC-MS/MS API 5000) to simultaneously quantify morphine, morphine 3-β-D-glucuronide, morphine 6-β-D-glucuronide, midazolam, 1- hydroxymidazolam, 4-hydroxymidazolam, fentanyl and nor-fentanyl in samples of human plasma has been developed [28]. The technique will also be expanded to analyse propofol, thiopentone and dexmedetomidine. Antibiotic concentrations in the collected plasma samples will be determined by separate validated chromatographic assay (HPLC and LC-MS/MS) methods. All samples will be assayed alongside calibration standards and quality control samples, and met the acceptance criteria.

### Statistical and pharmacokinetic analysis

This study aims to explain the variability of PK parameters between patients. Previous experience has shown that 8–12 patients are sufficient for to meet this objective [29]. Plots of drug doses over time for each patient will be used to visually identify trends and outliers. We will perform population PK modelling for each study drug and metabolite of interest using a non-linear mixed effects modelling approach (NONMEM®; Version 6.1, GloboMax LLC, Hanover, MD, USA) as previously described [30-32]. The residuals of the models will be checked to verify model adequacy and influential observations and subjects will be identified. The models will be used to create plots of the predicted mean doses over time. The models will also be used to determine if significant associations exist between the demographic or clinical variables and the pharmacokinetics. Any variables found to have a significant effect on the pharmacokinetics of the drug, will be incorporated into the final pharmacokinetic model. For example, if age is shown to be an important predictor of dose, then we will plot the predicted doses by age group. After developing and testing these PK models, we aim to perform Monte Carlo dosing simulations, which can then form the basis for dosing guidelines for antibiotics and sedative use in patients on ECMO.

### Ethical considerations

Multi-site ethics approval has been obtained (HREC/11/QPCH/121) for the 5 sites in Australia and Lower South Regional Ethics Committee approval for the site in New Zealand (LRS/12/06/020).

### Collaborating organisations

This multi-centre study is co-ordinated by The Critical Care Research Group at The Prince Charles Hospital in Brisbane, Australia. This group will collaborate closely with The Burns Trauma and Critical Care Research Centre, and The Centre for Integrated Preclinical Drug Development, The University of Queensland in Brisbane for antibiotic and sedative drug assays.

### Sample size and power

We estimate that a minimum of 12 patients for each study drug will be sufficient for population PK analysis. A minimum of 12 patients per antibiotic is based on data from previous non-interventional PK studies in critically ill patients [29-31].

### Discussion

This study will identify the sedative and antibiotic drugs whose PK are most influenced by the presence of by ECMO. It will also inform the development of strategies for drug administration using PK and pharmacodynamic principles in critically ill patients receiving ECMO. A lack of understanding of the impact of ECMO on drug Vd and CL may increase the likelihood of therapeutic failure or drug toxicity [33-35]. PK modelling is crucial to drug safety. This study aims to provide the key information for development of evidence-based dosing schedules and sedation protocols for use by clinicians caring for patients receiving ECMO. This study will be complimented by PK studies in the simulated ECMO circuits and ovine models of ECMO [36] which are currently being conducted by the same group. Using the correct sedative agent at an appropriate dose will minimise ICU morbidity, thereby improving patient outcomes [37]. Similarly, the right dose of the right antibiotic [38,39] will not only improve microbiological and clinical cure rates in an individual patient, but may also reduce the emergence of multi-resistant organisms.

### Abbreviations

ECMO: Extracorporeal membrane oxygenation; ICU: Intensive care unit; PK: Pharmacokinetics; Vd: Volume of distribution; CL: Clearance; RASS: Richmond Agitation Sedation Scale; BIS: Bispectral index; SOFA: Sequential Organ Failure Assessment; APACHE: Acute Physiology and Chronic Health Evaluation; HPLC: High performance liquid chromatography; LC-MS/MS: Liquid chromatography tandem mass spectrometry; RRT: Renal replacement therapy.

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# CHAPTER 5

## DRUG AND CIRCUIT FACTORS

## 5.1 SEQUESTRATION OF DRUGS IN THE CIRCUIT MAY LEAD TO THERAPEUTIC FAILURE DURING EXTRACORPOREAL MEMBRANE OXYGENATION.

### 5.1.1 Introduction to this publication

This chapter, for the first time reported significant antibiotic and sedative drug losses in ECMO circuit components used in adult patients. The circuits were primed with fresh whole human blood and this experiment was used to study drug and circuit interactions in the absence of a critically ill patient. Also, by performing drug stability testing in controls, this experiment overcame the short comings of previous neonatal experiments. This experiment also identified lipophilicty as a key factor in determining circuit sequestration of antibiotic and sedative drugs. Although this was previously reported in neonatal studies that used older generation circuits, there were no reports on drug disposition in new generation circuits at the time of this publication. This study also helped the choice of study drugs for the ovine experiments.

CRITICAL CARE

## RESEARCH

# Sequestration of drugs in the circuit may lead to therapeutic failure during extracorporeal membrane oxygenation

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## Abstract

**Introduction:** Extracorporeal membrane oxygenation (ECMO) is a supportive therapy, with its success dependent on effective drug therapy that reverses the pathology and/or normalizes physiology. However, the circuit that sustains life can also sequester life-saving drugs, thereby compromising the role of ECMO as a temporary support device. This *ex vivo* study was designed to determine the degree of sequestration of commonly used antibiotics, sedatives and analgesics in ECMO circuits.

**Methods:** Four identical ECMO circuits were set up as per the standard protocol for adult patients on ECMO. The circuits were primed with crystalloid and albumin, followed by fresh human whole blood, and were maintained at a physiological pH and temperature for 24 hours. After baseline sampling, fentanyl, morphine, midazolam, meropenem and vancomycin were injected into the circuit at therapeutic concentrations. Equivalent doses of these drugs were also injected into four polyvinylchloride jars containing fresh human whole blood for drug stability testing. Serial blood samples were collected from the ECMO circuits and the controls over 24 hours and the concentrations of the study drugs were quantified using validated assays.

**Results:** Four hundred samples were analyzed. All study drugs, except meropenem, were chemically stable. The average drug recoveries from the ECMO circuits and the controls at 24 hours relative to baseline, respectively, were fentanyl 3% and 82%, morphine 103% and 97%, midazolam 13% and 100%, meropenem 20% and 42%, vancomycin 90% and 99%. There was a significant loss of fentanyl ($p = 0.0005$), midazolam ($p = 0.01$) and meropenem ($p = 0.006$) in the ECMO circuit at 24 hours. There was no significant circuit loss of vancomycin at 24 hours ($p = 0.26$).

**Conclusions:** Sequestration of drugs in the circuit has implications on both the choice and dosing of some drugs prescribed during ECMO. Sequestration of lipophilic drugs such as fentanyl and midazolam appears significant and may in part explain the increased dosing requirements of these drugs during ECMO. Meropenem sequestration is also problematic and these data support a more frequent administration during ECMO.

## Introduction

Extracorporeal membrane oxygenation (ECMO) is increasingly being used in adult patients with cardiac or respiratory failure refractory to conventional therapy or with both. ECMO can be an effective bridge to recovery, clinical decision-making, long-term mechanical cardiac

support, and, less commonly, heart/lung transplantation [1]. Patients on ECMO receive multiple drugs that include sedatives and analgesics, antibiotics, anticoagulants, and vasoactive agents. The success of ECMO may rely on the successful use of these therapies. Although sedatives and vasoactive agents can be titrated to effect clinically, there are no reliable clinical markers to guide antibiotic therapy in critically ill patients. Antibiotics are commonly prescribed in patients on ECMO, and suboptimal therapy may result in therapeutic failure [2-5],

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adversely affecting patient outcomes. Despite the available endpoints for titration of sedation and analgesia in the intensive care unit [6] and efforts to minimize sedative drug use in this group [7], studies have reported escalating sedative doses over time in patients on ECMO [8-10].

There are limited data to guide drug therapy in adult patients receiving ECMO. Data from neonatal circuit experiments reveal significant sequestration of drugs in the ECMO circuit [11,12], and the extent of loss depends upon their physicochemical properties, type and age of the circuit, and the pumps used [10,13]. Pharmacokinetic (PK) studies in neonates [11,12] have consistently demonstrated increased volume of distribution (Vd) and decreased drug clearance (CL) during ECMO. Sequestration of drugs in the circuit appears to add to the increased Vd along with other factors related to critical illness, such as third spacing [11,14]. These studies in neonates highlight the important issue of altered PK during ECMO, but further extrapolation of the neonatal data to adult intensive care practice may not be relevant given the developmental and physiological differences between the two populations [15]. Systematic research in this area by using contemporary circuitry is required to develop evidence-based dosing guidelines for antibiotic therapy in adult patients receiving ECMO. The aim of this study was to describe the disposition of the analgesics fentanyl and morphine, the sedative agent midazolam, and the antibiotics meropenem and vancomycin in an *ex vivo* ECMO circuit model.

## Materials and methods
Ethics approval was obtained from the local Human Research Ethics Committee (HREC/12/QPCH/90).

### Extracorporeal membrane oxygenation circuits
The methods for the development of our *ex vivo* model of ECMO have been published previously [16]. Four permanent life support (PLS) ECMO circuits (Maquet Cardiopulmonary AG, Rastatt, Germany) were used. Each circuit consisted of Bioline tubing™, a PLS Quadrox D oxygenator, and RotaFlow pump head. A reservoir bladder (R-38; Medtronic Pty Ltd, Minneapolis, MN, USA) was added to allow fluid sampling from the closed circuit. The circuits were primed with 900 mL of Plasmalyte P-148 (Baxter Healthcare, Toongabbie, New South Wales, Australia) and then exchanged for 500 mL of Albumex 4 (Human Albumin 40 g/L; CSL Limited, Broadmeadows, Victoria, Australia). Porcine mucous heparin (Pfizer Australia, West Ryde, New South Wales, Australia) was added to the circuits (5,000 U). Fresh whole human blood (less than 5 days old, mean volume of 420 ± 52 mL, provided by Australian Red Cross Blood Service, Melbourne, Victoria, Australia) was used

for the final prime, and the circuits were pressurized to obtain post-oxygenator pressures of 230 to 250 mm Hg.

The final volume of the pressurized circuit was 668 ± 1.7 mL, and the mean hemoglobin value was 64 ± 13 g/L. The mean total protein and albumin concentration in the circuit were 33 ± 2.5 g/L and 25 ± 0.9 g/L, respectively. Activated clotting time was maintained between 220 and 250 seconds. A centrifugal pump (Jostra RotaFlow; Maquet Cardiopulmonary AG) was used to maintain a circuit flow rate of 4 to 5 L/minute. Oxygen tension in the circuit blood was maintained between 150 and 200 mm Hg. Circuit temperature was maintained at 37°C. Carbon dioxide gas or sodium bicarbonate solution was added to the circuit to maintain the pH of the circulating blood in the range of 7.25 to 7.55. Fentanyl (20 μg), morphine (100 μg), midazolam (100 μg), meropenem (10 mg), vancomycin (40 mg), propofol (1 mg), dexmedetomidine (5 μg), thiopentone (20 mg), ceftriaxone (50 mg), linezolid (10 mg), ciprofloxacin (5 mg), fluconazole (10 mg), and caspofungin (5 mg) were injected post-oxygenator as a single bolus. The drugs with known incompatibilities to study drugs (for example, gentamicin and ticarcillin/clavulunate) were excluded. These bolus doses were selected to produce concentrations similar to clinical concentrations. Larger doses were used for the drugs that exhibit high protein binding.

### Controls
Four polyvinylchloride jars with tight caps were filled with 50 mL of fresh human whole blood. Unfractionated heparin (500 U) was added to the jars for anticoagulation. Fentanyl (1.5 μg), morphine (7.5 μg), midazolam (7.5 μg), meropenem (0.75 mg), vancomycin (3 mg), propofol (75 μg), dexmedetomidine (0.375 μg), thiopentone (1.5 mg), ceftriaxone (3.75 mg), linezolid (0.75 mg), ciprofloxacin (0.375 mg), fluconazole (0.75 mg), and caspofungin (0.375 mg) were added to the control jars after collection of baseline blood samples. These amounts were chosen in order to produce study drug concentrations that were similar to those achieved in the ECMO circuit. The jars were then placed in an incubator at 37°C and rocked continuously to ensure even distribution of the drugs.

### Blood sample collection
Post-oxygenator blood was collected into lithium heparin tubes (5 mL) at baseline and at 2, 5, 15, 30, 60, 120, and 360 minutes and at 12 and 24 hours after addition of the drugs to the circuit. Blood samples (5 mL) were also obtained from the control jars at time intervals identical to that of the circuit. All blood samples were stored on ice and centrifuged (10 minutes at 3,000*g*), and the plasma was separated and stored in clean pre-labeled polypropylene cryogenic vials and stored at -80°C until analysis.

## Measurement of drugs in plasma samples

An on-line solid-phase extraction (SPE) Symbiosis Pharma system (Spark Holland, Emmen, The Netherlands) was used to extract the analytes of interest (fentanyl, morphine, and midazolam) and two internal standards (morphine-d3 and 1-hydroxymidazolam-d5) from plasma samples simultaneously [17]. Mass spectrometry in ESI (electrospray ionization) mode (API 5500; AB Sciex, Framingham, MA, USA) triple quadropole system was used as the detector. Liquid chromatography and extraction method were created by Symbiosis Pro for Analyst (version 2.1.0.0) and submitted to the MS controlling software (Analyst 1.5.1). Meropenem and vancomycin concentrations in the collected plasma samples were determined by separate validated chromatographic assay methods. Meropenem and the internal standard (cefotaxime) were detected by ultraviolet absorbance at 304 nm. Vancomycin analysis was by liquid chromatography-tandem mass spectrometry on an Applied Biosystems API2000 (Applied Biosystems, Foster City, CA, USA) with Shimadzu autosampler (Shimadzu Corporation, Kyoto, Japan). Vancomycin and the internal standard (teicoplanin) were detected by positive-mode MRM (multiple reaction monitoring). All samples were assayed alongside calibration standards and quality control samples and met the acceptance criteria.

## Statistical analysis

Linear mixed effects modeling was used to examine the change in concentration over time. This model accounts for the repeated responses from the same circuit by using a random intercept. The mixed effects model was fitted by using the R statistical software [18] version 2.13.2 and the 'lme4' library. The concentration-versus-time curves (mean ± standard error of the mean) were plotted by using GraphPad Prism version 5.03 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

The *ex vivo* circuits were maintained under physiological conditions for 24 hours with no complications during the run. Four hundred samples (80 per drug) were analyzed. The changes in drug concentrations in the *ex vivo* model are summarized in Figures 1 and 2, and the actual drug concentrations determined in plasma samples from the control jars and the ECMO circuits are presented in Table 1. In this paper, only the data for fentanyl, morphine, midazolam, meropenem, and vancomycin are presented. Validated assays are being developed for the remaining study drugs, and the results will be made available in due course.

Testing confirmed that all baseline plasma samples were free of study drugs. There were no statistically significant differences in drug recoveries between the four circuits. The mean drug recoveries from the circuits and the control jars at 24 hours relative to baseline were, respectively, fentanyl 3% and 82%, morphine 103% and 97%, midazolam 13% and 100%, meropenem 20% and 42%, and vancomycin 90% and 99%. Up to 70% of fentanyl and 50% of midazolam were lost in the circuit within the first hour of ECMO run. Fentanyl levels were undetectable in the circuit by 24 hours. This may be related to the lipophilicty of these drugs. Morphine, which is less lipophilic than fentanyl, was stable in both the circuit and the controls. Antibiotics were less significantly affected. The hydrophilic and minimally protein-bound drug meropenem was stable in the circuit and the controls in the first 120 minutes, and 62% of the drug was recovered from the circuit at 6 hours. This was statistically significant ($P = 0.01$) even after accounting for spontaneous degradation (21%). There was no significant loss of the moderately protein-bound hydrophilic drug vancomycin in the circuit at 12 hours ($P = 0.41$) or 24 hours ($P = 0.26$).

## Discussion

This is the first systematic investigation of drug disposition in the adult ECMO circuitry. The findings highlight the role of the circuit in altering the PK of sedative, analgesic, and antibiotic drugs during ECMO and clearly show that there is considerable between-drug variability in the degree of drug sequestration. Drugs that are unstable at physiological temperature (meropenem) and lipophilic drugs (fentanyl and midazolam) were more significantly affected. These findings may have significant implications for both the choice and the dosing of a particular drug prescribed during ECMO. Given the ongoing exteriorization of blood onto the circuit during ECMO, *in vivo* instability of drugs may also play a significant role in apparent PK during ECMO. By excluding the patient factors, this *ex vivo* model provides evidence that the adult ECMO circuit is not simply a benign conduit for blood but actively modulates drug PK.

The circuit factors were identical for all drugs. In this context, it is difficult to determine which of the drug factors contributed to the significant disparity in the degree of drug sequestration in the circuit and *ex vivo* stability. Differences in molecular size and lipophilicity and the differences in protein binding may all have contributed to the findings. This is important as a blanket increase in doses of all antibiotic drugs to avoid under-dosing without identifying the drugs that are most sequestered by the ECMO circuit may potentially result in drug toxicity. Similarly, drug sequestration in the circuit may also explain the increasing sedation requirements seen in patients on ECMO [8,9]. Using sedative and analgesic agents that are highly sequestered in the circuit may necessitate the use of very high doses of these drugs to
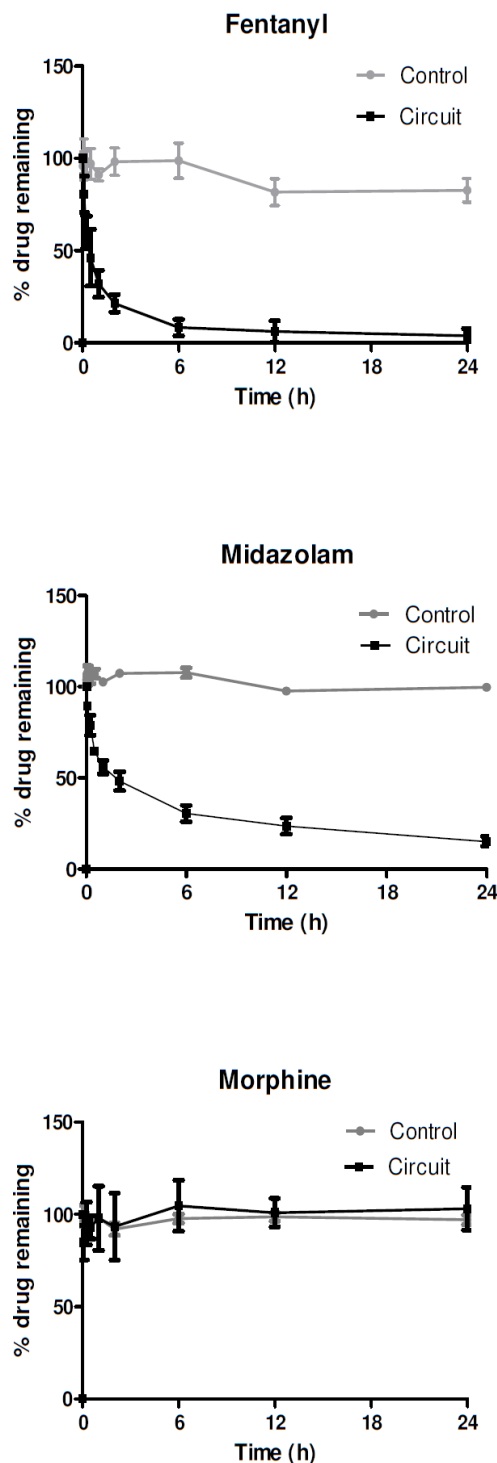
**Figure 1 Percentage of drug remaining in extracorporeal membrane oxygenation circuits and the controls plotted against time**. Lipophilic drugs such as fentanyl and midazolam were significantly sequestered in the circuit despite being stable in the controls. Morphine was relatively stable in both controls and the circuits.

achieve the desired pharmacological effect and may add to the associated morbidity [6]. This calls for further research in this area to improve drug prescription during ECMO.

Given that meropenem and vancomycin both rely on time-dependent bacterial killing, the data presented here on altered antibiotic concentrations may be clinically relevant and require evaluation in a clinical PK study. Meropenem is degraded and sequestered significantly in the circuit beyond 4 to 6 hours. Hence, a more frequent dosing or use of higher doses may be required to maximize the time above minimum inhibitory concentration of the pathogen [19] as demonstrated in a recent clinical study [20]. Furthermore, administration of meropenem by infusion is questionable given the instability issues at room temperatures [21]. The utility of more stable carbapenem antibiotics such as doripenem may have to be explored in future studies. Clinically, there are no data on meropenem PK in patients receiving ECMO. Neonatal studies have uniformly shown an increase in Vd for vancomycin and a lower CL and consequently a longer vancomycin half-life [22,23]. Similarly, PK studies in neonates have shown increased Vd and decreased CL for morphine, midazolam, and their metabolites during ECMO [24,25]. The extent to which these PK alterations during ECMO are related to sequestration of these drugs in the circuit is currently unclear.

Studies in the neonatal ECMO circuits have demonstrated significant sequestration of sedative and antibiotic drugs in the circuit. Also, there is drug sequestration variability based on the different circuits, oxygenators, and pumps used [13]. A recent *in vitro* study [13] reported meropenem and vancomycin recovery of 89% and 67% at 180 minutes in neonatal circuits that used a centrifugal pump and polypropylene hollow-fiber membrane oxygenators. Whereas the meropenem recovery at 180 minutes is comparable to our results, vancomycin recovery was much lower in the neonatal circuits. In contrast, the fentanyl and midazolam circuit losses seen in this study are consistent with the results of the neonatal circuit studies [13,26-28]. Morphine appears to be relatively stable in both neonatal and adult ECMO circuits and may be the preferred analgesic during ECMO. Future clinical studies should compare the efficacy of different classes of drugs to rationalize sedation and analgesia during ECMO.

Studies that compare drug losses in clinically used versus new neonatal circuits have demonstrated significant variability in drug sequestration between the used and new circuits [10,13,28]. Consequently, it is still unclear whether there is saturation of the drug-binding sites in the ECMO circuit over time. Similarly, the effect

**Figure 2 Percentage of meropenem and vancomycin remaining in extracorporeal membrane oxygenation circuits and the controls plotted against time**. Meropenem was unstable in the controls; however, the extracorporeal membrane oxygenation circuit losses significantly exceeded the levels of degradation seen in the controls at 6 hours. Vancomycin was stable in both controls and the circuits.

of priming with various solutions on drug sequestration is not well characterized in the available literature. Drug sequestration in blood-primed circuits has been shown to be much lower than that in crystalloid-primed circuits [27]. It is possible that some of the blood components may compete with drugs for circuit-binding sites. Even though ECMO circuits are not routinely primed with blood prior to their use in adult patients, the circuits get primed with the patient's own blood once ECMO is commenced.

In our study, we tried to replicate the clinical situation *ex vivo* which allowed us to study the interactions between the drug and the device in the absence of disease-related factors which independently can significantly alter PK [29,30]. Repeat dose experiments are required in long-term model systems to estimate the degree of circuit

saturation with time. The concurrent presence of several other physically compatible study drugs in the circuit and control jars mimicked the clinical scenario in which patients receive these drugs concurrently, but the drugs may have had an impact on competitive binding to blood proteins or the circuit components. The presence of a reservoir bladder may have influenced the circuit drug losses. Similarly, quantification of drug lost in control jars because of binding of drugs to the polypropylene container was not feasible. However, the results confirm the findings of neonatal ECMO circuit studies.

## Conclusions

This *ex vivo* study highlights the role of the ECMO circuit in altering PK during ECMO. These alterations are more pronounced for lipophilic drugs and may result in

**Table 1 Measured study drug concentrations in the control jars and the extracorporeal membrane oxygenation circuits at 5 minutes and at 1, 6, 12, and 24 hours**

| Drug, units of concentration | 5 minutes | | 1 hour | | 6 hours | | 12 hours | | 24 hours | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | E | C | E | C | E | C | E | C | E |
| Fentanyl, ng/mL | | | | | | | | | | |
| Median | 27 | 1 | 25 | 0.4 | 26 | 0.1 | 22 | 0.1 | 22 | 0 |
| Range | 26-29 | 1-1 | 24-25 | 0.2-0.5 | 26-28 | 0-0.2 | 20-24 | 0-0.2 | 20-24 | 0-0.1 |
| Morphine, ng/mL | | | | | | | | | | |
| Median | 136 | 7 | 139 | 8 | 137 | 8 | 139 | 8 | 135 | 8 |
| Range | 134-140 | 6-7 | 132-141 | 7-9 | 134-140 | 8-9 | 135-143 | 8-8 | 134-141 | 7-9 |
| Midazolam, ng/mL | | | | | | | | | | |
| Median | 134 | 175 | 131 | 117 | 138 | 59 | 124 | 42 | 128 | 31 |
| Range | 126-155 | 144-356 | 124-137 | 109-261 | 127-147 | 54-124 | 117-132 | 41- 96 | 120-131 | 30-61 |
| Vancomycin, μg/mL | | | | | | | | | | |
| Median | 71 | 73 | 70 | 73 | 72 | 71 | 72 | 71 | 69 | 67 |
| Range | 66-80 | 67-83 | 69-76 | 70-86 | 67-76 | 69-74 | 68-74 | 68-71 | 67-70 | 64-70 |
| Meropenem, μg/mL | | | | | | | | | | |
| Median | 18 | 15 | 17 | 14 | 14 | 10 | 11 | 6 | 7 | 3 |
| Range | 17-18 | 11-16 | 16-18 | 10-15 | 13-15 | 7-11 | 11-11 | 4-8 | 7-8 | 2-5 |

C, control jars; E, extracorporeal membrane oxygenation circuits.

therapeutic failure. Morphine may be a useful alternative to fentanyl in a patient with escalating sedative and analgesic drug requirements. Less lipophilic sedative agents may have to be considered in patients receiving unusually high doses of midazolam. Given the instability issues and circuit sequestration, meropenem may have to be dosed more frequently or in higher doses pending future clinical PK studies. Vancomycin is less significantly affected, and therapeutic drug monitoring as currently practiced can guide optimal treatment. PK studies in adult patients on ECMO are indicated for future research in order to generate the data to guide antibiotic, sedative, and analgesic therapy during ECMO.

## Key messages

- Lipophilic drugs appear to be more significantly sequestered in the ECMO circuit, although further study with different lipophilic drugs is required to confirm this observation.
- Fentanyl and midazolam are more significantly sequestered than morphine.
- Meropenem may have to be administered more frequently during ECMO.
- Physical instability of meropenem may affect its delivery by a continuous infusion.
- Sequestration of drugs in the circuit may have implications on both the choice and dosing of a particular drug prescribed during ECMO.

## Abbreviations

CL: clearance; ECMO: extracorporeal membrane oxygenation; PK: pharmacokinetics; PLS: permanent life support; Vd: volume of distribution.

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## Authors' contributions

KS designed and coordinated the study, collected and analyzed data, and developed the manuscript for publication. CIM provided technical assistance in setting up the circuits. SF helped with procuring study drugs and dispensing them. AGB helped with statistical analysis. SG assayed study drugs midazolam, morphine, and fentanyl. SCW assisted with the control experiments and antibiotic drug assays. MTS and JAR helped with study design, data analysis, and manuscript preparation. DVM, YLF, and JFF assisted with study design, resources, and manuscript preparation. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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## 5.2 PROTEIN-BOUND DRUGS ARE PRONE TO SEQUESTRATION IN THE EXTRACORPOREAL MEMBRANE OXYGENATION CIRCUIT: RESULTS FROM AN *EX VIVO* STUDY.

This experiment highlighted the importance of drug protein binding on drug disposition in ECMO circuits and also identified that drug factors lipophilicity and protein binding can have an additive effect with the drugs that are highly lipophilic and protein bound demonstrating the greatest loss in ex vivo ECMO circuits.

**CRITICAL CARE**

# Protein-bound drugs are prone to sequestration in the extracorporeal membrane oxygenation circuit: results from an *ex vivo* study

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## Abstract

**Introduction:** Vital drugs may be degraded or sequestered in extracorporeal membrane oxygenation (ECMO) circuits, with lipophilic drugs considered to be particularly vulnerable. However, the circuit effects on protein-bound drugs have not been fully elucidated. The aim of this experimental study was to investigate the influence of plasma protein binding on drug disposition in *ex vivo* ECMO circuits.

**Methods:** Four identical ECMO circuits comprising centrifugal pumps and polymethylpentene oxygenators and were used. The circuits were primed with crystalloid, albumin and fresh human whole blood and maintained at a physiological pH and temperature for 24 hours. After baseline sampling, known quantities of study drugs (ceftriaxone, ciprofloxacin, linezolid, fluconazole, caspofungin and thiopentone) were injected into the circuit to achieve therapeutic concentrations. Equivalent doses of these drugs were also injected into four polypropylene jars containing fresh human whole blood for drug stability testing. Serial blood samples were collected from the controls and the ECMO circuits over 24 hours, and the concentrations of the study drugs were quantified using validated chromatographic assays. A regression model was constructed to examine the relationship between circuit drug recovery as the dependent variable and protein binding and partition coefficient (a measure of lipophilicity) as explanatory variables.

**Results:** Four hundred eighty samples were analysed. There was no significant loss of any study drugs in the controls over 24 hours. The average drug recoveries from the ECMO circuits at 24 hours were as follows: ciprofloxacin 96%, linezolid 91%, fluconazole 91%, ceftriaxone 80%, caspofungin 56% and thiopentone 12%. There was a significant reduction of ceftriaxone ($P = 0.01$), caspofungin ($P = 0.01$) and thiopentone ($P = 0.008$) concentrations in the ECMO circuit at 24 hours. Both protein binding and partition coefficient were highly significant, with the model possessing a high coefficient of determination ($R^2 = 0.88$, $P < 0.001$).

**Conclusions:** Recovery of the highly protein-bound drugs ceftriaxone, caspofungin and thiopentone was significantly lower in the ECMO circuits at 24 hours. For drugs with similar lipophilicity, the extent of protein binding may determine circuit drug loss. Future clinical population pharmacokinetic studies should initially be focused on drugs with greater lipophilicity and protein binding, and therapeutic drug monitoring should be strongly considered with the use of such drugs.

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## Introduction

Extracorporeal membrane oxygenation (ECMO) is establishing itself as a viable ultimate support therapy for patients with severe cardiorespiratory failure resulting from a variety of clinical conditions, and its scope continues to expand [1-3]. Patients on ECMO receive multiple drugs in an attempt to either reverse the underlying pathology or to minimise and/or treat complications. In venovenous ECMO, a high proportion of the native cardiac output is required to pass through the oxygenator to achieve adequate systemic oxygenation. In venoarterial ECMO, the ECMO circuit flows may exceed native cardiac output [4]. This transit of blood through the extracorporeal circuit may result in degradation and/or sequestration of circulating compounds, including administered drugs [5].

In addition, ECMO is associated with significant pharmacokinetic (PK) alterations [6], most important of which is an increased volume of distribution ($V_D$) and decreased drug clearance (CL). From a PK point of view, the addition of an extracorporeal circuit that can sequester and/or degrade drugs during transit, as well as modulate their $V_D$ and CL, which presents a significant challenge. The drug, device and disease factors affecting PK during ECMO are very difficult to characterise in a critically unwell patient, and, as such, laboratory-based research that mimics the clinical scenario [7] should be used to fully understand the complex mechanisms behind the PK alterations.

Drug factors such as protein binding and lipophilicity play a key role in their absorption, distribution, metabolism and excretion. Drugs are transported partly as unbound drug and partly reversibly bound to blood components such as plasma proteins and blood cells. The unbound drug then diffuses to extravascular or tissue sites, where the pharmacologic effects are observed. The dynamic relationship between unbound drug concentrations in the blood and tissue sites determines the overall efficacy of the drug. The distribution of the drug and its tissue penetration are determined mainly by the extent of protein binding, degree of ionization, and lipophilicity [8]. Lipophilicity is the main determinant of a drug's permeability. It is traditionally assessed by measuring the drug distribution between immiscible phases of *n*-octanol and water. The ratio of a drug's concentration in *n*-octanol and water is referred to as the *partition coefficient* (*P*), the logarithm of which (log *P*) is commonly used to describe the lipophilicity of therapeutic drugs [9].

ECMO circuits, by binding both circulating proteins and the drugs, can therefore significantly influence the PK of administered drugs in critically ill patients. However, most available data on disposition of drugs in ECMO circuits are derived from neonatal studies that have used older generation of ECMO circuits [6]. Data from these studies reveal significant sequestration of drugs in the ECMO circuit [6,10], with the extent of loss dependent upon their physicochemical properties, type and age of the circuit and the pumps used [11,12]. There are limited data from studies based on circuits used in adult patients. Even though lipophilicity of drugs is widely believed to be a major drug-related factor for circuit sequestration [5,11], the implication of protein binding on circuit disposition has not been fully elucidated.

To address this, we undertook drug disposition studies in contemporary ECMO circuitry in adult patients using an *ex vivo* model of ECMO. We hypothesised that lipophilic and protein-bound drugs are more prone to sequestration in ECMO circuits. We have previously reported results for five drugs (meropenem, vancomycin, fentanyl, midazolam and morphine [5]), which highlighted the role of drug stability and lipophilicity in determining circuit drug loss. In this article, we present results for another six study drugs (ceftriaxone, ciprofloxacin, linezolid, fluconazole, caspofungin and thiopentone) and describe the influence of protein binding on drug disposition in ECMO circuits.

## Materials and methods

Ethical approval was obtained from the Research, Ethics and Governance Unit, The Prince Charles Hospital, Metro North Hospital & Health Service, Brisbane, Australia (HREC/12/QPCH/90). Informed consent was not relevant, as no human subjects were enrolled in this study. The methods have been published previously [5,13], and therefore only a brief overview of the methods is presented here.

### Extracorporeal membrane oxygenation circuits

Four pulse life support (PLS) ECMO circuits were used. They consisted of Bioline tubing (Netafim, Fresno, CA, USA), a QUADROX D oxygenator and RotaFlow pump head (MAQUET Cardiopulmonary, Hirrlingen, Germany). A bladder reservoir (R-38; Medtronic, Minneapolis, MN, USA) was added to provide compliance to the circuit and allow multiple fluid sampling from the closed circuit. The circuits were primed with Plasma-Lyte P-148 (Baxter Healthcare, Toongabbie, Australia) and then exchanged with ALBUMEX 4 (human albumin 40 g/L; CSL, Parkville, Australia) and fresh human whole blood (less than 5 days old, mean volume $420 \pm 52$ ml, provided by the Australian Red Cross Blood Service). Porcine mucous heparin (Pfizer Australia, West Ryde, Australia) was added to the circuits (5,000 U).

The final volume of the pressurised circuit was $668 \pm 1.7$ ml. Activated clotting time was maintained between 220 and 250 seconds. The circuit flow rate, oxygen tension and temperature were kept between 4 and 5 L/min, between 150 and 200 mmHg and at 37°C, respectively, to maintain the pH of the circulating blood in the range of 7.20 to 7.55 by addition of carbon dioxide gas to the

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circuit or by modulating fresh gas flows. Fentanyl (20 µg), morphine (100 µg), midazolam (100 µg), meropenem (10 mg), vancomycin (40 mg), propofol (1 mg), dexmedetomidine (5 µg), thiopentone (20 mg), ceftriaxone (50 mg), linezolid (10 mg), ciprofloxacin (5 mg), fluconazole (10 mg) and caspofungin (5 mg) were injected postoxygenator as a single bolus. The drugs with known incompatibilities to study drugs (for example, gentamicin and ticarcillin/clavulanate) were excluded. These bolus doses were selected to produce concentrations similar to clinical concentrations. Larger doses were used for the drugs that exhibit high protein binding.

### Controls

Four polypropylene jars with tight caps were filled with 50 ml of fresh human whole blood, and 500 U of unfractionated heparin were added to the jars for anticoagulation. Fentanyl (1.5 µg), morphine (7.5 µg), midazolam (7.5 µg), meropenem (0.75 mg), vancomycin (3 mg), propofol (75 µg), dexmedetomidine (0.375 µg), thiopentone (1.5 mg), ceftriaxone (3.75 mg), linezolid (0.75 mg), ciprofloxacin (0.375 mg), fluconazole (0.75 mg) and caspofungin (0.375 mg) were added to the control jars after collection of baseline blood samples. These quantities were chosen to produce study drug concentrations similar to those achieved in the ECMO circuit. The jars were then placed in an incubator at 37°C and rocked continuously to ensure even distribution of the drugs.

### Blood sample collection

Postoxygenator blood was collected into lithium heparin tubes (5 ml) at baseline and at 2, 5, 15 and 30 minutes and 1, 2, 6, 12 and 24 hours after addition of the drugs to the circuit. Blood samples (5 ml) were also obtained from the control jars at time intervals identical to those of the circuit. All blood samples were stored on ice prior to centrifugation (10 minutes at 3,000 $g$), and the plasma was separated and stored in clean, prelabelled polypropylene cryogenic vials at −80°C until analysis.

### Measurement of drugs in plasma samples

A robotic online solid-phase extraction (SPE) Symbiosis Pharma system (Spark Holland, Emmen, The Netherlands) was used to extract the thiopental and thiopental-d5 (internal standard) from plasma samples. The SPE was conducted using a HySphere C18 cartridge (Spark Holland), and the analytes eluted from the cartridges were directly transferred to an XTerra MS C18 column (Waters, Milford, MA, USA). Mass spectrometry (MS) in electrospray ionization in negative mode (QTRAP 5500; AB SCIEX, Concord, ON, Canada) was used as the detector. The liquid chromatography and extraction methods used were created by Symbiosis Pro for Analyst (version 2.1.0.0; Spark Holland) and submitted to the MS controlling software (Analyst 1.6).

For antibiotic analysis, plasma sample aliquots (100 µl) were combined with an internal standard before protein precipitation by addition of trichloroacetic acid (ciprofloxacin) or acetonitrile (ceftriaxone, caspofungin, linezolid and fluconazole). Ceftriaxone supernatant was washed with dichloromethane prior to instrumental analysis. Ceftriaxone and ciprofloxacin were analysed on a Prominence high-performance liquid chromatography system (Shimadzu, Kyoto, Japan). Caspofungin, Linezolid and Fluconazole were analysed on a Nexera-8030+ ultra-high-performance liquid chromatography tandem MS (Shimadzu, Kyoto, Japan). All separations were performed by reverse-phase chromatography. Ciprofloxacin was detected by fluorescence, ceftriaxone by ultraviolet detection, and caspofungin, linezolid and fluconazole by triple-quadrupole MS. All assays were validated and conducted according to the US Food and Drug Administration guidance on bioanalysis [14].

### Statistical analysis

The data consisted of a longitudinal and correlated time series. For each drug in both the control and experimental (circuit) assays, the drug assayers were assumed to be independent of one another. For continuous data, normality was checked using a Shapiro-Wilk test. Non-normally distributed data were transformed. The data were analysed using a time series in a generalized linear model with a normal link function. The results are reported as the mean ± standard deviation (SD) for normally distributed data, median (interquartile range) for non-normal or categorical data and the proportion—either fractional or as a percentage—for binary data. For the purposes of analysis, all drug levels were referenced to the concentration of that drug at zero hours (baseline) and are reported as the percentage change from that baseline.

An ordinary (least-squares) regression model was constructed to examine the relationship between the two explanatory variables, protein-bound fraction ($F_B$) and log partition coefficient (log $P$) and the outcome variable, fraction of drug remaining at 24 hours ($F_{C24}$). Model coefficients and their 95% confidence intervals are reported. The final model was also tested for fit to the data (adjusted $R^2$), and its residuals were examined for normality and homoscedasticity. Throughout, the level of significance was set at $P <0.05$. STATA™ software (version 12.0; StataCorp, College Station, TX, USA) was used for all analyses.

### Results

The *ex vivo* circuits were maintained under physiological conditions for 24 hours without complications. The mean (SD) total protein and albumin concentrations in the circuits were 33 (2.5) g/L and 25 (0.9) g/L, respectively. The measured mean (SD) pH in the individual

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circuits over the 24-hour period were 7.20 (0.4), 7.33 (0.15), 7.39 (0.3) and 7.26 (0.14). A total of 480 samples (80 per drug) were analysed. The changes in drug concentrations relative to the baseline over time are summarised in Figure 1. Testing confirmed that all baseline plasma samples (prior to study drug injection into the circuit) were free of study drugs. There were no statistically significant differences in individual study drug recoveries between the four circuits or controls. The mean drug recoveries from the circuits and the control jars at 24 hours relative to baseline were, respectively, 80% and 102% for ceftriaxone, 96% and 119% for ciprofloxacin, 91% and 102% for linezolid, 91% and 102% for fluconazole, 56% and 99% for caspofungin, and 12% and 102% for thiopentone. The reduction in ceftriaxone ($P = 0.01$), caspofungin ($P = 0.01$) and thiopentone ($P = 0.008$) concentrations in the ECMO circuit at 24 hours were all significant. Although there was some variability in pH between circuits, there was no significant independent effect of pH on individual drug disposition in the circuits.

Drug physicochemical data were obtained from the DrugBank online database [15] and then correlated with circuit drug behaviour. The relationships of study drug lipophilicity and protein binding with circuit drug concentrations are summarised in Table 1 and Figure 2. A linear regression model was constructed to examine the association between log $P$ and the $F_B$ in the prediction of $F_{C24}$. In this model, both predictors were highly significant, with model diagnostics revealing normally distributed, homoscedastic residuals. The following model equation was used:

$$F_{C24} = 1.21 - 0.17 \log P - 0.69 F_B (R_2 = 0.88).$$

The confidence intervals and associated *P*-values for the coefficients were as follows:

$$\text{Log } P[-0.17, -0.13], P < 0.001$$

$$F_B[-0.86, -0.52], P < 0.001$$



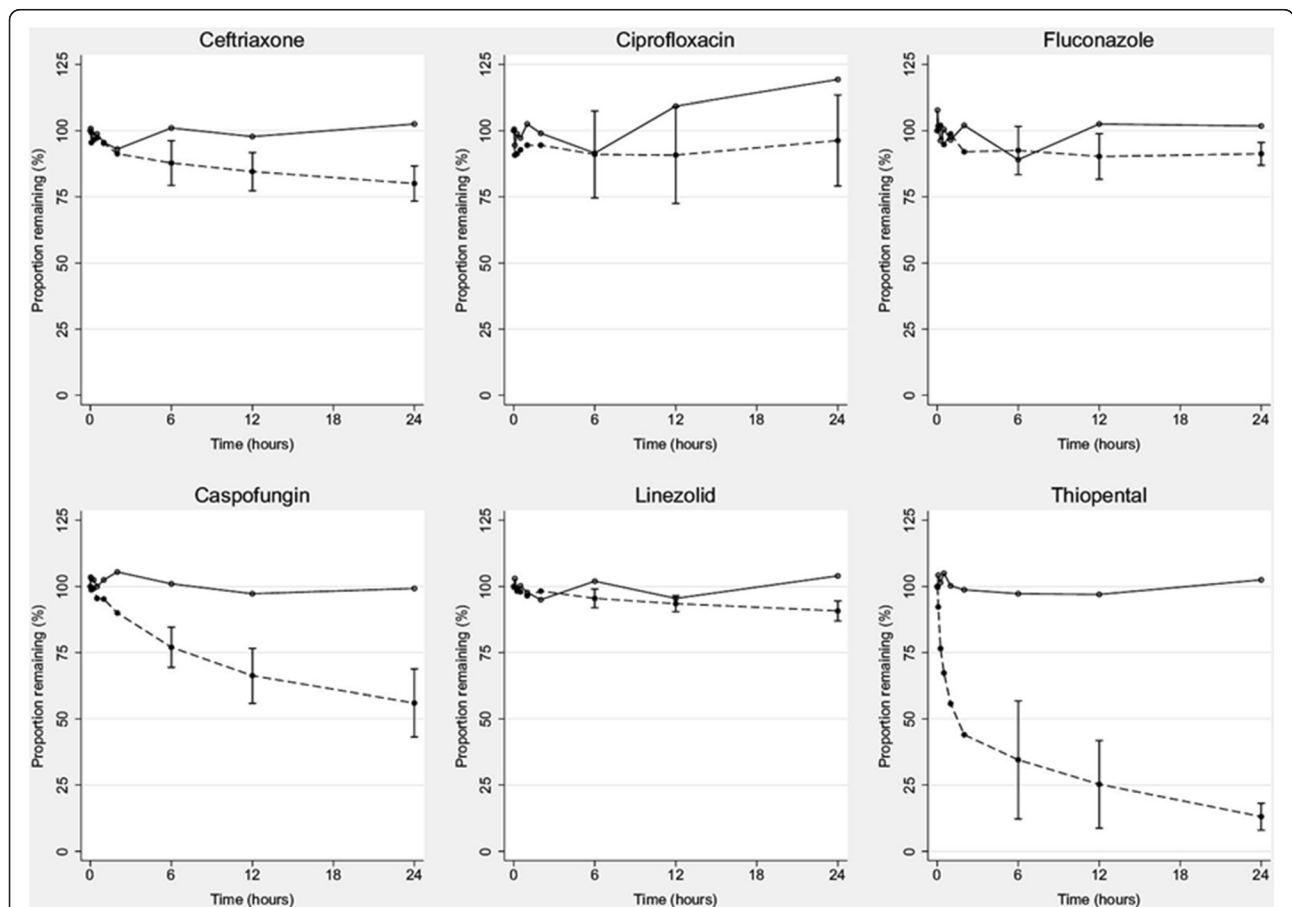**Figure 1 Percentages of drug remaining in *ex vivo* extracorporeal membrane oxygenation circuits and the controls plotted against time.** For clarity, 95% confidence intervals are shown only for the experimental (circuit) group at times 6, 12 and 24 hours. The control group is identified by the continuous lines and open symbols. The circuit group is identified by the dashed lines and solid symbols.

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**Table 1 Drug recoveries in *ex vivo* circuits and controls relative to baseline and their relationship to lipophilicity and protein-binding characteristics[a]**

| Drug | Mean (SD) drug recovery (%) from controls at 24 hr | Mean (SD) drug recovery (%) from circuits at 24 hr | Lipophilicity (log *P*) | Protein binding (%) |
|---|---|---|---|---|
| Ciprofloxacin | 119 (4) | 96 (17) | 2.3 | 20 to 40 |
| Fluconazole | 102 (1) | 91 (4) | 0.4 | 12 |
| Linezolid | 102 (4) | 91 (4) | 0.9 | 31 |
| Ceftriaxone | 102 (1) | 80 (6) | −1.7 | 95 |
| Caspofungin | 99 (8) | 56 (13) | 0.1 | 97 |
| Thiopentone | 102 (8) | 12 (5) | 2.3 | 80 |
| Fentanyl* | 82 (6.3) | 3 (3.8) | 3.9 | 85 |
| Midazolam* | 100 (3.6) | 13 (2) | 3.9 | 92 |
| Meropenem* | 42 (1.5) | 20 (7) | −0.6 | 2 |
| Vancomycin* | 98 (9) | 91 (11) | −3.1 | 55 |
| Morphine* | 103 (11) | 97 (2.6) | 0.8 | 30 |

[a]Log *P*, Log; SD, Standard deviation. Drugs that are significantly lost in the extracorporeal membrane oxygenation circuit are highlighted. Asterisks indicate previously published data from the same experiment [5].

## Discussion

This systematic investigation provides useful insights into the drug factors that influence drug disposition in circuitry currently used in adult patients undergoing ECMO. Based on a representative group of study drugs with diverse lipophilicity and protein-binding characteristics, this study demonstrates the importance of drug factors altering PK during ECMO. More important, the results indicate that for a given degree of lipophilicity, the extent of protein binding may determine circuit drug disposition. This is highly relevant, as the PK of highly protein-bound drugs are significantly affected during critical illness [16,17], and therapeutic drug monitoring (TDM) for many of these drugs is not routinely available at the current time.

The findings also highlight that there is considerable between-drug variability in the degree of drug sequestration. Drugs with significantly reduced concentrations at 24 hours were either highly protein-bound (>80%), highly lipophilic (log *P* >2.3) or both. As previously reported [5], meropenem (protein binding: 2%, log *P*: −0.6) was the only drug that did not conform to this trend, and its circuit loss can be attributed to its instability at physiological temperature [5,18]. Most other drugs that do not exhibit extremes of protein binding or lipophilicity remained relatively stable in the *ex vivo* ECMO circuit. Thus, drug stability at room temperature and at 37°C is also an important consideration for drugs prescribed during ECMO.

For a given solubility characteristic, the degree of protein binding appeared to be the main determinant of circuit drug concentration. For example, although ciprofloxacin and thiopentone have similar lipophilicity (log *P*: 2.3), greater reductions in 24-hour plasma concentrations were observed for thiopentone (88%), the more protein-bound

drug as compared with ciprofloxacin (4%). For the hydrophilic drugs vancomycin and ceftriaxone (log *P*: −3.1 and −1.7, respectively), protein binding (55 and 83% to 95%, respectively) once again appeared to be the key determinant of circuit drug recovery (91% and 80%, respectively) at 24 hours.

The mean (±SD) total protein and albumin concentrations (33 ± 2.5 g/L and 25 ± 0.9 g/L, respectively) in the *ex vivo* circuit were quite similar to what is encountered in critically unwell patients [19]. As unbound study drug concentrations were not measured, it remains unclear whether protein-bound or -unbound fractions are more susceptible to circuit degradation and/or sequestration. In one study, there was a more significant loss of ampicillin (a relatively hydrophilic and less protein-bound drug) in neonatal *ex vivo* crystalloid-primed circuits [20] than in blood-primed circuits (72% vs. 15% lost at 24 hours). This indicates that the ECMO circuits can bind both proteins and drugs, and it is unclear if there is any competitive binding between them and, if so, whether such a phenomenon is concentration-dependent. Thus, the net circuit loss of a drug may represent a balance between binding to circuit components versus extent of protein binding. In addition, similar to their critically ill counterparts [16], patients receiving ECMO have physiological alterations that may influence protein binding, and a resulting increase in unbound drug fraction may enhance circuit losses [6]. This may, in part, explain the high $V_D$ reported for drugs in patients receiving ECMO.

It is unclear if protein binding and lipophilicity have an additive effect on circuit drug sequestration, as some of the greatest decrements in circuit drug concentrations (>80%) reported at 24 hours [5] relate to drugs that have high degrees of both lipophilicity and protein binding (fentanyl, midazolam and thiopentone). This may be further

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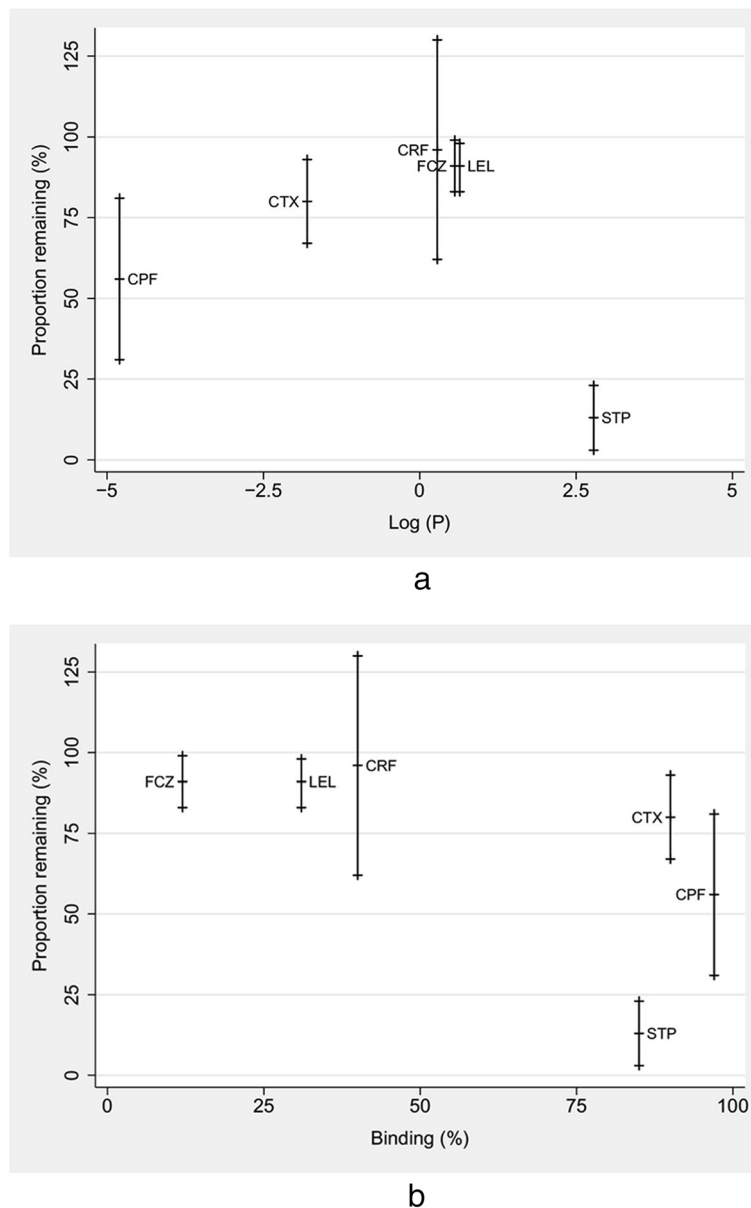


**Figure 2 Recovery of drugs in percent from extracorporeal membrane oxygenation circuit at 24 hours. (a)** Lipophilicity expressed as log partition coefficient (log *P*) values. **(b)** Protein binding expressed as percentage. For each drug, the mean concentration is indicated by a crossbar and the upper and lower 95% confidence intervals are indicated by crosses. FCZ, Fluconazole; LEL, Linezolid; CRF, Ciprofloxacin; STP, Sodium thiopentone; CTX, Ceftriaxone; CPF, Caspofungin.

substantiated by the fact that the less protein-bound drug ciprofloxacin (despite having a lipophilicity similar to that of thiopental) remained relatively stable in the circuit. The mechanisms that independently lead to circuit sequestration of a highly protein-bound drug are currently unclear. In a study using *ex vivo* neonatal circuits [21], up to 80% of the lipophilic and highly protein-bound drug fentanyl was lost in ECMO circuits without oxygenators at 6 hours, and addition of an oxygenator to the circuit only increased the losses by another 6%. It is possible that circuit sites

that bind albumin and other circulating proteins upon priming or after passage of patients' own blood may in turn bind to the administered drugs that exhibit high protein binding. Studies in which researchers have compared drug losses in clinically used versus new neonatal circuits have demonstrated significant variability in drug sequestration between the used and new circuits [11,12,22]. Consequently, it is still unclear if saturation of the drug-binding sites in the ECMO circuit over time occurs. Given that ECMO therapy may continue for many weeks, the time

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taken for saturation of both the protein- and drug-binding sites in the ECMO circuit also remains a subject for future studies. This could potentially be investigated with repeat dose experiments in a similar *ex vivo* model.

Studies in neonatal ECMO circuits have also demonstrated variable sequestration of drugs based on the different circuits, oxygenators and pumps used [11]. Even though these studies clearly identify lipophilicity as a factor for circuit drug sequestration, there are no published experiments that explore the impact of protein binding to the extent described in this study. Wildschut *et al.* [11] reported an 84% recovery for hydrophilic drug cefazolin (protein binding of 84%) at 3 hours in circuits with centrifugal pumps and polypropylene hollow fibre oxygenators. With silicone membrane oxygenators, the drug recoveries observed in blood-primed circuits by Mehta *et al.* for ampicillin, cefazolin and voriconazole were 85%, 79% and 29%, respectively. Although these three drugs exhibit contrasting degrees of lipophilicity (log *P*: −2, −1.5 and 1.0, respectively) and protein binding (25%, 84% and 58%, respectively), it should be noted that the least protein-bound and lipophilic drug of the three drugs—ampicillin—had the best recovery profile at 24 hours, despite its instability issues.

This *ex vivo* study has some limitations. The concurrent presence of several other physically compatible study drugs in the circuit and control jars mimicked the clinical scenario where patients receive these drugs concurrently, but it may have had an impact on competitive binding to blood proteins or the circuit components. Although there was some variability in pH between circuits, there was no significant independent effect of change in pH on individual drug disposition in the circuits, and similar drug loss trends were observed in all circuits. A reservoir bladder was necessary to allow removal of multiple blood samples from the otherwise non-compliant circuit, which may have influenced the circuit drug loss. Similarly, quantification of drug lost in control jars due to binding of drugs to the polypropylene container was not feasible, although this is suspected of being negligible because the surface area of the control experiment was significantly less.

The findings of this study may have significant implications for both the choice and the dosing of an individual drug prescribed during ECMO. Although any drug can be affected, these findings will inform the design of future clinical PK studies [23] that are the next logical step in the evaluation of the impact of the circuit and drug factors on PK in critically unwell patients receiving ECMO and in the development of robust dosing guidelines. Given that most of these drugs are highly relevant for this patient population, TDM, where available, is also strongly recommended, pending clinical PK data.

## Conclusions

This *ex vivo* study highlights the role of the ECMO circuit and drug factors in altering PK during ECMO. In addition to previously identified drug factors such as instability and lipophilicity, this study highlights the influence of protein biding on drug disposition in ECMO circuits. The drugs that are most significantly affected need expedited evaluation in clinical population PK studies and in further mechanistic studies in animal models so that the *in vivo* impact of such circuit drug losses are fully elucidated. Such mechanistic and clinical PK data can then assist the development of meaningful dosing simulations and robust dosing guidelines for the prescription of antibiotic and sedative drugs given during ECMO.

## Key messages

- Drug stability, lipophilicity and protein binding are the three key drug factors that influence drug disposition in ECMO circuits.
- Protein-bound drugs appear to be more significantly sequestered in *ex vivo* ECMO circuits.
- When multiple drugs with similar degrees of protein binding are administered, circuit drug loss is determined by degree of lipophilicity and vice versa.
- Sequestration of drugs in the circuit may have implications on both the choice and dosing of a particular drug prescribed during ECMO.

### Abbreviations
CL: Clearance; ECMO: Extracorporeal membrane oxygenation; $F_B$: Protein-bound fraction; $F_{C24}$: Fraction of the drug remaining in the circuit at 24 hours; Log *P*: Log partition coefficient; PK: Pharmacokinetics; PLS: Pulse life support; SD: Standard deviation; SPE: Solid-phase extension; TDM: Therapeutic drug monitoring; $V_D$: Volume of distribution.

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# CHAPTER 6

# CRITICAL ILLNESS AND CIRCUIT FACTORS

## 6.1 PHARMACOKINETICS OF MIDAZOLAM AND ITS METABOLITES IN HEALTHY AND CRITICALLY ILL OVINE MODELS OF EXTRACORPOREAL MEMBRANE OXYGENATION.

### 6.1.1 Introduction to this submitted manuscript

This chapter describes the results from an *in vivo* ovine experiment that was designed to test the combined effects of ECMO and critical illness on PK. This experiment was critical as it is ethically impossible to study PK in a healthy human volunteer on ECMO. The protein-bound and lipophilic drug midazolam was used to compare PK in healthy and critically ill sheep receiving ECMO with critical illness being the study variable in question. Interestingly, the addition of critical illness had limited additional influence on midazolam PK in this experiment and both healthy and critically ill sheep had significantly increased peripheral Vd after commencement of ECMO. This finding is important as up to 72% midazolam was lost in the circuit during ex vivo experiments and this may have played a significant part in altering PK even in *in vivo* conditions despite the other perturbations to PK that may arise from critical illness itself.

### 6.1.2 Submitted version of manuscript

### Abstract

**Introduction:** Patients on extracorporeal membrane oxygenation (ECMO) often receive sedation at doses much higher than their critically ill counterparts who are not on ECMO. However, the mechanisms behind this are not clear. This study aimed to compare pharmacokinetics of midazolam and its metabolites, 1-hydroxy midazolam and 4-hydroxy midazolam between healthy and critically ill sheep undergoing ECMO.

**Methods:** Twelve healthy sheep were anesthetised and mechanically ventilated. Smoke inhalation acute lung injury (S-ALI) was induced in five sheep after which they were ventilated for 2 hours prior to commencing venovenous ECMO. Similarly, the 7 healthy sheep were ventilated for two hours prior to initiation of venovenous ECMO. In addition to intravenous anaesthetic agents (alfaxalone and ketamine), the sheep also received intravenous midazolam (bolus then continuous infusion) prior to and after commencement of ECMO. Serial blood samples were collected and midazolam and metabolite assays performed using validated chromatographic methods. Pharmacokinetic analysis was undertaken using non-compartmental and population methods.

**Results:** There were no significant differences in average areas under the curve (AUC) $_{0-t}$ for midazolam (17028 vs. 15255 mg.hr/L, p=0.71), 1-hydroxy midazolam (4438 vs. 3168 mg.hr/L, p=0.26) and 4-hydroxy midazolam (1578 vs.1141 mg.hr/L, p=0.20) between healthy and SA-ALI sheep. The $AUC_{0-t}$ ratios for the 1-hydroxy midazolam and 4-hydroxy midazolam relative to the parent compound in healthy and SA-LI sheep were respectively 0.28 vs. 0.23 (p=0.28) and 0.11 vs. 0.11(p=0.44). There was a significant reduction in peripheral volumes of distribution for midazolam in both healthy and S-ALI sheep upon commencement of ECMO [pre ECMO 191 (147 – 306) L vs. 86 (66 – 137) L on commencing ECMO, p=0.015)].

**Conclusion(s):** The significant reduction in peripheral volume of distribution of midazolam seen upon commencing ECMO in both healthy and S-ALI sheep indicates sequestration of midazolam in ECMO circuits. The influence of critical illness itself on midazolam or metabolite pharmacokinetics was not significant and did not affect overall drug/metabolite exposure in S-ALI sheep. Clinical PK studies in ECMO patients are indicated to not only confirm these findings but also to evaluate the pharmacodynamic significance of our findings.

**Key words:** Pharmacodynamics; sedation and analgesia; therapeutic failure; volume of distribution; clearance

**Introduction**

The scope of extracorporeal membrane oxygenation (ECMO) is expanding and it is being increasingly used in adult patients with acute severe cardiorespiratory failure who are unresponsive to conventional treatment[2,56]. The spectrum of available extracorporeal life support (ECLS) techniques[2] are being increasingly applied in tailored fashion to bridge eligible patients to recovery, further mechanical assist device or transplantation. Sedation and analgesia play a vital role in management of these patients. Although, changing paradigms explore the potential for ambulatory ECLS, some patients still need optimal sedation to maintain ECMO flows and oxygenation[57,58]. This is particularly relevant in patients with severe respiratory failure with high cardiac output who rely on relatively high circuit flows to ensure oxygenation[57,58]. Adequate sedation is also necessary to alleviate physical and psychological distress and to prevent accidental circuit related complications such as dislodgement /displacement or obstruction all of which may have catastrophic consequences[11].

Patients on ECMO often have unusually high sedation requirements when compared with their critically ill counterparts[11] and the mechanisms behind this are still unclear. Studies have demonstrated a decrement in sedative drug and metabolite concentrations upon commencement of ECMO[9] and such a phenomenon in adult patients may be largely attributed to circuit sequestration of sedative drugs as the haemodilution that results upon commencement of ECMO is not significant enough to explain the reductions in sedative drug concentrations. Experiments in ECMO circuits have confirmed that sedative drug sequestration can occur even in modern day circuitry and centrifugal pumps[10,59].

Apart from sequestering drugs, the ECMO circuits may also contribute to significant pathophysiological and pharmacokinetic (PK) perturbations[12] in a critically ill patient. Available neonatal data[22] and emerging data in adult patients suggests that these pharmacokinetic changes may be more pronounced in patients on ECMO[11,16,60]. Increased volume of distribution (Vd) and decreased clearance (CL) of various antibiotic drugs during ECMO have been consistently reported in the neonatal literature[22]. These alterations may result from drug sequestration in the circuit[24], physiologic effects of the extracorporeal circuit and from the critical illness itself [60]. However, because these data are from critically ill patients, they probably reflect the combined effects of the ECMO circuit and critical illness on pharmacokinetics. Given that it is not possible to obtain pharmacokinetic data from healthy human subjects on ECMO, studies examining the effect of ECMO circuit on pharmacokinetics in healthy animals are desirable[51] and may further our understanding of the reasons for increased sedation in ECMO patients.

This study was designed to compare the PK of midazolam and its metabolites in healthy and critically ill sheep receiving ECMO.

**Materials and methods**

*Animal preparation:*

Ethics approval was obtained form the local Animal Research Ethics Committee (approval no. 1100000053). Our validated model of ovine ECMO has been published previously[51-53]. In an appropriately equipped theatre, twelve healthy sheep after overnight fasting were anesthetised and mechanically ventilated with a Hamilton Galileo ventilator (Hamilton Medical AG). Haemodynamic monitoring included invasive arterial blood pressure monitoring in the facial artery and continuous measurements of the central venous pressure, mixed venous saturation and cardiac output using a pulmonary artery catheter. Smoke inhalation acute lung injury (S-ALI) was induced in five sheep using a validated technique,[61] after which they were ventilated for another 2 hours prior to commencing venovenous ECMO. Similarly, the 7 healthy sheep were ventilated for 2 hours prior to initiation of venovenous ECMO. A 21 Fr (50 cm) femoral Carmeda Bioactive Surface coated (CBAS®) venous cannula (Medtronic Inc, Minneapolis, MN, USA) was inserted into the right IJV using a Seldinger technique and positioned in the proximal inferior vena cava (IVC). A 19 Fr (50 cm) Carmeda coated femoral venous cannula was used for return blood and also inserted in the right IJV and positioned at the mid right atrium. Intra cardiac echocardiography was used to confirm cannula position. The clamped ECMO circuit was connected to the access and return cannulae. Pump speeds were titrated to target flows at least 2/3$^{rd}$ of pre-ECMO cardiac output (or 60–80 mL/kg). The sheep were maintained on ECMO for 24 hours and then euthanised. Serial blood sampling was performed for midazolam and metabolite assays (at baseline, prior to and after commencement of ECMO) and subsequent PK analysis.

*Drug administration and PK sampling*

In addition to intravenous anaesthetic agents (alfaxalone 4–6 mg/kg/h and ketamine 3–5 mg/kg/h), all the sheep also received intravenous midazolam (0.5mg/kg bolus followed by 0.25 mg/kg/h) prior to and after commencement of ECMO. The animals did not receive any pharmacological paralysis. Blood samples were drawn at the baseline and at 15, 30 45, 60, 90,120 minutes after initiation of midazolam infusion and at 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 720 minutes after commencement of ECMO.

*Midazolam and metabolite assay*

Aliquots of plasma samples (150 µL) was mixed with 150 µL of 1-hydroxymidazolam-d5 solution (50 ng/mL) (Internal standards) and were shaken vigorously for 5 min before

transferring to the 96-well plate. Samples were diluted 3 and 30 times with blank plasma to fit inside the calibration range for all analytes. The on-line Solid phase extraction (SPE) Symbiosis System (SPARK Holland, Netherland) was used to extract all compounds of interest and the internal standards simultaneously from plasma. Mass spectrometer QTrap 5500 (AB Sciex, concord, ON, Canada) was used as the detector. The calibration range for midazolam, 1-hydroxymidazolam and 4-hydroxymidazolam were: 0.5-100, 2.5-500 and 1-200 ng/mL, respectively[54].

### *Statistical analysis*

Statistical analyses were performed using the SPSS 13.0 for Windows NT software package (SPSS Inc. 2004). Discrete variables were expressed as counts (percentage) and continuous variables as means ± SD. Demographics and clinical differences between study-groups were assessed using a chi-square, Fisher's exact test, Student's t-test, or Mann-Whitney U test, as appropriate. A $p < 0.05$ was considered to be statistically significant. We used a mixed model with a random intercept to describe the mean differences in physiology between healthy and SALI over 1 to 24 hours. This included a linear time trend using each sheep's baseline (time zero) as a covariate.

### Pharmacokinetic Analysis

The pharmacokinetics of midazolam, 1-OH-midazolam and 4-OH midazolam were calculated by using both non-compartmental and population methods.

### *Non-compartmental Analysis*

The area under the concentration-time curve from 0 h to the end of the sampling time (t; $AUC_{0-t}$) was calculated using the trapezoidal rule. The formation of the metabolites was described using the ratio of $AUC_{0-t}$ for each metabolite to the corresponding $AUC_{0-t}$ of the parent compound.

### *Population Pharmacokinetics Analysis*

The concentration-time data for midazolam in sheep plasma were fitted using Non-Linear Mixed-Effects Modeling (NONMEM version 7.2, Globomax LLC, Hanover, USA) [30]. A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM (http://wfn.sourceforge.net). Data were analyzed using the first-order conditional estimation method with interaction (ADVAN3). Between subject variability (BSV) was calculated using an exponential variability model and was assumed to follow a log normal distribution. Residual unexplained variability (RUV) was modeled using an exponential error model. Visual inspection of diagnostic scatter plots and the NONMEM objective function value (OFV) were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program on the basis of a $\chi2$ test of the difference in OFV. A

decrease in the OFV of 3.84 units ($p < 0.05$) was considered statistically significant. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model. Specifically, we calculated midazolam CL, central volume of distribution ($V_1$), inter-compartmental clearance ($Q$) and peripheral volume of distribution ($V_2$). The final base model was evaluated by performing a visual predictive check (VPC), and by nonparametric bootstrapping.

## Results

Seven healthy sheep and five S-ALI sheep completed the 24-hour ECMO run without any complications. Baseline demographic and physiologic data from all 12 sheep prior to smoke inhalation and ECMO are presented in Table 1. Data on the mean differences in physiological data between healthy compared with S-ALI sheep are presented in Table 2. Mean arterial pressure, 24 h fluid balance, haemoglobin, pH, lactate, total protein and albumin varied significantly ($p<0.05$) between the two groups.

| | Group 1 (n=7) | Group 2 (n=5) | p-value |
|---|---|---|---|
| Weight (kg) | 48.5 (4.6) | 49.6 (4.6) | |
| Heart rate (bpm) | 112(9) | 118 (13) | 0.44 |
| Mean Arterial BP(mmHg) | 116 (7) | 114 (8) | 0.7 |
| Mean PAP(mmHg) | 24.6 (3) | 21.4 (3.5) | 0.14 |
| CVP(cm $H_2O$) | 15 (2) | 12 (3) | 0.21 |
| CCO (L/min) | 5.6 (0.8) | 5.0 (0.5) | 0.24 |
| SvO2 (%) | 78 (8) | 81 (4) | 0.46 |
| PEEP (cm $H_2O$) | 7.9(2.7) | 7.0(2.7) | 0.6 |
| Respiratory Rate (per min) | 11.0(6.0) | 12(6) | 0.9 |
| pO2(mmHg) | 455(165) | 517 (41) | 0.37 |
| pCO2(mmHg) | 42 (4) | 43 (4) | 0.61 |
| Haemoglobin(g/L) | 7.0 (1.2) | 7.8 (1.8) | 0.47 |
| pH | 7.4 (0.03) | 7.4 (0.05) | 0.98 |
| Temperature($^O$C) | 38.3 (0.6) | 38.2 (0.5) | 0.81 |
| Lactate(mmol/L) | 1.3 (0.45) | 1 (0.27) | 0.3 |
| Albumin (g/L) | 37(3) | 38(0.4) | 0.5 |
| Total Protein (g/L) | 70(6) | 71(2) | 0.95 |
| Blood Urea (mmol/L) | 8.8(2.8) | 7.3(1.7) | 0.3 |
| Serum Creatinine (µmol/L) | 86(15) | 88(12) | 0.87 |
| Serum Bilirubin(µmol/L) | 2.23(0.7) | 1.96 (0.9) | 0.57 |
| Alanine amino transferase (U/L) | 12(2) | 13(1.6) | 0.44 |

Table 1. Baseline demographic and physiology data compared between sheep allocated to healthy ECMO group (Group1,n=7) and or smoke inhalation acute lung injury and ECMO group (Group 2, n=5) prior to commencement venovenous ECMO. BP- blood pressure, PAP-pulmonary artery pressure, CVP-central venous pressure CCO- continuous cardiac

output, Svo2- mixed venous oxygen saturation, PEEP-positive end expiratory pressure, pO2 –partial pressure of oxygen, pCO2-partial pressure of carbon dioxide.

| Variable | Mean | Lower | Upper | p.value |
|---|---|---|---|---|
| Heart rate | 7.48 | −2.58 | 17.53 | 0.205 |
| Mean Arterial BP | −23.15 | −32.04 | −14.23 | 0.001 |
| Mean PAP | 2.22 | −1.61 | 6.05 | 0.315 |
| CVP | 2.30 | −0.69 | 5.30 | 0.191 |
| CCO | −1.22 | −2.31 | −0.12 | 0.077 |
| SvO2 | −2.06 | −6.69 | 2.57 | 0.435 |
| PEEP | −23.55 | −182.66 | 135.53 | 0.792 |
| Respiratory Rate (Sheep) | 608.52 | −377.44 | 1600.11 | 0.285 |
| Tidal Volume | −1350.09 | −2450.88 | −249.36 | 0.05 |
| FiO2 | 4.42 | −111.00 | 119.90 | 0.945 |
| pO2 | 22.65 | −17.46 | 62.77 | 0.328 |
| pCO2 | 1.57 | −1.20 | 4.33 | 0.325 |
| Running Fluid Balance | 4805.78 | 3083.25 | 6528.30 | <0.001 |
| ctHb | 2.24 | 1.78 | 2.69 | <0.001 |
| pH | −0.05 | −0.08 | −0.03 | 0.004 |
| Temperature | −0.07 | −0.28 | 0.13 | 0.486 |
| Lac | 0.76 | 0.46 | 1.06 | 0.001 |
| Midazolam dose/h | −6.04 | −24.37 | 12.29 | 0.559 |
| Urine Output | −23.64 | −90.27 | 42.98 | 0.529 |
| Albumin | −14.11 | −16.67 | −11.46 | <0.001 |
| ALT | 7.24 | −4.99 | 19.46 | 0.303 |
| AST | 95.65 | −17.49 | 208.58 | 0.153 |
| Bilirubin D | −0.60 | −1.98 | 0.80 | 0.45 |
| Bilirubin T | −0.34 | −1.58 | 0.91 | 0.632 |
| Creatinine | 0.29 | −13.33 | 13.90 | 0.97 |
| Total protein | −25.00 | −30.42 | −19.60 | <0.001 |
| Urea | 1.07 | −0.19 | 2.32 | 0.159 |
| Urine Creatinine | −3372.93 | −11346.98 | 4601.12 | 0.456 |

Table 2. Mean difference in smoke inhalation acute lung injury (S-ALI) group (n=5) compared with healthy ECMO group (n=7) over the sampling interval. Using a mixed model with a random intercept for each sample. Results presented as mean difference and 95% confidence intervals. Including a linear time trend and using each sheep's baseline (time zero) as a covariate. BP- blood pressure, PAP-pulmonary artery pressure, CVP-central venous pressure CCO- continuous cardiac output, Svo2- mixed venous oxygen saturation, PEEP-positive end expiratory pressure, Fio2-fraction of inspired oxygen, pO2 –

partial pressure of oxygen, pCO2-partial pressure of carbon dioxide, Hb –haemoglobin, Lac –Lactate, ALT –alanine amino transferase,  AST-aspartate amino transferase.

The concentration time curves for midazolam, 1- and 4-OH midazolam are plotted in Figure 1. The mean (±SD) steady-state concentrations (last concentrations during continuous infusion) of midazolam, 1-OH-midazolam and 4-OH midazolam in the healthy and S-ALI sheep were 1.16 (.52), 0.33 (.09) and 0.15 (.07) mg/L and 1.49 (.76), 0.28(.20) and .12(.05) mg/L respectively and the differences were not statistically significant.

The average $AUC_{0-t}$ for the healthy sheep for the measured molecules was 17028, 4438 and 1578 mg.hr/L for the healthy sheep and 15255, 3168 and 1141 mg.hr/L for the S-ALI sheep (p=0.71, 0.26, 0.20 respectively).The $AUC_{0-t}$ ratios for the 1-OH-midazolam and 4-OH midazolam relative to the parent compound were 0.28 and 0.11 respectively in the healthy sheep and 0.23 and 0.10 in the S-ALI sheep suggesting little PK differences resulting from the S-ALI ( p=0.28 and 0.44).  The ratios of 1and 4 –OH midazolam and midazolam concentrations in both healthy and S-ALI sheep over time are presented in Figure 2.

Differences in areas under the curve and log-transformed areas under the curve ratios for midazolam and its metabolites between healthy and S-ALI sheep using healthy sheep on ECMO as the reference group are presented in Tables 3 and 4.

| Drug/metabolite | Group | Mean | Lower | Upper | p-value |
|---|---|---|---|---|---|
| Midazolam | SALI | -1773.25 | -12085.42 | 8538.92 | 0.71 |
| 1-OH midazolam | SALI | -1269.73 | -3678.01 | 1138.56 | 0.26 |
| 4-OH midazolam | SALI | -436.68 | -1152.34 | 278.98 | 0.20 |

Table 3. Difference in areas under the curve and for midazolam and its metabolites between healthy and smoke inhalation acute lung injury (SALI) sheep (n=5) using healthy sheep on ECMO (n=7) as the reference group Results are presented as mean differences and 95% confidence intervals.
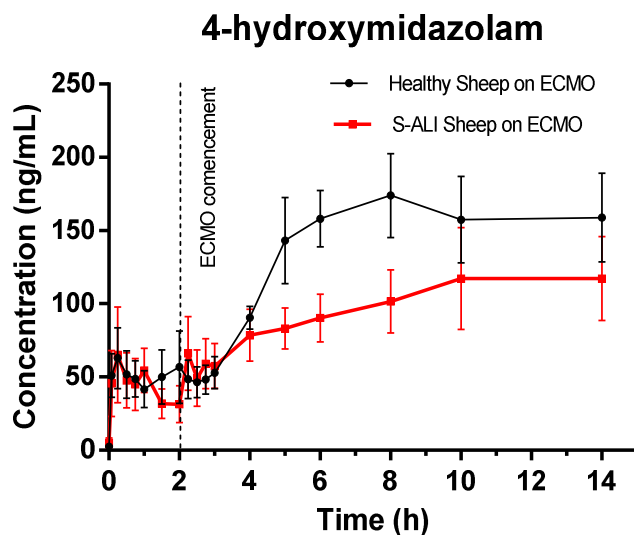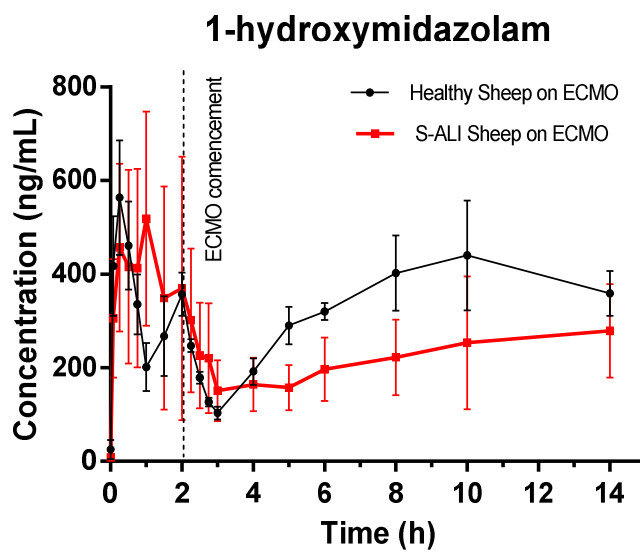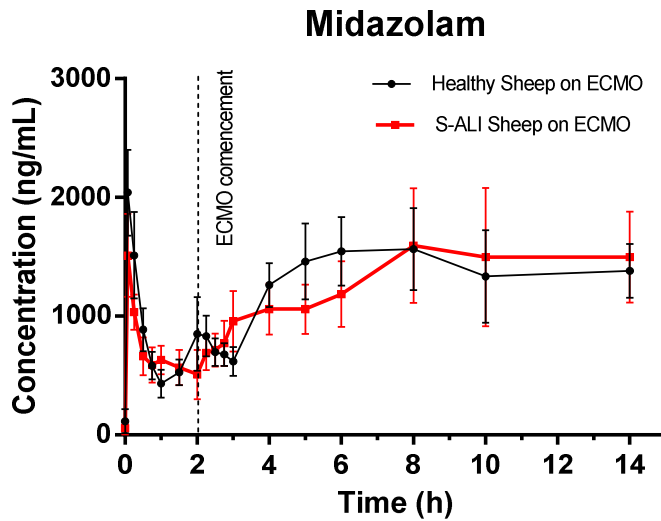
Figure 1. Concentration time curves for midazolam, 1- hydroxy midazolam and 4 –hydroxy midazolam. All sheep received 0.5mg/kg bolus followed by 0.25 mg/kg/h of midazolam by a continuous infusion for 2 hours prior to commencement of ECMO.

# 1-OH-MDZ/MDZ
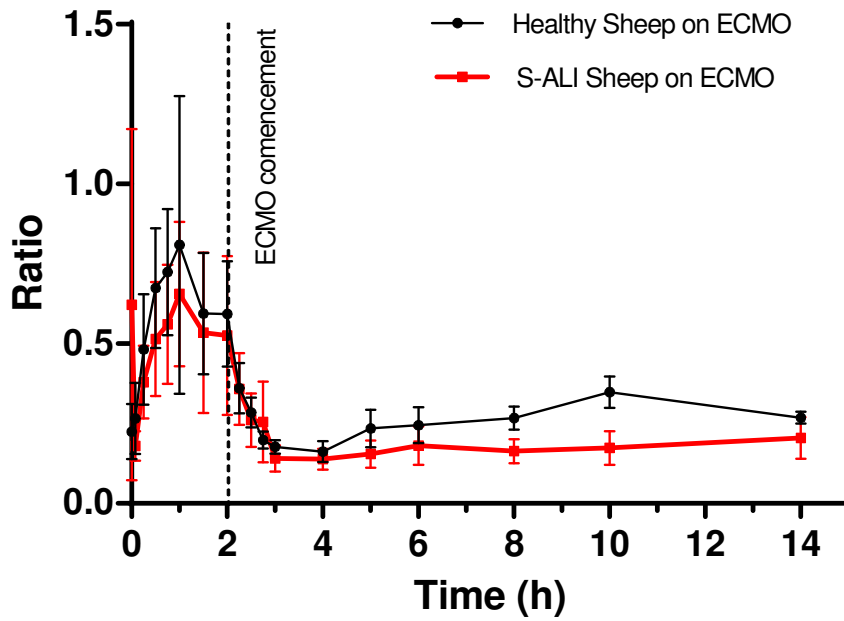
# 4-OH-MDZ/MDZ
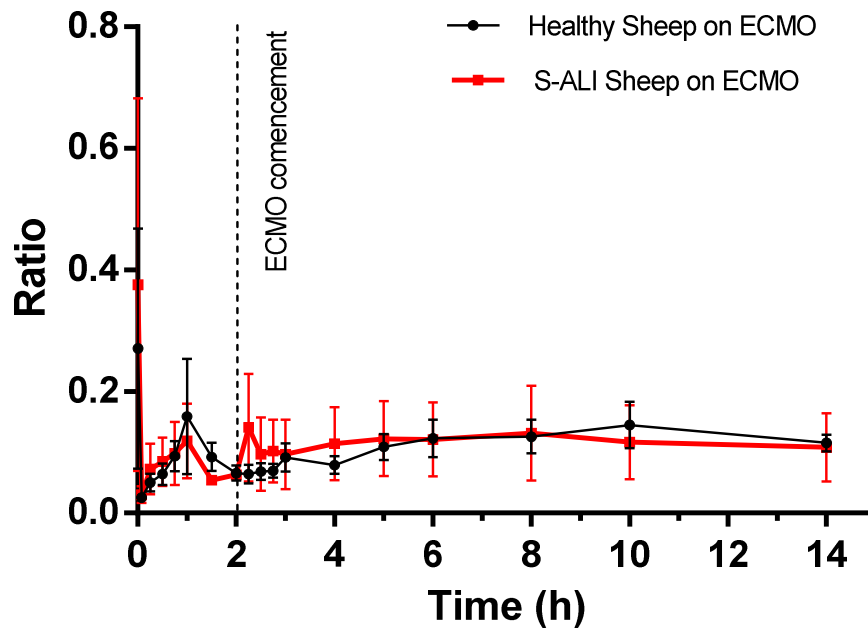
Figure 2. Ratio of parent 1and 4 –hydroxy midazolam and midazolam concentrations in both healthy (n=7) and smoke inhalation acute lung injury (SALI) sheep (n=5) plotted against time.

| Metabolite/drug | Group | Mean | Lower | Upper | p-value |
|---|---|---|---|---|---|
| 1-OH MDZ/MDZ | SALI | -0.43 | -1.29 | 0.42 | 0.28 |
| 4-OH MDZ/MDZ | SALI | -0.32 | -1.22 | 0.58 | 0.45 |

Table 4.   Difference in log-transformed areas under the curve ratios for 1-hydroxy midazolam (1-OH MDZ) and 4-hydroxy midazolam (4-OH MDZ) relative to midazolam (MDZ) between healthy and smoke inhalation acute lung injury (S-ALI) sheep (n=5) using healthy sheep on ECMO (n=7)as the reference group. Results are presented as mean differences and 95% confidence intervals.

*Pharmacokinetic model building*

The time-course of midazolam in plasma was best described by a two-compartment linear model with exponential residual error and BSV on CL, Q and $V_2$. This model included zero order input of drug into the central compartment. The goodness of fit plots were acceptable and suggested that the model described the data sufficiently (population predicted versus observed concentrations $R^2$ = 0.50; and individual predicted versus observed concentrations $R^2$ = 0.78). After screening all relevant biologically plausible covariates and body weight, only the presence of ECMO was included on $V_2$ in the final model. When this covariate was added, the objective function value reduced statistically significantly ($p<0.05$) and improved the goodness of fit plots. The objective function value for the final covariate model was -200.020. The median (IQR) population PK parameter estimates for the final covariate model were: CL 3.58 (2.64 – 6.05) L/h, $V_1$ 8.45 (no BSV included) L and Q 44.9 (23.7 – 75.9) L/h and $V_2$ 191.3 (147.1 – 305.7) L. The model predicted V2 pre-ECMO commencement and then afterward was 55% lower, 191.3 (147.1 – 305.7) L and 86.0 (66.1 – 137.4) L respectively which was statistically significant ($p=0.015$).

**Discussion**

This PK study mechanistically confirms the independent effects of ECMO on midazolam PK in the *in vivo* setting. This is important as midazolam PK estimated in critically ill patients on ECMO cannot distinguish the contributing impact of the host pathophysiology, ECMO circuit or the drug factors. For the severity of illness seen in the S-ALI sheep on ECMO, the effects of critical illness itself on midaozlam PK was modest. However, a significant increase in peripheral Vd in both healthy and critically ill sheep on ECMO is an interesting finding.   This reveals midazolam sequestration/inactivation in the circuit and highlights the "compartmental" effect the ECMO circuit has on drug PK. This finding also is

in keeping with significant midazolam losses seen in ECMO circuits in *ex vivo* experiments that used both adult and neonatal circuit components[10,24,59].

An increase in peripheral Vd for midazolam in both healthy and S-ALI sheep upon commencement of ECMO was the key finding in this study. There was no significant difference in exposure to midzolam and its metabolites between groups. Midazolam is a highly plasma –protein bound drug (92%) and relatively lipophilic, both of which shown to be significant risk factors for sequestration[10,59] in the ECMO circuit resulting in an increased peripheral Vd. Equally, larger Vd and accumulation of midazolam in peripheral body tissues have been reported in the critically ill population. However, this does not explain an increased Vd seen in healthy sheep upon initiating ECMO as these sheep did not manifest any physiologic signs of systemic inflammation or organ dysfunction post initiation. Increased Vd is also not explained by the degree of haemodiluation that may have occurred by addition of the primed ECMO circuit (average prime volume 667±20 mL) to a large animal. Similarly, despite the inflammation, shock and significant hypoproteinaemia, the S-ALI ECMO sheep exhibited PK characteristics similar to those of healthy sheep on ECMO. All of these findings indicate that midazolam sequestration in the circuit may play a significant role in PK and hence pharmacodynamics (PD) during ECMO. There was no significant difference in midazolam or metabolite CL in both groups and this may be attributed to insignificant differences in organ failures between the groups. Midazolam undergoes hydroxylation by the hepatic cytochrome P 450 (CYP) system to form three metabolites that are excreted through the renal system[62]. The active metabolite of significance 1-OH midazolam is 20% less potent than the parent drug with a half life of 0.8-1 h in patients with intact renal function. The glucouronidation of 1-OH midazolam results in less potent 1-OH midazolam glucuronide which is also really excreted and prone to accumulation. Although, the ECMO circuit can independently reduce drug and metabolite CL even in patients with normal renal function, renal dysfunction is not uncommon during ECMO[63]. The eventual target site concentrations of midazolam and its metabolites during ECMO are determined not only by dosing and method of administration but also by the magnitude of the increase in Vd and decreased CL in an individual patient.

These findings are clinically relevant as they show that altered PK may have to be considered whilst planning sedation or whilst assessing patient's neurological status. The ECMO circuit by sequestering large amounts of the sedative drug and its metabolites may actively modulate PK/PD. This may manifest clinically as higher than usual midazolam dose requirement to achieve the desired sedative effect on ECMO. Equally, patients may

take longer than usual to wake and clinicians should be cautious especially when they deduce futility of ongoing care based on neurological status in the absence of significant central nervous system pathology on imaging.

The study groups were well separated physiologically from a compromised hemodynamic and respiratory function point of view but the biochemical differences in hepatic and renal functions were non- significant during the sampling interval. It should be noted that ,the degree of hepatic dysfunction that need to present in a critically ill patient[64] to significantly impact midazolam metabolism  is unclear and is not easy to quantify this based on biochemical tests[65]. Hepatic blood flows and critical illness may also impact CYP activity[66], even when biochemical liver derangements are not apparent. Occasionally, ECMO patients (especially those ones supported with venoarterial ECMO for cardiac failure) may have significant biochemical derangement in hepatic function.   Available data does not indicate a significant difference in sedation requirement between patients on venovenous and venoarterial ECMO[11]. Given the most venovenous ECMO patients have reasonably preserved hepatic function based on biochemical tests, absence of significant liver dysfunction in the S-ALI animal may limit the interpretation of our PK data.

Although there are no PK data for midazolam and its metabolites from healthy subjects undergoing ECMO to compare, the available corresponding PK data from critically ill patients on ECMO concurs with the findings of our study. Neonates on ECMO have been shown to develop an increased Vd upon commencement of ECMO and an increasing CL for both midazolam and 1-OH midazolam. Ahsman et a[43]l reported a greater than 3-fold increase in central Vd (1.43 to 4.86 L/Kg) and a 3-fold increase in CL with in the first 5 days (up to 0.46 and 1.77 L /h /Kg) upon commencement of venoarterial ECMO. Increased central Vd for midazolam upon commencement venovenous ECMO (0.81 ± 0.5 and 4.1 ± 0.5 L/Kg) was also reported by Mulla et al [67]in neonates.  In these studies, volume expansion from addition of a primed circuit, presence of a critical illness and circuit binding of drugs were hypothesised to be possible mechanisms behind an increased Vd[43]. Increased CL was attributed to maturity of organs, disease progression and possible stabilisation of blood flow especially in neonates on venoarterial ECMO[43]. A reduction in midazoalm and its metabolite level with an associated increase in bispectral index scores and awakening has also been described in adult patients upon commencement of ECMO[9]. These data demonstrate that the PK of midazolam and its metabolites during ECMO is significantly affected by circuit sequestration of the drug in addition to illness related factors. Given that lipophilic and protein-bound drugs are preferentially sequestered in ECMO circuits[10,59], the available drug choices for sedation during ECMO in adult patients

may have to be carefully considered based on physicochemical properties until population PK data becomes available for commonly used drugs. This may also apply for analgesic agents as drugs such as fentanyl [10], which are highly sequestered in the ECMO circuit.

A short acting, potent, less protein bound drug with limited lipophilicity, that has no active metabolites and minimal other systemic effects, with rapid offset and onset of action may be an ideal agent for sedation and analgesia during ECMO. Not many current drugs have such favourable physicochemistry and most highly protein bound sedative drugs are also lipophilic. Equally, the lipophilic drugs are more likely to readily penetrate and distribute in effect sites within the central nervous system[68,69]. This along with the fact that sedative drugs can be titrated to clinical effect would mean that relatively less protein bound and lipophilic sedative and analgesic drugs may have to be simply used in higher doses than usual to provide sedation and analgesia in ECMO patients. These doses may often exceed the maximum recommended doses and raise safety concerns. Clinical PK/PD studies[55] are indicated to establish the ideal sedative agent for use during ECMO.

This PK study has limitations. Midazolam PK data was not available from non ECMO controls. Although using animals as their own controls for PK sampling prior to ECMO commencement was a positive, the sampling interval (2 h) prior to ECMO commencement may be considered too short to attain a steady state. There were no PD endpoints and midazolam was administered as a constant dose infusion as the study entirely focussed on PK of midazolam and its metabolites. It should be noted that ECMO may induce PD alterations which are beyond the scope of this study.

## Conclusion

An increase in peripheral Vd was observed upon commencement of ECMO in both healthy and critically ill sheep. The overall exposure to midazolam and its metabolites 1 and 4 –OH midazolam did not differ significantly. The PK changes observed in healthy sheep provide preliminary insights into the role of the ECMO circuit itself in exerting a compartmental effect and modulating PK during ECMO.

## Key messages

- Midazolam sequestration in ECMO circuits affects PK significantly
- There was an increase in peripheral Vd upon commencement of ECMO
- No significant effects of critical illness on midazolam PK was observed in this study
- These findings explain the increased sedation requirement seen during ECMO

- Clinical studies are indicated to further confirm these findings and to develop sedation protocols for ECMO patients

**Transparency declarations:**

None to declare

## 6.2 CAN PHYSICOCHEMICAL PROPERTIES OF ANTIMICROBIALS BE USED TO PREDICT THEIR PHARAMCOKINETICS DURING EXTRACORPOREAL MEMBRANE OXYGENATION? ILLUSTRATIVE DATA FROM OVINE MODELS.

### *6.2.1 Introduction to this publication*

This chapter describes the results of a mechanistic investigation into altered PK of antibiotics during ECMO. Using ovine models of increasing complexity and eight antibiotic study drugs with a range of lipophilicty and protein binding, this study for the first time highlighted the utility of these physicochemical properties in predicting their PK in ECMO patients. The findings of this study confirm the findings of *ex vivo* studies in ECMO circuits and provide preliminary evidence that drug circuit interactions play a key role in altering PK in a critically ill patient. This supports the hypothesis that drug and circuit factors may in play an independent role in altering antibiotic PK in critically ill patients on ECMO.

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# Can physicochemical properties of antimicrobials be used to predict their pharmacokinetics during extracorporeal membrane oxygenation? Illustrative data from ovine models

Kiran Shekar[1,2]*, Jason A. Roberts[3], Adrian G. Barnett[4], Sara Diab[1], Steven C. Wallis[3], Yoke L. Fung[5] and John F. Fraser[1]

## Abstract

**Introduction:** Ex vivo experiments in extracorporeal membrane oxygenation (ECMO) circuits have identified octanol-water partition coefficient (logP, a marker of lipophilicity) and protein binding (PB) as key drug factors affecting pharmacokinetics (PK) during ECMO. Using ovine models, in this study we investigated whether these drug properties can be used to predict PK alterations of antimicrobial drugs during ECMO.

**Methods:** Single-dose PK sampling was performed in healthy sheep (HS, $n = 7$), healthy sheep on ECMO (E24H, $n = 7$) and sheep with smoke inhalation acute lung injury on ECMO (SE24H, $n = 6$). The sheep received eight study antimicrobials (ceftriaxone, gentamicin, meropenem, vancomycin, doripenem, ciprofloxacin, fluconazole, caspofungin) that exhibit varying degrees of logP and PB. Plasma drug concentrations were determined using validated chromatographic techniques. PK data obtained from a non-compartmental analysis were used in a linear regression model to predict PK parameters based on logP and PB.

**Results:** We found statistically significant differences in pH, haemodynamics, fluid balance and plasma proteins between the E24H and SE24H groups ($p < 0.001$). logP had a strong positive linear relationship with steady-state volume of distribution ($V_{ss}$) in both the E24H and SE24H groups ($p < 0.001$) but not in the HS group ($p = 0.9$) and no relationship with clearance (CL) in all study groups. Although we observed an increase in CL for highly PB drugs in ECMO sheep, PB exhibited a weaker negative linear relationship with both CL (HS, $p = 0.01$; E24H, $p < 0.001$; SE24H, $p < 0.001$) and $V_{ss}$ (HS, $p = 0.01$; E24H, $p = 0.004$; SE24H, $p = 0.05$) in the final model.

**Conclusions:** Lipophilic antimicrobials are likely to have an increased $V_{ss}$ and decreased CL during ECMO. Protein-bound antimicrobial agents are likely to have reductions both in CL and $V_{ss}$ during ECMO. The strong relationship between lipophilicity and $V_{ss}$ seen in both the E24H and SE24H groups indicates circuit sequestration of lipophilic drugs. These findings highlight the importance of drug factors in predicting antimicrobial drug PK during ECMO and should be a consideration when performing and interpreting population PK studies.

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## Introduction

With refinements in technology, extracorporeal membrane oxygenation (ECMO) and extracorporeal life support (ECLS) in general now represent a significant development in intensive care practice [1–4]. A wide range of acutely ill patients with cardiorespiratory failure are now being successfully rescued with ECLS therapies, but clinicians await definitive evidence supporting their use. Invasive ECLS therapies such as ECMO are complex supportive interventions, and outcomes rely not only on technology but also on user experience [5]; optimisation of other aspects of intensive care unit (ICU) management, established processes and other available services in each centre; and optimisation of pharmacotherapy to minimise and/or treat complications [6].

A variety of infectious and non-infectious conditions may result in severe cardiorespiratory failure, and an infection or sepsis is no longer considered a contraindication for ECMO [7]. Similarly, patients on ECMO may develop a variety of ICU-acquired infections that may necessitate antimicrobial therapy. Optimal antimicrobial therapy in these patients is a balance between potency, bacterial susceptibility and exposure [8, 9]. The authors of a recent review identified 30.1 infections per 1000 days of ECMO among patients with infections who were experiencing prolonged ICU and hospital lengths of stay [10]. The authors of another review [11] identified a total of 2418 infections in 20,741 (12 %) ECMO cases, with increased morbidity seen in patients with infections. Antimicrobial therapy is commonly prescribed in ECMO patients, and optimisation of dosing is central not only to improving patient outcomes but also to minimising the emergence of microbial resistance [8].

However, ECMO is known to induce significant pharmacokinetic (PK) alterations [12] in critically ill patients who already exhibit significantly altered PK [13], raising concerns of therapeutic failure or toxicity. Neonatal studies have shown major variations in antibiotic PK during ECMO [12, 14–16], and there is an emerging body of literature to support this in adult patients [17–20]. The interaction between the drug, the ECMO device and the disease are complex; hence clinical population PK studies alone may not be able to advance understanding of mechanisms behind altered PK in ECMO patients. This calls for systematic investigation [21] of each of these factors. To this end, experimental studies [22, 23] using circuit components used in adults have shown significant drug sequestration in ECMO circuits based on physicochemical properties of the drug, such as drug stability, octanol-water coefficient (logP, a marker of lipophilicity) and protein binding (PB).

Ex vivo experimental conditions are quite different from in vivo scenarios. The addition of an extracorporeal circuit to a critically ill patient may result in profound PK alterations, and appreciating the relative contributions of drug, device and disease factors to altered PK is challenging. Building on the data derived from ex vivo circuit studies, we aimed to develop PK models for antibiotic study drugs that exhibit wide a range of logP and PB in ambulatory healthy sheep (HS) as well as in healthy and critically ill sheep on ECMO. We hypothesised that the drug properties logP and PB can be used to predict PK alterations of antimicrobial drugs during ECMO.

## Methods

Ethical approval was obtained from the Queensland University of Technology Animal Ethics Committee (approval number 1100000053) and the University of Queensland Animal Ethics Committee (approval number QUT/194/12). All experimentation was done in accordance with the National Health and Medical Research Council's Australian Code for the Care and Use of Animals for Scientific Purposes, Eighth Edition (2013) (https://www.nhmrc.gov.au/book/australian-code-care-and-use-animals-scientific-purposes-8th-edition-2013).

### Pharmacokinetic sampling
#### Healthy ambulatory sheep
Seven HS weighing 46–51 kg were housed in a metabolic cart amongst a larger flock, with free access to food and water. Two three-lumen central venous catheters were inserted in the left and right internal jugular veins (IJVs) while the animals were under local anaesthesia for drug administration and PK sampling. The catheters were secured with adhesive glue and a sleeve dressing around the neck. Study drugs were infused for 30 minutes, and serial blood samples were obtained for drug assays using validated chromatographic methods and subsequent PK analysis.

#### Healthy sheep on ECMO
We performed PK sampling in seven healthy sheep on extracorporeal membrane oxygenation (E24H). A detailed description of our ovine model of venovenous ECMO is provided elsewhere [21, 24]. Briefly, a central venous line was placed in the right IJV while the animals were under local anaesthesia. Alfaxalone, ketamine and midazolam were used for induction and maintenance of anaesthesia. Buprenorphine 0.01 mg/kg was used for supplemental analgesia. Sheep were intubated and ventilated with a Hamilton Galileo ventilator (Hamilton Medical AG, Bonaduz, Switzerland). The facial artery was cannulated for invasive arterial blood pressure monitoring. A pulmonary arterial catheter provided continuous measurements of central venous pressure, mixed venous oxygen saturation and continuous cardiac output (CCO).

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Cannulation for ECMO was performed with the animals in supine position. A 21-French (50 cm) CARMEDA BioActive Surface–coated (CBAS®; Carmeda, Upplands Väsby, Sweden) venous cannula (Medtronic, Minneapolis, MN, USA) was inserted into the right IJV using a Seldinger technique and positioned using intracardiac echocardiography (ICE) [25] in the proximal inferior vena cava. A 19-French (50 cm) CARMEDA-coated femoral venous cannula was used for return blood and was inserted in the right IJV and positioned at the superior vena cava right atrium using ICE. ECMO pump speeds were titrated to target flows at least two-thirds of pre-ECMO CCO (or 60–80 ml/kg). Immediately upon commencement of ECMO, study drugs were infused for 30 minutes and serial blood samples were obtained for drug assays using validated chromatographic methods and subsequent PK analysis.

### Smoke inhalation acute lung injury sheep on ECMO
We performed PK sampling in six sheep with smoke inhalation acute lung injury on ECMO (SE24H). The anaesthesia and ECMO techniques we used are described in the previous section. Smoke inhalation acute lung injury (S-ALI) was induced using a validated, reproducible technique previously published [26]. Briefly, a stainless steel plate was heated to 750 °C and placed on top of 8 g of cotton in a cup. The smoke resulting from combustion collected in the bellows of the purpose-built device was delivered to the sheep by manual compression (tidal volume [$V_T$], 10–12 ml/kg) to achieve a carboxyhaemoglobin content of 45–50 %. The sheep were ventilated using Acute Respiratory Distress Syndrome Network criteria ($V_T$ 4–6 ml/kg, positive end-expiratory pressure 10–15 cm $H_2O$) for lung-protective ventilation [27]. Once ECMO was established, study drugs were infused for 30 minutes and serial blood samples were obtained for drug assays using validated chromatographic methods and subsequent PK analysis.

### Study drugs, drug administration and pharmacokinetic sampling
Following baseline sampling, study drugs in identical doses were administered to the HS, E24H and SE24H groups. The chosen anti-infective study drugs exhibit a wide range of logP and PB (Table 1). The intravenous (IV) study drugs (doses, administration techniques) used were meropenem (500 mg, bolus), ceftriaxone (500 mg, IV bolus), gentamicin (240 mg, slow IV bolus), vancomycin (500 mg in 50 ml 0.9 % saline, IV for 30 minutes), fluconazole (100 mg in 50 ml of 0.9 % saline, IV for 30 minutes), caspofungin (50 mg in 100 ml of 0.9 % saline, IV for 30 minutes), ciprofloxacin (100 mg in 50 ml of 0.9 % saline, IV for 30 minutes) and doripenem (500 mg in 100 ml of 0.9 % saline, IV for 30 minutes). Serial blood samples

**Table 1** Lipophilicity and protein binding characteristics of study drugs

| Study drug | Lipophilicity (logP) | Protein binding (%) |
|---|---|---|
| Ceftriaxone | −1.7 | 95 |
| Ciprofloxacin | 2.3 | 20–40 |
| Caspofungin | 0.1 | 97 |
| Fluconazole | 0.4 | 11–12 |
| Gentamicin | −3.1 | 0–30 |
| Meropenem | −0.6 | 2 |
| Doripenem | 0.7 | 8 |
| Vancomycin | −3.1 | 55 |

A higher numeric value for octanol-water partition coefficient (logP) indicates greater lipophilicity [29]

(2 ml) were obtained at 15, 30, 45, 60, 90, 180, 360, 480 and 720 minutes after commencement of antibiotic drug infusions for drug assays and subsequent PK analysis.

### Antimicrobial drug assays
Meropenem, doripenem, ceftriaxone and vancomycin analysis was done using high-performance liquid chromatography (HPLC) on a Prominence Ultra Fast system (Shimadzu, Kyoto, Japan) with ultraviolet light detection at 304 nm (meropenem and doripenem) and 230 nm (ceftriaxone and vancomycin). Ciprofloxacin was analysed on a Prominence HPLC system with fluorescence detection at 278 nm (excitation) and 456 nm (emission). Caspofungin, gentamicin and fluconazole analysis was carried out using liquid chromatography–tandem mass spectrometry on a Shimadzu Nexera-8030+ system with detection by positive mode multiple reaction monitoring. Samples were prepared by protein precipitation with trichloroacetic acid (ciprofloxacin and gentamicin), acetonitrile (caspofungin and fluconazole) or acetonitrile with dichloromethane washing (meropenem, ceftriaxone, vancomycin and doripenem). Chromatography was carried out using reversed-phase C18 HPLC columns (meropenem, ceftriaxone, vancomycin, doripenem, ciprofloxacin), reversed-phase C8 HPLC columns (caspofungin, fluconazole) or high-performance liquid chromatography (HPLC) (gentamicin). All methods were validated according to the guidelines of the US Food and Drug Administration [28]. All samples were assayed with internal standards, alongside calibration standards and quality control samples, and met the acceptance criteria.

### Statistical analysis and pharmacokinetic modelling
Discrete variables were expressed as count (percentage) and continuous variables as mean ± SD. Demographics and clinical differences between study groups were assessed using a $\chi^2$ test, Fisher's exact test or Student's *t* test, as appropriate. $p < 0.05$ was considered statistically significant.

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**Table 2** Demographic and physiologic data at baseline after initiation of anaesthesia, mechanical ventilation and haemodynamic monitoring and before smoke inhalation and commencement of ECMO

|  | Group | Mean | SD | *p* Value |
|---|---|---|---|---|
| Weight, kg | E24H | 48.5 | 4.6 | 0.84 |
|  | SE24H | 49.6 | 4.4 |  |
| Heart rate, beats/min | E24H | 116 | 13 | 0.78 |
|  | SE24H | 118 | 11 |  |
| Mean arterial BP, mmHg | E24H | 116.1 | 6.8 | 0.91 |
|  | SE24H | 115.7 | 8.1 |  |
| Mean PAP, mmHg | E24H | 24.6 | 2.8 | 0.06 |
|  | SE24H | 21.2 | 3.2 |  |
| CVP, cmH$_2$O | E24H | 15.6 | 2.8 | 0.06 |
|  | SE24H | 12.2 | 3.2 |  |
| CCO, L/min | E24H | 5.44 | 0.93 | 0.33 |
|  | SE24H | 5.03 | 0.46 |  |
| SvO$_2$, % | E24H | 78.8 | 7.1 | 0.31 |
|  | SE24H | 82.0 | 4.1 |  |
| PEEP, cmH$_2$O | E24H | 8.1 | 2.6 | 0.67 |
|  | SE24H | 7.5 | 2.7 |  |
| Respiratory rate, breaths/min | E24H | 10.9 | 6.0 | 0.6 |
|  | SE24H | 12.5 | 5.2 |  |
| Fluid balance, ml | E24H | 681 | 376 | 0.11 |
|  | SE24H | 926 | 68 |  |
| Haemoglobin, g/L | E24H | 7.0 | 1.2 | 0.47 |
|  | SE24H | 7.6 | 1.7 |  |
| pH | E24H | 7.385 | 0.031 | 0.62 |
|  | SE24H | 7.397 | 0.052 |  |
| Body temperature, °C | E24H | 38.26 | 0.61 | 0.67 |
|  | SE24H | 38.13 | 0.50 |  |
| Lactate, mmol/L | E24H | 1.34 | 0.44 | 0.17 |
|  | SE24H | 1.07 | 0.24 |  |
| Midazolam dose, mg/h | E24H | 14.4 | 1.2 | 0.17 |
|  | SE24H | 15.0 | 0.0 |  |
| Urine output, ml/h | E24H | 69 | 44 | 0.87 |
|  | SE24H | 74 | 68 |  |
| Albumin, g/L | E24H | 37.16 | 2.53 | 0.33 |
|  | SE24H | 38.15 | 0.86 |  |
| Alanine aminotransferase (U/L) | E24H | 11.7 | 2.1 | 0.51 |
|  | SE24H | 12.4 | 1.7 |  |
| Serum bilirubin, μmol/L | E24H | 2.16 | 0.69 | 0.75 |
|  | SE24H | 2.03 | 0.74 |  |
| Serum creatinine, μmol/L | E24H | 88 | 15 | 0.94 |
|  | SE24H | 87 | 11 |  |

**Table 2** Demographic and physiologic data at baseline after initiation of anaesthesia, mechanical ventilation and haemodynamic monitoring and before smoke inhalation and commencement of ECMO *(Continued)*

| Total protein, g/L | E24H | 71.7 | 6.4 | 0.78 |
|---|---|---|---|---|
|  | SE24H | 71.0 | 1.9 |  |
| Urine creatinine, μmol/L | E24H | 10,172 | 3798 | 0.17 |
|  | SE24H | 15,541 | 8009 |  |

*BP* blood pressure, *PAP* pulmonary arterial pressure, *CVP* central venous pressure, *CCO* continuous cardiac output, *SvO$_2$* mixed venous oxygen saturation, *PEEP* positive end-expiratory pressure
Data presented are derived from comparison of the results between groups: healthy sheep on extracorporeal membrane oxygenation (E24H) (*n* = 7), sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation (SE24H) (*n* = 6)

A linear mixed effects model was used to examine changes in concentration over time whilst controlling for repeated results from the same sheep. The result adjusts for changes over time and repeated results from the same sheep. The concentration versus time curves (mean ± SEM) were plotted using GraphPad Prism version 5.03 software (GraphPad Software, La Jolla, CA, USA). PK analysis of antibiotic concentrations was undertaken using a non-compartmental approach. All statistical analyses were done using R version 3.1.2 software (R Foundation for Statistical Computing, Vienna, Austria).

We compared the statistical data between the three groups using a box plot. To look for a difference in the mean statistics between groups, we used a linear model with group as the dependent variable. Because the PK data were strongly positively skewed, we log-transformed them before building regression models. Regression models were derived to examine the differences in the following PK parameters between the three study groups: area under the curve (AUC), mean resident time, clearance (CL), steady-state volume of distribution ($V_{ss}$), maximum plasma concentration and minimum plasma concentration. A linear regression analysis was used to predict PK parameters based on drug properties. logP data for the individual drugs are available from the University of Alberta DrugBank website [29].

## Results

We observed no complications during the ECMO run. We found no significant differences between the physiologic variables at the baseline (Table 2). Differences in physiologic variables between the E24H and SE24H groups are presented in Table 3. We found statistically significant differences in pH, haemodynamics, fluid balance and plasma proteins between the E24H and SE24H groups (*p* < 0.001).

Sixteen hundred samples were analysed for study drug concentrations. Concentration versus time curves for

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**Table 3** Mean differences in Physiologic parameters of E24H and SE24H groups during the pharmacokinetic sampling interval

| Variable | Mean | Lower | Upper | p Value |
|---|---|---|---|---|
| Heart rate | 1.41 | −9.54 | 12.36 | 0.815 |
| Mean arterial BP | −23.83 | −31.35 | −16.25 | <0.001 |
| Mean PAP | 1.77 | −0.83 | 4.37 | 0.234 |
| CVP | 0.93 | −3.00 | 4.86 | 0.669 |
| CCO | −2.09 | −3.30 | −0.87 | 0.01 |
| SvO$_2$ | −0.78 | −3.31 | 1.74 | 0.585 |
| PEEP | 2.09 | 0.66 | 3.52 | 0.02 |
| Respiratory rate (sheep) | 2.33 | 0.18 | 4.48 | 0.068 |
| Tidal volume | −38.24 | −135.38 | 58.87 | 0.479 |
| FiO$_2$ | 7.77 | −2.04 | 17.69 | 0.151 |
| paO$_2$ | 48.05 | 0.43 | 95.67 | 0.053 |
| paCO$_2$ | 3.12 | −0.28 | 6.52 | 0.115 |
| Running fluid balance | 4604.14 | 2779.38 | 6428.89 | <0.001 |
| ctHb | 2.00 | 1.34 | 2.66 | <0.001 |
| pH | −0.07 | −0.10 | −0.03 | 0.003 |
| Body temperature | −0.06 | −0.38 | 0.27 | 0.754 |
| Lactate | 0.71 | 0.26 | 1.16 | 0.013 |
| Midazolam dose per hour | −5.97 | −21.37 | 9.42 | 0.485 |
| Urine output | 6.68 | −54.88 | 68.25 | 0.843 |
| Albumin | −14.91 | −16.70 | −13.10 | <0.001 |
| ALT | 5.12 | −6.25 | 16.49 | 0.419 |
| AST | 82.87 | −23.66 | 189.39 | 0.175 |
| Bilirubin (Direct) | −0.48 | −1.68 | 0.73 | 0.476 |
| Bilirubin (Total) | −0.12 | −0.71 | 0.50 | 0.823 |
| Creatinine | 0.56 | −11.52 | 12.64 | 0.933 |
| Total protein | −26.60 | | | <0.001 |
| Urea | 0.79 | −0.47 | 2.06 | 0.271 |
| Urine creatinine | −4327.47 | −9508.52 | 974.49 | 0.322 |

*BP* blood pressure, *PAP* pulmonary arterial pressure, *CVP* central venous pressure, *CCO* continuous cardiac output, *SvO$_2$* mixed venous oxygen saturation, *PEEP* positive end-expiratory pressure, *FiO$_2$* fraction of inspired oxygen, *paO$_2$* partial pressure of oxygen, *paCO$_2$* partial pressure of carbon dioxide, *ctHb* concentration of total blood haemoglobin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase
The analysis was carried out using a mixed model with a random intercept for each sample. The results are presented as mean difference and 95 % confidence intervals, including a linear time trend and using each sheep's baseline (time 0) as a covariate

the study antibiotics are shown in Fig. 1. A summary of PK parameters estimated using a non-compartmental analysis is presented in Table 4. Significant differences in AUC between groups were found for ciprofloxacin, gentamicin and caspofungin. For ciprofloxacin, the most lipophilic drug studied, there was a significant difference in $V_{ss}$ between the E24H and SE24H groups ($p = 0.004$). For relatively protein-bound drugs, there was a trend towards increased $V_{ss}$ only in the SE24H group compared with HS group. However, an increase in CL was seen in

both the E24H and SE24H groups compared with the HS group for vancomycin ($p = 0.02$ for both), ceftriaxone ($p = 0008$ and $p = 0.05$, respectively) and caspofungin ($p < 0.001$ for both), which are relatively more protein-bound.

Scatterplots and linear regression of both CL and $V_{ss}$ against logP and PB are presented in Figs. 2 and 3. Table 5 shows regression parameters for predicting study drug PK using logP and PB. PB exhibited a weaker negative linear relationship with CL (HS, $p = 0.01$; E24H, $p < 0.001$; SE24H, $p < 0.001$) and with $V_{ss}$ (HS, $p = 0.01$; E24H, $p = 0.004$; SE24H, $p = 0.05$). Despite an increased CL for more protein-bound study drugs, PB in itself was a predictor of decreased CL in all study groups (Table 5). logP had a strong positive linear relationship with $V_{ss}$ in both E24H and SE24H ($p < 0.001$) but not in HS ($p = 0.9$). There was no significant association of logP with CL (HS, $p = 0.55$; E24H, $p = 0.74$; SE24H, $p = 0.24$).

## Discussion
In this study, we systematically investigated the effects of the ECMO circuit on PK in HS and the combined effects of ECMO circuit and critical illness on PK in S-ALI sheep receiving ECMO. In addition, by using antimicrobials with a range of logP and PB, we were also able to investigate the relative contributions of drug, circuit and disease factors influencing PK during ECMO.

There was some expected variability in PK parameters between the groups. Overall, the main findings of the study are that (1) a significant increase in $V_{ss}$ for lipophilic drugs that was observed only in the ECMO sheep and (2) protein-bound drugs exhibited decreased CL and CL was also more significantly reduced in ECMO sheep. These findings are significant, as they conform to PK alterations described in neonates in the clinical ECMO setting and to emerging PK data in adults, and they provide further insights into mechanisms behind these PK alterations.

Although an increase in $V_{ss}$ during ECMO has been described clinically [12] for many antimicrobial and sedative drugs, the relative contribution of critical illness, circuit and drug factors towards this phenomenon is largely unclear. Systemic inflammation, capillary leak syndrome and hypoproteinaemia during critical illness can result in a significantly increased $V_{ss}$ [13]. Similarly, sequestration of drugs in ECMO circuits may lead to a further increase in $V_{ss}$. Equally, a reduction in drug CL during critical illness may result from renal and hepatic dysfunction [13]. This study confirms both these findings. An increase in $V_{ss}$ for lipophilic drugs occurred in both E24H and SE24H but not in HS, clearly highlighting the role of circuit drug sequestration. For all study drugs except ciprofloxacin, we found no significant difference in $V_{ss}$ between the E24H and SE24H groups,

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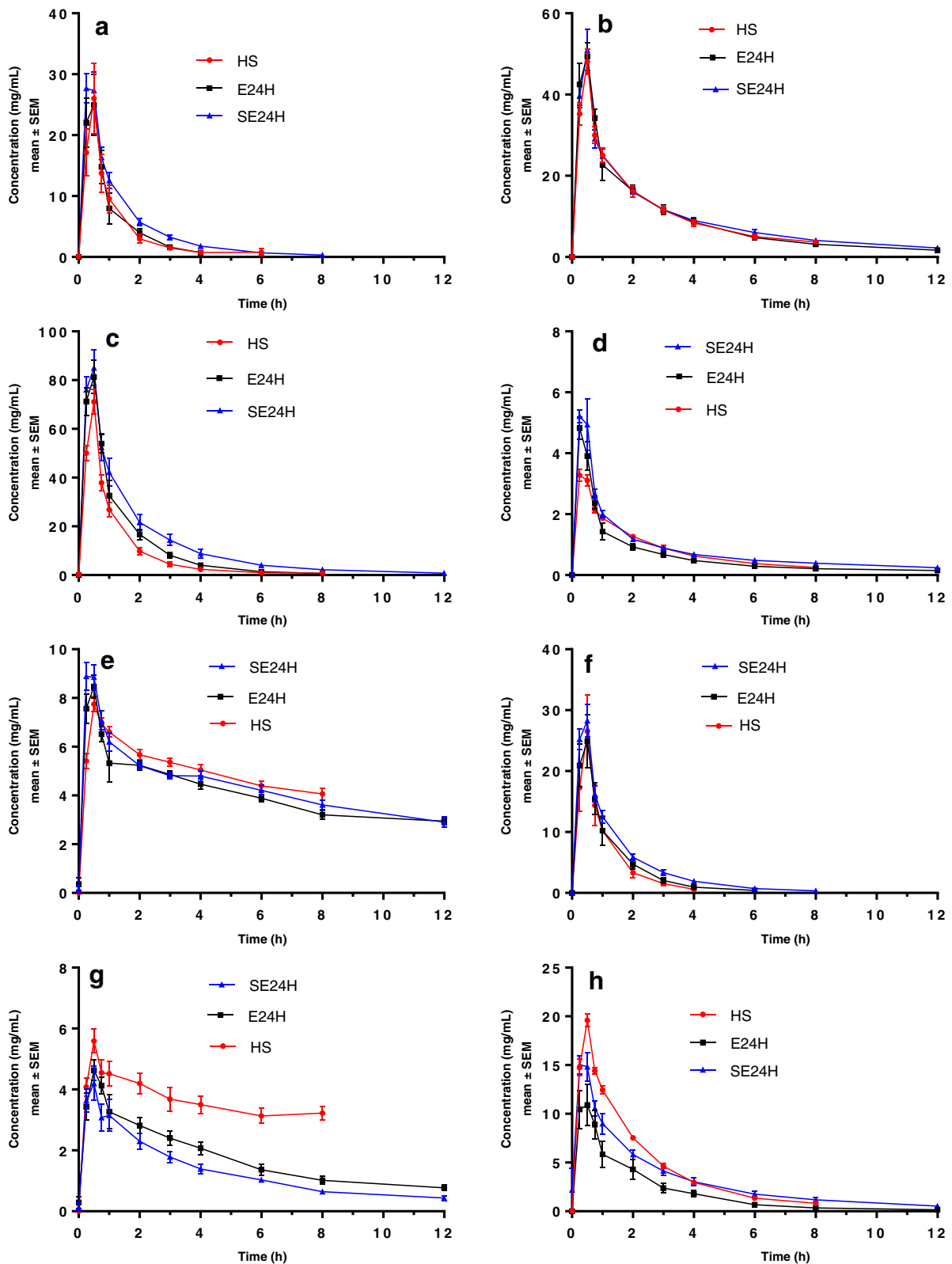Page 6 of 11



**Fig. 1** Concentration versus time curves for study drugs. **a** Meropenem. **b** Vancomycin. **c** Ceftriaxone. **d** Ciprofloxacin. **e** Fluconazole. **f** Doripenem. **g** Caspofungin. **h** Gentamicin. *E24H* healthy sheep on extracorporeal membrane oxygenation, *HS* healthy sheep, *SE24H* sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation

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**Table 4** Non-compartmental pharmacokinetic estimates for eight study drugs for all three study groups

| Study drug | Group | $C_{max}$ (mg/L) | $C_{min}$ (mg/L) | $AUC_{0-\infty}$ (mg/h/L) | $V_{ss}$ (L) | Clearance (L/h) | MRT (h) |
|---|---|---|---|---|---|---|---|
| Ceftriaxone | HS | 71 (14) | 0.0 (0.1) | 202 (46) | 9.0 (1.8) | 2.6 (0.6) | 3.5 (0.2) |
| | E24H | 86 (15) | 1.2 (1.4) | 135 (74)[a] | 6.3 (1.4)[a] | 4.3 (1.4)[a] | 1.7 (0.8)[a] |
| | SE24H | 85 (18) | 1.0 (0.3)[b,c] | 142 (39) | 7.4 (1.8) | 3.6 (0.8) | 2.1 (0.6)[b] |
| Vancomycin | HS | 48 (9) | 0.0 (0) | 180 (37) | 12.4 (2.6) | 2.8 (0.7) | 4.5 (0.2) |
| | E24H | 52 (10) | 1.59 (0.9)[a] | 131 (60)[a] | 15.7 (3.9) | 4.0 (1.1)[a] | 4.0 (0.6) |
| | SE24H | 51 (12) | 2.2 (0.4)[b] | 116 (20)[b] | 19.3 (3.3)[b] | 3.9 (0.6)[b] | 4.9 (0.7)[c] |
| Gentamicin | HS | 20 (2) | 0.0 (0) | 78 (7) | 13.0 (1.3) | 3.1 (0.3) | 4.3 (0.1) |
| | E24H | 12 (6)[a] | 0.3 (0.3)[a] | 25 (12)[a] | 34 (23.5)[a] | 12.1 (7.4)[a] | 2.7 (0.5)[a] |
| | SE24H | 16 (2)[b] | 0.6 (0.2)[b,c] | 39 (6.5)[b] | 20.1 (2.9)[b] | 5.9 (1.0)[b,c] | 3.5 (0.7)[b,c] |
| Meropenem | HS | 26 (16) | 0.0 (0.1) | 71 (45) | 91.0 (138) | 20.9 (28) | 3.9 (0.8) |
| | E24H | 27 (13) | 0.5 (0.4) | 36 (26) | 16.5 (3.0) | 13.8 (4.9) | 1.4 (0.9)[a] |
| | SE24H | 29 (7) | 0.3 (0.1) | 39 (9.0) | 19.9 (3.7) | 13.2 (2.4) | 1.6 (0.3)[b] |
| Doripenem | HS | 26 (17) | 0 (0) | 73 (47) | 63.8 (83) | 17.6 (22) | 3.5 (0.2) |
| | E24H | 30 (6) | 0 (0) | 42 (20) | 17.1 (2.8) | 13.5 (4.6) | 1.5 (0.9)[a] |
| | SE24H | 28 (6) | 0 (0) | 39 (8) | 20.2 (3.0) | 13.1 (2.4) | 1.6 (0.2)[b] |
| Ciprofloxacin | HS | 3.3 (0.5) | 0 (0) | 13.6 (1.8) | 31.9 (4.4) | 7.2 (0.9) | 4.5 (0.3) |
| | E24H | 5.1 (1.1)[a] | 0.1 (0.1)[a] | 8.3 (1.5)[a] | 39.0 (7.6)[a] | 11.8 (2.5)[a] | 3.5 (1.2)[a] |
| | SE24H | 5.8 (1.2)[b] | 0.1 (0.1)[b,c] | 10.2 (1.5)[b,c] | 52.7 (9.1)[b,c] | 8.2 (1.2)[c] | 6.4 (0.5)[b,c] |
| Fluconazole | HS | 7.7 (0.9) | 0 (0) | 48.2 (6.2) | 13.3 (2.2) | 1.2 (0.3) | 12.0 (5.8) |
| | E24H | 9.1 (1.2)[a] | 2.6 (0.8)[d] | 51.0 (5.0) | 16.7 (2.8)[a] | 1.0 (0.3) | 17.1 (5.5) |
| | SE24H | 9.2 (1.4)[b] | 2.9 (0.5)[b] | 52.3 (3.5) | 17.7 (3.4)[b] | 0.8 (0.5)[b] | 33.7 (29)[b] |
| Caspofungin | HS | 5.7 (1.0) | 0 (0) | 33.8 (7.3) | 10.0 (2.5) | 0.8 (0.1) | 12.9 (2.6) |
| | E24H | 4.8 (0.8) | 0.7 (0.2)[a] | 22.3 (6.6)[a] | 14.4 (5.0)[a] | 1.9 (0.4)[a] | 7.6 (1.9)[a] |
| | SE24H | 4.3 (1.3)[b] | 0.4 (0.2)[b,c] | 15.5 (3.7)[b,c] | 18.8 (8.4)[b] | 2.8 (0.9)[b,c] | 7.9 (6.4)[b] |

*HS* healthy sheep ($n = 7$), *E24H* healthy sheep on extracorporeal membrane oxygenation ($n = 7$), *SE24H* sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation ($n = 6$), *AUC* area under the curve, *MRT* mean resident time, $V_{ss}$ steady-state volume of distribution, $C_{max}$ maximum plasma concentration, $C_{min}$ minimum plasma concentration
[a]Statistically significant results for E24H group compared with HS group
[b]Statistically significant results for SE24H group compared with HS group
[c]Statistically significant differences between E24H and SE24H groups

hence the additional influence of critical illness, if at all, in increasing $V_{ss}$ was less apparent. The reasons behind a greater $V_{ss}$ seen in the case of ciprofloxacin in the SE24H group relative to the E24H group is probably a result of decreased CL in the SE24H group and may indicate altered hepatic metabolism. It should be noted that there was no biochemical evidence of any significant hepatic dysfunction in our model. Clinicians should consider circuit sequestration and alterations in hepatic function when prescribing lipophilic antibiotics. In patients with presumably preserved hepatic function, lipophilic antibiotics may have to be prescribed in higher doses. These findings need further validation in clinical PK studies.

Even though protein-bound drugs have previously been shown to be sequestered in ECMO circuits under physiologic conditions [23] with expected increased $V_{ss}$, we observed no significant increase in $V_{ss}$ for these drugs in the present study. However, there was a trend towards increased $V_{ss}$ for protein-bound drugs in the SE24H group. This may have resulted from reduced plasma protein concentrations in the SE24H group. The difference in blood pH between the SE24H and E24H groups was significant and may have affected PB [30, 31] and circuit sequestration. Given that unbound drug concentrations were not measured, further interpretation of these data is not possible. From a general PK perspective, protein-bound drugs are expected to have a relatively lower $V_{ss}$, and during critical illness and ECMO there is a potential for this to increase due to circuit sequestration and other critical illness–induced PK alterations [13]. The net increase in $V_{ss}$ in a critically ill patient on ECMO is therefore challenging to predict on the basis of mechanistic studies alone. Clinical population PK studies are therefore indicated.

Decreases in CL of antimicrobial and other drugs during ECMO have been reported in previous clinical
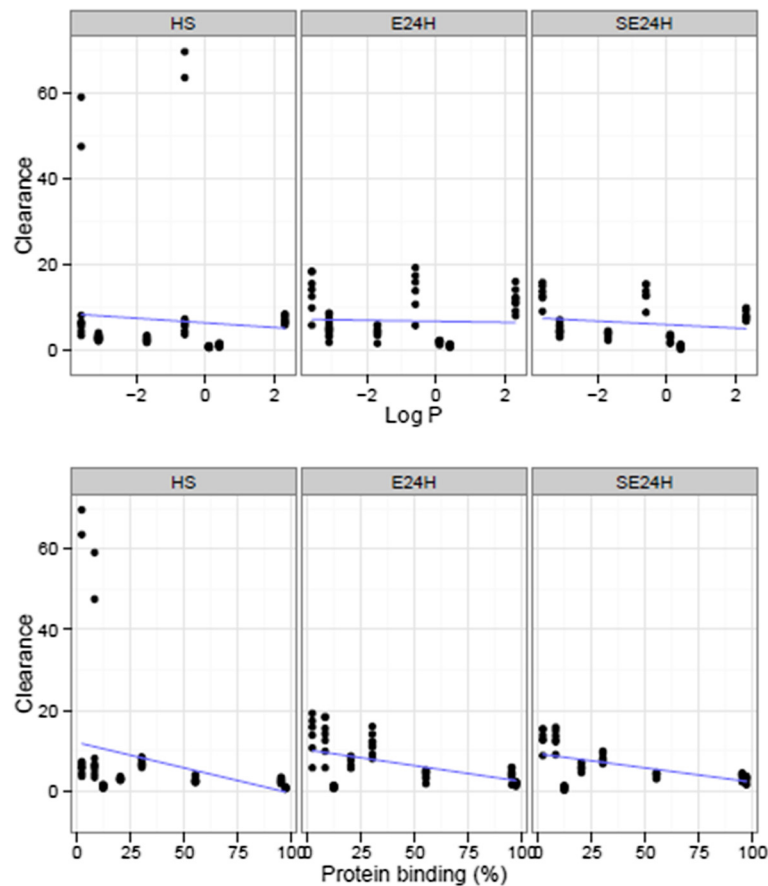
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**Fig. 2** Scatterplots and regression of clearance against octanol-water partition coefficient and protein binding by groups. Healthy sheep (HS, $n = 7$), healthy sheep on extracorporeal membrane oxygenation (E24H, $n = 7$), sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation (SE24H, $n = 6$)

studies [12]. Antimicrobial CL could not be predicted on the basis of logP in the present study, which suggests that the CL for lipophilic drugs may depend largely on critical illness factors and hepatic drug metabolism. Sequestration of drugs in the ECMO circuit by itself is unlikely to play any significant role in reducing CL for lipophilic drugs. However, it should be noted that alterations in hepatic blood flow [32] and hepatic dysfunction may occur in patients before initiation of ECMO or during ECMO (especially during venoarterial ECMO initiated in patients with severe cardiac failure), which may then adversely affect hepatic metabolism of lipophilic drugs and result in decreased CL. The degree of biochemical hepatic derangement in the SE24H group that received venovenous ECMO for predominant respiratory failure may not have been sufficient to influence metabolism of lipophilic drugs significantly.

Even though protein-bound drugs appeared to have more significantly reduced CL in ECMO sheep in the final model, we observed increased CL in both healthy and critically ill sheep on ECMO for relatively more protein-bound drugs (55 % for vancomycin, 95 % for ceftriaxone and 97 % for caspofungin) compared with HS. Interestingly, these three drugs also demonstrated a trend towards an increased $V_{ss}$ during ECMO, especially in the SE24H group. This is an interesting finding, given that protein-bound drugs have been shown to have a greater propensity for sequestration in ECMO circuits in the ex vivo setting. This relative increase in CL and a trend towards an increased $V_{ss}$ for more protein-bound drugs in ECMO sheep may indicate circuit sequestration. Equally, an increase in plasma unbound fraction of these drugs due to heparin displacement [33] may also have contributed to increased CL and $V_{ss}$ for these drugs. Although this increased CL was apparent in our ovine ECMO models with relatively preserved renal function, this may be of less significance in critically ill patients with significant renal dysfunction or those on continuous renal replacement therapy (CRRT). For example, no significant impact of ECMO on vancomycin CL was observed in a recent clinical population PK study by Donadello et al. [18]. The sheep had normal renal function, at least
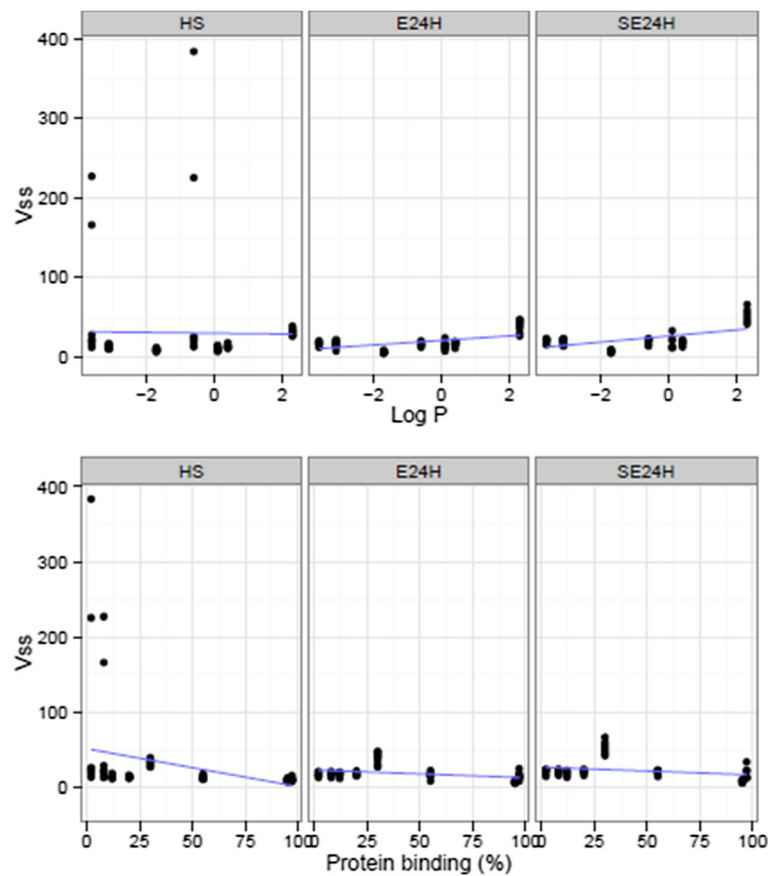
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**Fig. 3** Scatterplots and regression of steady-state volume of distribution ($V_{ss}$) against octanol-water partition coefficient and protein binding by groups. Healthy sheep (HS, $n = 7$), healthy sheep on extracorporeal membrane oxygenation (E24H, $n = 7$), sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation (SE24H, $n = 6$)

**Table 5** Linear regression parameters for predicting PK parameters using drug properties

| Group | Dependent | Independent | Mean | Lower | Upper | p Value |
|---|---|---|---|---|---|---|
| HS | CL | logP | −0.54 | −2.37 | 1.29 | 0.556 |
| E24H | CL | logP | −0.12 | −0.86 | 0.62 | 0.744 |
| SE24H | CL | logP | −0.41 | −1.09 | 0.28 | 0.235 |
| HS | CL | PB | −0.13 | −0.22 | −0.03 | 0.01 |
| E24H | CL | PB | −0.08 | −0.11 | −0.04 | <0.001 |
| SE24H | CL | PB | −0.07 | −0.10 | −0.04 | <0.001 |
| HS | $V_{ss}$ | logP | −0.48 | −8.43 | 7.46 | 0.903 |
| E24H | $V_{ss}$ | logP | 2.85 | 1.73 | 3.97 | <0.001 |
| SE24H | $V_{ss}$ | logP | 3.84 | 2.18 | 5.50 | <0.001 |
| HS | $V_{ss}$ | PB | −0.50 | −0.92 | −0.09 | 0.017 |
| E24H | $V_{ss}$ | PB | −0.10 | −0.17 | −0.04 | 0.004 |
| SE24H | $V_{ss}$ | PB | −0.10 | −0.21 | 0.00 | 0.056 |

*PB* protein binding, *logP* octanol-water partition coefficient (measure of drug lipophilicity)

Separate results for each group are presented for healthy sheep (HS, $n = 7$), healthy sheep on extracorporeal membrane oxygenation (E24H, $n = 7$), sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation (SE24H, $n = 6$) and pharmacokinetic (PK) parameters clearance (CL) and steady-state volume of distribution ($V_{ss}$)

biochemically, as opposed to 7 of 11 patients who received CRRT in the above-mentioned study. This is an important point to note because kidney injury and relatively lower CL for vancomycin achieved on CRRT may have negated an increase in CL during ECMO.

Although ex vivo studies confirm relative stability of vancomycin in ECMO circuits, they do not replicate the in vivo situation. A recent ex vivo study showed that, with drugs with similar PB, lipophilicity becomes the determinant of eventual circuit loss. Vancomycin, although relatively protein-bound (55 %), is hydrophilic. Hence, it is possible that, in the in vivo setting, there is a greater propensity for hydrophilic protein-bound drugs to undergo circuit sequestration. Appropriately powered clinical population PK studies in which investigators compare vancomycin PK in ECMO patients with and without preserved renal function are needed to address this further, and such studies are currently underway [34].

In summary, sequestration of lipophilic antibiotics plays an important role in increasing their $V_{ss}$ during ECMO. CL of lipophilic drugs is largely dependent on hepatic drug metabolism, which can be significantly

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affected in a subgroup of ECMO patients receiving venoarterial ECMO for cardiac failure. Although more protein-bound drugs were found to have relatively higher CL in this study, PB in isolation may not be a reliable predictor of CL. Patients on ECMO may have significant renal dysfunction, which is more likely to influence the net CL than sequestration alone. Overall, ECMO appears to decrease antimicrobial CL. These findings need further validation in clinical studies, and such studies are currently underway [34].

This animal study has limitations. Apart from inherent PK variability that is expected in a small sample, the distribution, metabolism and excretion processes in sheep may differ from those of humans. Despite the SE24H group's development of severe cardiorespiratory failure following S-ALI, the degree of hepatic and renal dysfunction may not have been sufficient to more fully elucidate the full impact of critical illness of PK. However, the changes in PK due to critical illness are very well described, and the use of a model with no advanced end-organ failures that is designed to more fully examine the circuit–drug interactions is justified. Also, this study was directed more at observing relative PK changes between groups and the effects of drug factors logP and PB on antibiotic PK.

## Conclusions

Lipophilic antimicrobial agents are likely to have an increased $V_{ss}$ and decreased CL during ECMO. Protein-bound antibiotics are likely to have reductions in both CL and $V_{ss}$ during ECMO. The strong relationship between logP and $V_{ss}$ during ECMO indicates circuit sequestration of lipophilic drugs. These findings highlight the importance of drug factors in predicting antibiotic drug PK during ECMO and should be a consideration when performing and interpreting population PK studies.

## Key messages

- Sequestration of lipophilic antibiotics results in increased $V_{ss}$ on ECMO.
- Lipophilic drugs exhibit a larger $V_{ss}$ during ECMO, and lipophilicity by itself has little impact on drug CL.
- Protein-bound drugs may have decreased $V_{ss}$ and CL during ECMO.
- Higher doses of lipophilic antibiotics may be indicated in patients with intact hepatic function.
- Lipophilicity and PB are useful drug factors to use in predicting antibiotic PK during ECMO.

## Abbreviations

ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BP: blood pressure; CL: clearance; $C_{max}$: maximum plasma concentration; $C_{min}$: minimum plasma concentration; CCO: continuous cardiac output; CRRT: continuous renal replacement therapy; ctHb: concentration of total blood haemoglobin; CVP: central venous pressure; E24H: healthy sheep on extracorporeal membrane oxygenation; ECLS: extracorporeal life support; ECMO: extracorporeal membrane oxygenation; $FiO_2$: fraction of inspired oxygen; HPLC: high-performance liquid chromatography; HS: healthy sheep; ICE: intra-cardiac echocardiography; ICU: intensive care unit; IJV: internal jugular vein; IV: intravenous; logP: octanol-water partition coefficient; MRT: mean resident time; $paCO_2$: partial pressure of carbon dioxide; $paO_2$: partial pressure of oxygen; PAP: pulmonary arterial pressure; PB: protein binding; PEEP: positive end-expiratory pressure; PK: pharmacokinetic(s); S-ALI: smoke inhalation acute lung injury; SE24H: sheep with smoke inhalation acute lung injury on extracorporeal membrane oxygenation; $SvO_2$: mixed venous oxygen saturation; $V_{ss}$: steady-state volume of distribution; $V_T$: tidal volume.

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# CHAPTER 7
# CLINICAL TRANSLATION

## 7.1 THE COMBINED EFFECTS OF EXTRACORPOREAL MEMBRANE OXYGENATION AND RENAL REPLACEMENT THERAPY ON MEROPENEM PHARAMCOKINETICS: A MATCHED COHORT STUDY.

### 7.1.1 Introduction to this publication

This chapter focuses on the translational aspects of the ECMO PK project. Meropenem PK data was compared between critically ill patients with and without ECMO in order to relatively quantify the clinical magnitude of altered PK. Meropenem was chosen deliberately as it is hydrophilic, less protein bound and renally excreted and therefore is more likely to be significantly affected by critical illness factors such as systemic inflammation and capillary leak. This was also based on published preliminary data that showed major alterations in meropenem PK (not included in this thesis) and risks of under dosing with use of standard doses (1g q 8h). However, the population PK results were surprising and indicated that even standard dosing of meropenem may provide usually targeted meropenem plasma minimum inhibitory concentrations of ≥ 2 mg/L in most patients even when renal functions were preserved and whilst on renal replacement therapy. Using sophisticated dosing simulations, plasma meropenem concentrations in ECMO patients with or without dialysis dependent renal failure were able to be predicted.

**CRITICAL CARE**

# The combined effects of extracorporeal membrane oxygenation and renal replacement therapy on meropenem pharmacokinetics: a matched cohort study

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## Abstract

**Introduction:** The scope of extracorporeal membrane oxygenation (ECMO) is expanding; however, optimal drug prescription during ECMO remains a developing science. Currently, there are no clear guidelines for antibiotic dosing during ECMO. This open-label, descriptive, matched-cohort pharmacokinetics (PK) study aimed to compare the PK of meropenem in ECMO patients to critically ill patients with sepsis not receiving ECMO (controls).

**Methods:** Eleven adult patients on ECMO (venovenous (VV) ECMO, n = 6; venoarterial (VA) ECMO, n = 5) receiving intravenous (IV) meropenem were included. Meropenem plasma concentrations were determined using validated chromatography. Population PK analysis was performed using non-linear mixed effects modelling. This data was compared with previously published meropenem PK data from 10 critically ill adult patients not on ECMO (preserved renal function (n = 5) or receiving renal replacement therapy (RRT) (n = 5). Using these data, we then performed Monte Carlo simulations (n = 1,000) to describe the effect of creatinine clearance on meropenem plasma concentrations.

**Results:** In total, five (two VV, three VA) out of eleven ECMO patients received RRT. The other six patients (four VV, two VA) had no significant impairment in renal function. A two-compartment model adequately described the data. ECMO patients had numerically higher volume of distribution (0.45 ± 0.17 versus 0.41 ± 0.13 L/kg, P = 0.21) and lower clearance compared to controls (7.9 ± 5.9 versus 11.7 ± 6.5 L/h, P = 0.18). Variability in meropenem clearance was correlated with creatinine clearance or the presence of RRT. The observed median trough concentrations in the controls were 4.2 (0.0 to 5.7) mg/L. In ECMO patients, while trough meropenem concentrations >2 mg/L were achieved in all patients, a more aggressive target of >8 mg/L for less susceptible microorganisms was observed in only eight out of eleven patients, with five of them being on RRT.

**Conclusions:** ECMO patients exhibit high PK variability. Decreased meropenem CL on ECMO appears to compensate for ECMO and critical illness-related increases in volume of distribution. Routine target concentrations >2 mg/L are maintained with standard dosing (1 g IV 8-hourly). However, an increase in dose may be necessary when targeting higher concentrations or in patients with elevated creatinine clearance.

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## Introduction

Extracorporeal membrane oxygenation (ECMO) is being increasingly used in adult patients with acute severe cardiorespiratory failure as a supportive therapy [1,2]. Prolonged support for bridge to recovery or transplantation is now possible. While ECMO sustains life, stabilises physiology and allows time for definitive management, little is known about the independent effects of ECMO on antibiotic pharmacokinetics (PK). ECMO is thought to further complicate the PK alterations seen during critical illness [3], which appears to manifest as increased volume of distribution (Vd) and decreased clearance (CL) [4]. *Ex vivo*, animal [5,6] and clinical studies [7] are currently underway to further investigate the PK changes seen during ECMO and to develop evidence-based dosing guidelines. Simulated *ex-vivo* studies that utilised adult circuitry [8] have demonstrated significant antibiotic drug sequestration in the circuit based on physicochemical properties of individual drugs. However, the PK data on antibiotics in critically ill adult patients is limited with available studies indicating significant PK alterations [3,9,10]. This is concerning as the risks of suboptimal drug dosing (both under- and overdosing) are profound in this complex group of patients who have high infection-related mortality.

A significant number of patients receive ECMO for severe cardiac and/or respiratory failure resulting from infectious aetiologies. Patients may develop new infection during ECMO support. Studies indicate that infections occur frequently during ECMO and infections/colonisation with multi-drug-resistant organisms is not uncommon [11-13]. Gram-negative bacteria are responsible for significant proportions of these infections acquired during ECMO [11]. Meropenem is used as an empirical or targeted broad-spectrum antibiotic in this setting. It is a minimally protein-bound and hydrophilic drug that undergoes significant sequestration/degradation in *ex vivo* ECMO circuit models [8]. In this setting, one would anticipate profound alterations in meropenem PK in patients on ECMO, although to date, we are unaware of any studies to guide meropenem dosing in adults on ECMO.

This open-label, descriptive, matched-cohort PK study aimed to describe single-dose meropenem PK during ECMO using critically ill patients with sepsis and not receiving ECMO as controls.

## Materials and methods

### Participants and data collection

This study was conducted at a 650-bed university-affiliated tertiary referral hospital. The ICU is a 27-bed mixed ICU with a predominantly cardio-thoracic cohort. There is an antibiotic stewardship programme with twice weekly ward rounds by an infectious diseases physician. Infection control practices include review of all healthcare-associated bacteraemia and multiple-resistant organism screening.

Ethics approval was obtained from the Prince Charles Hospital Ethics Committee, Brisbane, QLD, Australia (HREC/11/QPCH/121). Informed consent was obtained from the study participants or surrogate decision makers as applicable. The study protocol has been published and detailed methodology, inclusion and exclusion may be found elsewhere [7]. Eligible patients ≥18 years of age and receiving meropenem during their ECMO therapy were recruited. Known allergy to study drug, pregnancy, serum bilurubin concentration >150 μmol/L, ongoing massive blood transfusion requirement (>50% blood volume transfused in the previous 8 hours) and therapeutic plasma exchange in the preceding 24 hours were exclusion criteria. Data related to patient demographics, renal and hepatic function, details of ECMO and renal replacement therapy (RRT) were collected.

### Details of ECMO and RRT support

Patients received either venovenous (VV) or peripheral venoarterial (VA) ECMO as clinically indicated. The standardised ECMO circuitry comprised of Bioline tubing, Quadrox D oxygenator and a centrifugal pump (Jostra Medizintechnik AG, Hirrlingen, Germany). The prime volume was 668 mL and the circuits were freshly primed with Plasmalyte 148 (Baxter, Sydney, NSW, Australia) followed by Albumex 4% (human albumin, 40 g/L; CSL Bioplasma, Melbourne, VIC, Australia). RRT was provided as extended daily diafiltration (EDD-f) to ECMO patients using a Fresenius haemodialysis machine (4008 s ARrT plus, Fresenius Medical Care, Bad Homburg, Germany) that was connected to the post-oxygenator site of the ECMO circuit using Fresenius AV600S filters. The blood flow (200 to 300 mL/min) and dialysate flow rates (200 mL/min) and duration were standardised (6 to 8 hours).

Continuous venovenous haemofiltration (CVVHF) was performed in the control group RRT patients [14] using the Nephral ST500 (AN69 hollow-fibre) filter with a surface area of 2.15 m$^2$. All patients were initiated on the CVVHF at least 8 hours prior to the sampling period. The ultrafiltrate rate was set between 66 and 100 mL/min, with a target blood flow rate of 250 mL/min.

### Controls

Previously published meropenem PK data were used for the historical controls (n = 10). Five patients with sepsis and no renal dysfunction receiving intermittent infusions of meropenem were included [15] from one study. The remaining five patients were the first five recruited to a PK study (n = 10) examining meropenem PK in high-volume continuous RRT [14].

### Meropenem dosing and measurements

Meropenem dosing in ECMO patients was at the discretion of the clinician, based on the clinical context and

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unit guidelines. The following meropenem doses were administered prior to PK sampling in the ECMO patients; 1 g intravenous (IV) bolus and 1 g IV q8h (n = 8), 1.5 g IV bolus and 1 g IV q8h (n = 2), 2 g IV bolus and 1 g IV q8h (n = 1). None of the RRT-dependent patients received an additional dose post RRT. Doses were reconstituted in 10 mL of diluent and given as IV bolus infusion in 50 mL over 30 minutes. The control patients [15] with preserved renal function (*n* = 5) were given a 1.5 g meropenem first dose (in 10 mL of water-for-injection infused by central line over 5 minutes) and then 1 g (in 10 mL of water-for-injection infused by central line over 3 minutes) every 8 hours. Controls with impaired renal function on high-volume CVVHF [14] received meropenem as 1 g (in 20 mL of water-for-injection infused by central line over 3 minutes) every 8 hours.

Blood sampling in ECMO patients was undertaken at predose, 15, 30, 45, 60, 120, 180, 360 and 480 minutes. In controls with preserved renal function [15], samples were collected at predose, 3, 5, 7, 10 15, 20, 30, 45, 60, 90, 150, 240, 360 and 480 minutes. In controls on CVVHF [14], sampling was performed at predose, and at 15, 30, 45, 60, 120, 240, and 480 minutes. All samples were immediately refrigerated at 4°C, and plasma was separated and frozen at 80°C within 24 hours of sample collection. The blood samples were centrifuged at 3,000 rpm for 10 minutes.

Meropenem analysis was conducted on a Shimadzu Prominence high-performance liquid chromatography (HPLC) system (Shimadzu Corp, Kyoto, Japan) with a Waters XBridge C18 column stationary phase (Waters Corp, Milford, MA, USA). The mobile phase was 4% acetonitrile/96% phosphate buffer 50 mM at pH 2.5 and the eluent was measured by UV at 304 nm. The internal standard for the HPLC assay was ertapenem. HPLC assays had inter- and intra-day reproducibility of 5.6% and 0.6%, respectively. The limit of quantification for meropenem was 1.0 mg/L and the coefficient of correlation for the assay was 1.000.

### Population pharmacokinetic analysis

The concentration-time data for meropenem in plasma were fitted using a non-linear mixed-effects modeling approach (NONMEM version 7.3, Globomax LLC, Hanover, MD, USA) [30]. A Digital Fortran compiler was used and the runs were executed using Wings for NONMEM [16]. Data were analysed using the first-order conditional estimation method with interaction (ADVAN3). Between-subject variability (BSV) was calculated using an exponential variability model and was assumed to follow a log-normal distribution. Residual unexplained variability (RUV) was modeled using a combined exponential and additive random error model. Visual inspection of

diagnostic scatter plots and the NONMEM objective function value (OFV) were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program on the basis of a $\chi2$ test of the difference in OFV. A decrease in the OFV of 3.84 units ($P$ <0.05) was considered statistically significant. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model. Specifically, we calculated central volume of distribution (Vc), peripheral volume of distribution (Vp), total indexed volume of distribution (Vd), inter-compartmental clearance (Q) and meropenem CL using NONMEM.

### Population pharmacokinetic model diagnostics

Visual inspection of diagnostic scatter plots and the NONMEM OFV were used to evaluate goodness of fit. Statistical comparison of nested models was undertaken in the NONMEM program using log-likelihood ratios, which are assumed to be chi-square distributed. On the basis of a $\chi2$ test of the difference in OFV, a decrease in the OFV of 3.84 units ($P$ <0.05) for one degree of freedom was considered statistically significant. Decreases in BSV of one of the parameters of at least 10% were also accepted for inclusion of a more complicated model.

### Population pharmacokinetic covariate screening

Covariate model building was performed in a stepwise fashion with forward inclusion and backward deletion based upon the aforementioned model selection criteria. Age, sex, weight, serum creatinine concentration, Cockroft-Gault-calculated creatinine clearance (CrCL) as well as presence of ECMO and RRT were evaluated as covariates.

### Population pharmacokinetic bootstrap

A non-parametric bootstrap method (n = 1,000) was used to study the uncertainty of the pharmacokinetic parameter estimates in the final model. From the bootstrap empirical posterior distribution, we have been able to obtain the 95% confidence interval (2.5 to 97.5% percentile) for the parameters, as described previously [17].

### Dosing simulations

We performed Monte Carlo simulations (n = 1,000) to describe the effect of five different CrCL on meropenem concentrations in a 50-year-old, 80 kg male receiving ECMO. The CrCL simulated were at 20, 50, 80, 120 and 180 mL/min. We simulated the following doses 1 g IV 8-hourly, 500 mg IV 8-hourly and 2 g IV 8-hourly. While interpreting the simulations, a trough meropenem concentration of 2 mg/L and 8 mg/L was considered optimal for treating susceptible and less susceptible pathogens, respectively [18].

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## Statistical analysis

Statistical analyses were performed using the SPSS 13.0 for Windows NT software package (SPSS Inc., Chicago, IL, USA, 2004). Discrete variables were expressed as counts (percentage) and continuous variables as means ± standard deviation (SD) or median (25th to 75th percentiles). Demographics and clinical differences between study groups were assessed using a chi-square, Fisher's exact test, Student's *t* test, or Mann-Whitney *U* test, as appropriate. A *P* <0.05 was considered to be statistically significant.

## Results

Five (two VV, three VA) out of eleven ECMO patients received RRT. The other six patients (four VV, two VA) had no significant impairment in renal and hepatic functions, based on routine biochemical parameters. The indications for ECMO included pneumonia, septic shock (n = 7); cardiogenic shock (n = 2); sickle-cell crisis (n = 1); primary graft dysfunction post lung transplant (n = 1). The median sequential organ failure assessment scores were not significantly different between the controls and ECMO patients (7 [3-15] vs. 13 [9-15,17,18], respectively, *P* = 0.14). The demographic and clinical data are summarised in Table 1. Median time to PK sampling in ECMO patients was 2 days (1 to 7).

## Meropenem concentrations and PK parameters in controls and ECMO patients

The median observed peak concentrations ($C_{max}$) and trough meropenem concentrations ($C_{min}$) in controls were 65.4 (58.7 to 74.4) mg/L and 4.2 (0.0 to 5.7) mg/L respectively. The ECMO group achieved a median $C_{max}$ of 55.3 (37.8 to 60.4) mg/L and a $C_{min}$ of 7.2 (4.0 to 17.2) mg/L; 10 out of 11 ECMO patients maintained a $C_{min}$ >2 mg/L between doses. ECMO patients had a numerically higher, but non-statistically significant volume of distribution (0.45 ± 0.17 vs. 0.41 ± 0.13 L/kg, *P* = 0.21) and lower clearance compared to controls (7.9 ± 5.9 vs. 11.7 ± 6.5 L/h, *P* = 0.18). In ECMO patients, while trough meropenem concentrations of >2 mg/L were achieved in all patients, a more aggressive strategy of >8 mg/L targeting less susceptible microorganisms was maintained only in eight out of eleven patients, with five of them being on RRT.

## Pharmacokinetic model building

The time course of plasma meropenem concentrations was best described by a two-compartment linear model with combined residual error and BSV on Vc, Vp and CL. This model included zero order input of drug into the central compartment. The mean parameter estimates from the final covariate model as well as the 95% confidence intervals from all bootstrap runs are shown in Table 2. The goodness-of-fit plots are shown in Figure 1.

After screening all relevant biologically plausible covariates, the following covariates were included in the final model. RRT was included for CL with Cockroft-Gault CrCL for CL in patients not receiving RRT. When

### Table 1 Demography and severity of illness data

| | Controls | | ECMO | |
| --- | --- | --- | --- | --- |
| | No RRT | RRT | No RRT | RRT |
| | (n = 5) | (n = 5) | (n = 6) | (n = 5) |
| Male/Female | 3/2 | 3/2 | 1/5 | 3/2 |
| Age (years) | 55.0 (48–61) | 56 (46–66) | 29 (16–46) | 38 (23–56) |
| Total body weight (kg) | 80 (75–85) | 70 (60–100) | 69 (60–80) | 70 (70–76) |
| Mechanical ventilation | 5/5 | 5/5 | 5/5 | 5/5 |
| Type of ECMO (VA/VV) | 0/0 | 0/0 | 3/3 | 2/3 |
| Day 1 SOFA score | 3 (3–4) | 15 (14–16) | 9 (7–14) | 16 (13–17) |
| Plasma creatinine concentration (μmol/L) | 73 (55–101) | na[*] | 75 (44–82) | na[*] |
| Creatinine clearance (mL/min) | 106 (98–127) | na[*] | 108 (65–183) | na[*] |
| RRT mode | - | CVVH | - | EDD-f |
| Serum bilirubin (μmol/L) | 9 (5–23) | 93 (36–115) | 23 (9–73) | 58 (34–134) |
| Serum albumin (g/L) | 22 (18–36) | 26 (23–36) | 31 (27–35) | 24 (22–32) |
| Serum proteins (g/L) | 56 (55–70) | 62 (60–65) | 49 (46–54) | 44 (34–56) |
| Meropenem daily dose (g) | 1 q 8 h | 1 q 8 h | 1 q 8 h | 1q 8 h |
| Plasma C max (mg/L) | 93 (74–119) | 58 (52–68) | 42 (27–56) | 59 (50–86) |
| Plasma C min (mg/L) | 0 (0–2) | 7.5 (5–18) | 4.9 (2–10) | 18 (7–43) |

[*]Patients RRT dependent. The biochemical indices were measured on day of pharmacokinetic sampling. Data are presented as median (IQR). ECMO, extracorporeal membrane oxygenation; RRT, renal replacement therapy; VA, venoarterial; VV, venovenous; SOFA, sequential organ failure assessment; CVVH, continuous venovenous haemofiltration; EDD-f, extended daily diafiltration.

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**Table 2 Mean parameter estimates and bootstrap mean (95% confidence interval) estimates for the final covariate model**

| Parameter | Model | Bootstrap | |
|---|---|---|---|
| | Mean | Mean | 95% confidence interval |
| | | | 2.5% | 97.5% |
| Fixed effects | | | | |
| CL (L/h) | 5.1 | 5.4 | 3.7 | 7.4 |
| Vc (L) | 18.7 | 18.2 | 13.0 | 21.0 |
| Vp (L) | 13.2 | 13.6 | 11.3 | 15.9 |
| Q (L/h) | 21.0 | 24.2 | 12.8 | 37.0 |
| CL$_{CRCL}$ | 1.89 | 1.85 | 0.99 | 2.65 |
| Random effects BSV (% CV) | | | | |
| CL (L/h) | 51.6 | 52.2 | 37.9 | 66.6 |
| Vc (L) | 45.8 | 48.4 | 32.1 | 69.9 |
| Vp (L) | 28.7 | 16.2 | 0.2 | 42.1 |
| Random error | | | | |
| RUV (% CV) | 13.7 | 13.3 | 10.1 | 17.6 |
| RUV (SD, mg/L) | 2.3 | 1.87 | 1.02 | 2.70 |

CL, clearance; Vc, volume of distribution of the central compartment; Vp, volume of distribution of the peripheral compartment; Q, inter-compartmental clearance; CL$_{CRCL}$, the fractional effect of CrCL on CL for patients not receiving RRT; BSV, between-subject variability; RUV, residual unexplained variability.
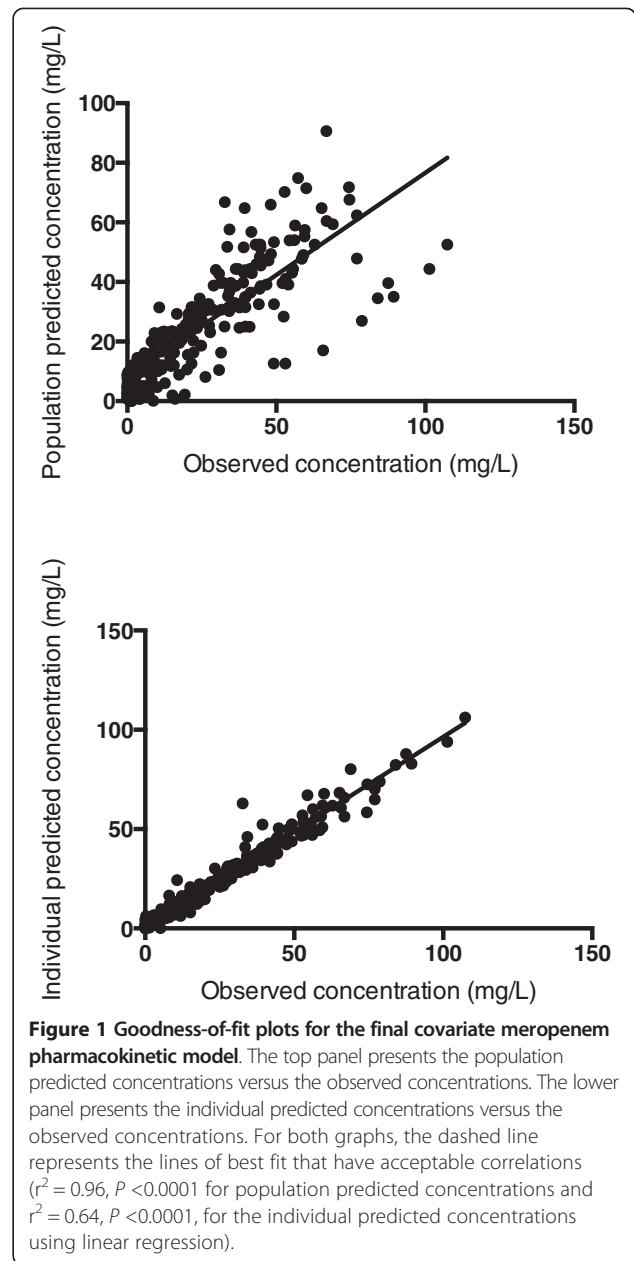
each covariate was sequentially added to the parameters, the OFV reduced statistically significantly ($P <0.05$) and the goodness-of-fit plots improved. Inclusion of ECMO as a covariate on any parameter did not improve the goodness of fit of the model nor was it statistically significant. The final covariate model for the two-compartment meropenem model was represented by the following equation:

$$\text{TVCL} = \theta_1 \cdot (\text{CL}_{RRT}) + \theta_1 \cdot (\text{CL}_{NORRT} * \text{CrCL})$$

Where TVCL is the typical value of meropenem clearance where CL$_{RRT}$ is 0 for patients not receiving RRT and CL$_{NORRT}$ is 0 for patients receiving RRT. CrCL is Cockroft-Gault-calculated creatinine clearance and $\theta_1$ is the typical population value for clearance.

## Dosing simulations

Figure 2 (a-c) shows the mean concentration-time curves for the 1,000 simulated patients for each dose and CrCL and highlights the importance of CrCL in meropenem dosing. Table 3 reports the mean and 10th percentile trough concentrations for the simulated regimens. This table demonstrates the wide PK variability present in the studied patients as evidenced by the profound difference in the values described. From this data, patients with the following CrCL should receive the corresponding doses to ensure 90% of patients maintain concentrations above 2 mg/L throughout the entire dosing interval, 20 to



**Figure 1 Goodness-of-fit plots for the final covariate meropenem pharmacokinetic model**. The top panel presents the population predicted concentrations versus the observed concentrations. The lower panel presents the individual predicted concentrations versus the observed concentrations. For both graphs, the dashed line represents the lines of best fit that have acceptable correlations ($r^2 = 0.96$, $P <0.0001$ for population predicted concentrations and $r^2 = 0.64$, $P <0.0001$, for the individual predicted concentrations using linear regression).

50 mL/min - 500 mg 8-hourly, 80 to 180 mL/min - 1 g 8-hourly, >180 mL/min - 2 g 8-hourly.

## Discussion

This study provides preliminary evidence that standard meropenem dosing (1 g IV 8-hourly) as an intermittent bolus infusion in ECMO patients is likely to result in drug concentrations sufficient to treat highly susceptible Gram-negative pathogens. Conventional-dose meropenem should achieve a time over minimal inhibitory concentration (T$_{>MIC}$) of 100%, assuming a minimum inhibitory concentration (MIC) of 2 mg/L (the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for

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**Figure 2 Simulated mean meropenem logarithmic concentrations in a critically ill patient on ECMO with CrCL of 20, 50, 80, 120 and 180 mL/min for (a) 500 mg IV 8-hourly, (b) 1 g IV 8-hourly and (c) 2 g IV 8-hourly.** CrCL, creatinine clearance; ECMO, extracorporeal membrane oxygenation.

**Table 3 The effect of changing creatinine clearance on mean (50th percentile) and 10th percentile trough concentrations from the simulated ECMO patients (n = 1,000) receiving various meropenem doses**

| Creatinine clearance | Concentration percentile | Dose | | |
| --- | --- | --- | --- | --- |
| | | 500 mg 8-hrly | 1 g 8-hrly | 2 g 8-hrly |
| 20 | 50th | 18.9 | 26.4 | 76.4 |
| | 10th | 3.6 | 16.2 | 14.6 |
| 50 | 50th | 14.5 | 19.7 | 58.8 |
| | 10th | 2.6 | 9.8 | 10.5 |
| 80 | 50th | 10.0 | 14.8 | 39.7 |
| | 10th | 1.3 | 4.8 | 5.1 |
| 120 | 50th | 7.6 | 11.1 | 30.0 |
| | 10th | 0.7 | 2.5 | 2.7 |
| 180 | 50th | 5.6 | 7.9 | 21.7 |
| | 10th | 0.4 | 0.7 | 0.9 |

These simulations assume that no significant accumulation of meropenem occurred in study population. ECMO, extracorporeal membrane oxygenation.

*Pseudomonas aeruginosa*). However, when treating less susceptible *P. aeruginosa* ($MIC_{90}$ 8 mg/L) and *Acinetobacter* species ($MIC_{90}$ 16 mg/L) higher meropenem doses would have to be considered especially in patients with elevated CrCL. Given that, patients on ECMO have decreased CL in most cases [3], standard dosing is likely to achieve target plasma concentrations in most patients. This is important considering the potential clinical and bacteriological benefits of maintaining 100% $T_{>MIC}$ in critically ill patients [19].

This study uses meropenem plasma concentration data from four different patient populations to perform robust dosing simulations and to provide preliminary insights into the incremental effects of critical illness, ECMO and RRT on meropenem PK. The plasma concentrations observed in ECMO patients reflect a balance between the independent alterations in Vd and CL that occur in the presence of critical illness [20], organ failures and ECMO [3]. Interestingly in this study, routinely targeted meropenem plasma concentrations (>2 mg/L) were maintained with standard dosing, both in ECMO patients on RRT and those with preserved renal function. However, plasma meropenem concentrations were significantly higher in the RRT group when compared to patients with preserved renal function. This is important as standard dose adjustments for renal impairment (for example IV 500 mg 8-hourly or 1 g 12-hourly) in these patients receiving RRT may potentially result in under dosing. Equally, use of higher than standard doses may precipitate the risk of toxicity. Therapeutic drug monitoring where available may further improve the safety and efficacy of meropenem dosing during ECMO [4,21].

ECMO patients demonstrated reduced meropenem CL and an increased Vd when compared with controls, but these changes were not statistically significant. This trend is consistent with the PK changes expected during ECMO based on available literature [3]. An increase in Vd resulting from critical illness [20] and sequestration in the ECMO circuit [8] can significantly affect plasma concentrations probably of meropenem, a hydrophilic with limited protein binding and predominant renal CL. Equally, AKI is common in patients on ECMO, with incidence as high as 70% to 85% in single-centre studies [22]. The Extracorporeal Life Support Organisation (ELSO) Registry data [23] suggests that up to 46% of patients on VV ECMO and 44% on VA ECMO may require some form of RRT during the ECMO run. There is significant variability in mode of RRT used in ECMO patients and this may appear to limit the generalisability

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of our results. This to an extent has been overcome with our dosing simulations that account for a range (20 to 180 mL/min) of net CrCL (native and RRT) achieved in ECMO patients However, dosing may not be entirely based on CL achieved during RRT and possible residual renal CL or extra renal CL may have to be considered. This is of high relevance during ECMO as meropenem can undergo significant degradation/sequestration during their transit through the ECMO circuit. Although upregulated non-renal elimination is possible for ciprofloxacin in renally impaired patients [24], there is no data to support this in the case of meropenem.

In this study the estimated median meropenem CL seen in controls on CVVHF was 3.5 (3 to 4) L/h. There is no reliable data on meropenem CL during EDD-f even in non-ECMO patients and it is highly likely that this will be greater than seen with high-volume CVVHF. Despite RRT partially compensating for decreased drug CL in patients with ECMO and acute kidney injury (AKI), they maintained significantly higher meropenem concentrations during the entire dosing interval with standard dosing when compared with patients without RRT and the controls. Our simulations confirm that a meropenem dose of 500 mg - 1 g 8-hourly will provide a plasma concentration >2 mg/mL in 90% of the patients with CrCL ranging from 20 to 180 mL/min. Given the relatively wide therapeutic index of meropenem, highly variable CrCL between ECMO patients based on modality and intensity of RRT used, loss in the ECMO circuit and preponderance of less susceptible organisms in this population especially with prolonged ECMO support, a dose of 1 g 8-hourly may be considered appropriate till more PK data becomes available. Meropenem accumulation and under dosing are still potential concerns in patients with extremes of CL and these high-risk groups need to be specifically addressed in future PK studies in this population.

The findings of this study contradict the available sparse data pertaining to meropenem PK during ECMO. To our knowledge, there are no previously published PK studies in neonatal or adult patients on ECMO. A recent case report [9] indicated heightened meropenem CL (20.8 L/h) and Vd (0.56 L/kg) during ECMO and RRT and a high-dose meropenem infusion was utilised to maintain optimal concentrations. However, the CL for meropenem in the current study was significantly lower $(7.3 \pm 5.6$ L/h) and the Vd was highly comparable $(0.53 \pm 0.17$ L/kg). However, it should be noted that meropenem is unstable at 37°C and ongoing exteriorization of blood during ECMO may lead to a degree of spontaneous degradation, which can be erroneously interpreted as increased CL. While it is challenging to arrive at any strong conclusions based on these data, it appears that eventual success of meropenem regimens during ECMO

may rely more on the CL that occurs in an individual patient.

There is emerging data to suggest that the commonly used dose of meropenem (1 g 8-hourly) may be sufficient to treat an unselected population of septic critically ill patients not receiving ECMO or RRT [25]. In this setting, the risk of under dosing with 1 g 8-hourly dosing in critically ill patients on ECMO and with preserved renal function appears small and augmented renal clearance [26] has not yet been described in this population. However, patients on peripheral VA ECMO in whom oxygenated blood is being returned into iliac artery or distal aorta may experience very high non-pulsatile renal blood flows and whether this result in heightened CL in patients with preserved renal function needs further evaluation.

The current study has limitations. Characterizing altered PK in patients receiving RRT while on ECMO can be complex. Variability in the techniques used for RRT and ECMO is a significant limitation in the generalisability of our results. Future studies should further investigate the effects of type and intensity of RRT chosen on meropenem PK during ECMO. Despite the ECMO population being small and heterogeneous, our model could accurately predict drug concentrations in ECMO patients and controls and discriminated well for RRT. This study does not address the pharmacodynamic aspects of meropenem therapy and no meaningful clinical outcome measures can be generated from the small sample. The non-ECMO patients selected were considered to be optimal controls for patients on ECMO who exhibited systemic inflammatory syndrome with or without clear evidence of infection. Systemic inflammation is known to significantly affect volume of distribution of the hydrophilic and renally excreted drugs such as meropenem [20]. Hepatic and renal function, however, were not matched as a clear separation between controls and ECMO patients who had preserved renal function and ECMO patients who were dialysis dependent, desirable in the context of this study, which sought to highlight the influence of inflammation and illness, ECMO and RRT.

## Conclusions

In patients receiving meropenem on ECMO, standard dosing (1 g 8-hourly) should achieve routinely targeted plasma concentrations. However, an increase in dose may be necessary when targeting higher plasma concentrations (>4x MIC and 100% $T_{>MIC}$) and or in patients with elevated creatinine clearance. Future PK studies should validate these findings especially in ECMO patients with extremes of CrCL (<20 or >150 mL/min). Therapeutic drug monitoring where possible is recommended until robust dosing guidelines become available.

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## Key messages

- ECMO patients exhibit high PK variability.
- Standard meropenem dosing (1 g IV 8-hourly) during ECMO achieved usual target trough concentrations of >2 mg/L both in patients with preserved renal function and in those on RRT.
- Standard meropenem dosing (1 g IV 8-hourly) during ECMO may not achieve higher target MICs (>8 mg/L), especially in patients with preserved renal function.
- Clinicians need to consider the presence of ECMO, renal function or RRT and microbiological characteristics when choosing doses for patients
- Therapeutic drug monitoring (TDM) is recommended where possible

### Abbreviations

AKI: acute kidney injury; BSV: between-subject variability; CL: clearance; CrCL: creatinine clearance; CVVHF: continuous venovenous haemofiltration; ECMO: extracorporeal membrane oxygenation; EDD-f: extended daily diafiltration; HPLC: high-performance liquid chromatography; IV: intravenous; MIC: minimum inhibitory concentration; OFV: objective function value; PK: pharmacokinetics; Q: inter-compartmental clearance; RRT: renal replacement therapy; RUV: residual unexplained variability; TDM: therapeutic drug monitoring; VA: venoarterial; Vc: central volume of distribution; Vd: volume of distribution; Vp: peripheral volume of distribution; VV: venovenous.

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# CHAPTER 8

# CONCLUDING DISCUSSION AND FUTURE RESEARCH

## 8.1. KEY FINDINGS

- Significant PK alterations may occur during ECMO in adult patients even when modern technology is used as evidenced by suboptimal plasma antibiotic concentrations and increased sedative requirements in adult patients on ECMO.

- Escalating sedative doses in ECMO patients are associated with decrements in plasma sedative drug concentrations and an increase in BIS.

- An incremental research plan as utilised in this research constitutes a rational, holistic research approach to a complex problem.

- Drug factors stability, lipophilicty and protein binding are the key determinants of drug loss in ECMO circuits.

- When multiple drugs with similar degrees of protein binding are administered, circuit drug loss is determined by degree of lipophilicity and vice versa.

- For the relatively lipophilic, protein bound drugs such as midazolam; the effects of ECMO on PK may be more significant than those induced by critical illness depending on the extent of organ failure present in the patient.

- Drug properties lipophilicty and protein binding can be used to predict antimicrobial PK during ECMO.

- For hydrophilic and less protein bound drugs such as meropenem, an increase in Vd appears to be offset by a decreased CL in the presence of ECMO resulting in usual target minimum inhibitory concentrations (≥ 2 mg/L) aimed at less resistant organisms.

- Given that lipophilic and protein bound drugs are most likely to be affected by both the presence of ECMO circuit and critical illness itself, clinical population PK studies should prioritise these drugs to obtain vital PK data and inform dosing.

- Future antibiotic and sedative dosing approaches in ECMO patients should consider drug factors, circuit–drug interactions, disease factors and available data from early clinical population PK studies until robust dosing guidelines become available.

- Population PK studies and dosing simulations will remain a vital tool until TDM is readily available

- ECMO is also likely to alter host pathophysiology significantly and the PD consequences of which are poorly understood and remain a subject for future research.

## 8.2 IMPLICATIONS OF THIS PROJECT ON CLINICAL PRACTICE

This research originated at the patient bedside and the findings are imminently translatable as and when clinical population PK data becomes available for the study drugs. The clinical data will be further complimented by the mechanistic data and will facilitate the development of dosing guidelines for antibiotic and sedative drugs during ECMO. The guideline development process and possible recommendations are outlined in the subsequent sections. Optimal dosing of the study drugs in ECMO patients has the potential to improve patient outcomes both in terms of survival and long term quality of life. Optimal sedation and development of sedation protocols can minimise sedative use and reduce short and long term morbidity. Optimal antibiotic dosing will not only improve outcomes in patients but may also reduce the burden of antimicrobial resistance and benefit society in general. The dosing recommendations will need further prospective validation in future clinical studies. Most importantly, clinicians will now know what they are dealing with when it comes to altered PK during ECMO in adult patients.

## 8.3 CONCLUDING SUMMARY

This research originated from a real world clinical problem. Given the complex interactions between drug, device and disease factors that may affect PK during ECMO, a comprehensive research plan outlined in the thesis was the only logical way to deconstruct the key determinants of altered PK. Based on this research, the ability to predict PK based on lipophilicity and protein binding characteristics of drugs can be a significant step forward. This will help identifying drugs that are most affected by ECMO and prioritise them for future population PK studies. Until robust dosing guidelines become available, clinicians may use the physicochemical profile of antibiotic and sedative drugs and available data on antibiotic drug dosing in critically ill as a guide when dosing patients on ECMO.

## 8.4 FUTURE DIRECTIONS

### 8.4.1 Completion of clinical PK study

The multi-centre study ASAP ECMO is currently recruiting patients and recruitment is nearing completion for some study drugs such as ceftriaxone, vancomycin. The samples will be analysed for plasma drug concentrations sin batches and PK data upon analysis will be published prospectively. More ECMO centres have expressed interest in this study, which will further assist with patient recruitment.

### 8.4.1 Development of dosing guidelines

Although necessary collaborations are in place to ensure robust dosing guidelines for antibiotic and sedative drug dosing in adults patients on ECMO are developed in a timely manner, population PK data for all drugs will only be available at the end of the clinical study. In the meantime clinicians will be guided by ongoing publication of PK data for individual drugs. A systems approach outlined by the NHMRC will serve as a useful template for guideline development. A schematic presentation of guideline development process is provided in Figure 9.

The members of this research team are ECMO and PK experts and play key roles within International ECMO Network (www.internationalecmonetwork.org), Extracorporeal Life Support Organisation, Australia New Zealand Intensive Care Society, European Society of Intensive Care Medicine and European Society of Clinical Microbiology and Infectious Diseases. This will further facilitate guideline development and translation process.

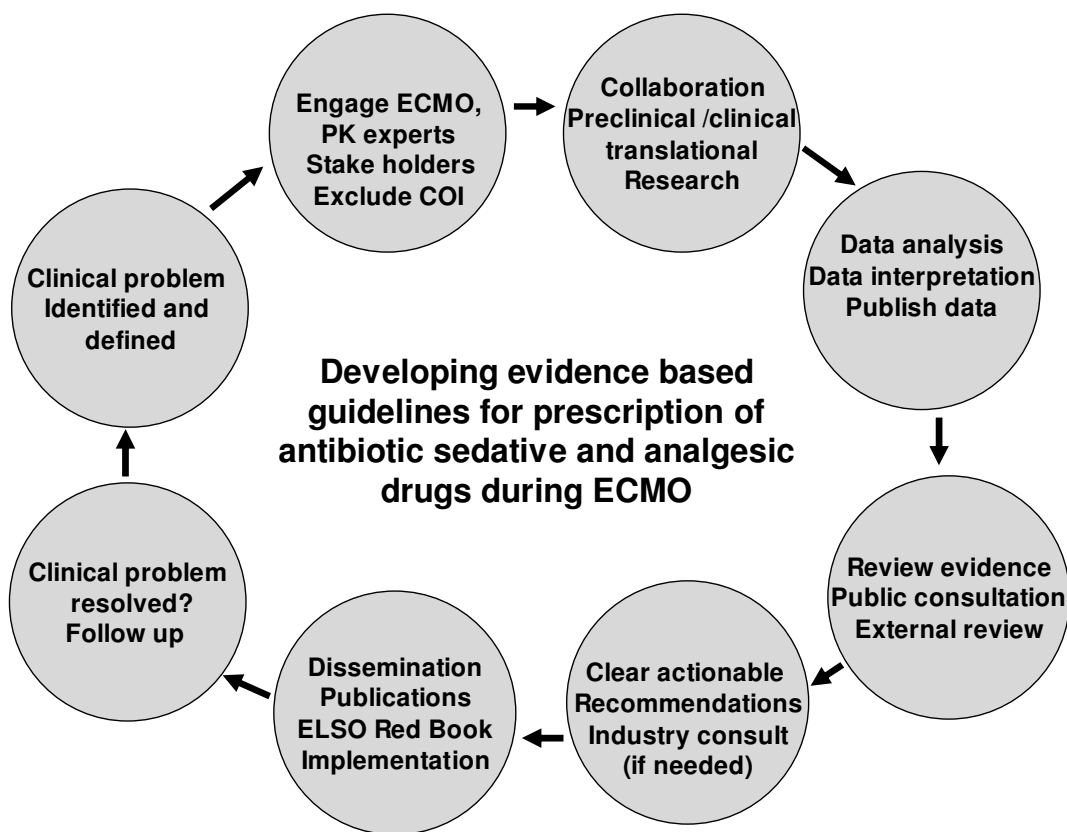It is anticipated the recommendations will pertain to:



**Figure 3. Guideline development process**

### 8.4.1.1 Choice of drug:

Physicochemical properties of drugs along with patient and microbiological factors are key factors when prescribing for patients on ECMO. For example, midazolam and fentanyl may not be ideal for ECMO patients as they are highly sequestered in the circuit.

### 8.4.1.2 Dose, mode of administration (bolus vs. infusions) and dosing intervals:

Adjusting the method of administration can minimise the effect of PK variability and hence will be considered during our guideline development. For example, preliminary data[8] shows that in a patient supported with venovenous ECMO and RRT, meropenem administered as a continuous infusion resulted in target concentrations (~8 mg/L). However, subsequent population study (Chapter 7) found that meropenem in standard doses should achieve usual target MIC. Thus population PK data, dosing simulations and Bayesian dosing approaches (including adaptive feedback control algorithms) will increase the likelihood of successful drug therapy.

### 8.4.1.3 Dose adjustments for RRT during ECMO:

No drug dosing guidelines during RRT and ECMO exist. As described in (Chapter 7) Standard doses of meropenem achieved acceptable plasma meropenem concentrations even in ECMO patients receiving RRT and dose adjustments during RRT are probably not required. These findings can be further substantiated when population Pk data becomes available fro all study antibiotics.

### 8.4.1.4 Development of ECMO specific sedation protocols:

Preliminary data[9,11](Fig 2) highlights that the sedation protocols designed for general critically ill patients are not ideal for patients on ECMO due to significantly altered PK of sedative and analgesic drugs. The knowledge gained from future research will assist development of specific sedation protocols for patients on ECMO

## 8.4.2 Refinements in ECMO circuitry

ECMO circuits have evolved significantly; however they continue to sequester vital drugs. It is important to acknowledge that drug sequestration is not the predominant issue with circuits. Bleeding and thrombosis remain major circuit related complications and optimising anticoagulation in these patients can be a difficult balancing act. Technological advancements should be more directed at these aspects first. Exteriorisation of blood onto foreign surfaces comes at a physiologic cost and it may be nearly impossible to completely overcome this. However, research into engineering aspects of circuit design such as

choice of materials used, coating, flow dynamics etc are areas where there is potential to minimise drug sequestration in ECMO circuit.

### 8.4.3 Drug development

Altering drug physicochemistry just to minimise circuit sequestration is not without challenges. Even minor modifications to physicochemical properties of drugs can result in major alterations in safety and efficacy of that drug. For example, a less Lipophilic and protein –bound sedative drug may be ideal for ECMO patients based on the findings in this thesis. However, a hydrophilic drug may not penetrate the blood-brain barrier as effectively as a lipophilic drug and most available sedative agents are lipophilic and protein-bound. Thus, it appears that altering chemistry of currently available drugs in order to minimise circuit sequestration is probably not practical. However, as newer drugs become available, the findings of this research can be applied to those drugs to determine their suitability for use during ECMO.

### 8.4.4 Investigating PD during ECMO

This research focuses predominantly on the PK of antibiotic and sedative drugs. However, these Pk alterations do not fully explain the clinical findings. For example, ECMO patients often need higher sedative doses compared with critically ill patients not on ECMO despite the degree of other organ failures being similar. While circuit sequestration may partly explain this, it is possible that ECMO may also alter the PD responses to sedative drugs. The probable mechanisms may include alterations to the blood-brain barrier, hepatic and renal perfusion and hepatic metabolism.  The effects of a non–pulsatile circulation that may result from venoarterial ECMO on pathophysiology and PD responses remain largely unclear. A deeper understanding altered PD may help further optimise drug dosing during ECMO. Building such PD models is challenging and a comprehensive research plan similar to the one used in this thesis may have to be utilised to study the interactions between the drug, devise and the disease that result in altered PD.

### 8.4.5 More detailed understanding of pathophysiology of ECMO

ECMO can independently alter pathophysiology in a critically ill Advancements in circuit technology may have minimised these alterations to an extent, but it is highly likely that the bio-synthetic interface created during ECMO may significantly influence inflammation, regional blood flows, the microcirculation and hence end organ function. Despite significant advances in our understanding of critical illness and related PK alterations,

there is a paucity of knowledge when it comes to pathophysiology of ECMO. Preclinical and clinical research aimed at understanding the altered pathophysiology has significant implications for the field of extracorporeal life support and is necessary to refine the ECMO technology and its clinical application further. Such research will allow any significant ECMO induced physiologic covariates to be included in PK/PD models and will further compliment the findings of this research.

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# APPENDIX 1: MANUSCRIPTS DURING CANDIDATURE NOT RELEVANT TO THESIS

1. Shruti Rateesh, **Kiran Shekar**, Rishendran Naidoo, Dolly Mithal, Balu Bhaskar. Use of Extracorporeal Membrane Oxygenation for mechanical circulatory support in a patient with 5-fluorouracil -induced Acute Heart Failure. Circ Heart Fail. 2015 Mar;8(2):381-3.

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4. Marie Cooke, Claire Rickard, Ivan Rapchuk, **Kiran Shekar**, Andrea P Marshall, Tracy Comans, Suhail Doi, John McDonald, Amy Spooner . PC6 acupoint stimulation for the prevention of post cardiac surgery nausea and vomiting: a protocol for a two-group, parallel, superiority randomised clinical trial. BMJ Open 11/2014; 4(11):e006179

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12. **Shekar K**, Fraser JF. Ventilator-Induced Lung Injury. N Engl J Med. 2014 Mar 6;370(10):979

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14. Platts DG, Thomson B, **Shekar K**, Fraser JF. Quantification of Differential ECMO Return Flow through an Axillary Artery Anastomosis Graft using Spectral Doppler Echocardiography. Cardiovascular Medicine 2014;17(2):48–50

15. David Platts, Andrew Hilton, Sara Diab, Charles McDonald, **Kiran Shekar**, Saul Chemonges, Kimble Dunster, Matthew Tunbridge, Darryl J Burstow and John F Fraser. A Novel Echocardiographic Imaging Technique, Intracatheter Echocardiography, to Guide Venovenous Extracorporeal Membrane Oxygenation Cannulae Placement in a Validated Ovine Model. Intensive Care Medicine Experimental 2014, 2:2

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22. Hayes RA, **Shekar K**, Fraser JF. Is hyperoxaemia helping or hurting patients during extracorporeal membrane oxygenation? Review of current literature. Perfusion 2013. Jan 15. [Epub ahead of print]

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24. D. Platts, S. Diab, C. MacDonald, M. Tunbridge, S. Chemonges, K. Dunster, **K. Shekar**, D. Burstow, D. Mullany, J. Fraser. .The Impact of Continuous Flow From Venovenous Extracorporeal Membrane Oxygenation Cannulae on Tricuspid Valve Geometry and Function. Heart Lung &amp Circulation 01/2013; 22, Supplement 1:S199

25. D. Platts, J. Cafaro, A. Maurice, K. Dunster, S. Diab, J. Fraser, **K. Shekar**, D. Burstow, L. Fung, J. Chan. Temporal Changes in Left Ventricular Radial and Circumferential Strain During Venovenous Extra Corporeal Membrane Oxygenation Following Acute Lung Injury in an Ovine Model. Heart Lung &amp Circulation 01/2013; 22, Supplement 1:S198

26. D. Platts, A. Hilton, S. Diab, C. MacDonald, M. Tunbridge, S. Chemonges, K. Dunster, **K. Shekar**, D. Burstow, J. Fraser . Feasibility of a Novel Echocardiographic Imaging Technique, Intracatheter Echocardiography, to Guide Venovenous ECMO Cannulae Placement in a Validated Ovine Model. Heart Lung & Circulation 01/2013; 22, Supplement 1:S180.

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28. Platts D, **Shekar K**, Senz A, Thomson B. Massive bilateral pulmonary emboli, paradoxical embolus and the knot of life. Eur Heart J. 2012 Dec;33(24):3077

29. Mullany DV, Bull T, Hunt B, **Shekar K**, Thomson B, Fraser JF, Ziegenfuss M. Outcomes of the first 30 cases of the Adult ECMO Program: Strategies to manage the 'learning curve" and implications for ICU risk adjustment models. Crit Care Resusc (2012) June; 14(2):119 – 129.

30. Chaudhuri A, **Shekar K**, Coulter C. Post-operative deep sternal wound infections: making an early microbiological diagnosis. Eur J Cardiothorac Surg. 2012 Jun; 41(6):1304-8.

31. Kumar R, **Shekar K**, Widdicombe N, Fraser J. Donation after cardiac death in Queensland: review of the pilot project. Anaesth Intensive Care. 2012 May; 40(3):517-22.

# APPENDIX 2: ORAL PRESENTATIONS BY THE CANDIDATE RELEVANT TO THE THESIS

## 2.1 INVITED LECTURES AND COMMENTARY

1. ASAP-ECMO study presented for endorsement. International ECMO Network scientific meeting .Milan February 2015.

2. Australian Society of Anaesthetists National Scientific Congress, Gold Coast, October 4th - 7th 2014. Building a bridge between the bench and the bedside.

3. EURO ELSO meeting , May 22-24 ,2014 Paris .Invited lecture "sedation under ECMO"

4. EURO ELSO meeting, May 22-24, 2014 Paris. "sedation and pain control of your ECMO patient"

5. ANZ ECMO update. Annual ECMO meeting. RPA Sydney 2013 September. "Is ECMO short changing your patient"

6. ASAP ECMO study update, 15 th Annual Meeting on Clinical Trials in Intensive Care (ANZICS) 7-9 March 2013.

7. Pharmacokinetics in ECMO. 4th Alfred ICU International Symposium on ECMO & VAD Support in Critical Care. Melbourne, Australia 18-19 July 2012.

8. ASAP-ECMO - understanding pharmacotherapy during ECLS.14th Annual Meeting on Clinical Trials in Intensive Care (ANZICS) 9 - 11 March 2012.

9. Invited commentary. ECMO: bringing patients back from respiratory failure .September 2012 www.thelancet.com/respiratory.

10. PK and ECMO: Can ECMO result in treatment failure by drug adsorption. 2nd ECMO and Mechanical Circulatory Support Special Interest Group, Brisbane, Australia. 12 October 2011.

11. PK and ECMO: Can ECMO result in treatment failure by drug adsorption. 2nd ECMO and Mechanical Circulatory Support Special Interest Group, Brisbane, Australia. 12 October 2011.

## 2.2. ORAL ABSTRACTS

1. **Kiran Shekar**, Jason A Roberts, Sussan Ghassabian, Kimble R Dunster,Sara Diab, Charles I McDonald, Yoke Lin Fung, Daniel V Mullany, Maree T Smith, John F Fraser. Greater exposures to midazolam seen during ECMO in critically ill sheep

when compared with ECMO in healthy sheep. Australia and New Zealand Society of Intensive Care Medicine ASM 2014

2. **Shekar K**, Roberts JA, Diab S, Dunster KR, , Ghassabian S, McDonald CI, Fung YL, Mullany DV, Platts DG, Smith MT, Fraser JF. Altered PK of midazolam and its metabolites in healthy and critically ill ovine models of ECMO. The Prince Charles Hospital Research Forum 2014.

3. **Kiran Shekar**, Jason A Roberts, Sussan Ghassabian, Charles I McDonald, Yoke Lin Fung, Daniel V Mullany, Maree T Smith, John F Fraser. Are Protein bound drugs more prone to sequestration in ECMO circuits: Results from an ex vivo experiment. Australia and New Zealand Society of Intensive Care Medicine ASM 2014

4. **Kiran Shekar**, John F Fraser, Sussan Ghassabian, Kimble R Dunster, Sara Diab, Charles I McDonald, Lin Fung, Daniel V Mullany, Maree T Smith, Jason A Roberts. ECMO had a greater influence on midazolam PK when compared with critical illness in an ovine model. Extracorporeal Life Support Organisation , Michigan 2014

5. **Shekar K**, Roberts JA, McDonald CI, Ghassabian S, Wallis SC, Fisquet S, Mullany DV, Smith MT, Fung YL , Fraser JF. Protein bound drugs more prone to sequestration in ECMO circuits. The Prince Charles Hospital Research Forum 2014

6. **Kiran Shekar**, Michelle Chew, Sara Diab, Kimble Dunster, Gabriela Simonova, Sam Foley, Margaret Passmore, Lin Fung, Michael Reade, John Fraser. A novel large animal model to investigate the tissue effects of fluid and blood resuscitation in sepsis. ANZICS 2013

7. **Kiran Shekar**, John F Fraser, Daniel V Mullany, Steven C Wallis, Jason A Roberts. Standard meropenem dosing during ECMO provides optimal meropenem concentrations both in patients with preserved renal functions and in those receiving renal replacement therapy. Australia and New Zealand Society of Intensive Care Medicine. 2013

# APPENDIX 3: POSTER PRESENTATIONS BY THE CANDIDATE RELEVANT TO THE THESIS

1. **Kiran Shekar**, Jason A Roberts, Sussan Ghassabian, Charles I McDonald, Yoke Lin Fung, Daniel V Mullany, Maree T Smith, John F Fraser. Protein bound drugs more prone to sequestration in ECMO circuits: Results from an ex vivo experiment. Extracorporeal Life Support Organisation , Michigan 2014

2. Michael C. Reade, Natasha van Zyl, Elissa M. Milford, **Kiran Shekar**, John Paul Tung, Kimble R. Dunster, Sara Diab, John F Fraser. Validation of an ovine model of Acute Traumatic Coagulopathy. Military Health System Research Symposium (MHSRS), Florida 18-21 August 2014

3. Yi-Chin Tsai, Andrie Stroble, Livia Willams, Marc Ziengenfuss, Lisa Nicotra, Elizabeth Ryan, James McGree, Peter Tesar, **Kiran Shekar** . Tracheostomy: A Risk Factor for Deep Sternal Wound Infection after Cardiac Surgery – An Experience of Single Cardiothoracic Surgical Unit

4. Platts DG, Sim B, Turnbridge M, Daib S, Dunster K, **Shekar K**, Burstow D, Maybauer M, Chan J, Fraser JF. Feasibility of Perflutren Microsphere Contrast Transthoracic Echocardiography in Assessment of Right Ventricular Endocardial Definition During VenoVenous Extra Corporeal Membrane Oxygenation in an Ovine Model. World Heart Federation's World Congress of Cardiology 2014

5. Passmore M, Fung YL, Dunster KR, Diab S, **Shekar K**, Fraser JF. Lung inflammatory changes in an ovine model of VV ECMO. American Thoracic Society 2014

6. Yuen A, Gregory S, **Shekar K**, Fraser JF. Pulsing a rotary blood pump during mechanical circulatory support may result in increased thromboembolic or bleeding risks. ANZICS 2013

7. Passmore M, Fung YL, Dunster KR, Diab S, **Shekar K**, Fraser JF. ECMO contributes to lung injury in an ovine model of VV ECMO. APELSO 2013

8. **Shekar K**, Diab S, Dunster KD, Roberts JA, McDonald CI, Passmore M, Platts DG, Simonova G, Foley S, Wallis SC, Fung YL, Smith MT, Fraser JF. ECMO has more profound influence on Ciprofloxacin pharmacokinetics in critically ill sheep when compared to healthy sheep. APELSO 2013

9. Platts DG, McDonald CI, **Shekar K**, Diab S, Dunster KS, Burstow D, Chan J, Fraser JF. Quantification of perflutren microsphere destruction during transit through an ex vivo ECMO circuit. APELSO 2013

10. Foley SR, Fung YL, Simonova G, Solano S, Diab S, Dunster KD, McDonald CI, **Shekar K**, Fraser JF. Acute lung injury compounds ECMO induced changes to haemostasis in an ovine model. APELSO 2013

11. Estensen K, **Shekar K**, Robins E, McDonald CI, Fraser JF. Disposition of macro and micronutrients in an ex vivo ECMO circuit. APELSO 2013

12. Platts DG, Sim B, Turnbridge M, Daib S, Dunster K, **Shekar K**, Burstow D, Maybauer M, Chan J, Fraser JF. Perfutren microsphere contrast transthoracic echocardiography improves endocardial definition during VV ECMO in a validated ovine model. APELSO 2013

13. Platts DG, Diab S, McDonald CI, Turnbridge M, Chemonges S, Dunster K**, Shekar K** , Burstow D, Mullany DV, Fraser JF. The impact of continuous flow from VV ECMO cannulae on tricuspid valve geometry and function. APELSO 2013

14. Platts DG, Cafaro J, Maurice A, Dunster K, Diab S, Fraser JF, **Shekar K**, Burstow D, Mullany DV, Chan J. Feasibility of left ventricular strain assessment using velocity vector imaging coupled with contrast enhanced transthoracic echocardiography in a VV ECMO ovine model. APELSO 2013

15. Platts DG, Cafaro J, Maurice A, Dunster K, Diab S, Fraser JF, **Shekar K**, Burstow D, Mullany DV, Chan J. Temporal changes in left ventricular radial and circumferential strain during VV ECMO following acute lung injury in an ovine model. APELSO 2013

16. Hayes R, Foley S, **Shekar K**, Diab S, Dunster K, Fraser JF. Hyperoxaemia adversely affects adenosine diphosphate induced platelet aggregation during ECMO in ovine models. APELSO 2013

17. Turnbridge M, Sim B, Diab S, Dunster K, McDonald CI, Platts DG, Foley S, Simonova G, Tung JP, **Shekar K**, Fraser JF. Stored and fresh blood transfusions have similar effects on systemic and pulmonary haemodynamics and pulmonary compliance characteristics during VV ECMO in a validated ovine model. APELSO 2103

18. **Shekar K**, Fraser JF, Mullany DV, Wallis SC, Roberts JA. Optimal meropenem exposure may be achieved with standard doses in patients receiving ECMO. APELSO 2013

19. **Kiran Shekar**, John F Fraser, Daniel V Mullany, Steven C Wallis, Jason A Roberts. Standard meropenem dosing during ECMO provides optimal meropenem

concentrations both in patients with preserved renal functions and in those receiving renal replacement therapy. Australia and New Zealand Society of Intensive Care Medicine. 2013

20. Dan Mullany, Marc Ziegenfuss, **Kiran Shekar** et al. SOFA Scores and Outcomes in ECMO patients: when is it too late? College of Intensive Care Medicine of Australia and New Zealand ASM 2013.

21. David Platts, James Cafaro , Andrew Maurice,  Kimble Dunster, Sara Diab, John Fraser,  **Kiran Shekar**,  Darryl Burstow, Lin Fung,  Jonathan Chan. Temporal Changes in Left Ventricular Radial And Circumferential Strain during venovenous ECMO Following Acute Lung Injury in an Ovine Model. American Society of Echocardiography ASM, Minneapolis, June 2013.

22. David Platts, James Cafaro , Andrew Maurice,  Kimble Dunster, Sara Diab, John Fraser,  **Kiran Shekar**,  Darryl Burstow, Daniel Mullany, Jonathan Chan. Feasibility of Left Ventricular Strain Assessment using Velocity Vector Imaging Coupled with Contrast Enhanced Transthoracic Echocardiography in a venovenous ECMO Ovine Model American Society of Echocardiography ASM, Minneapolis, June 2013.

23. David Platts, Beatrice Sim, Matthew Tunbridge, Sara Diab, Kimble Dunster, **Kiran Shekar**, Darryl Burstow, Marc Maybauer, John Chan, John Fraser. Perflutren Microsphere Contrast Transthoracic Echocardiography (CE) Improves Endocardial Definition During venovenous ECMO in an Ovine Model. American Society of Echocardiography ASM, Minneapolis, June 2013.

24. David Platts, Andrew Hilton, Sara Diab, Charles MacDonald, Mathew Tunbridge, Saul Chemonges, Kimble Dunster, **Kiran Shekar**, John Fraser. Feasibility of a novel echocardiographic imaging technique, intracatheter echocardiography (iCATHe), to guide venovenous ECMO cannulae placement. American Society of Echocardiography ASM, Minneapolis, June 2013.

25. David Platts, Sara Diab, Charles MacDonald, Mathew Tunbridge, Saul Chemonges, Kimble Dunster, **Kiran Shekar**, Darryl Burstow, John Fraser. The impact of continuous flow from venovenous extracorporeal membrane oxygenation (VV ECMO) cannulae on tricuspid valve geometry and function. American Society of Echocardiography ASM, Minneapolis, June 2013.

26. **Shekar K**, Buschel R, Kermeen F. Intravenous Sildenafil- New role for a not so new drug? PHSANZ Annual Scientific meeting, November 16, 2012, Sydney, NSW

27. **Shekar K**, Buschel R, Kermeen F. Intravenous Sildenafil- New role for a not so new drug? 5th World Symposium on Pulmonary Hypertension, Nice, February 27-28/March 1, 2013.

28. Cree M, Foenander D, Cornmell G, Richards B, Ziegenfuss M, Gordon G, Willis N, **Shekar K**. Do we need an intensive care clinical information system? Where? When? ANZICS ASM Oct 2012.

29. **Shekar K**, Fraser JF, Fung YL. Is morphine superior to fentanyl for analgesia during extracorporeal oxygenation in adult patients? 60th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Brisbane, Australia. 16-19 August 2012.

30. **Shekar K**, Fraser JF. Altered antibiotic pharmacokinetics during extracorporeal membrane oxygenation may cause therapeutic failure. 60th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Brisbane, Australia. 16-19 August 2012.

31. **Shekar K**, Fraser J, Mullany D, Barnett A, Fisquet S, Corley A, Spooner A, Bull T, Dean C, Canning M. Do patients receiving extracorporeal membrane oxygenation require different analgaesic and sedative doses? Medicines Management 2011, the 37th Society of Hospital Pharmacists of Australia National Conference, Hobart, Australia. 10-13 November 2011.

32. **Shekar K**, McDonald C, Fisquet S, Mullany D, Barnett A, Wallis S, Ghassabian S, Fung L, Roberts J. Sequestration of up to eighty percent meropenem in the circuit may cause treatment failure in patients receiving extracorporeal membrane oxygenation. Medicines Management 2011, the 37th Society of Hospital Pharmacists of Australia National Conference, Hobart, Australia. 10-13 November 2011.

33. **Shekar K**, McDonald C, Fisquet S, Mullany D, Barnett A, Wallis S, Ghassabian S, Fung L, Roberts J. Sequestration of Meropenem in the circuit may cause treatment failure in patients receiving Extra-corporeal Membrane Oxygenation.28th Annual Scientific Meeting of the Australian and New Zealand College of Perfusion, Sydney, Australia. 3-5 November 2011.

34. **Shekar K**, McDonald C, Fisquet S, Mullany D, Barnett A, Wallis S, Ghassabian S, Roberts J, Fung L, Fraser J. Do patients receiving extracorporeal membrane oxygenation require different analgesic and sedative doses? 2011 ANZICS/ACCCN Intensive Care Annual Scientific Meeting, Brisbane, Australia. 13-15 October 2011

35. **Shekar K**, McDonald C, Fisquet S, Mullany D, Barnett A, Wallis S, Ghassabian S, Fung L, Roberts J. Sequestration of up to eighty percent meropenem in the circuit

may cause treatment failure in patients receiving extracorporeal membrane oxygenation. 2011 ANZICS/ACCCN Intensive Care Annual Scientific Meeting, Brisbane, Australia. 13-15 October 2011.

36. **Shekar K**, Fung YL, Diab S, Mullany DV, McDonald CI, Dunster KR, Fisquet S, Platts DG, Stewart D, Wallis S. Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit-host interactions. College of Intensive Care Medicine, ASM 2012

# APPENDIX 4: ETHICS APPROVALS

1. Ex vivo circuit experiments using human blood .The Prince Charles Hospital Research Ethics Committee. (HREC/12/QPCH/90)

2. In vivo ovine studies and ex vivo circuit experiments that utilise sheep blood .Queensland University of Technology Research Ethics Committee (approval no. 1100000053)

3. Multi-site ethics approval for the clinical studies in Australia. The Prince Charles Hospital Ethics Committee (HREC/11/QPCH/121)

4. Single-site ethics approval for the clinical study in New Zealand. Auckland City Hospital Research Ethics Committee (LRS/12/06/020)

5. Ethics approval for retrospective data analysis of sedation (Chapter 1.3) in ECMO patients (HREC/11/QPCH/3)

6. Ethics approval for PK sampling and publication of pilot data (Chapter1.1, sections 1.1.3.1 and 1.1.3.3 ) from ECMO patients (HREC/11/QPCH/121)

# APPENDIX 5: GRANT FUNDING

## 5.1. GRANT FUNDING RELEVANT TO THIS THESIS

1. **Shekar K**, Fraser JF, Roberts JA. Antibiotic, sedative and analgesic drug pharmacokinetics during ovine extracorporeal membrane oxygenation (ASAP ECMO) -Understanding altered pharmacokinetics to improve patient outcomes. The Prince Charles Hospital Foundation. (2014) $99,620.94

2. Fraser JF, Gregory S, **Shekar K**, Olive C, Tansley G, Platts D, Tung JP, McGiffin D, Thomson B, Bull T. Advanced Cardio-respiratory Therapies Improving OrgaN Support (ACTIONS). TPCHF- Program grant (2014) $600,000.00

3. **Shekar K**, Fraser JF, Roberts JA. Antibiotic, sedative and analgesic drug pharmacokinetics during extracorporeal membrane oxygenation (ASAP ECMO) - Understanding altered pharmacokinetics to improve patient outcomes. The Prince Charles Hospital Foundation $99,958

4. Mullany DV, **Shekar K**, Roberts JA, Smith MT, Fraser JF. . Antibiotic, sedative and analgesic drug pharmacokinetics during ovine extracorporeal membrane oxygenation (ASAP ECMO) -Understanding altered pharmacokinetics to improve patient outcomes. Australian and New Zealand College of Anaesthetists(ANZCA) project grant (2012)$35,000

5. **Shekar K**, Fraser JF, Roberts JA, Smith MT. Antibiotic, sedative and analgesic drug pharmacokinetics during ovine extracorporeal membrane oxygenation (ASAPECMO) -Understanding altered pharmacokinetics to improve patient outcomes. Intensive Care Foundation project grant (2012) $30,000

6. **Shekar K**, Fraser JF, Roberts JA, Smith MT, Fung L. Antibiotic, sedative and analgesic drug pharmacokinetics during ovine extracorporeal membrane oxygenation (ECMO) -Understanding altered pharmacokinetics to improve patient outcomes. The Prince Charles Hospital Foundation. (2012) $91,899.95.

7. **Shekar K**, Roberts JA, Smith MT, Fraser JF, McDonald C. Sedative and analgesic drug disposition during simulated extracorporeal membrane oxygenation. The Prince Charles Hospital Foundation (2011) $9997.84.

8. **Shekar K**, Roberts JA, Smith MT, Fraser JF, McDonald C. Disposition of sedative, analgesic and antibiotic drugs during simulated extracorporeal membrane oxygenation. Intensive Care Foundation (2011) $10,909.09.

9. Mullany D, **Shekar K**, Roberts JA, Smith MT, Fraser JF. Disposition of sedative, analgesic and antibiotic drugs during simulated extracorporeal membrane oxygenation Australian and New Zealand College of Anaesthetists (2011) $35,000.

## 5.2 OTHER GRANT FUNDING DURING CANDIDATURE

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# APPENDIX 6: STATISTICAL AND PK MODELING APPROACH

Linear and non-linear mixed effects modeling, non-compartmental and compartmental PK model building techniques were effectively used during data analysis in this research. In addition, regression analysis was used to estimate the relationships among a dependent variable and one or more independent variables. The statistical software used for these analyses have been described in the relevant chapters. These published, validated techniques have been extensively used in previous research. However a brief overview of these techniques is provided below.

## 6.1. Mixed effects modelling

PK studies invariably involve repeated measurements on each subject over time or space and mixed model analysis provides a general, flexible approach in these situations, because it allows a wide variety of correlation patterns (or variance-covariance structures) to be explicitly modeled. The term mixed model refers to the use of both fixed and random effects in the same analysis. Fixed effects have levels that are of primary interest and would be used again if the experiment were repeated. Random effects have levels that are not of primary interest, but rather are thought of as a random selection from a much larger set of levels. Subject effects are almost always random effects, while treatment levels are almost always fixed effects. Mixed effects models flexibly give correct estimates of treatment and other fixed effects in the presence of the correlated errors that arise from a data hierarchy[70].

## 6.2. Pharmacokinetic modeling

### 6.2.1. Non-compartmental models

PK data can be analysed by either model fitting using nonlinear regression analysis or non-compartmental analysis techniques (NCA). The method applied usually depends on what is required from the analysis. If the primary requirement is to determine the degree of exposure following administration of a drug (such as AUC), and other PK parameters, such as CL, elimination half-life, T (max), C (max), etc., then NCA is the preferred method because it requires fewer assumptions than model-based approaches. In this research, the NCA methods used applied the trapezoidal rule for measurements of the area under the plasma concentration-time curve[72].

## 6.2.2. Compartmental models

A compartmental analysis is based on development of PK models. A PK model is a mathematical representation of the passage and fate of the drug through the body and contains variables (e.g. dose, times of doses and times at which blood samples are taken) and constants called parameters (e.g. clearance and volume of distribution) that quantify the drug disposition. This approach assumes that there are some underlying physiological processes that underpin the PK of the drug[73]. In this approach the body is divided into a series of linked homogenous compartments that represent the disposition of the drug.  For example, the models built during this research factored the physiologic perturbations that may arise in the presence of critical illness and ECMO. The mechanistic PK data from simulated ECMO circuit experiments and ovine models further assisted the choice of covariates to be included in those models.  Compartmental approaches reliable predictive properties and are generally independent of the size of the drug dose and dosing interval and allow extrapolation to a similar patient population that may receive the drug in the future. Bootstrapping refers to a method to obtain a measure of reliability of predictions made in part from fits of individual drug level data with a PK model, and to help clarify parameter identifiability for such models[71,74]. This is important because this translational research aims to develop evidence based guidelines based on the PK data generated through the clinical population PK study (ASAP ECMO).

## *6.3. Population PK analysis*

Population PK is the study of PK at the population level, in which data from all individuals in a population are evaluated simultaneously using a nonlinear mixed-effects model. "Nonlinear" refers to the fact that the dependent variable (e.g., drug concentration) is nonlinearly related to the model parameters and independent variable(s). "Mixed-effects" refers to the parameterization: parameters that do not vary across individuals are referred to as "fixed effects," parameters that vary across individuals are called "random effects." The five key aspects to developing a population PK model are, (i) data, (ii) structural model, (iii) statistical model, (iv) covariate models, and (v) modeling software. Structural models describe the typical concentration time course within the population. Statistical models account for "unexplainable" (random) variability in concentration within the population (e.g., between-subject, between-occasion, residual, etc.). Covariate models explain variability predicted by subject characteristics (covariates). Nonlinear mixed effects modeling software brings data and models together, implementing an estimation method

for finding parameters for the structural, statistical, and covariate models that describe the data[71].

### 6.4. Dosing simulations

The practical and financial difficulties of performing PK studies in critically ill patients mean that analyses to maximize data such as Monte Carlo simulation (MCS) are highly valuable. MCS uses computer software to perform virtual clinical trials. The building blocks for MCS are: firstly, a robust population PK model from the patient population of interest; secondly, descriptors of the effect of covariates that influence the PK parameters; thirdly, description of the susceptibility of bacteria to the antibiotic and finally a PK/PD target associated with antibiotic efficacy. Such analyses can then inform dosing requirements, which can be used to have a high likelihood of achieving PK/PD targets for organisms with a range of MICs[75]. For example, MCS was effectively used in Chapter 7 where in plasma meropenem concentrations were simulated at various CrCL and meropenem doses. Dosing simulations will be invaluable tools for development of drug dosing guidelines through this research.

# APPENDIX 7: ASAP ECMO INVESTIGATORS

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# APPENDIX 8: ERRATA

**Chapter 4.1**

 i.  Page 46: The phrase 'multi-centre' in the article abstract to be read as 'multicentre'

 ii.  Page 52, Figure 6. The label insert 'oxygentor' to be read as 'oxygenator'.