ISSN 2220-3141 (online)

World Journal of *Critical Care Medicine*

World J Crit Care Med 2013 November 4; 2(4): 21-64

Baishideng Publishing Group
www.wjgnet.com

WJCCM World Journal of Critical Care Medicine

A peer-reviewed, online, open-access journal of critical care medicine

Editorial Board

2011-2015

The *World Journal of Critical Care Medicine* Editorial Board consists of 246 members, representing a team of worldwide experts in critical care medicine. They are from 45 countries, including Argentina (2), Australia (8), Austria (2), Bangladesh (1), Belgium (3), Brazil (4), Canada (7), China (23), Croatia (1), Cuba (1), Denmark (1), Egypt (4), Finland (1), France (8), Germany (11), Greece (9), Hungary (1), India (10), Iran (2), Ireland (1), Israel (6), Italy (14), Japan (6), Jordan (1), Mexico (1), Morocco (1), Netherlands (4), New Zealand (3), Norway (1), Poland (1), Portugal (4), Russia (1), Saudi Arabia (3), Singapore (1), Slovenia (1), South Africa (1), Spain (7), Sweden (1), Switzerland (3), Thailand (1), Tunisia (1), Turkey (3), United Kingdom (8), United States (72), and Uruguay (1).

Thailand

Viroj Wiwanitkit, *Bangkok*

Tunisia

Mabrouk Bahloul, *Sfax*

Turkey

Yusuf Kenan Coban, *Malatya*
Bensu Karahalil, *Ankara*
Ali Nayci, *Mersin*

United Kingdom

Sammy Al-Benna, *Nottingham*
Giles N Cattermole, *London*
Frantisek Duska, *Nottingham*
James Nicholas Fullerton, *London*
Christina Jones, *Prescot*
Sameer Khan, *Middlesbrough*
George Ntoumenopoulos, *London*
Cecilia O'Kane, *Belfast*

United States

Edward Abraham, *Winston-Salem*
Bernard R Bendok, *Chicago*
Michael Blaivas, *Atlanta*
Charles D Boucek, *Pittsburgh*
Marcia Leigh Brackbill, *Winchester*
Ronald A Bronicki, *Houston*
Robert C Cantu, *Concord*
Marylou Cardenas-Turanzas, *Houston*
Archana Chatterjee, *Omaha*
Paul A Checchia, *St. Louis*
Rubin Issam Cohen, *New Hyde Park*
Stephen Cohn, *San Antonio*
Donald Edward Craven, *Burlington*
Ruy J Cruz Jr, *Pittsburgh*
Francis C Dane, *Roanoke*
Marc de Moya, *Boston*
Steven M Donn, *Ann Arbor*
Christopher P Farrell, *Wynnewood*
Marco Fernández, *Nashville*
Kevin Foster, *Phoenix*
Barry D Fuchs, *Philadelphia*
Richard P Gonzalez, *Mobile*
Kenneth W Gow, *Seattle*
Alan H Hall, *Laramie*
Jijo John, *Oklahoma City*
Lewis J Kaplan, *New Haven*
Jason N Katz, *Chapel Hill*
Salah Georges Keyrouz, *Little Rock*
Deborah A Kuhls, *Las Vegas*
Gregory Luke Larkin, *New Haven*
Christos Lazaridis, *Charleston*
James Anthony Lin, *Los Angeles*
Yahia M Lodi, *Syracuse*
Roger M Loria, *Richmond*
Aigang Lu, *Cincinnati*
Rudolf Lucas, *Augusta*
O John Ma, *Portland*
Robert T Mallet, *Fort Worth*
William T McGee, *Springfield*
Mark G McKenney, *Miami*
Michael Moussouttas, *Philadelphia*
Oliver Hans-Josef Muensterer, *Birmingham*
Rahul Nanchal, *Milwaukee*
Michael Steven Niederman, *Mineola*
Gary Frank Nieman, *Syracuse*
James Martin O'Brien, *Columbus*
Martin Oudega, *Pittsburgh*
Catherine Mobley Preissig, *Duluth*
Virginia Prendergast, *Phoenix*
Ramesh Raghupathi, *Philadelphia*
Miren Ava Schinco, *Jacksonville*
Carl Ivan Schulman, *Miami*
L Keith Scott, *Shreveport*
Kevin Navin Sheth, *Baltimore*
Jenni Short, *Salina*
Ronald Fong Sing, *Charlotte*
Philip Charles Spinella, *St. Louis*
Robert M Starke, *Charlottesville*
Stanislaw Peter A Stawicki, *Columbus*
David Christopher Stockwell, *Washington*
Stanislav Svetlov, *Gainesville*
Maged A Tanios, *Long Beach*
Neal James Thomas, *Hershey*
Nancy Moon Tofil, *Birmingham*
Balagangadhar R Totapally, *Miami*
Steven Nicholas Vaslef, *Durham*
Joseph Clark Watson, *Falls Church*
John Stephen Wilgis, *Orlando*
David Conrad Willms, *San Diego*
Haodong Xu, *Rochester*
Xiao-Ming Xu, *Indianapolis*
Midori Anne Yenari, *San Francisco*

Uruguay

William Manzanares, *Montevideo*

WJCCM
World Journal of Critical Care Medicine

Contents

Quarterly Volume 2 Number 4 November 4, 2013

Contents

World Journal of Critical Care Medicine
Volume 2 Number 4 November 4, 2013

AIM AND SCOPE

World Journal of Critical Care Medicine (*World J Crit Care Med*, *WJCCM*, online ISSN 2220-3141, DOI: 10.5492) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJCCM covers topics concerning severe infection, shock and multiple organ dysfunction syndrome, infection and anti-infection treatment, acute respiratory distress syndrome and mechanical ventilation, acute kidney failure, continuous renal replacement therapy, rational nutrition and immunomodulation in critically ill patients, sedation and analgesia, cardiopulmonary cerebral resuscitation, fluid resuscitation and tissue perfusion, coagulant dysfunction, hemodynamic monitoring and circulatory support, ICU management and treatment control, and application of bronchofiberscopy in critically ill patients.

We encourage authors to submit their manuscripts to *WJCCM*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

INDEXING/ABSTRACTING

World Journal of Critical Care Medicine is now indexed in Digital Object Identifier.

NAME OF JOURNAL
World Journal of Critical Care Medicine

ISSN
ISSN 2220-3141 (online)

LAUNCH DATE
February 4, 2012

FREQUENCY
Quarterly

World Journal of Critical Care Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-6555-7188
Telephone: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
November 4, 2013

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5492/wjccm.v2.i4.21

World J Crit Care Med 2013 November 4; 2(4): 21-28
ISSN 2220-3141 (online)

REVIEW

Computerized decision support in adult and pediatric critical care

Cydni N Williams, Susan L Bratton, Eliotte L Hirshberg

Cydni N Williams, Susan L Bratton, Eliotte L Hirshberg, Department of Pediatrics, University of Utah, Pediatric Critical Care, Salt Lake City, UT 84158, United States
Eliotte L Hirshberg, Division of Pulmonary and Critical Care Medicine, Intermountain Medical Center, Murray, UT 84157, United States
Author contributions: All listed authors contributed to the design, conception, research, analysis, and writing of this paper.
Correspondence to: Cydni N Williams, MD, Department of Pediatrics, University of Utah, Pediatric Critical Care, 295 Chipeta Way, Salt Lake City, UT 84158, United States. cydni.willams@hsc.utah.edu
Telephone: +1-405-7605837 Fax: +1-801-5818686
Received: June 14, 2013 Revised: August 2, 2013
Accepted: August 20, 2013
Published online: November 4, 2013

Abstract

Computerized decision support (CDS) is the most advanced form of clinical decision support available and has evolved with innovative technologies to provide meaningful assistance to medical professionals. Critical care clinicians are in unique environments where vast amounts of data are collected on individual patients, and where expedient and accurate decisions are paramount to the delivery of quality healthcare. Many CDS tools are in use today among adult and pediatric intensive care units as diagnostic aides, safety alerts, computerized protocols, and automated recommendations for management. Some CDS use have significantly decreased adverse events and improved costs when carefully implemented and properly operated. CDS tools integrated into electronic health records are also valuable to researchers providing rapid identification of eligible patients, streamlining data-gathering and analysis, and providing cohorts for study of rare and chronic diseases through data-warehousing. Although the need for human judgment in the daily care of critically ill patients has limited the study and realization of meaningful improvements in overall patient outcomes, CDS tools continue to evolve and integrate into the daily workflow of clinicians, and will likely provide advancements over time. Through novel technologies, CDS tools have vast potential for progression and will significantly impact the field of critical care and clinical research in the future.

Key words: Clinical decision support systems; Critical care; Computers; Computer-assisted decision making

Core tip: Computerized decision support (CDS) is increasingly utilized in both adult and pediatric critical care. Improvements in care have been shown in areas including guideline adherence and reduction of medical errors, but reports of meaningful improvements in patient outcome have been scarce to date. However, with technology improvements and widespread acceptance of tools, CDS has the potential to revolutionize critical care medicine with improved diagnosis, monitoring, risk prediction, and treatment. Improvements in multiple aspects of patient care through CDS tools can lead to better patient outcomes.

Williams CN, Bratton SL, Hirshberg EL. Computerized decision support in adult and pediatric critical care. *World J Crit Care Med* 2013; 2(4): 21-28 Available from: URL: http://www.wjgnet.com/2220-3141/full/v2/i4/21.htm DOI: http://dx.doi.org/10.5492/wjccm.v2.i4.21

INTRODUCTION

Decision support tools have been used by the medical profession for decades and evolved with technology to become largely computer based and widely accessible to all clinicians in the form of smart phone applications,

web-based search engines, online references and journal access, and bedside tools incorporated into daily clinical practice. The potential for further advancements in biomedical informatics to improve healthcare quality is vast and increasingly studied at the patient care level and in research[1]. The goal of clinical decision support is to provide current and pertinent knowledge to clinicians to aid patient care at the exact time of care delivery[2]. Computerized technology provides the tools to facilitate timely delivery of this knowledge to bedside clinicians. Computerized decision support (CDS) systems have been implemented by hospitals internationally targeting important goals including improved diagnostic accuracy, error reduction, delivery of preventative care, and better patient outcomes[3]. As the field of medicine continues to increase in complexity, these tools are likely to become further integrated into patient care, as well as provide substantial resources for clinical research.

Intensive care unit (ICU) clinicians are in unique environments where vast amounts of information are collected and displayed by computerized systems, and where expedient, accurate diagnosis and treatment may profoundly affect quality of care and patient outcomes. ICU clinicians are tasked daily to manage large volumes of data from multiple sources and incorporate this data into patient-specific decisions. Given the unique position of ICU clinicians, CDS will likely become central to delivery of critical care in the coming years. However, inter-provider decision variability, lack of universal diagnostic and therapeutic protocols for many common diagnoses, and the demand for real-time individual variation at the bedside provide challenges for CDS design in critical care. In this paper, we give an overview of CDS history in clinical medicine, discuss different types of CDS tools, review some current applications in adult and pediatric critical care, address advantages and limitations to CDS tool use, and discuss the potential of CDS for critical care in the future.

HISTORY AND OVERVIEW OF COMPUTERIZED DECISION SUPPORT

CDS is the most efficient form of decision support and is designed to improve the quality of healthcare delivery, assist nurses and physicians in clinical decision making, and reduce variation[1,2]. CDS tools have evolved over time in both content and theoretical design for many healthcare related functions commonly used today: alert, diagnosis, reminder, suggestion, interpretation, prediction, critique, and assistance[4]. The concept of computer aided diagnosis in medicine was introduced as early as by Ledley *et al*[5]. Warner *et al*[6] presented a Bayesian theory based system for diagnosing congenital heart disease relying on inputted signs and symptoms. Design of CDS tools has evolved beyond rule-based tools to contain more complex mathematical models incorporating multiple static and dynamic factors rather than just the presence or absence of a variable. Despite the many advantages of CDS tools, widespread acceptance by clinicians across healthcare disciplines remains variable.

Computerized order entry (CPOE) and electronic health records (EHR) represent forms of computer assistance used in healthcare systems worldwide. CPOE and EHR centralize information and CDS can be incorporated into these technologies. CPOE and EHR with integrated CDS enables provision of abnormal lab value and allergy alerts, antibiotic choice assistance, vaccination reminders, mortality prediction tools, compliance with protocols and care guidelines, and suggestions for therapeutic interventions at the bedside[4,7,8]. The warehousing of information in EHR and other computer databases with CDS enables research advancement as databases can be linked and analysis of previously unrecognized relationships between patients and disease states explored[9].

Bedside computer monitoring devices can also be considered a form of CDS and have evolved from display tools to alarm systems and clinical assistance tools. For example, electrocardiography machines now provide a tracing as well as an interpretation. This type of analysis, using various inputs and known associations to generate a weighted output, is known as a neural network and is commonly utilized in both waveform analysis and mortality risk assessment tools[9]. Another type of CDS tool with increasing medical use is fuzzy logic; this permits use of ambiguous and imprecise data in logic control when constructing objective outputs. Applications include mechanical ventilation control, oxygen titration, and medication administration for blood pressure regulation[9]. Belief networks, another type of CDS tool, are algorithms derived from probability trees describing relationships of variables in a system to each other. Belief networks often utilize one of three models: simulation, mathematical, or statistical[10]. Belief networks can be designed to assist clinicians in real-time clinical decision making, and such belief networks have also been used to determine prognosis following head injury[9]. Effective computer decision support tools require high data integration accuracy and quality meshed with error free logic, ease of use, and explicit communication[11].

Experience with decision support and decision making in medicine

Current examples of accepted CDS tools include mortality prediction tools, such as the acute physiology and chronic health evaluation (APACHE) and pediatric risk of mortality (PRISM) scores. These have been validated and revalidated providing accurate mortality risk prediction and are routinely employed to generate risk adjusted mortality estimates to assess ICU performance[12,13]. CDS tools used in outpatient care document improved adherence to recommended vaccine schedules and adherence to recommended asthma care[14,15]. CPOE, with integrated CDS, decreases medical errors and improves pharmacy costs over time[16-20]. CDS tools have improved care in time-sensitive disease states including septic shock[21].

Table 1 Applications of computerized decision support in adult, pediatric and neonatal critical care

Type of support tool	Example or subject
Adult critical care	
Diagnostic	DXplain[28]
	Mortality and length of stay prediction[9,29]
Alert and reminder	Ventilator induced lung injury[84]
	Blood pressure variability while on vasopressors[32]
	Adverse drug reactions[19]
	Drug induced thrombocytopenia[30]
	Epidural hematoma with neuraxial anesthesia[31]
Protocol/procedure	Acute respiratory distress syndrome[33-35]
	Sepsis[21]
	VTE prophylaxis and events in trauma patients[37]
	VTE prophylaxis[38,39]
	Tidal volume during mechanical ventilation[36]
Management	Ventilator fraction of inspired oxygen[41]
	Pressure support ventilation[42]
	Antibiotic recommendation[43,44]
	Blood glucose control[46-48]
	Sepsis[21]
	Heparin dosing after myocardial infarction[45]
Research	Mortality prediction[29]
	Prediction of fluid requirement[53]
	Predictive alerts for hemodynamic instability[49-51]
	Ventilator settings[76]
	Prediction of dialysis need[52]
	Insulin e-protocol[54,85]
Pediatric and neonatal critical care	
Diagnostic	ISABEL (www.isabelhealthcare.com)[59,86]
	SimulConsult (www.simulconsult.com)[87]
	MEDITEL pediatric diagnostic system[58]
	Outcome prediction and severity of illness[55-57]
Alert and Reminder	Drug interaction[62]
	Prescription errors and adverse drug events[18,61]
	Parenteral nutrition orders[61]
	NICU pulse oximeter[60]
Protocol/Procedure	Blood transfusions[63]
	Medications[61]
	Parenteral nutrition[64]
Management	Oxygen in ventilated newborns[68]
	Antibiotic recommendation[44,65]
	Blood glucose control[69]
	Medication information databases[88]
	Medication dosing calculators[61]
	Ventilator management in neonates[66,67]
Research	Virtual PICU (www.picu.net)[89]
	Pediatric cardiac care consortium[70]
	Acuity scoring systems for quality improvement[56,57]
	Ventilator settings in neonates[71]
	Neonatal seizure detection[72]
	Glycemic control[90]

VTE: Venous thromboembolism; PICU: Pediatric intensive care unit; NICU: Neonatal intensive care unit.

Hunt *et al*[3] conducted a literature review on > 60 studies evaluating decision support tools to determine if these systems impacted patient care and found that CDS tools consistently enhanced performance for drug dosing and preventative care. Similarly, improvements in practitioner performance are noted in a review by Garg *et al*[22] with implementation of CDS systems.

Research on the effectiveness of CDS tools for more advanced clinical decisions in disciplines such as critical care are limited. Decisions rely heavily on clinical judgment and provider knowledge, and in the ICU environment, decisions are often affected by uncertainty. Clinical uncertainty among diagnoses and therapies makes conclusive decisions challenging[23]. Use of computer protocols or automated systems is still considered investigational; however, computers could assist clinicians' decisions by providing probabilistic estimates for diagnosis, choice of therapy, and survival[10]. Additionally, little is known about physician and nurse utilization or opinions of CDS tools[1]. To accurately assess the potential impact of a CDS tool by a clinical parameter, such as patient outcome, widespread tool use and acceptance is required[11]. Currently, use of CDS remains variable across different healthcare professionals and clinical situations[1,23,24]. Furthermore, an unappreciated challenge to CDS tools is that clinical decisions often incorporate patient and provider preferences. Some might term this phenomenon "the art of medicine." Therefore, it is not surprising that individual clinicians might resist incorporation of automated decision trees into their daily practice.

Despite advancements in the field, many pitfalls in both design and implementation of CDS tools occur and are multifactorial. In the review by Hunt *et al*[3] only 1 of 5 diagnostic aides showed a quantifiable benefit and in the review by Garg *et al*[22] only 4 of 10 diagnostic tools showed patient benefit. Diagnostic aides may be limited by variations within a diagnosis between patients and by the uncertainty of symptom variables collected from patients and inputted into systems. Also, measurement of meaningful outcomes in these studies is difficult when the intervention is designed to improve workflow and reduce barriers to guideline compliance. Other reports found no improvement or worsening in patient outcomes and costs after implementation of computerized systems[25-27]. Many reports have suggested that failure of some CDS systems is related to problems with implementation and not to content. Several studies highlighted learned lessons from failed implementation and suggested strategies for improved success. Seamless integration with existing systems and clinician workflow, limiting alarms in a system to prevent alert fatigue, and proper training before and after implementation are particularly important[1,7]. Additionally, the complex nature of human decision making adds confusion to measuring the effectiveness of CDS tools as many healthcare decisions are unstructured with high levels of uncertainty and depend on the judgment of the decision maker[1].

CURRENT APPLICATIONS OF CDS IN ADULT AND PEDIATRIC CRITICAL CARE

CDS systems in critical care continue to advance and are beginning to show improvements in care for both adult and pediatric ICU patients. A large amount of data is available for each ICU patient, and CDS tools

are designed to assist the clinician in incorporating this multitude of data into patient specific therapeutic plans. Examples of CDS systems in adult critical care are provided in Table 1. Diagnostic support tools are available to assist in disease identification and also for using symptoms and patient condition on admission to predict outcome[9,28,29]. Ranson's criteria and various APACHE models are examples that have been validated using real time data to predict mortality risk in critically ill patients[29]. Alert support tools are used to improve workflow, warn practitioners of adverse drug reactions[19,30], and to notify practitioners of potential adverse consequences of an ordered therapy, such as anticoagulation[31,32]. This type of system decreases reported patient complications from drug-drug interactions and adverse drug events in the ICU following implementation[19]. In the prospective cohort study by Bertsche *et al*[19], implementation of a CDS program showed significant decreases in drug-drug interactions and in adverse events related to drug-drug interactions, including prolonged QT interval and hypokalemia. Additionally, CDS improves adherence to protocols for mechanical ventilation[33-36], sepsis[21], and venous thromboembolism prevention[37-39], and can improve patient care. Such protocol use in critical care standardizes treatments of common physiologic states and is often central to quality improvement efforts in the ICU[40]. Tafelski *et al*[21] demonstrated significantly increased adherence to standard care protocols for sepsis following implementation of CDS, and additionally reported a significant association between mortality and adherence to those care protocols. CDS is also used to aide patient management independent of protocols by recommending suggestions for ventilator settings and weaning[41,42], antibiotic assistance[43,44], and medication dosing. Mungall *et al*[45] found significant improvement in achieving desired anticoagulation goals when using a CDS tool for heparin dosing following tissue plasminogen activator treatment in myocardial infarction compared to the standard nomogram. Blood glucose control is a commonly investigated area for support tools, and studies report more consistent target glucose levels and few adverse events with these tools[46-48]. CDS is also used for research in improving mortality risk estimation[29], prediction of hemodynamic instability[49-51], and in forecasting the need for therapies in the ICU, such as dialysis[52,53]. CDS tools can also reduce variability of clinical decisions during critical care research, therefore enabling replicable experimental methods and reproducible results[54].

Pediatric and neonatal ICUs are also utilizing CDS tools with increasing frequency, and specific examples are provided in Table 1. Multiple support tools are available to aide in diagnosis, classification of disease severity, and outcome prediction[55-59]. ISABEL is one such diagnostic aide that is commercially available as a standalone product or for integration into existing EHR systems and has shown good sensitivity for common pediatric diagnoses[59]. PRISM models and score for acute neonatal physiology (SNAP) models are validated tools for mortality risk prediction in pediatric and neonatal patients[55,56]. CDS alerts improve patient safety and are used to warn of drug interactions and adverse events and to improve the specificity of monitor alarms[18,60-62]. Kadmon *et al*[18] found alert CDS tools integrated with CPOE significantly decreased dosing order errors and potential adverse events in a pediatric ICU. Similarly, use of these tools reduced parenteral nutrition order errors in the neonatal ICU[61]. Similar to adult tools, CDS in pediatrics provides improved adherence to care protocols for blood transfusion, parenteral nutrition, and medication orders[61,63,64]. Adams *et al*[63] found a significant reduction in pediatric blood transfusions, consistent with best practice guidelines, when CDS was added to CPOE. Pediatric CDS tools also assist patient care by providing antibiotic assistance[65], medication dosing calculators[61], and ventilator management suggestions[66-68]. These management tools have improved attainment of target oxygen saturations in newborns and target blood glucose concentrations in critically ill children[68,69]. CDS tools are also used for pediatric and neonatal research on a variety of topics, including seizure detection and quality improvement[56,57,70-72].

BARRIERS TO WIDESPREAD ACCEPTANCE OF CDS IN THE ICU

CDS tools are not uniformly incorporated into critical care units. There are many barriers to widespread acceptance, including style of implementation, variability in provider preference, and perceived lack of generalizability to patient populations. Use of CDS tools is largely optional and determined by either provider preference or group consensus and a cultural shift must occur to ensure broad utilization[11]. Additionally, the formation of CDS tools through integration of independent systems, such as EHR, with probability estimates from different ICUs is complex and dependent on the quality and generalizability of the data collected[29]. Likewise, data used to create a protocol often rely on imperfect data, such as from meta-analyses, that individual clinicians may determine are not generalizable to their patients[73,74].

Even the use of simple computer protocols for care items like ventilator weaning can ignite objection from clinicians who value the importance of individual patient specific decision making. Some argue CDS tools overly standardize medicine and fail to satisfy the complex nature of ICU decision making. Proponents cite the unique processing capabilities of computer networks and the advantages of analyzing several data points simultaneously[29]. CDS tools also allow for programming models that can respond to patient specific states and data[54]. CDS tools are meant to support, not replace, clinical decisions and can expand limited human recall by presenting several data points simultaneously. The successful use of CDS tools in the ICU relies heavily on the preferences of clinicians and on the specific contexts

and degree of uncertainty present for a given clinical decision[1].

Additionally, some failures with CDS tools have been noted in the literature. Han *et al*[27] reported an unexpected increase in mortality associated with implementation of a CPOE program with integrated CDS due to delays in medication ordering, dispensing, and administration to critically ill patients. These delays were linked to unanticipated delays in workflow with early implementation. The published failures highlight the importance of proper design, implementation, and deployment of CDS tools. Mitigation of changes to clinician workflow and widespread user acceptance are important to production of a successful CDS tool.

POTENTIAL FOR CDS IN CRITICAL CARE

Advancements in computer technology and mathematics have already led to improved technology for aides in critical care, but have the potential to enhance clinician performance and patient care even more. Bedside monitors collect vast amounts of information that are currently analyzed at discrete time periods by clinicians. Neural networks and fuzzy logic systems are two types of tools that can be integrated into these bedside alarms to provide continuous analysis and potentially identify patterns consistent with various diagnoses, such as cardiac ischemia and hypovolemia[9]. Evaluation of hemodynamic data for prediction of instability and hypotension is an ongoing area of research that could translate into bedside tools in the future[49-51].

Continuous electroencephalogram (EEG) is a commonly used critical care tool from which patients and clinicians may benefit from rapid identification of seizures or prediction of seizures before they occur. Retrospective evaluation of EEG data by various mathematical techniques has shown good detection of seizure and identification of pre-ictal states minutes to hours prior to onset of seizure activity; no prospectively evaluated models have proven effective, though new methods are being researched[75]. Fuzzy logic controllers could also be used with bedside devices to provide automatic adjustment of ventilators or dialysis machines by integration of patient specific information and programmed logic controllers[9].

CDS for mechanical ventilation in adults and children has already shown good agreement with clinician recommendations[71,76,77]. In the future, these CDS tools could provide independent control of ventilator settings based on patient specific data. CDS tools also have the potential to manage decisions regarding titration of medications or weaning of support devices, thereby freeing the clinician's mind to direct the overall care of a patient. CDS incorporated into CPOE could also be used to decrease unnecessary testing or to enhance the proper selection of available tests, such as radiologic exams, based on patient information[78]. Incorporation of belief networks and neural networks into existing EHR could also provide tools for identifying diseases or estimating the probability a patient will develop a disease, such as sepsis or acute respiratory distress syndrome[9]. As septic shock is a disease with time-sensitive implications for outcome, use of prediction tools could alert clinicians to high risk patients that may benefit from additional or different therapies[79]. Additionally, the adoption of CDS linked into EHR systems could identify patients presenting to small facilities with time-sensitive diagnoses and disseminate ICU protocols to providers lacking in-house critical care specialists.

In addition to identification of disease for clinical support, CDS tools integrated into existing EHR or databases can rapidly identify patients for inclusion into research studies[80]. Utilization of CDS in this way has the potential to increase recruitment numbers, especially among studies with time dependent inclusion criteria. CDS can also provide automatic data capture for research studies by tracking patient information and automatically transmitting it to a central data coordination center, saving coordinator time and potentially costs[11]. This automatic capture can also be used to operate research protocols, potentially improving compliance[11]. CDS can standardize co-intervention control during multicenter prospective clinical trials. Co-interventional control improves the signal to noise ratio on pertinent clinical questions, thereby standardizing clinical experimental methods and enhancing the probability of accurate trial results[81,82]. Finally, increased use of CDS with EHR and data warehousing provides opportunities for collecting information across many institutions. This data provides cohorts for research on rare diseases or chronic diseases that could close existing gaps in medical evidence and improve care for patients[83].

CONCLUSION

Computerized decision support systems are becoming increasingly common in medicine, though barriers to widespread acceptance continue to exist. Studies have shown benefits to their use in a variety of applications, but research regarding improvement in patient outcome is limited. Studies have also shown that careful and proper implementation is crucial to the success of these systems. Critical care physicians are in unique environments where the use of CDS could play a significant role in patient safety and outcome over the coming years. CDS has the potential to provide improved care standardization, faster diagnosis and treatment, reduced medical errors, improved health care costs, and unique research opportunities that could all translate into improved patient outcomes over time. Advancements are occurring in the field of CDS and promise to improve current technologies and to yield exciting new technologies for clinicians in the future.

REFERENCES

1 **Weber S**. Clinical decision support systems and how critical care clinicians use them. *J Healthc Inf Manag* 2007; **21**: 41-52

[PMID: 17583167]
2 **Avansino J**, Leu MG. Effects of CPOE on provider cognitive workload: a randomized crossover trial. *Pediatrics* 2012; **130**: e547-e552 [PMID: 22891236 DOI: 10.1542/peds.2011-3408]
3 **Hunt DL**, Haynes RB, Hanna SE, Smith K. Effects of computer-based clinical decision support systems on physician performance and patient outcomes: a systematic review. *JAMA* 1998; **280**: 1339-1346 [PMID: 9794315 DOI: 10.1001/jama.280.15.1339]
4 **Ramnarayan P**, Britto J. Paediatric clinical decision support systems. *Arch Dis Child* 2002; **87**: 361-362 [PMID: 12390900 DOI: 10.1136/adc.87.5.361]
5 **Ledley RS**, LUSTED LB. Reasoning foundations of medical diagnosis; symbolic logic, probability, and value theory aid our understanding of how physicians reason. *Science* 1959; **130**: 9-21 [PMID: 13668531 DOI: 10.1126/science.130.3366.9]
6 **Warner HR**, TORONTO AF, VEASEY LG, STEPHENSON R. A mathematical approach to medical diagnosis. Application to congenital heart disease. *JAMA* 1961; **177**: 177-183 [PMID: 13783190 DOI: 10.1001/jama.1961.03040290005002]
7 **Mitchell JA**, Gerdin U, Lindberg DA, Lovis C, Martin-Sanchez FJ, Miller RA, Shortliffe EH, Leong TY. 50 years of informatics research on decision support: what's next. *Methods Inf Med* 2011; **50**: 525-535 [PMID: 22146915 DOI: 10.3414/ME11-06-0004]
8 **Johnson KB**, Feldman MJ. Medical informatics and pediatrics. Decision-support systems. *Arch Pediatr Adolesc Med* 1995; **149**: 1371-1380 [PMID: 7489077 DOI: 10.1001/archpedi.1995.02170250077014]
9 **Hanson CW**, Marshall BE. Artificial intelligence applications in the intensive care unit. *Crit Care Med* 2001; **29**: 427-435 [PMID: 11269246 DOI: 10.1097/00003246-200102000-00038]
10 **Mallach EG**. Understanding Decision Support Systems and Expert Systems. Chicago, IL: Irwin, 1994
11 **Clemmer TP**. Computers in the ICU: where we started and where we are now. *J Crit Care* 2004; **19**: 201-207 [PMID: 15648035 DOI: 10.1016/j.jcrc.2004.08.005]
12 **Keegan MT**, Gajic O, Afessa B. Severity of illness scoring systems in the intensive care unit. *Crit Care Med* 2011; **39**: 163-169 [PMID: 20838329 DOI: 10.1097/CCM.0b013e3181f96f81]
13 **Pollack MM**, Ruttimann UE, Getson PR. Pediatric risk of mortality (PRISM) score. *Crit Care Med* 1988; **16**: 1110-1116 [PMID: 3048900 DOI: 10.1097/00003246-198811000-00006]
14 **Bell LM**, Grundmeier R, Localio R, Zorc J, Fiks AG, Zhang X, Stephens TB, Swietlik M, Guevara JP. Electronic health record-based decision support to improve asthma care: a cluster-randomized trial. *Pediatrics* 2010; **125**: e770-e777 [PMID: 20231191 DOI: 10.1542/peds.2009-1385]
15 **Zhu VJ**, Grannis SJ, Tu W, Rosenman MB, Downs SM. Evaluation of a clinical decision support algorithm for patient-specific childhood immunization. *Artif Intell Med* 2012; **56**: 51-57 [PMID: 22633492 DOI: 10.1016/j.artmed.2012.04.004]
16 **Potts AL**, Barr FE, Gregory DF, Wright L, Patel NR. Computerized physician order entry and medication errors in a pediatric critical care unit. *Pediatrics* 2004; **113**: 59-63 [PMID: 14702449 DOI: 10.1542/peds.113.1.59]
17 **Lehmann CU**, Kim GR. Computerized provider order entry and patient safety. *Pediatr Clin North Am* 2006; **53**: 1169-1184 [PMID: 17126689 DOI: 10.1016/j.pcl.2006.09.006]
18 **Kadmon G**, Bron-Harlev E, Nahum E, Schiller O, Haski G, Shonfeld T. Computerized order entry with limited decision support to prevent prescription errors in a PICU. *Pediatrics* 2009; **124**: 935-940 [PMID: 19706588 DOI: 10.1542/peds.2008-2737]
19 **Bertsche T**, Pfaff J, Schiller P, Kaltschmidt J, Pruszydlo MG, Stremmel W, Walter-Sack I, Haefeli WE, Encke J. Prevention of adverse drug reactions in intensive care patients by personal intervention based on an electronic clinical decision support system. *Intensive Care Med* 2010; **36**: 665-672 [PMID: 20143221 DOI: 10.1007/s00134-010-1778-8]
20 **Shekelle P**, Morton SC, Keeler EB. Costs and Benefits of Health Information Technology. Evidence Report/Technology Assessment No. 132. (Prepared by the Southern California Evidence-based Practice Center under Contract No. 290-02-0003.) AHRQ Publication No.106-E006. Rockville, MD: Agency for Healthcare Research and Quality, 2006
21 **Tafelski S**, Nachtigall I, Deja M, Tamarkin A, Trefzer T, Halle E, Wernecke KD, Spies C. Computer-assisted decision support for changing practice in severe sepsis and septic shock. *J Int Med Res* 2010; **38**: 1605-1616 [PMID: 21309474 DOI: 10.1177/147323001003800505]
22 **Garg AX**, Adhikari NK, McDonald H, Rosas-Arellano MP, Devereaux PJ, Beyene J, Sam J, Haynes RB. Effects of computerized clinical decision support systems on practitioner performance and patient outcomes: a systematic review. *JAMA* 2005; **293**: 1223-1238 [PMID: 15755945 DOI: 10.1001/jama.293.10.1223]
23 **Henry SB**. Effect of level of patient acuity on clinical decision making of critical care nurses with varying levels of knowledge and experience. *Heart Lung* 1991; **20**: 478-485 [PMID: 1894528]
24 **Grier MR**. Information processing in nursing practice. *Annu Rev Nurs Res* 1984; **2**: 265-287 [PMID: 6568118]
25 **Al-Dorzi HM**, Tamim HM, Cherfan A, Hassan MA, Taher S, Arabi YM. Impact of computerized physician order entry (CPOE) system on the outcome of critically ill adult patients: a before-after study. *BMC Med Inform Decis Mak* 2011; **11**: 71 [PMID: 22098683 DOI: 10.1186/1472-6947-11-71]
26 **Teufel RJ**, Kazley AS, Ebeling MD, Basco WT. Hospital electronic medical record use and cost of inpatient pediatric care. *Acad Pediatr* 2012; **12**: 429-435 [PMID: 22819201 DOI: 10.1016/j.acap.2012.06.004]
27 **Han YY**, Carcillo JA, Venkataraman ST, Clark RS, Watson RS, Nguyen TC, Bayir H, Orr RA. Unexpected increased mortality after implementation of a commercially sold computerized physician order entry system. *Pediatrics* 2005; **116**: 1506-1512 [PMID: 16322178 DOI: 10.1542/peds.2005-1287]
28 **Berner ES**, Webster GD, Shugerman AA, Jackson JR, Algina J, Baker AL, Ball EV, Cobbs CG, Dennis VW, Frenkel EP. Performance of four computer-based diagnostic systems. *N Engl J Med* 1994; **330**: 1792-1796 [PMID: 8190157 DOI: 10.1056/NEJM199406233302506]
29 **Barnato AE**, Angus DC. Value and role of intensive care unit outcome prediction models in end-of-life decision making. *Crit Care Clin* 2004; **20**: 345-362, vii-viii [PMID: 15183207 DOI: 10.1016/j.ccc.2004.03.002]
30 **Harinstein LM**, Kane-Gill SL, Smithburger PL, Culley CM, Reddy VK, Seybert AL. Use of an abnormal laboratory value-drug combination alert to detect drug-induced thrombocytopenia in critically Ill patients. *J Crit Care* 2012; **27**: 242-249 [PMID: 22520497 DOI: 10.1016/j.jcrc.2012.02.014]
31 **Gupta RK**.Using An Electronic Clinical Decision Support System to Reduce the Risk of Epidural Hematoma. *Am J Ther* 2012; Epub ahead of print [PMID: 22878410 DOI: 10.1097/MJT.0b013e31825a3631]
32 **Giuliano KK**, Jahrsdoerfer M, Case J, Drew T, Raber G. The role of clinical decision support tools to reduce blood pressure variability in critically ill patients receiving vasopressor support. *Comput Inform Nurs* 2012; **30**: 204-209 [PMID: 22228216 DOI: 10.1097/NCN.0b013e3182418c39]
33 **Henderson S**, East TD, Morris AH, Gardner RM. Performance evaluation of computerized clinical protocols for management of arterial hypoxemia in ARDS patients. Proc 13th Annu Symp Comp Appl Med Care. Washington, DC: IEEE Computer Society Press, 1989: 588-592
34 **McKinley BA**, Moore FA, Sailors RM, Cocanour CS, Marquez A, Wright RK, Tonnesen AS, Wallace CJ, Morris AH, East TD. Computerized decision support for mechanical ventilation of trauma induced ARDS: results of a random-

ized clinical trial. *J Trauma* 2001; **50**: 415-424; discussion 425 [PMID: 11265020 DOI: 10.1097/00005373-200103000-00004]
35 **East TD**, Heermann LK, Bradshaw RL, Lugo A, Sailors RM, Ershler L, Wallace CJ, Morris AH, McKinley B, Marquez A, Tonnesen A, Parmley L, Shoemaker W, Meade P, Thaut P, Hill T, Young M, Baughman J, Olterman M, Gooder V, Quinn B, Summer W, Valentine V, Carlson J, Steinberg K. Efficacy of computerized decision support for mechanical ventilation: results of a prospective multi-center randomized trial. *Proc AMIA Symp* 1999; 251-255 [PMID: 10566359]
36 **Eslami S**, Abu-Hanna A, Schultz MJ, de Jonge E, de Keizer NF. Evaluation of consulting and critiquing decision support systems: effect on adherence to a lower tidal volume mechanical ventilation strategy. *J Crit Care* 2012; **27**: 425.e1-425.e8 [PMID: 22172793 DOI: 10.1016/j.jcrc.2011.07.082]
37 **Haut ER**, Lau BD, Kraenzlin FS, Hobson DB, Kraus PS, Carolan HT, Haider AH, Holzmueller CG, Efron DT, Pronovost PJ, Streiff MB. Improved prophylaxis and decreased rates of preventable harm with the use of a mandatory computerized clinical decision support tool for prophylaxis for venous thromboembolism in trauma. *Arch Surg* 2012; **147**: 901-907 [PMID: 23070407 DOI: 10.1001/archsurg.2012.2024]
38 **Kucher N**, Koo S, Quiroz R, Cooper JM, Paterno MD, Soukonnikov B, Goldhaber SZ. Electronic alerts to prevent venous thromboembolism among hospitalized patients. *N Engl J Med* 2005; **352**: 969-977 [PMID: 15758007 DOI: 10.1056/NEJMoa041533]
39 **Adams P**, Riggio JM, Thomson L, Brandell-Marino R, Merli G. Clinical decision support systems to improve utilization of thromboprophylaxis: a review of the literature and experience with implementation of a computerized physician order entry program. *Hosp Pract* (1995) 2012; **40**: 27-39 [PMID: 23086092 DOI: 10.3810/hp.2012.08.987]
40 **Button P**. Collaboration strategies for CDS success. *Health Manag Technol* 2012; **33**: 12-13 [PMID: 22908438]
41 **Karbing DS**, Allerød C, Thorgaard P, Carius AM, Frilev L, Andreassen S, Kjaergaard S, Rees SE. Prospective evaluation of a decision support system for setting inspired oxygen in intensive care patients. *J Crit Care* 2010; **25**: 367-374 [PMID: 20149586 DOI: 10.1016/j.jcrc.2009.12.013]
42 **Bouadma L**, Lellouche F, Cabello B, Taillé S, Mancebo J, Dojat M, Brochard L. Computer-driven management of prolonged mechanical ventilation and weaning: a pilot study. *Intensive Care Med* 2005; **31**: 1446-1450 [PMID: 16132889 DOI: 10.1007/s00134-005-2766-2]
43 **Yong MK**, Buising KL, Cheng AC, Thursky KA. Improved susceptibility of Gram-negative bacteria in an intensive care unit following implementation of a computerized antibiotic decision support system. *J Antimicrob Chemother* 2010; **65**: 1062-1069 [PMID: 20215130 DOI: 10.1093/jac/dkg058]
44 **Mullett CJ**, Thomas JG, Smith CL, Sarwari AR, Khakoo RA. Computerized antimicrobial decision support: an offline evaluation of a database-driven empiric antimicrobial guidance program in hospitalized patients with a bloodstream infection. *Int J Med Inform* 2004; **73**: 455-460 [PMID: 15171986 DOI: 10.1016/j.ijmedinf.2004.04.002]
45 **Mungall DR**, Anbe D, Forrester PL, Luoma T, Genovese R, Mahan J, LeBlanc S, Penney JB. A prospective randomized comparison of the accuracy of computer-assisted versus GUSTO nomogram--directed heparin therapy. *Clin Pharmacol Ther* 1994; **55**: 591-596 [PMID: 8181203 DOI: 10.1038/clpt.1994.73]
46 **Eslami S**, de Keizer NF, Dongelmans DA, de Jonge E, Schultz MJ, Abu-Hanna A. Effects of two different levels of computerized decision support on blood glucose regulation in critically ill patients. *Int J Med Inform* 2012; **81**: 53-60 [PMID: 22071011 DOI: 10.1016/j.jimedinf.2011.10.004]
47 **Mann EA**, Jones JA, Wolf SE, Wade CE. Computer decision support software safely improves glycemic control in the burn intensive care unit: a randomized controlled clinical study. *J Burn Care Res* 2011; **32**: 246-255 [PMID: 21240001 DOI: 10.1097/BCR.0b013e31820aaebf]
48 **Pielmeier U**, Rousing ML, Andreassen S, Nielsen BS, Haure P. Decision support for optimized blood glucose control and nutrition in a neurotrauma intensive care unit: preliminary results of clinical advice and prediction accuracy of the Glucosafe system. *J Clin Monit Comput* 2012; **26**: 319-328 [PMID: 22581038 DOI: 10.1007/s10877-012-9364-y]
49 **Eshelman LJ**, Lee KP, Frassica JJ, Zong W, Nielsen L, Saeed M. Development and evaluation of predictive alerts for hemodynamic instability in ICU patients. *AMIA Annu Symp Proc* 2008; 379-383 [PMID: 18999006]
50 **Donald R**, Howells T, Piper I, Chambers I, Citerio G, Enblad P, Gregson B, Kiening K, Mattern J, Nilsson P, Ragauskas A, Sahuquillo J, Sinnott R, Stell A. Early warning of EUSIG-defined hypotensive events using a Bayesian Artificial Neural Network. *Acta Neurochir Suppl* 2012; **114**: 39-44 [PMID: 22327662 DOI: 10.1007/978-3-7091-0956-48]
51 **Lee J**, Mark RG. An investigation of patterns in hemodynamic data indicative of impending hypotension in intensive care. *Biomed Eng Online* 2010; **9**: 62 [PMID: 20973998 DOI: 10.1186/1475-925X-9-62]
52 **Verplancke T**, Van Looy S, Steurbaut K, Benoit D, De Turck F, De Moor G, Decruyenaere J. A novel time series analysis approach for prediction of dialysis in critically ill patients using echo-state networks. *BMC Med Inform Decis Mak* 2010; **10**: 4 [PMID: 20092639 DOI: 10.1186/1472-6947-10-4]
53 **Celi LA**, Hinske LC, Alterovitz G, Szolovits P. An artificial intelligence tool to predict fluid requirement in the intensive care unit: a proof-of-concept study. *Crit Care* 2008; **12**: R151 [PMID: 19046450 DOI: 10.1186/cc7140]
54 **Morris AH**, Orme J, Rocha BH, Holmen J, Clemmer T, Nelson N, Allen J, Jephson A, Sorenson D, Sward K, Warner H. An electronic protocol for translation of research results to clinical practice: a preliminary report. *J Diabetes Sci Technol* 2008; **2**: 802-808 [PMID: 19885263]
55 **Richardson DK**, Corcoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. *J Pediatr* 2001; **138**: 92-100 [PMID: 11148519 DOI: 10.1203/00006450-199804001-01347]
56 **Marcin JP**, Pollack MM. Review of the acuity scoring systems for the pediatric intensive care unit and their use in quality improvement. *J Intensive Care Med* 2007; **22**: 131-140 [PMID: 17562737 DOI: 10.1177/0885066607299492]
57 **Marcin JP**, Pollack MM. Triage scoring systems, severity of illness measures, and mortality prediction models in pediatric trauma. *Crit Care Med* 2002; **30**: S457-S467 [PMID: 12528788 DOI: 10.1097/00003246-200211001-00011]
58 **Barness LA**, Tunnessen WW, Worley WE, Simmons TL, Ringe TB. Computer-assisted diagnosis in pediatrics. *Am J Dis Child* 1974; **127**: 852-858 [PMID: 4600619]
59 **Bavdekar SB**, Pawar M. Evaluation of an Internet delivered pediatric diagnosis support system (ISABEL) in a tertiary care center in India. *Indian Pediatr* 2005; **42**: 1086-1091 [PMID: 16340049]
60 **Bosque EM**. Symbiosis of nurse and machine through fuzzy logic: improved specificity of a neonatal pulse oximeter alarm. *ANS Adv Nurs Sci* 1995; **18**: 67-75 [PMID: 8585709]
61 **Stultz JS**, Nahata MC. Computerized clinical decision support for medication prescribing and utilization in pediatrics. *J Am Med Inform Assoc* 2012; **19**: 942-953 [PMID: 22813761 DOI: 10.1136/amiajnl-2011-000798]
62 **De Giorgi I**, Guignard B, Fonzo-Christe C, Bonnabry P. Evaluation of tools to prevent drug incompatibilities in paediatric and neonatal intensive care units. *Pharm World Sci* 2010; **32**: 520-529 [PMID: 20556656 DOI: 10.1007/s11096-010-9403-z]
63 **Adams ES**, Longhurst CA, Pageler N, Widen E, Franzon D, Cornfield DN. Computerized physician order entry with decision support decreases blood transfusions in children.

Pediatrics 2011; **127**: e1112-e1119 [PMID: 21502229 DOI: 10.1542/peds.2010-3252]
64 **Peverini RL**, Beach DS, Wan KW, Vyhmeister NR. Graphical user interface for a neonatal parenteral nutrition decision support system. *Proc AMIA Symp* 2000; 650-654 [PMID: 11079964]
65 **Mullett CJ**, Evans RS, Christenson JC, Dean JM. Development and impact of a computerized pediatric antiinfective decision support program. *Pediatrics* 2001; **108**: E75 [PMID: 11581483 DOI: 10.1542/peds.108.4.e75]
66 **Carlo WA**, Pacifico L, Chatburn RL, Fanaroff AA. Efficacy of computer-assisted management of respiratory failure in neonates. *Pediatrics* 1986; **78**: 139-143 [PMID: 3523414]
67 **Arrøe M**. A computerized aid in ventilating neonates. *Comput Biol Med* 1991; **21**: 15-21 [PMID: 2044357 DOI: 10.1016/0010-4825(91)90031-4]
68 **Sun Y**, Kohane I, Stark AR. Fuzzy logic assisted control of inspired oxygen in ventilated newborn infants. *Proc Annu Symp Comput Appl Med Care* 1994; 757-761 [PMID: 7950026]
69 **Faraon-Pogaceanu C**, Banasiak KJ, Hirshberg EL, Faustino EV. Comparison of the effectiveness and safety of two insulin infusion protocols in the management of hyperglycemia in critically ill children. *Pediatr Crit Care Med* 2010; **11**: 741-749 [PMID: 20543759 DOI: 10.1097/PCC.0b013e3181e88cfb]
70 **Pyles LA**, Hills CM, Larson VE, Moller JH. Pediatric Cardiac Care Consortium: an instrument for evidence-based clinical decision support. *J Cardiovasc Transl Res* 2009; **2**: 219-224 [PMID: 20559990 DOI: 10.1007/s12265-009-9091-z]
71 **Tehrani FT**, Abbasi S. Evaluation of a computerized system for mechanical ventilation of infants. *J Clin Monit Comput* 2009; **23**: 93-104 [PMID: 19263230 DOI: 10.1007/s10877-009-9170-3]
72 **Temko A**, Thomas E, Marnane W, Lightbody G, Boylan G. EEG-based neonatal seizure detection with Support Vector Machines. *Clin Neurophysiol* 2011; **122**: 464-473 [PMID: 20713314 DOI: 10.1016/j.clinph.2010.06.034]
73 **Tobin MJ**, Jubran A. Meta-analysis under the spotlight: focused on a meta-analysis of ventilator weaning. *Crit Care Med* 2008; **36**: 1-7 [PMID: 18007269 DOI: 10.1097/01.CCM.0000297883.04634.11]
74 **Marrie TJ**. Blood cultures in ambulatory patients who are discharged from emergency with community-acquired pneumonia. *Can J Infect Dis* 2004; **15**: 21-24 [PMID: 18159439 DOI: 10.1097/01.CCM.0000297958.82589.E2]
75 **Binder DK**, Haut SR. Toward new paradigms of seizure detection. *Epilepsy Behav* 2013; **26**: 247-252 [PMID: 23246145 DOI: 10.1016/j.yebeh.2012.10.027]
76 **Karbing DS**, Allerød C, Thomsen LP, Espersen K, Thorgaard P, Andreassen S, Kjærgaard S, Rees SE. Retrospective evaluation of a decision support system for controlled mechanical ventilation. *Med Biol Eng Comput* 2012; **50**: 43-51 [PMID: 22105216 DOI: 10.1007/s11517-011-0843-y]
77 **Jouvet PA**, Payen V, Gauvin F, Emeriaud G, Lacroix J. Weaning children from mechanical ventilation with a computer-driven protocol: a pilot trial. *Intensive Care Med* 2013; **39**: 919-925 [PMID: 23361631 DOI: 10.1007/s00134-013-2837-8]
78 **Zafar HM**, Mills AM, Khorasani R, Langlotz CP. Clinical decision support for imaging in the era of the Patient Protection and Affordable Care Act. *J Am Coll Radiol* 2012; **9**: 907-918.e5 [PMID: 23206649 DOI: 10.1016/j.jacr.2012.09.014]
79 **Herasevich V**, Afessa B, Chute CG, Gajic O. Designing and testing computer based screening engine for severe sepsis/septic shock. *AMIA Annu Symp Proc* 2008; 966 [PMID: 18999146]
80 **Schmickl CN**, Shahjehan K, Li G, Dhokarh R, Kashyap R, Janish C, Alsara A, Jaffe AS, Hubmayr RD, Gajic O. Decision support tool for early differential diagnosis of acute lung injury and cardiogenic pulmonary edema in medical critically ill patients. *Chest* 2012; **141**: 43-50 [PMID: 22030803 DOI: 10.1378/chest.11-1496]
81 **Morris AH**. Computerized protocols and bedside decision support. *Crit Care Clin* 1999; **15**: 523-545, vi [PMID: 10442261 DOI: 10.1016/S0749-0704(05)70069-5]
82 **Morris AH**, East TD, Wallace CJ, Franklin M, Heerman L, Kinder T, Sailor M, Carlson D, Bradshaw R. Standardization of clinical decision making for the conduct of credible clinical research in complicated medical environments. *Proc AMIA Annu Fall Symp* 1996; 418-422 [PMID: 8947700]
83 **Fiks AG**, Grundmeier RW, Margolis B, Bell LM, Steffes J, Massey J, Wasserman RC. Comparative effectiveness research using the electronic medical record: an emerging area of investigation in pediatric primary care. *J Pediatr* 2012; **160**: 719-724 [PMID: 22364853 DOI: 10.1016/j.jpeds.2012.01.039]
84 **Herasevich V**, Tsapenko M, Kojicic M, Ahmed A, Kashyap R, Venkata C, Shahjehan K, Thakur SJ, Pickering BW, Zhang J, Hubmayr RD, Gajic O. Limiting ventilator-induced lung injury through individual electronic medical record surveillance. *Crit Care Med* 2011; **39**: 34-39 [PMID: 20959788 DOI: 10.1097/CCM.0b013e3181fa4184]
85 **Thompson BT**, Orme JF, Zheng H, Luckett PM, Truwit JD, Willson DF, Duncan Hite R, Brower RG, Bernard GR, Curley MA, Steingrub JS, Sorenson DK, Sward K, Hirshberg E, Morris AH. Multicenter validation of a computer-based clinical decision support tool for glucose control in adult and pediatric intensive care units. *J Diabetes Sci Technol* 2008; **2**: 357-368 [PMID: 19885199]
86 Isabel Healthcare Ltd. Isabel the diagnosis checklist. Available from: URL: http:\\www.isabelhealthcare.com. Accessed April 25, 2013
87 SimulConsult Inc. SimulConsult: A Simultaneous Consult on Your Patient's Diagnosis. Available from: URL: http:\\www.simulconsult.com. Accessed April 25, 2013
88 **Benavides S**, Polen HH, Goncz CE, Clauson KA. A systematic evaluation of paediatric medicines information content in clinical decision support tools on smartphones and mobile devices. *Inform Prim Care* 2011; **19**: 39-46 [PMID: 22118335]
89 L.K. Whittier Foundation. The Laura P. and Leland K. Whittier Virtual Pediatric Intensive Care Unit (vPICU). Available from: URL: http:\\www.picu.net. Accessed April 25, 2013
90 **Agus MS**, Steil GM, Wypij D, Costello JM, Laussen PC, Langer M, Alexander JL, Scoppettuolo LA, Pigula FA, Charpie JR, Ohye RG, Gaies MG. Tight glycemic control versus standard care after pediatric cardiac surgery. *N Engl J Med* 2012; **367**: 1208-1219 [PMID: 22957521 DOI: 10.1056/NEJMoa1206044]

P- Reviewers: Aoyagi K, Hsu CW, Moghazy A
S- Editor: Gou SX **L- Editor**: A **E- Editor:** Liu XM

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5492/wjccm.v2.i4.29

World J Crit Care Med 2013 November 4; 2(4): 29-39
ISSN 2220-3141 (online)

REVIEW

Extracorporeal membrane oxygenation for pediatric respiratory failure: History, development and current status

Anna Maslach-Hubbard, Susan L Bratton

Anna Maslach-Hubbard, Susan L Bratton, Division of Critical Care Medicine, Department of Pediatrics, University of Utah, Salt Lake City, UT 84158, United States

Author contributions: Maslach-Hubbard A and Bratton SL contributed equally to the work and both participated in research, drafting and editing the review.

Correspondence to: Dr. Anna Maslach-Hubbard, MD, Division of Critical Care Medicine, Department of Pediatrics, University of Utah, 295 Chipeta Way, PO Box 581289, Salt Lake City, UT 84158, United States. anna.hubbard@hsc.utah.edu

Telephone: +1-801-5877560 Fax: +1-801-5818686

Received: August 8, 2013 Revised: September 21, 2013

Accepted: October 17, 2013

Published online: November 4, 2013

Abstract

Extracorporeal membrane oxygenation (ECMO) is currently used to support patients of all ages with acute severe respiratory failure non-responsive to conventional treatments, and although initial use was almost exclusively in neonates, use for this age group is decreasing while use in older children remains stable (300-500 cases annually) and support for adults is increasing. Recent advances in technology include: refinement of double lumen veno-venous (VV) cannulas to support a large range of patient size, pumps with lower prime volumes, more efficient oxygenators, changes in circuit configuration to decrease turbulent flow and hemolysis. Veno-arterial (VA) mode of support remains the predominant type used; however, VV support has lower risk of central nervous injury and mortality. Key to successful survival is implementation of ECMO before irreversible organ injury develops, unless support with ECMO is used as a bridge to transplant. Among pediatric patients treated with ECMO mortality varies by pulmonary diagnosis, underlying condition, other non-pulmonary organ dysfunction as well as patient age, but has remained relatively unchanged overall (43%) over the past several decades. Additional risk factors associated with death include prolonged use of mechanical ventilation (> 2 wk) prior to ECMO, use of VA ECMO, older patient age, prolonged ECMO support as well as complications during ECMO. Medical evidence regarding daily patient management specifically related to ECMO is scant, it usually mirrors care recommended for similar patients treated without ECMO. Linkage of the Extracorporeal Life Support Organization dataset with other databases and collaborative research networks will be required to address this knowledge deficit as most centers treat only a few pediatric respiratory failure patients each year.

Key words: Respiratory failure; Pediatrics; Extracorporeal life support; Veno-arterial; Veno-venous

Core tip: Extracorporeal membrane oxygenation (ECMO) is a very important mode of support for patients of all ages with acute severe respiratory failure, non-responsive to conventional treatments. Goal of this review is to describe evolution of ECMO support for respiratory failure, changes and advances in technology, epidemiology, outcomes and care of pediatric respiratory failure patients. Also, we would like to describe changes in modes of support and although veno-arterial (VA) mode of support remains the predominant type used, veno-venous (VV) support is increasingly used especially in older children and adults. We described advantages and limitations of VV ECMO comparing to VA support.

Maslach-Hubbard A, Bratton SL. Extracorporeal membrane oxygenation for pediatric respiratory failure: History, development and current status. *World J Crit Care Med* 2013; 2(4): 29-39 Available from: URL: http://www.wjgnet.com/2220-3141/full/v2/i4/29.htm DOI: http://dx.doi.org/10.5492/wjccm.v2.i4.29

BACKGROUND

Extracorporeal membrane oxygenation (ECMO), a form of prolonged cardiopulmonary bypass (CPB), has been used to "rescue" patients suffering from severe cardiopulmonary failure unresponsive to conventional therapies for over 30 years. ECMO development has been guided by the Extracorporeal Life Support Organization (ELSO)[1], an International Consortium of Health Care Centers that voluntarily contribute detailed data to a registry supporting the vast majority of ECMO clinical research. ELSO also develops and disseminates standards and guidelines for the member programs and sponsors medical education[2,3]. In addition to supporting patients with acute, severe respiratory failure failing conventional management, there are new applications for ECMO recently reported. These include support of patients with chronic respiratory failure as a bridge to transplant[4,5] as well as cardiopulmonary support for organ donation after circulatory determination of death both *in vivo*[6] and *ex vivo*[7] Although ECMO is currently used in both adults and children, the pioneering initial application of ECMO was for neonates with severe respiratory failure.

This focus on newborns was because initial applications in patients with respiratory failure[8] found that veno-arterial (VA) bypass dramatically improved survival in "moribund" infants, while the first randomized trial in adults with severe hypoxic respiratory failure reported dismal overall survival (9%) that was not improved by ECMO support[9]. The initial success in newborns was because ECMO support could interrupt the spiral of unrelenting hypoxia and acidosis from severe pulmonary hypertension, while adults with hypoxic respiratory failure treated in the original study reported by Hardart *et al*[10] had already suffered severe ventilator induced lung injury (VILI) that was not reversible. Early recognition that ECMO support is only potentially effective, when implemented before irreversible organ injury develops, became the key to successful ECMO patient selection. Another lesson learned from early reports was that potential candidates should not have high risk of severe bleeding complications. Premature newborns with gestational age < 35 wk suffered high rates of severe of intraventricular hemorrhage that continues to limit ECMO use for premature infants.

EPIDEMIOLOGY OF NEONATAL RESPIRATORY FAILURE AND ECMO

In the 2013 ELSO international summary 26205 of 53190 (49%) of all patients treated with ECMO reported to ELSO were neonates with respiratory failure, and patients less than a month of age comprised 60 percent of patients treated with ECMO if neonates with cardiac failure (9%) or failed cardiopulmonary resuscitation (3%) are also considered[3].

However, ECMO use to treat severe neonatal respiratory failure peaked in 1992 and steadily declined since, as advances in other less invasive therapies occurred[11,12] such as high frequency oscillatory ventilation[13-15], inhaled nitric oxide[15-20], surfactant[21,22] and maternal antibiotic therapy[23,24]. In 2011, neonatal respiratory failure accounted for 24% of cases reported to ELSO. The neonatal respiratory failure population for whom the annual cases treated with ECMO have remained relatively unchanged over time are infants with congenital diaphragmatic hernia and unfortunately approximately 40%-50% mortality persists among these patients[3,25-27]. Understanding the contribution of reversible pulmonary hypertension relative to lung hypoplasia in these newborns continue to complicate prognosis assessment.

EPIDEMIOLOGY OF PEDIATRIC RESPIRATORY AND ECMO

ELSO annual reports of ECMO use to support pediatric patients with respiratory failure have remained fairly stable over last 5 years (300-500 cases/year) with some transient increases during years of severe influenza outbreaks[3,28-31]. Compared to neonates much less ECMO experience involves pediatric respiratory failure and no clinical trials have established efficacy in this patient group. A United States study led by James Fackler[32] was initiated, but concurrent changes in critical care practices resulted in lower pediatric mortality from acute hypoxic respiratory failure and providers were largely unwilling to enroll patients in an ECMO trial. However, studies of pediatric hypoxic respiratory failure found that centers with ECMO available had lower mortality among patients with respiratory failure compared to centers treating patients without ECMO available for select cases[32]. Furthermore, recent randomized trial in adult ARDS patients reported by Peek *et al*[33] found that adults transferred to a centralized ECMO center died less frequently when both standardized conventional care and "rescue" ECMO was available compared to patients treated at multiple centers with only conventional care as a treatment option.

Trials in the United Kingdom in both neonates[34,35] and adults[33] have shown that ECMO is an effective treatment for severe respiratory failure compared to conventional support with acceptable cognitive and functional status. The remaining studies in this review refer to children treated with ECMO for respiratory failure but lack key information including: the number of children with a given pulmonary process at risk for severe respiratory failure compared to the number treated with ECMO. The unanswered questions are would a child with a unique set of clinical and demographic features survive without ECMO yesterday, today and tomorrow?

ORIGINAL TECHNOLOGY

Initial ECMO support relied exclusively on VA support typically with arterial (carotid) and venous (internal jugular) cannula in the neck with the distal end of the carotid artery permanently ligated (Figure 1). The cannula

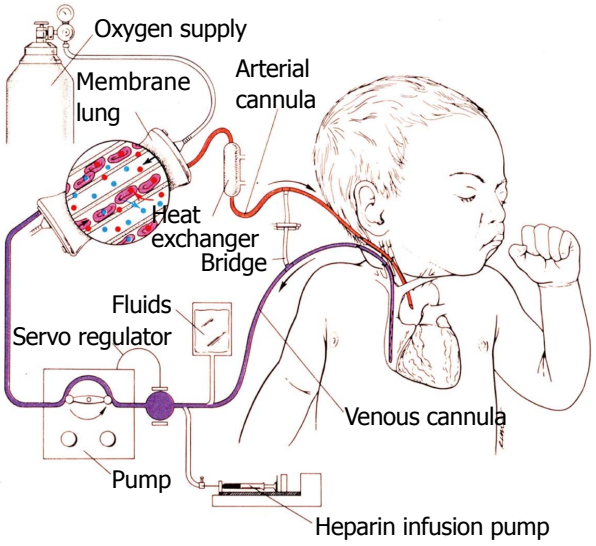

Figure 1 Classic veno arterial extracorporeal membrane oxygenation in an infant with cannulation of the right internal jugular vein draining blood by gravity to a roller pump that generates non pulsatile flow to a membrane oxygenator. Oxygenated blood is returned to the aorta by a catheter inserted in the right internal carotid artery.

drained venous blood by gravity into a reservoir (bladder) and a roller pump provided non-pulsatile propelled blood flow through an oxygenator and returned blood to the infant's aorta. With this configuration, venous drainage can be augmented by elevating the patient on multiple cushions to increase the pressure gradient between the patient and the ECMO bladder enhancing circulating volume. Systemic anti-coagulation was primarily monitored with activated clotting times. The blood moved through a circuit, and diffusion of gases occurred across a semi-permeable membrane with oxygenation limited by time in the oxygenator membrane, so larger membranes are required to support larger patients. A blood primed bridge existed in the circuit so that flow could be diverted away from the patient to preserve the circuit from clotting when testing to see if the patient was ready for ECMO support to be removed.

TECHNICAL PROBLEMS

The technical limitations to early ECMO primarily revolved around complications due to inadequate flow and oxygen delivery, hemolysis, and anti-coagulation (clot and bleeding complications). Arterial cannulation for infants is primarily limited to either accessing the heart and great vessels either *via* a sternotomy or open cannulation of the carotid because extremity vessels are inadequate until the child is ambulatory at which time the femoral artery is an option. Infants usually tolerate loss of a unilateral carotid artery, but adults are at greater risk of hemispheric stroke complicating carotid artery cannulation and ligation. Like other neonates treated with CPB[36-39], the majority of critically ill neonates treated with ECMO survive with subtle cognitive deficits, but approximately 10%-15% of survivors manifest more severe neurologic complications and central nervous system complications also increase mortality risk[40-47].

CHANGES AND ADVANCEMENTS IN TECHNOLOGY IN ECMO FOR RESPIRATORY FAILURE

As care for patients with respiratory failure evolved during last 30 years, technological advances in ECMO have also changed to enhance safety, accessibility and facilitate its use, contributing to decreased morbidity and improved survival of patients requiring ECMO support[48].

Major advances in ECMO deployment paralleled advances in conventional CPB including cannulas, pumps, oxygenator bubble detectors and heparin bonded circuits which decrease platelet activation, circuit clotting and prolong the circuit life. There is an overall tendency to use smaller circuit size decreasing priming volume, and exposure to foreign material and blood products.

CANNULAE

Double lumen venous cannulae were initially developed for use in neonates and small infants for veno -venous (VV) support, while multiple single lumen venous cannulas were used for VV support in toddlers, older children, adolescent and adults with respiratory failure. Newer double lumen cannulas with better flow profiles and less blood recirculation have increased ease of VV use in older children.

VV double lumen catheter provides drainage of SVC and IVC and more directed "arterial flow" towards tricuspid valve but require echocardiography or fluoroscopy for initial placemen[49,50]. Insertion of these cannulas can be done percutaneously, theoretically decrease risk of infection and bleeding. In adult patients, use of these cannulas is associated with less sedation use and active rehabilitation of patients during ECMO support[51,52]. Finally manufacture of double lumen catheters for older patients made this technique possible for all size patients[53,54]. However rigorous evaluation of these catheters compared to VV ECMO accomplished with use of multiple venous catheters is needed. Although the catheters appear to decrease reperfusion and provide adequate flow, reported complications include right heart perforation during insertion[55].

PUMPS

The majority of programs traditionally used roller-head (semi-occlusive) pumps, but use of smaller, low-friction centrifugal pump has increased over last several years. These pumps have potential advantages compared to roller-head pumps where circuit flow is dependent on gravity drainage and a reservoir or "bladder" is required. These mechanical features increase turbulent flow, and tubing

rupture can occur so longer circuit tubing is needed to "walk the raceway". Thanks to magnetic drives centrifugal pumps enable use of shorter tubing and smaller priming volume. Hemolysis and renal injury appear to be more common with centrifugal pumps especially in neonates. However, hemolysis can occur with both pump types[56-59].

OXYGENATORS

Initial silicon oxygenators had very large surface areas, which were more difficult to prime and debubble and furthermore their use was associated with a large inflammatory response. Polypropylene, hollow fiber devices were developed and appeared to elicit less inflammation but have a tendency to leak plasma, which decreased oxygenator life span. Some ECMO programs used the hollow fiber devices for rapid deployment and reserved the silicon oxygenators for replacement after ECMO initiation. The newest polymethyl pentene, nonporous hollow fiber oxygenators, are now widely used, provide very efficient gas exchange, with low resistance to flow and use smaller priming volumes[59-61].

CIRCUIT CONFIGURATION AND ANTICOAGULATION

Attention to flow monitoring and pressure changes across the circuit prompted modification to decrease clotting and hemolysis. Removal of the bridge blood prime during ECMO initiation potentially could lower risk of embolic events during trials off when the circuit flow is decreased and the bridge "flashed" periodically. Heparin coated tubing often decreases requirement for anticoagulation use during the initial hours after ECMO initiation, which can be especially important for patients with bleeding complications and requiring surgical procedures.

Anticoagulation strategies to prevent circuit clotting vary widely between institutions. Currently no consensus exists regarding the best way to manage anticoagulation and blood product administration for patients supported on ECMO[62-64]. All centers use unfractionated heparin as the primary anticoagulant, while few centers reported use of alternative anticoagulation agents (direct thrombin inhibitors: Argatroban and Lepirudin or Bivalirudin) when heparin-induced thrombocytopenia is suspected.

Likewise practice varies regarding anticoagulation monitoring. ACT remains the predominant diagnostic test utilized to monitor and adjust anticoagulation, but more specific tests including anti-factor Xa level, antithrombin Ⅲ (AT Ⅲ) as well as thromboelastograms are used with increasing frequency at many centers. No studies have demonstrated advantages of one test over another either in complications or cost of monitoring.

VENO VENOUS ECMO *VS* VENO ARTERIAL

Recognition that profound respiratory failure frequently could be supported with VV ECMO (Table 1), a mode where deoxygenated blood from the patient and oxygenated blood from the ECMO circuit mix in the venous circulation and any venous clots are "filtered" by the patient's native lungs. Thus for this approach to succeed in pediatric patients, native cardiac output must be adequate and in order to limit VILI, medical providers must accept relative hypoxia in some patients as a treatment goal (saturations 75%-85%).

VV ECMO can be accomplished using two separate venous catheters or a double lumen catheter. However, recirculation of blood can complicate both blood saturation monitoring and limits effective blood flow. As described above, double lumen catheter improvements decreases recirculation and improved patient arterial oxygenation when optimally positioned.

While extracorporeal support for patients with severe respiratory failure is implemented as a life saving and lung protective measure, it also carries significant risks of complications. Most commonly described and feared are neurological complications: intracranial hemorrhage, thromboembolic or ischemic strokes and seizures as they can have a profound impact on the patient's overall outcome[42-47]. These complications are related to mode of support, with VV ECMO thought to be safer from thromboembolic stand point. Also sparing of the carotid artery, which remains the most common arterial cannulation site in children with respiratory failure, could contribute to less neurological injury.

In a single center report comparing neonates who survived ECMO, infants treated with VV had significantly lower risk evidence of embolic brain lesions compared to those supported with VA when patients were routinely imaged with MRI[43]. Similarly analysis of ELSO data for pediatric patients with respiratory failure compared to patients found lower rates of central nervous system radiological injury and seizures for children treated with VV compared to VA, however, reports to ELSO, review clinical data and ascertainment of these complications likely vary by reporting center and asymptomatic patients are not routinely evaluated[65]. These findings are also similar to reports of patients supported with VA ECMO to treated failed CPR in adults[66] and children[67-69].

Surveillance of brain function among pediatric patients supported on ECMO varies. Although most centers routinely follow serial cranial ultrasound studies in infants with an open fontanel, imaging of the brain among older patients is often reserved for patients with new neurological deficits. Thus avoidance of prolonged neuromuscular blockage and minimal sedation when possible enhances neurologic monitoring for frequent patient assessment. However assessment of sedated patients if often difficult, and a quarter of pediatric patients with intracranial pathology detected by CT did not have clinical evidence of neurological compromise[70].

Rates of other commonly reported complications during ECMO support including renal failure; bleeding and infection differ by support mode. Development of acute kidney injury (AKI) and fluid overload are common

Table 1 Extracorporeal membrane oxygenation limitations and advantages comparing veno venous to veno arterial support

Factors	Veno venous	Veno arterial
Systemic emboli	Lower rate unless intra cardiac shunt present	Increased rate of stroke and seizures with carotid cannulation, risk increases with patient age Limb ischemia with femoral arterial cannulation
Cardiopulmonary support	Does not provide direct hemodynamic support Lower systemic oxygenation Increased rate of hypertension during ECMO Usually requires some degree of pulmonary gas exchange and lung recruitment Indirect support with more oxygenated blood provided to pulmonary circulation	Provides full hemodynamic support High systemic oxygenation Non pulsatile flow More commonly used with severe air leak
Organ injury	Less acute kidney injury- preserved pulsatile blood flow Less central nervous system injury risk	More acute kidney injury More central nervous system injury risk
Monitoring	Mixed venous oxygen saturation less reliable due to recirculation	Reliable mixed venous saturation measurements
Bleeding	Increased cannula site bleeding	More bleeding with multiple site cannulation and femoral arterial cannulation compared to carotid
Infection	Less risk with percutaneous and single cannula use	Greater rates of infection
Rehabilitation	Less sedation use if adequate oxygen delivery possible Mobilization of patients more feasible with single catheter neck catheter	

ECMO: Extracorporeal membrane oxygenation.

among patients supported on ECMO and especially renal failure is seen more commonly during VA support[3]. It is impossible to assess how much ECMO support contributes to renal failure distinct from injury related to pre-ECMO events. Overzealous fluid removal with diuretics or continuous renal replacement therapy (CRRT), lack of pulsatile flow with VA support and hemolysis all may contribute to development of renal insufficiency[71-75]. A recent report by Wolf *et al*[76] of patients with congenital heart disease supported on ECMO, highlighted that early implementation of CRRT and too aggressive fluid removal may lead to intravascular volume depletion, aggravate AKI and worsen outcome.

As with other modes of mechanical support, ECMO also carries risk of acquired infection during the bypass run. In 2010 the ELSO international summary data reported the incidence of culture-proven bacterial infection increased with age: 6.1 % in neonates, 18.7% in children and 20.5% in adults and was associated with increased mortality[77,78]. Duration of catheter use, ECMO to support CPR, VA mode were all associated with increased infection risk[79,80].

Bleeding on ECMO is the most frequently described complication resulting from necessity of continuous anti-coagulation to prevent circuit clotting and thromboembolic events. Surgical cannulation site and other procedural incisions are the most commonly reported bleeding sites. Rollins *et al*[65] evaluated bleeding from surgical cannula site among pediatric patients receiving ECMO for respiratory failure and reported hemorrhage rates were significantly greater for VV (19%) than VA (15%) but among arterial cannula site rates of hemorrhage were greater in the femoral artery (28%) compared to carotid artery (15%).

Trials off VV support are simplified compared to VA because the oxygenator can be "isolated" from the patient at full flow by simply removing the gas source. Trials off VA support increase risk of clot development and emboli due to both stasis with lower pump flows, changes in blood volume relative to heparin dosing and flushing the bridge, which leads to turbulent flow. Finally, among patients treated with VA ECMO for respiratory failure, infants, children and adults have higher mortality compared to patients supported with VV[3,48]; however, this observation is likely confounded at least in part by severity of illness as high need for vasoactive medications and cardiopulmonary arrest immediately preceding initiation of ECMO are viewed by many critical care physicians as a contraindication for VV ECMO use.

OUTCOME/MORTALITY

Because the ELSO registry and other administrative data sets do not systematically collect information regarding disability, hospital mortality is most commonly reported. Among patients treated with ECMO mortality varies by pulmonary diagnosis, underlying condition, other non-pulmonary organ dysfunction as well as patient age[48,81], but has remained relatively unchanged overall (43%) over the past several decades. Although survival among patients without concurrent non-pulmonary disease has improved, ECMO has increasingly been reserved for medically complex patients with acute other organ failure and/or underlying chronic diseases (Table 2). This is logical as ECMO continues to be a "rescue" therapy and mortality from acute respiratory failure in children has declined concurrent with recognition of successful strategies to decrease VILI as well as other improvements in critical care[82-84]. Indirect evidence that "gentle" ventilation has been embraced by the pediatric critical care community is provided by the most recent ELSO registry analysis, in which the duration of pre-ECMO mechanical

Table 2 Factors associated with hospital mortality among pediatric patients receiving extracorporeal membrane oxygenation for respiratory failure

Factors	Increased survival	Increased death
Age	Younger	Age > 10 yr
Pulmonary process	Asthma	Pertussis
	Viral pneumonia/bronchiolitis	Sepsis
	Aspiration pneumonia	Opportunistic infections
Organ dysfunction		Renal failure/dialysis
		Liver injury
		Immune impairment/deficiency
		Cardiac arrest prior to ECMO
Severity of ventilator associated lung injury prior to ECMO		Severe acidosis
		High mean airway pressure
		Duration ventilation > 14 d
Mode	VV	VA
Complications during ECMO		Infection
		Stroke
		Cardiac arrest
		Organ failure

VV: Veno venous; VA: Veno arterial; ECMO: Extracorporeal membrane oxygenation.

ventilation was no longer associated with lower survival until ECMO deployment occurred at 2 wk or more after initiation of mechanical ventilation[48,85].

ECMO use and prognosis in pediatric patients with intractable respiratory failure differs from use in newborns in several obvious (*e.g.,* range of patient size, age, survival) and less obvious ways (reversibility of disease process). Like neonates, use of VV ECMO is increasing but remains less common than VA support. Recent advances in availability of double lumen catheters that decrease recirculation for use in patients of all sizes may continue to enhance use of VV; however, VV cannulation is technically more difficult and associated with increased cannulation site bleeding and flow issues[65]. VV support should be the default choice for pediatric respiratory failure because central nervous system injury is less common, and recent ELSO analysis did not find increased mortality from a "failed" trial of VV requiring conversion to VA support. Among pediatric patients who require VA, firm recommendations cannot be made regarding arterial site. Although carotid cannulation is avoided in adults due to concern for hemispheric stroke, insufficient data are available in older children to make firm recommendations when children requiring VA support should transition to femoral artery use rather than carotid[65]. When using the femoral artery for VA ECMO, a bypass graft or reperfusion catheter to maintain adequate limb perfusion is often required.

Other factors associated with increased survival among pediatric ECMO patients with respiratory failure include: young age, obstructive lung diseases compared to restrictive processes (*e.g.,* asthma or bronchiolitis *vs* acute lung injury), and primary lung injury compared to secondary lung injury (*e.g.,* pneumonia *vs* sepsis)[48,86,87]. Risk of mortality increases for potential ECMO patients with acute respiratory failure if they have either pre-existing chronic non-pulmonary organ failure or develop additional acute organ failure before or after institution of ECMO. Patients who can tolerate net fluid loss while receiving ECMO support have lower risk of death[88], while development of renal failure during ECMO has repeatedly been associated with increased mortality[71-75]. It remains unclear if early fluid removal on ECMO improves outcome. Recently Selewski *et al*[75] examined the association between fluid overload and outcomes in pediatric ECMO patients receiving continuous renal replacement therapy (CRRT). Among patients treated with ECMO, 28% received concurrent CRRT and survival was significantly lower (34% *vs* 58%) compared to those not treated with CRRT. However, it is likely that those receiving CRRT had greater severity of illness and greater risk of death.

As expected patients with underlying immune disorders or an active malignancy have lower survival compared to patients with intact immune function[48]. Although profound immunosuppression was once considered an absolute contraindication, now decisions regarding whether a child is an ECMO candidate include nuisances such as the anticipated duration of neutropenia, other organ failure and ultimate cancer prognosis. Although numbers of patients treated with ECMO after hematopoietic stem cell transplantation are few, some cases have survived to hospital discharge; however, long term cancer survival was not reported.

Complications occurring after institution of ECMO that reflect either inadequate cardiorespiratory support or end organ failure arising from pre-ECMO injury have consistently been associated with increased risk of mortality[48,87]. Thus care to avoid complications during ECMO may increase survival. Although most pediatric patients with respiratory failure are able to be liberated from ECMO within 3 wk, survival decreases with pro-

longed duration; however, no studies have enumerated factors that clearly predict death and typically support is continued till either complications ensue causing multiple organ dysfunction or pulmonary status improves and the patient can be supported without ECMO. Survival among patients treated with ECMO for longer than 3 wk decreases to 38%.

CARE OF PEDIATRIC RESPIRATORY FAILURE ECMO PATIENTS

Many management aspects of respiratory failure for patients treated with ECMO are not based on medical evidence specific to ECMO care, but mirrors care recommended for similar patients treated without ECMO. However, ECMO does affect management. For instance some medications bind to or interact with the ECMO circuit (*e.g.,* fentanyl) or clearance may be altered by addition of dialysis (*e.g.,* barbiturates) so critical care teams should include expertise from a pharmacologist. Certainly patients must be able to cooperate with care without risk of unplanned medical appliance dislodgement. Some pediatric age patients require high levels of sedation; however, when possible neuromuscular blocking agents (NMBA) should be avoided and sedation minimized to allow spontaneous coughing, optimize respiratory secretion clearance and decrease respiratory and peripheral muscle de-conditioning. In addition, reduced use of deep sedation and NMBA will allow better assessment of patient neurological status[62,89]. As with survivors of any critical illness, ECMO survivors may be left with significant psychological and functional disabilities[90]. Recently more attention is paid to early recognition of possible neurological injury which should lead to early implementation of rehabilitation. Although implementation of physical therapy (PT) for patients while still on ECMO is very challenging due to increased risk of acute decompensation, medical device dislodgment , some authors suggest use of simple PT interventions to avoid motor and cardio-respiratory deconditioning[91]. Reports from multiple centers show that cooperative patients can assist with mobility including pulmonary rehabilitation while on ECMO without high risk of appliance movement and that prone positioning is routinely possible in pediatric patients[91-94]. However, it is unclear which components of rehabilitation are essential to maintain and improve functional outcomes (*e.g.,* passive limb range of motion, splinting, active limb exercises/strengthening, ambulation) are not known. If tolerated by the patient, rehabilitation should be continued through post-ECMO hospitalization period and following hospital discharge[94].

No studies have evaluated optimal nutrition goals, but many critical care providers consider enteral feeds preferable to intravenous nutrition. Our center routinely uses trans pyloric feeding tubes rather than nasogastric to decrease risk for aspiration of formula. The optimal transfusion threshold during ECMO also remains unclear. Especially for patients treated with VV ECMO, providers must weigh potential benefits from increased oxygen deliver and potential harm from increased fluid overload. Recent trends in both adult and pediatric critical care medicine have found similar patient outcomes with lower red blood cell administration thresholds[95,96]. Many providers now accept lower transfusion thresholds (hgb > 10 g/dL) for ECMO pediatric patients with normal arterial saturations but maintain higher thresholds for desaturated patients. Trending mixed venous desaturation is an additional measure to aid transfusion decisions. If the mixed venous saturation increases after a transfusion then a higher hemoglobin concentration may provide physiologic benefit However, ECMO patients who tolerate fluid loss either using diuretics or hemoconcentration have higher rates of survival[88,97].

The mechanical ventilator strategy for respiratory ECMO should limit VILI[98-100]. Maintaining lung recruitment is ideal unless severe air-leak syndrome has developed, our practice is to reduce positive end expiratory pressure till the leak is minimized. Otherwise, PEEP is maintained and a low stretch ventilation strategy initiated as the circuit removal of carbon dioxide is efficient. No studies have compared conventional to high frequency oscillation ventilation in ECMO patients but patient care and avoidance of NMBA is easier to achieve during conventional ventilation.

Other care should be directed to decrease complications. Because they are intensively monitored ECMO patients have increased risk of catheter associated blood stream infections[101] and other nosocomial infections. Development of other organ failure substantially increases mortality. ECMO should be removed as soon as pulmonary compliance and gas exchange have improved and the patient can be maintained on non-toxic ventilator settings.

CONCLUSION

ECMO continues to be used as a rescue therapy for increasingly complex pediatric patients with respiratory failure. VV ECMO should be the used as the default mode due to lower complication rates and many aspects of ideal care remain unstudied. Linkage of the ELSO dataset with other databases or collaborative research networks will be required to address this knowledge deficit as most centers treat only a few pediatric respiratory failure patients each year.

REFERENCES

1 Extracorporeal Life Support Organization. Available from: URL: http: //www.elsonet.org. Accessed 03-19-2013
2 ELSO Guidelines for ECMO Centers. Available from: URL: http: //www.elsonet.org/index.php/resources/guidelines.html. Accessed 03-19-2013
3 ECLS Registry Report: International Summary January 2013. Available from: URL: http: //www.elsonet.org/index.php/registry/statistics/limited.html
4 **Fuehner T**, Kuehn C, Hadem J, Wiesner O, Gottlieb J, Tudorache I, Olsson KM, Greer M, Sommer W, Welte T, Haverich

A, Hoeper MM, Warnecke G. Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. *Am J Respir Crit Care Med* 2012; **185**: 763-768 [PMID: 22268135 DOI: 10.1164/rccm.201109-1599OC]

5 **Diso D**, Anile M, Patella M, Pecoraro Y, Rendina EA, Carillo C, Russo E, Onorati I, Angioletti D, Ruberto F, Mazzesi G, Marullo AG, Frati G, Venuta F. Lung transplantation for cystic fibrosis: outcome of 101 single-center consecutive patients. *Transplant Proc* 2013; **45**: 346-348 [PMID: 23375321 DOI: 10.1016/j]

6 **Magliocca JF**, Magee JC, Rowe SA, Gravel MT, Chenault RH, Merion RM, Punch JD, Bartlett RH, Hemmila MR. Extracorporeal support for organ donation after cardiac death effectively expands the donor pool. *J Trauma* 2005; **58**: 1095-101; discussion 1101-2 [PMID: 15995454]

7 **Fondevila C**, Hessheimer AJ, Flores E, Ruiz A, Mestres N, Calatayud D, Paredes D, Rodríguez C, Fuster J, Navasa M, Rimola A, Taurá P, García-Valdecasas JC. Applicability and results of Maastricht type 2 donation after cardiac death liver transplantation. *Am J Transplant* 2012; **12**: 162-170 [PMID: 22070538 DOI: 10.1111/j.1600-6143.2011.03834.x]

8 **Bartlett RH**, Gazzaniga AB, Jefferies MR, Huxtable RF, Haiduc NJ, Fong SW. Extracorporeal membrane oxygenation (ECMO) cardiopulmonary support in infancy. *Trans Am Soc Artif Intern Organs* 1976; **22**: 80-93 [PMID: 951895]

9 **Zapol WM**, Snider MT, Hill JD, Fallat RJ, Bartlett RH, Edmunds LH, Morris AH, Peirce EC, Thomas AN, Proctor HJ, Drinker PA, Pratt PC, Bagniewski A, Miller RG. Extracorporeal membrane oxygenation in severe acute respiratory failure. A randomized prospective study. *JAMA* 1979; **242**: 2193-2196 [PMID: 490805 DOI: 10.1001/jama.1979.03300200023016]

10 **Hardart GE**, Fackler JC. Predictors of intracranial hemorrhage during neonatal extracorporeal membrane oxygenation. *J Pediatr* 1999; **134**: 156-159 [PMID: 9931522]

11 **Hintz SR**, Suttner DM, Sheehan AM, Rhine WD, Van Meurs KP. Decreased use of neonatal extracorporeal membrane oxygenation (ECMO): how new treatment modalities have affected ECMO utilization. *Pediatrics* 2000; **106**: 1339-1343 [PMID: 11099586]

12 **Roy BJ**, Rycus P, Conrad SA, Clark RH. The changing demographics of neonatal extracorporeal membrane oxygenation patients reported to the Extracorporeal Life Support Organization (ELSO) Registry. *Pediatrics* 2000; **106**: 1334-1338 [PMID: 11099585]

13 **deLemos R**, Yoder B, McCurnin D, Kinsella J, Clark R, Null D. The use of high-frequency oscillatory ventilation (HFOV) and extracorporeal membrane oxygenation (ECMO) in the management of the term/near term infant with respiratory failure. *Early Hum Dev* 1992; **29**: 299-303 [PMID: 1396256]

14 **Gerstmann DR**, Minton SD, Stoddard RA, Meredith KS, Monaco F, Bertrand JM, Battisti O, Langhendries JP, Francois A, Clark RH. The Provo multicenter early high-frequency oscillatory ventilation trial: improved pulmonary and clinical outcome in respiratory distress syndrome. *Pediatrics* 1996; **98**: 1044-1057 [PMID: 8951252]

15 **Clark RH**, Yoder BA, Sell MS. Prospective, randomized comparison of high-frequency oscillation and conventional ventilation in candidates for extracorporeal membrane oxygenation. *J Pediatr* 1994; **124**: 447-454 [PMID: 8120720]

16 **Kinsella JP**, Truog WE, Walsh WF, Goldberg RN, Bancalari E, Mayock DE, Redding GJ, deLemos RA, Sardesai S, McCurnin DC, Moreland SG, Cutter GR, Abman SH. Randomized, multicenter trial of inhaled nitric oxide and high-frequency oscillatory ventilation in severe, persistent pulmonary hypertension of the newborn. *J Pediatr* 1997; **131**: 55-62 [PMID: 9255192]

17 **Clark RH**, Kueser TJ, Walker MW, Southgate WM, Huckaby JL, Perez JA, Roy BJ, Keszler M, Kinsella JP. Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. Clinical Inhaled Nitric Oxide Research Group. *N Engl J Med* 2000; **342**: 469-474 [PMID: 10675427]

18 **Christou H**, Van Marter LJ, Wessel DL, Allred EN, Kane JW, Thompson JE, Stark AR, Kourembanas S. Inhaled nitric oxide reduces the need for extracorporeal membrane oxygenation in infants with persistent pulmonary hypertension of the newborn. *Crit Care Med* 2000; **28**: 3722-3727 [PMID: 11098980]

19 **Dobyns EL**, Anas NG, Fortenberry JD, Deshpande J, Cornfield DN, Tasker RC, Liu P, Eells PL, Griebel J, Kinsella JP, Abman SH. Interactive effects of high-frequency oscillatory ventilation and inhaled nitric oxide in acute hypoxemic respiratory failure in pediatrics. *Crit Care Med* 2002; **30**: 2425-2429 [PMID: 12441749]

20 **Kinsella JP**, Cutter GR, Walsh WF, Gerstmann DR, Bose CL, Hart C, Sekar KC, Auten RL, Bhutani VK, Gerdes JS, George TN, Southgate WM, Carriedo H, Couser RJ, Mammel MC, Hall DC, Pappagallo M, Sardesai S, Strain JD, Baier M, Abman SH. Early inhaled nitric oxide therapy in premature newborns with respiratory failure. *N Engl J Med* 2006; **355**: 354-364 [PMID: 16870914]

21 Collaborative European Multicenter Study Group. Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international randomized clinical trial. *Pediatrics* 1988; **82**: 683-691 [PMID: 2903480]

22 **Liechty EA**, Donovan E, Purohit D, Gilhooly J, Feldman B, Noguchi A, Denson SE, Sehgal SS, Gross I, Stevens D. Reduction of neonatal mortality after multiple doses of bovine surfactant in low birth weight neonates with respiratory distress syndrome. *Pediatrics* 1991; **88**: 19-28 [PMID: 2057268]

23 **Mercer BM**, Arheart KL. Antimicrobial therapy in expectant management of preterm premature rupture of the membranes. *Lancet* 1995; **346**: 1271-1279 [PMID: 7475723]

24 **Mercer BM**, Miodovnik M, Thurnau GR, Goldenberg RL, Das AF, Ramsey RD, Rabello YA, Meis PJ, Moawad AH, Iams JD, Van Dorsten JP, Paul RH, Bottoms SF, Merenstein G, Thom EA, Roberts JM, McNellis D. Antibiotic therapy for reduction of infant morbidity after preterm premature rupture of the membranes. A randomized controlled trial. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *JAMA* 1997; **278**: 989-995 [PMID: 9307346]

25 **Seetharamaiah R**, Younger JG, Bartlett RH, Hirschl RB. Factors associated with survival in infants with congenital diaphragmatic hernia requiring extracorporeal membrane oxygenation: a report from the Congenital Diaphragmatic Hernia Study Group. *J Pediatr Surg* 2009; **44**: 1315-1321 [PMID: 19573654]

26 **Wynn J**, Krishnan U, Aspelund G, Zhang Y, Duong J, Stolar CJ, Hahn E, Pietsch J, Chung D, Moore D, Austin E, Mychaliska G, Gajarski R, Foong YL, Michelfelder E, Potolka D, Bucher B, Warner B, Grady M, Azarow K, Fletcher SE, Kutty S, Delaney J, Crombleholme T, Rosenzweig E, Chung W, Arkovitz MS. Outcomes of congenital diaphragmatic hernia in the modern era of management. *J Pediatr* 2013; **163**: 114-119.e1 [PMID: 23375362 DOI: 10.1016/j.jpeds.2012.12.036]

27 **Stevens TP**, Chess PR, McConnochie KM, Sinkin RA, Guillet R, Maniscalco WM, Fisher SG. Survival in early- and late-term infants with congenital diaphragmatic hernia treated with extracorporeal membrane oxygenation. *Pediatrics* 2002; **110**: 590-596 [PMID: 12205265]

28 **Pham T**, Combes A, Rozé H, Chevret S, Mercat A, Roch A, Mourvillier B, Ara-Somohano C, Bastien O, Zogheib E, Clavel M, Constan A, Marie Richard JC, Brun-Buisson C, Brochard L. Extracorporeal membrane oxygenation for pandemic influenza A(H1N1)-induced acute respiratory distress syndrome: a cohort study and propensity-matched analysis. *Am J Respir Crit Care Med* 2013; **187**: 276-285 [PMID: 23155145 DOI: 10.1164/rccm.201205-0815OC]

29 **Kumar A**, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lac-

roix J, Stelfox T, Bagshaw S, Choong K, Lamontagne F, Turgeon AF, Lapinsky S, Ahern SP, Smith O, Siddiqui F, Jouvet P, Khwaja K, McIntyre L, Menon K, Hutchison J, Hornstein D, Joffe A, Lauzier F, Singh J, Karachi T, Wiebe K, Olafson K, Ramsey C, Sharma S, Dodek P, Meade M, Hall R, Fowler RA. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. *JAMA* 2009; **302**: 1872-1879 [PMID: 19822627 DOI: 10.1001/jama.2009.1496]

30 **Randolph AG**, Vaughn F, Sullivan R, Rubinson L, Thompson BT, Yoon G, Smoot E, Rice TW, Loftis LL, Helfaer M, Doctor A, Paden M, Flori H, Babbitt C, Graciano AL, Gedeit R, Sanders RC, Giuliano JS, Zimmerman J, Uyeki TM. Critically ill children during the 2009-2010 influenza pandemic in the United States. *Pediatrics* 2011; **128**: e1450-e1458 [PMID: 22065262 DOI: 10.1542/peds.2011-0774]

31 **Zangrillo A,** Biondi-Zoccai G, Landoni G, Frati G, Patroniti N, Pesenti A, Pappalardo F. Extracorporeal membrane oxygenation (ECMO) in patients with H1N1 influenza infection: a systematic review and meta-analysis including 8 studies and 266 patients receiving ECMO. *Crit Care* 2013; **17**: R30 [PMID: 23406535]

32 **Green TP**, Timmons OD, Fackler JC, Moler FW, Thompson AE, Sweeney MF. The impact of extracorporeal membrane oxygenation on survival in pediatric patients with acute respiratory failure. Pediatric Critical Care Study Group. *Crit Care Med* 1996; **24**: 323-329 [PMID: 8605808]

33 **Peek GJ**, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, Hibbert CL, Truesdale A, Clemens F, Cooper N, Firmin RK, Elbourne D. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. *Lancet* 2009; **374**: 1351-1363 [PMID: 19762075 DOI: 10.1016/S0140-6736(09)61069-2]

34 **Karimova A**, Brown K, Ridout D, Beierlein W, Cassidy J, Smith J, Pandya H, Firmin R, Liddell M, Davis C, Goldman A. Neonatal extracorporeal membrane oxygenation: practice patterns and predictors of outcome in the UK. *Arch Dis Child Fetal Neonatal Ed* 2009; **94**: F129-F132 [PMID: 18829617 DOI: 10.1136/adc.2008.141051]

35 **Petrou S**, Bischof M, Bennett C, Elbourne D, Field D, McNally H. Cost-effectiveness of neonatal extracorporeal membrane oxygenation based on 7-year results from the United Kingdom Collaborative ECMO Trial. *Pediatrics* 2006; **117**: 1640-1649 [PMID: 16651318]

36 **Sananes R**, Manlhiot C, Kelly E, Hornberger LK, Williams WG, MacGregor D, Buncic R, McCrindle BW. Neurodevelopmental outcomes after open heart operations before 3 months of age. *Ann Thorac Surg* 2012; **93**: 1577-1583 [PMID: 22541188 DOI: 10.1016/j.athoracsur.2012.02.011.]

37 **Neufeld RE**, Clark BG, Robertson CM, Moddemann DM, Dinu IA, Joffe AR, Sauve RS, Creighton DE, Zwaigenbaum L, Ross DB, Rebeyka IM. Five-year neurocognitive and health outcomes after the neonatal arterial switch operation. *J Thorac Cardiovasc Surg* 2008; **136**: 1413-1421, 1413-1421 [PMID: 19114183 DOI: 10.1016/j.jtcvs2008.05.011]

38 **Wypij D**, Newburger JW, Rappaport LA, duPlessis AJ, Jonas RA, Wernovsky G, Lin M, Bellinger DC. The effect of duration of deep hypothermic circulatory arrest in infant heart surgery on late neurodevelopment: the Boston Circulatory Arrest Trial. *J Thorac Cardiovasc Surg* 2003; **126**: 1397-1403 [PMID: 14666011]

39 **Tabbutt S**, Gaynor JW, Newburger JW. Neurodevelopmental outcomes after congenital heart surgery and strategies for improvement. *Curr Opin Cardiol* 2012; **27**: 82-91 [PMID: 22274574 DOI: 10.1097/HCO.0b013e328350197b]

40 **Costello JM**, O'Brien M, Wypij D, Shubert J, Salvin JW, Newburger JW, Laussen PC, Arnold JH, Fynn-Thompson F, Thiagarajan RR. Quality of life of pediatric cardiac patients who previously required extracorporeal membrane oxygenation. *Pediatr Crit Care Med* 2012; **13**: 428-434 [PMID: 22067987 DOI: 10.1097/PCC.0b013e318238ba21]

41 The collaborative UK ECMO (Extracorporeal Membrane Oxygenation) trial: follow-up to 1 year of age. *Pediatrics* 1998; **101**: E1 [PMID: 9521968]

42 **Friedman S**, Chen C, Chapman JS, Jeruss S, Terrin N, Tighiouart H, Parsons SK, Wilson JM. Neurodevelopmental outcomes of congenital diaphragmatic hernia survivors followed in a multidisciplinary clinic at ages 1 and 3. *J Pediatr Surg* 2008; **43**: 1035-1043 [PMID: 18558179 DOI: 10.1016/j.jpedsurg.2008.02.029]

43 **Rollins MD**, Yoder BA, Moore KR, Barnhart DC, Jones C, Null DM, DiGeronimo RJ. Utility of neuroradiographic imaging in predicting outcomes after neonatal extracorporeal membrane oxygenation. *J Pediatr Surg* 2012; **47**: 76-80 [PMID: 22244396 DOI: 10.1016/j.jpedsurg.2011.10.016]

44 **Hervey-Jumper SL**, Annich GM, Yancon AR, Garton HJ, Muraszko KM, Maher CO. Neurological complications of extracorporeal membrane oxygenation in children. *J Neurosurg Pediatr* 2011; **7**: 338-344 [PMID: 21456903 DOI: 10.3171/2011.1.PEDS10443]

45 **Hamrick SE**, Gremmels DB, Keet CA, Leonard CH, Connell JK, Hawgood S, Piecuch RE. Neurodevelopmental outcome of infants supported with extracorporeal membrane oxygenation after cardiac surgery. *Pediatrics* 2003; **111**: e671-e675 [PMID: 12777584]

46 **Danzer E**, Gerdes M, D'Agostino JA, Partridge EA, Hoffman-Craven CH, Bernbaum J, Rintoul NE, Flake AW, Adzick NS, Hedrick HL. Preschool neurological assessment in congenital diaphragmatic hernia survivors: outcome and perinatal factors associated with neurodevelopmental impairment. *Early Hum Dev* 2013; **89**: 393-400 [PMID: 23333410 DOI: 10.1016/j.earlhumdev.2012.12.009]

47 **Waitzer E**, Riley SP, Perreault T, Shevell MI. Neurologic outcome at school entry for newborns treated with extracorporeal membrane oxygenation for noncardiac indications. *J Child Neurol* 2009; **24**: 801-806 [PMID: 19196874 DOI: 10.1177/0883073808330765]

48 **Zabrocki LA**, Brogan TV, Statler KD, Poss WB, Rollins MD, Bratton SL. Extracorporeal membrane oxygenation for pediatric respiratory failure: Survival and predictors of mortality. *Crit Care Med* 2011; **39**: 364-370 [PMID: 20959787 DOI: 10.1097/CCM.0b013e3181fb7b35]

49 **Javidfar J**, Wang D, Zwischenberger JB, Costa J, Mongero L, Sonett J, Bacchetta M. Insertion of bicaval dual lumen extracorporeal membrane oxygenation catheter with image guidance. *ASAIO J* 2011; **57**: 203-205 [PMID: 21499077 DOI: 10.1097/MAT.0b013e3182155fee]

50 **de Bucourt M**, Teichgräber UK. Image guided placement of extracorporeal life support through bi-caval dual lumen venovenous membrane oxygenation in an interventional radiology setting--initial experience. *J Vasc Access* 2012; **13**: 221-225 [PMID: 22266596 DOI: 10.5301/jva.5000033]

51 **Camboni D**, Philipp A, Lubnow M, Bein T, Zausig Y, Hilker M, Flörchinger B, Rupprecht L, Keyser A, Kobuch R, Lunz D, Schopka S, Haneya A, Schmid C, Müller T. Extracorporeal membrane oxygenation by single-vessel access in adults: advantages and limitations. *ASAIO J* 2012; **58**: 616-621 [PMID: 22990284 DOI: 10.1097/MAT.0b013e31826a8a32]

52 **Reeb J**, Falcoz PE, Santelmo N, Massard G. Double lumen bi-cava cannula for veno-venous extracorporeal membrane oxygenation as bridge to lung transplantation in non-intubated patient. *Interact Cardiovasc Thorac Surg* 2012; **14**: 125-127 [PMID: 22108944 DOI: 10.1093/icvts/ivr046]

53 **Lazar DA**, Cass DL, Olutoye OO, Kim ES, Welty SE, Fernandes CJ, Lee TC. Venovenous cannulation for extracorporeal membrane oxygenation using a bicaval dual-lumen catheter in neonates. *J Pediatr Surg* 2012; **47**: 430-434 [PMID: 22325408 DOI: 10.1016/j.jpedsurg.2011.10.055]

54 **Fallon SC**, Shekerdemian LS, Olutoye OO, Cass DL, Zamora

IJ, Nguyen T, Kim ES, Larimer EL, Lee TC. Initial experience with single-vessel cannulation for venovenous extracorporeal membrane oxygenation in pediatric respiratory failure. *Pediatr Crit Care Med* 2013; **14**: 366-373 [PMID: 23548959 DOI: 10.1097/PCC.0b013e31828a70dc]

55 **Hirose H**, Yamane K, Marhefka G, Cavarocchi N. Right ventricular rupture and tamponade caused by malposition of the Avalon cannula for venovenous extracorporeal membrane oxygenation. *J Cardiothorac Surg* 2012; **7**: 36 [PMID: 22515939 DOI: 10.1186/1749-8090-7-36]

56 **Barrett CS**, Jaggers JJ, Cook EF, Graham DA, Rajagopal SK, Almond CS, Seeger JD, Rycus PT, Thiagarajan RR. Outcomes of neonates undergoing extracorporeal membrane oxygenation support using centrifugal versus roller blood pumps. *Ann Thorac Surg* 2012; **94**: 1635-1641 [PMID: 22921236 DOI: 10.1016/j.athoracsur.2012.06.061]

57 **Barrett CS**, Jaggers JJ, Cook EF, Graham DA, Yarlagadda VV, Teele SA, Almond CS, Bratton SL, Seeger JD, Dalton HJ, Rycus PT, Laussen PC, Thiagarajan RR. Pediatric ECMO outcomes: comparison of centrifugal versus roller blood pumps using propensity score matching. *ASAIO J* 2013; **59**: 145-151 [PMID: 23438777 DOI: 10.1097/MAT.0b013e31828387cd]

58 **Meyer AD**, Wiles AA, Rivera O, Wong EC, Freishtat RJ, Rais-Bahrami K, Dalton HJ. Hemolytic and thrombocytopathic characteristics of extracorporeal membrane oxygenation systems at simulated flow rate for neonates. *Pediatr Crit Care Med* 2012; **13**: e255-e261 [PMID: 22596067 DOI: 10.1097/PCC.0b013e31823c98ef]

59 **Khan S**, Vasavada R, Qiu F, Kunselman A, Undar A. Extracorporeal life support systems: alternative vs. conventional circuits. *Perfusion* 2011; **26**: 191-198 [PMID: 21227982 DOI: 10.1177/0267659110395060]

60 **Iwahashi H**, Yuri K, Nosé Y. Development of the oxygenator: past, present, and future. *J Artif Organs* 2004; **7**: 111-120 [PMID: 15558331]

61 **Lim MW**. The history of extracorporeal oxygenators. *Anaesthesia* 2006; **61**: 984-995 [PMID: 16978315]

62 **Bembea MM**, Annich G, Rycus P, Oldenburg G, Berkowitz I, Pronovost P. Variability in anticoagulation management of patients on extracorporeal membrane oxygenation: an international survey. *Pediatr Crit Care Med* 2013; **14**: e77-e84 [PMID: 23287906 DOI: 10.1097/PCC.0b013e31827127e4]

63 **Buck ML**. Control of Coagulation during Extracorporeal Membrane Oxygenation. *J Pediatr Pharmacol Ther* 2005; **10**: 26-35 [PMID: 23118623]

64 **Görlinger K**, Bergmann L, Dirkmann D. Coagulation management in patients undergoing mechanical circulatory support. *Best Pract Res Clin Anaesthesiol* 2012; **26**: 179-198 [PMID: 22910089]

65 **Rollins MD**, Hubbard A, Zabrocki L, Barnhart DC, Bratton SL. Extracorporeal membrane oxygenation cannulation trends for pediatric respiratory failure and central nervous system injury. *J Pediatr Surg* 2012; **47**: 68-75 [PMID: 22244395]

66 **Mateen FJ**, Muralidharan R, Shinohara RT, Parisi JE, Schears GJ, Wijdicks EF. Neurological injury in adults treated with extracorporeal membrane oxygenation. *Arch Neurol* 2011; **68**: 1543-1549 [PMID: 21825216 DOI: 10.1001/archneurol.2011.209]

67 **Thiagarajan RR**, Laussen PC, Rycus PT, Bartlett RH, Bratton SL. Extracorporeal membrane oxygenation to aid cardiopulmonary resuscitation in infants and children. *Circulation* 2007; **116**: 1693-1700 [PMID: 17893278]

68 **Barrett CS**, Bratton SL, Salvin JW, Laussen PC, Rycus PT, Thiagarajan RR. Neurological injury after extracorporeal membrane oxygenation use to aid pediatric cardiopulmonary resuscitation. *Pediatr Crit Care Med* 2009; **10**: 445-451 [PMID: 19451851 DOI: 10.1097/PCC.0b013e318198bd85]

69 **Raymond TT**, Cunnyngham CB, Thompson MT, Thomas JA, Dalton HJ, Nadkarni VM. Outcomes among neonates, infants, and children after extracorporeal cardiopulmonary resuscitation for refractory inhospital pediatric cardiac arrest: a report from the National Registry of Cardiopulmonary Resuscitation. *Pediatr Crit Care Med* 2010; **11**: 362-371 [PMID: 19924027 DOI: 10.1097/PCC.0b013e3181c0141b]

70 **Lidegran MK**, Mosskin M, Ringertz HG, Frenckner BP, Lindén VB. Cranial CT for diagnosis of intracranial complications in adult and pediatric patients during ECMO: Clinical benefits in diagnosis and treatment. *Acad Radiol* 2007; **14**: 62-71 [PMID: 17178367]

71 **Gadepalli SK**, Selewski DT, Drongowski RA, Mychaliska GB. Acute kidney injury in congenital diaphragmatic hernia requiring extracorporeal life support: an insidious problem. *J Pediatr Surg* 2011; **46**: 630-635 [PMID: 21496529 DOI: 10.1016/j.jpedsurg.2010.11.031]

72 **Meyer RJ**, Brophy PD, Bunchman TE, Annich GM, Maxvold NJ, Mottes TA, Custer JR. Survival and renal function in pediatric patients following extracorporeal life support with hemofiltration. *Pediatr Crit Care Med* 2001; **2**: 238-242 [PMID: 12793948]

73 **Paden ML**, Warshaw BL, Heard ML, Fortenberry JD. Recovery of renal function and survival after continuous renal replacement therapy during extracorporeal membrane oxygenation. *Pediatr Crit Care Med* 2011; **12**: 153-158 [PMID: 20453702 DOI: 10.1097/PCC.0b013e3181e2a596]

74 **Askenazi DJ**, Selewski DT, Paden ML, Cooper DS, Bridges BC, Zappitelli M, Fleming GM. Renal replacement therapy in critically ill patients receiving extracorporeal membrane oxygenation. *Clin J Am Soc Nephrol* 2012; **7**: 1328-1336 [PMID: 22498496]

75 **Selewski DT**, Cornell TT, Blatt NB, Han YY, Mottes T, Kommareddi M, Gaies MG, Annich GM, Kershaw DB, Shanley TP, Heung M. Fluid overload and fluid removal in pediatric patients on extracorporeal membrane oxygenation requiring continuous renal replacement therapy. *Crit Care Med* 2012; **40**: 2694-2699 [PMID: 22743776 DOI: 10.1097/CCM.0b013e318258ff01]

76 **Wolf MJ**, Chanani NK, Heard ML, Kanter KR, Mahle WT. Early renal replacement therapy during pediatric cardiac extracorporeal support increases mortality. *Ann Thorac Surg* 2013; **96**: 917-922 [PMID: 23915590 DOI: 10.1016/j.athoracsur.2013.05.056]

77 **Meyer DM**, Jessen ME, Eberhart RC. Neonatal extracorporeal membrane oxygenation complicated by sepsis. Extracorporeal Life Support Organization. *Ann Thorac Surg* 1995; **59**: 975-980 [PMID: 7695427]

78 **Douglass BH**, Keenan AL, Purohit DM. Bacterial and fungal infection in neonates undergoing venoarterial extracorporeal membrane oxygenation: an analysis of the registry data of the extracorporeal life support organization. *Artif Organs* 1996; **20**: 202-208 [PMID: 8694690]

79 **Bizzarro MJ**, Conrad SA, Kaufman DA, Rycus P. Infections acquired during extracorporeal membrane oxygenation in neonates, children, and adults. *Pediatr Crit Care Med* 2011; **12**: 277-281 [PMID: 20495508 DOI: 10.1097/PCC.0b013e3181e28894]

80 **Odetola F**, Custer JR. Infections acquired while on extracorporeal membrane oxygenation: navigating the maze. *Pediatr Crit Care Med* 2011; **12**: 353-355 [PMID: 21637145]

81 **Moler FW**, Custer JR, Bartlett RH, Palmisano JM, Akingbola O, Taylor RP, Maxvold NJ. Extracorporeal life support for severe pediatric respiratory failure: an updated experience 1991-1993. *J Pediatr* 1994; **124**: 875-880 [PMID: 8201470]

82 **Arnold JH**, Anas NG, Luckett P, Cheifetz IM, Reyes G, Newth CJ, Kocis KC, Heidemann SM, Hanson JH, Brogan TV, Bohn DJ. High-frequency oscillatory ventilation in pediatric respiratory failure: a multicenter experience. *Crit Care Med* 2000; **28**: 3913-3919 [PMID: 11153635]

83 **Zimmerman JJ**, Akhtar SR, Caldwell E, Rubenfeld GD. Incidence and outcomes of pediatric acute lung injury. *Pediatrics* 2009; **124**: 87-95 [PMID: 19564287 DOI: 10.1542/peds.2007-2462]

84 **Flori HR**, Glidden DV, Rutherford GW, Matthay MA. Pediatric acute lung injury: prospective evaluation of risk factors associated with mortality. *Am J Respir Crit Care Med* 2005; **171**: 995-1001 [PMID: 15618461]

85 **Brogan TV**, Zabrocki L, Thiagarajan RR, Rycus PT, Bratton SL. Prolonged extracorporeal membrane oxygenation for children with respiratory failure. *Pediatr Crit Care Med* 2012; **13**: e249-e254 [PMID: 22596069 DOI: 10.1097/PCC.0b013e31824176f4.]

86 **Smalley N**, MacLaren G, Best D, Paul E, Butt W. Outcomes in children with refractory pneumonia supported with extracorporeal membrane oxygenation. *Intensive Care Med* 2012; **38**: 1001-1007 [PMID: 22543425 DOI: 10.1007/s00134-012-2581-5]

87 **Brogan TV**, Thiagarajan RR, Rycus PT, Bartlett RH, Bratton SL. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. *Intensive Care Med* 2009; **35**: 2105-2114 [PMID: 19768656 DOI: 10.1007/s00134-009-1661-7]

88 **Swaniker F**, Kolla S, Moler F, Custer J, Grams R, Barlett R, Hirschl R. Extracorporeal life support outcome for 128 pediatric patients with respiratory failure. *J Pediatr Surg* 2000; **35**: 197-202 [PMID: 10693665]

89 **Abend NS**, Dlugos DJ, Clancy RR. A review of long-term EEG monitoring in critically ill children with hypoxic-ischemic encephalopathy, congenital heart disease, ECMO, and stroke. *J Clin Neurophysiol* 2013; **30**: 134-142 [PMID: 23545764 DOI: 10.1097/WNP.0b013e3182872af9]

90 **Madderom MJ**, Reuser JJ, Utens EM, van Rosmalen J, Raets M, Govaert P, Steiner K, Gischler SJ, Tibboel D, van Heijst AF, Ijsselstijn H. Neurodevelopmental, educational and behavioral outcome at 8 years after neonatal ECMO: a nationwide multicenter study. *Intensive Care Med* 2013; **39**: 1584-1593 [PMID: 23740280 DOI: 10.1007/s00134-013-2973-1]

91 **Thiagarajan RR**, Teele SA, Teele KP, Beke DM. Physical therapy and rehabilitation issues for patients supported with extracorporeal membrane oxygenation. *J Pediatr Rehabil Med* 2012; **5**: 47-52 [PMID: 22543892 DOI: 10.3233/PRM-2012-0195]

92 **Rahimi RA**, Skrzat J, Reddy DR, Zanni JM, Fan E, Stephens RS, Needham DM. Physical rehabilitation of patients in the intensive care unit requiring extracorporeal membrane oxygenation: a small case series. *Phys Ther* 2013; **93**: 248-255 [PMID: 23104895 DOI: 10.2522/ptj.20120336]

93 **Haefner SM**, Bratton SL, Annich GM, Bartlett RH, Custer JR. Complications of intermittent prone positioning in pediatric patients receiving extracorporeal membrane oxygenation for respiratory failure. *Chest* 2003; **123**: 1589-1594 [PMID: 12740278]

94 **Golej J**, Trittenwein G. Early detection of neurologic injury and issues of rehabilitation after pediatric cardiac extracorporeal membrane oxygenation. *Artif Organs* 1999; **23**: 1020-1025 [PMID: 10564309]

95 **Lacroix J**, Hébert PC, Hutchison JS, Hume HA, Tucci M, Ducruet T, Gauvin F, Collet JP, Toledano BJ, Robillard P, Joffe A, Biarent D, Meert K, Peters MJ. Transfusion strategies for patients in pediatric intensive care units. *N Engl J Med* 2007; **356**: 1609-1619 [PMID: 17442904]

96 **Hébert PC**, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweeddale M, Schweitzer I, Yetisir E. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med* 1999; **340**: 409-417 [PMID: 9971864]

97 **Valentine SL**, Sapru A, Higgerson RA, Spinella PC, Flori HR, Graham DA, Brett M, Convery M, Christie LM, Karamessinis L, Randolph AG. Fluid balance in critically ill children with acute lung injury. *Crit Care Med* 2012; **40**: 2883-2889 [PMID: 22824936]

98 **Fan E**, Villar J, Slutsky AS. Novel approaches to minimize ventilator-induced lung injury. *BMC Med* 2013; **11**: 85 [PMID: 23536968 DOI: 10.1186/1741-7015-11-85]

99 **Plataki M**, Hubmayr RD. The physical basis of ventilator-induced lung injury. *Expert Rev Respir Med* 2010; **4**: 373-385 [PMID: 20524920 DOI: 10.1586/ers.10.28.]

100 **Petrucci N**, De Feo C. Lung protective ventilation strategy for the acute respiratory distress syndrome. *Cochrane Database Syst Rev* 2013; **2**: CD003844 [PMID: 23450544 DOI: 10.1002/14651858.CD003844.pub4]

101 **Odetola FO**, Moler FW, Dechert RE, VanDerElzen K, Chenoweth C. Nosocomial catheter-related bloodstream infections in a pediatric intensive care unit: risk and rates associated with various intravascular technologies. *Pediatr Crit Care Med* 2003; **4**: 432-436 [PMID: 14525637]

P- Reviewers: Inaba H, Ntoumenopoulos G
S- Editor: Gou SX **L- Editor**: A **E- Editor:** Lu YJ

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5492/wjccm.v2.i4.40

World J Crit Care Med 2013 November 4; 2(4): 40-47
ISSN 2220-3141 (online)

MINI REVIEWS

Neurologic complications and neurodevelopmental outcome with extracorporeal life support

Amit Mehta, Laura M Ibsen

Amit Mehta, Laura M Ibsen, Division of Pediatric Critical Care Medicine, Department of Pediatrics, Oregon Health and Sciences University, Portland, OR 97239, United States
Author contributions: Mehta A, Ibsen LM wrote the paper.
Correspondence to: Amit Mehta, MD, Division of Pediatric Critical Care Medicine, Department of Pediatrics, Oregon Health and Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97239,
United States. mehtaa@ohsu.edu
Telephone: +1-503-4949000 Fax: +1-503-4944951
Received: July 10, 2013 Revised: July 27, 2013
Accepted: August 4, 2013
Published online: November 4, 2013

Abstract

Extracorporeal life support is used to support patients of all ages with refractory cardiac and/or respiratory failure. Extracorporeal membrane oxygenation (ECMO) has been used to rescue patients whose predicted mortality would have otherwise been high. It is associated with acute central nervous system (CNS) complications and with long- term neurologic morbidity. Many patients treated with ECMO have acute neurologic complications, including seizures, hemorrhage, infarction, and brain death. Various pre-ECMO and ECMO factors have been found to be associated with neurologic injury, including acidosis, renal failure, cardiopulmonary resuscitation, and modality of ECMO used. The risk of neurologic complication appears to vary by age of the patient, with neonates appearing to have the highest risk of acute central nervous system complications. Acute CNS injuries are associated with increased risk of death in a patient who has received ECMO support. ECMO is increasingly used during cardiopulmonary resuscitation when return of spontaneous circulation is not achieved rapidly and outcomes may be good in select populations. Economic analyses have shown that neonatal and adult respiratory ECMO are cost effective. There have been several intriguing reports of active physical rehabilitation of patients during ECMO support that is well tolerated and may improve recovery. Although there is evidence that some patients supported with ECMO appear to have very good outcomes, there is limited understanding of the long-term impact of ECMO on quality of life and long-term cognitive and physical functioning for many groups, especially the cardiac and pediatric populations. This deserves further study.

Key words: Respiratory failure; Cardiopulmonary resuscitation; Pediatrics; Extracorporeal life support; Congenital heart disease; Stroke

Core tip: Extracorporeal life support is used to support patients of all ages with refractory cardiac and/or respiratory failure. It is associated with acute central nervous system complications and with long- term neurologic morbidity. Many patients treated with extracorporeal membrane oxygenation (ECMO) have acute neurologic complications, including seizures, hemorrhage, infarction, and brain death. In this review paper, we review the incidence of and factors associated with neurologic complications associated with the use of ECMO and the associated long term neurologic outcomes of patients treated with ECMO.

Mehta A, Ibsen LM. Neurologic complications and neurodevelopmental outcome with extracorporeal life support. *World J Crit Care Med* 2013; 2(4): 40-47 Available from: URL: http://www.wjgnet.com/2220-3141/full/v2/i4/40.htm DOI: http://dx.doi.org/10.5492/wjccm.v2.i4.40

INTRODUCTION

Extracorporeal life support (ECLS) is used to provide pulmonary, cardiac, or cardiopulmonary support in neonate, pediatric, and adult patients refractory to con-

ventional management. Deployment of ELCS may be planned, urgent/emergent, or associated with cardiopulmonary resuscitation (ECPR). Various cannulation strategies exist with the dominant differentiating factor being veno-venous (VV) *vs* veno-arterial (VA) circuits. Survival has improved since extracorporeal membrane oxygenation (ECMO) was first utilized in the 1970s and indications and populations supported have expanded as techniques and strategies of deployment have evolved.

Patients supported by ECLS are at risk of neurologic injury from pre-ECLS factors including hypoxia, acidosis, hypotension and low cardiac output, infection, and organ failure, and from ECLS factors including hemorrhage, infarction, seizures, disrupted cerebral circulation, and development of new organ failure. Functional outcome after critical care, including ECLS support, is difficult to assess on a systematic basis, as follow up studies are limited and not standardized. Although children and adults are treated with ECMO, nearly 50% of treated patients are neonates[1] and assessing their long-term outcomes requires both time and an appropriate comparison population to account for severity of illness and social factors[2,3]. Neurodevelopmental and longitudinal outcomes after ECLS are most important to assess but are largely limited to neonatal and limited adult studies. More information is available on the incidence and risk factors for acute neurologic complications associated with ECLS.

The Extracorporeal Life Support Organization (ELSO) collects utilization, mortality, and complication data from 200 centers internationally on all technologies used to support cardiopulmonary function. Acute neurologic complications (seizures, infarction, hemorrhage, and brain death) are reported but functional outcome or disability is not. Administrative databases such as the Pediatric Health Information System or the Kids Inpatient Database also collect complication diagnoses but do not collect functional outcome specifically. Most reports of functional outcome after ECMO support are from single centers or multiple centers over a small time period[4-9].

ANATOMY AND PHYSIOLOGY OF NEUROLOGIC INJURY ASSOCIATED WITH ECLS

Neurologic injury associated with ECLS ranges from subtle neurocognitive defects to devastating intracranial hemorrhage to brain death. Injury may be sustained prior to ECLS support, during ECLS support, or after ECLS support has been discontinued. Neurologic complications reported to ELSO include hemorrhage and infarction documented by ultrasound and or computed tomography, clinical seizures, EEG documented seizures, and brain death (Table 1). The incidence of complications may be underreported due to the difficulty of obtaining reliable imaging in critically ill patients during extracorporeal support, and many patients who sustain neurologic injury likely have increased risk of death and may die without imaging[10-12].

Hemorrhage is generally poorly tolerated due to the anti-coagulation that is needed during ECLS support. Most common hemorrhagic injuries include intraventricular, intracerebral, and subdural hemorrhages. Risk factors for intraventricular hemorrhage have been most clearly demonstrated in neonatal patients[13]. Intra-cerebral hemorrhages are thought to be at significant risk of extension due to anti-coagulation but there is little published information on the natural history of intracranial pathology during ECMO[14].

Infarctions may be small and related to micro emboli of clot or air, or may be large and related to large embolic clots. The incidence of image documented infarction is roughly similar to that of hemorrhage. Clinical seizures occur in 5%-10% of neonatal and pediatric patients, and 1%-2% of adult patients[1]. The difference between the adult and pediatric incidence of seizures may be related to trends in VV versus VA cannulation techniques[12] or may be due to relative sparing of cerebral vessels given that adults are more likely to be cannulated through femoral vessels or may be due to increase likelihood of infants to seizures due to developmental vulnerability[15].

Hypoxic ischemic damage due to poor cardiac output or acidosis prior to, during, or after mechanical support is difficult to quantify and makes outcome studies difficult to interpret in the absence of appropriate control groups.

HISTORICAL TRENDS

ECMO was developed in the 1970's and although the first reported case was an adult, the first series of neonates[16,17] reported success whereas the first series of adults reported dismal survival (9%)[18]. It became clear that ECMO was efficacious for patients with reversible disease and that prematurity or other risk factors for bleeding posed a significant risk. The importance of early recognition of intraventricular hemorrhage in neonates became evident. It appears that after the first week of life premature infants have reduced risk of intracranial hemorrhage[19].

Neonatal respiratory ECMO volume peaked in the early 1990s and has remained relatively stable since the early 2000's. Overall survival has diminished likely reflecting advances in standard therapies and application of ECMO to more diverse and ill patients. Neonates with congenital diaphragmatic hernia continue to be a high-risk group with high mortality among respiratory patients. The majority of neonatal respiratory ECMO support is VA. Pediatric respiratory ECMO has increased substantially in the past 3 decades with a peak in 2009 coincident with the H1N1 influenza pandemic. About half of pediatric respiratory ECMO support is VA but there is increasing utilization of VV support modes. Adult ECMO was rarely performed until late in

Table 1 Survival and cumulative incidence of neurologic complications by age and indication for extracorporeal membrane oxygenation (Extracorporeal Life Support Organization 2013)

	Cumulative survival to discharge or transfer	2012 survival to discharge or transfer	Clinical seizures	Central nervous system hemorrhage
Neonatal				
Respiratory	75%	69%	9.20%	7.10%
Cardiac	40%	45%	7.20%	11.10%
ECPR	39%			
Pediatric				
Respiratory	56%	58%	5.70%	6.00%
Cardiac	49%	55%	6.80%	4.90%
ECPR	41%			
Adult				
Respiratory	55%	57%	1.10%	4.00%
Cardiac	39%	39%	2.00%	2.00%
ECPR	28%			

ECPR: Extracorporeal membrane oxygenation support initiated during cardiopulmonary resuscitation.

the 2000s and has also increased substantially in 2009, with further increases since. The significant majority of adult ECMO support is VV.

ECMO support for cardiac indications in all age groups has increased steadily since the early 1990s[1]. ECPR was first described in 1992[20] and its application has expanded substantially in the past several years though indications remain controversial.

NEUROLOGIC COMPLICATIONS AND NEURODEVELOPMENTAL OUTCOME BY AGE GROUP

Neonatal

Neonates have the highest rate of neurologic complications when examining the ELSO reports by patient age. Cumulative ELSO data on neonates who undergo ECMO for respiratory support report a 9.2% incidence of clinical seizures and a 7.1% incidence of intracranial hemorrhage[1]. Neonates who are undergoing support for cardiac indications have a 7.2% incidence of seizures and an 11.1% incidence of hemorrhage. An initial assessment of risk factors for intracranial hemorrhage included lower gestational age, sepsis, acidosis, coagulopathy, and inotropic support[13]. Gestational age less than 34 wk has repeatedly been associated with unacceptably high rates of intracranial hemorrhage[13,17]. In a recent analysis of the ELSO registry[21], for those neonatal patients supported with ECMO from 2005-2010, a 20% incidence of neurologic complications was noted. Risk factors for neurologic complications included birth weight less than three kg, gestational age less than 34 wk, need for CPR, acidosis, and use of VA ECMO support.

Studies of long-term neurodevelopmental outcomes of infants who undergo ECMO support suggest that impairment is similar to that of comparable conventionally treated infants. Single institutions series have noted impairment or disability in 20%-50% of surviving infants[4,22,23]. Factors that may influence the determination of disability include differences in socioeconomic status of those who complete follow-up[4], time from ECMO support to evaluation[22], and institutional selection criteria. Long-term outcome studies highlight the changing neurologic function over time, with some initial disabilities likely resolving, and new more subtle learning and language deficits emerging. The 1996 United Kingdom collaborative randomized trial of neonatal ECMO versus conventional management[24] demonstrated similar proportions of survival without disability at seven years of age, and overall severe disability is less common than that in earlier reports. Sensorineural hearing loss is increasingly recognized as an important consequence of ECMO[23,25]. Sensorineural hearing loss has been found to be associated with pre-ECLS seizures[26].

In neonates treated with ECMO, those with seizures were found to have lower IQ at preschool age than those without seizures[27]. In another small group of neonatal ECMO patients, seizures were found to be a risk factor for the subsequent development of cerebral palsy or developmental delay[28].

An understanding of baseline characteristics of patients undergoing ECMO including ECPR is critical to any evaluation of outcome. Patients who receive ECMO support have generally had periods of serious illness including underlying congenital heart disease or hypoxemia which may play an important role in modifying outcome. Long-term neurodevelopmental outcomes of children who undergo cardiopulmonary bypass for congenital heart disease reveal a range of outcomes including mild cognitive and psychomotor delays[29,30] as well as speech and language abnormalities[31] in a substantial proportion of survivors. Newborns with congenital heart disease have brain abnormalities including white matter injury prior to cardiac surgery[32].

Pediatric

Pediatric ECMO patients represent a population of patients that are substantially more varied than the neonatal group. Cumulative ELSO data on pediatric patients who undergo ECMO for respiratory support report a 5.7% incidence of clinical seizures and a 6% incidence

of intracranial hemorrhage. Pediatric cardiac ECMO patients have a slightly greater incidence (6.8%) of clinical seizures and a 4.9% incidence of hemorrhage[1]. Acute neurologic complications including CNS hemorrhage or infarct, and EEG-determined or clinically determined seizures are associated with reduced hospital survival[1,14]. Similar to neonates, risk factors for severe CNS complications in pediatric patients include metabolic acidosis, inotrope or vasopressor requirement, renal failure, cardiopulmonary resuscitation, or left ventricular assist device prior to initiation of ECMO[33].

There is little data on long-term neurodevelopmental outcomes in the extremely heterogeneous pediatric ECMO population[7]. Zabrocki *et al*[34] demonstrated that over a 15-year period from 1993-2007 the survival of pediatric patients supported for respiratory indications did not change, but ECMO was offered to increasingly medically complex patients, which could easily confound assessments of disability after ECMO.

Adult

Adults comprise the smallest, though growing, population of ECMO patients. Cumulative ELSO data on adult patients who undergo ECMO for respiratory support report a 1.1% incidence of clinical seizures and a 4% incidence of intracranial hemorrhage. Adults supported for cardiac indications have a 2% incidence of seizures and a 2% incidence of intracranial hemorrhage[1]. The lower incidence of severe CNS complications may be due to patient factors, selection factors, or technical factors. Adult patients are almost exclusively supported with VV ECMO and this has been shown to lower the rate of neurologic complications in pediatric patients[12] although this association may not remain consistent in adults[35]. When adult patients are supported with VA ECMO, cannulation of the cervical vessels is avoided, which may decrease the incidence of neurologic complications.

As for pediatric patients there is relatively little long-term functional outcome data for adult patients. The CESAR (Conventional ventilation or ECMO for Severe Adult Respiratory Failure) trial, demonstrated no difference in severe disability of any measure of health care quality between patients randomized to the consideration for ECMO group versus the control group[36].

Cardiac and ECPR outcome

The use of ECMO to support low cardiac output, failure to wean from bypass, or cardiac arrest has been steadily increasing for the past 20 years and during that time the use of ECPR has emerged as an important strategy. Although somewhat variable year to year, overall survival of ECMO for cardiac indications has not shown definitive trends over time and cumulative ELSO survival now averages 39% in adults, 49% in pediatric patients, and 39% in neonates. ECPR survival is slightly lower at 28% in adults, 41% in children, and 39% in neonates[1]. ECPR survival does not appear to have changed significantly over time[37,38]

The incidence of major neurologic morbidity in cardiac patients as reported to ELSO is highest in neonates, with 7.2% suffering seizures, 3.5% infarction, and 11.1% intracranial hemorrhage. Children have slightly lower incidence of seizures and hemorrhage and a slightly higher incidence of infarction. Adults have the lowest incidence of major neurologic morbidity with 2% suffering seizures, 3.7% infarction, and 2% hemorrhage. In all age groups those patients who suffer major neurologic complications have a lower hospital survival[1].

Several single institution studies have reported neurologic outcomes after cardiac ECMO[5,6,39-42]. Evaluations included chart review, telephone interviews, and neurodevelopmental testing but no studies that performed detailed neurologic and neurodevelopmental testing included control groups of normal infants or of infants who had similar cardiac defects or illnesses. Hospital survival was similar to that reported by ELSO and all series had patients who died following discharge and who were lost to follow-up. Time to evaluation was extremely variable ranging from months to years within individual cohorts. Those studies that performed detailed neurodevelopmental assessments[6,42] found 50% incidence of moderate to severe cognitive delay and 12%-25% incidence of neuromotor delay among long term survivors. Predictors of poor cognitive outcome included time to normalization of lactate, highest inotrope score, and chromosomal abnormality. As previously discussed, premorbid characteristics of cardiac patients undergoing ECMO support are critical to assessment of outcomes.

Significant neurologic complications during ECPR are more prevalent than during other support strategies, with a 12% incidence of seizures, 11.8% incidence of radiologic evidence of infarct or hemorrhage, and 11% incidence of brain death in one evaluation of the ELSO database[37]. Infarction or hemorrhage but not seizures were more common in nonsurvivors than survivors. Further evaluation of this data revealed that risk factors for major neurologic complication included acidosis, non-cardiac disease, pulmonary hemorrhage, need for dialysis, and CPR while on ECMO[11]. In this analysis, there was a trend towards less neurologic injury over time.

Limited long-term neurologic and neurodevelopmental outcome after ECPR data are available from several single institution series[38,43-48]. In these series survival to hospital discharge ranges from 33%-74%, with most ranging from 33%-41%. Some studies reported neurologic outcome based on chart review and some assigned Pediatric Cerebral Performance Category (PCPC) and Pediatric Outcome Performance Category (POPC) scores. None reported detailed neurodevelopmental evaluations[49] but the PCPC and POPC scores have been rigorously validated[50]. An early series[43] reported no change from baseline POPC or PCPC in 50% of patients, and several authors[38,46,48] reported post ECPR PCPC scores of 1-2 (normal or mild cerebral disability) in 3/4 of patients. A recent analysis of the National

Registry of Cardiopulmonary Resuscitation confirms favorable [PCPC 1-3 (normal, mild cerebral disability or moderate cerebral disability)] neurologic outcomes in at least 64% and up to 95% of ECPR survivors[51]. This is similar to an analysis of the National Registry of Cardiopulmonary Resuscitation database, which showed the outcome of pediatric survivors of in hospital cardiac arrest, where the overall hospital survival was lower than that usually reported for ECPR (27%) but 65% of surviving patients had good neurologic outcome[52].

The effect of duration of CPR on outcome, either survival or neurodevelopmental outcome is difficult to determine. Several studies report no correlation between duration of CPR and survival[38,43,44,51] and several report remarkable grossly normal survival after prolonged (> 60 min of CPR) in a few patients[43,46,51], yet this is not a universal finding[47]. Cardiac disease as the indication for ECPR appears to improve the odds of survival[51]. In general the term ECPR is reserved for those patients who do not achieve return of spontaneous circulation (ROSC) prior to cannulation. When evaluating these studies it is important to consider that the decisions to perform ECPR vary considerably between institutions and are not standardized. The development of rapid deployment teams, which could cannulate during CPR or after ROSC may lead to improved outcomes[53]. As with any form of ECMO support the premise of reversible disease as a prerequisite for ECMO support must be kept in mind.

QUALITY OF LIFE ASSESSMENT

Increasingly quality of life and functional and school related outcomes are appreciated as important indicators of the efficacy of critical care. Several reports have described relatively subtle cognitive, physical, and school related problems in survivors of pediatric critical care. A recent report showed that pediatric survivors or critical illness had verbal, spatial, and memory problems, attention and problem solving difficulty, and school performance following paediatric intensive care unit (PICU) admission[54]. A different study evaluated health care related quality of life and adaptive behavior functioning and found it to be significantly reduced in survivors of urgent PICU admission[55]. In this study prolonged ECMO support was one factor associated with reduced quality of life.

Looking at the issue of quality of life differently, a study that looked at patients with prolonged ICU stays (> 28 d) found that the majority of children (57%) had a normal quality of life, with 22.9% having impaired quality of life and 20% having poor quality of life[56]. In a single institution cohort of cardiac ECMO survivors physical health related quality of life was lower than that of the general population but similar to those with complex congenital heart disease. Psychosocial quality of life as reported by parents and by older surviving patients was similar to that of the general population[8].

There have been relatively few analyses of the cost-effectiveness of ECMO but there have been some positive reports. The United Kingdom Collaborative ECMO Trial was a randomized, controlled trial of neonatal respiratory ECMO. Over 7-year follow-up of enrolled subjects they found an incremental cost per disability-free life year gained to be below the nationally acceptable threshold[24]. Similarly the CESAR trial found referral to an ECMO center to be cost effective when evaluated in terms of quality of life year expense[36]. In a single institution evaluation of ECPR for patients with congenital heart disease, ECMO was found to be within acceptable cost efficacy[57].

TECHNICAL ISSUES INFLUENCING NEUROLOGIC OUTCOME

Along with evolving indications for ECMO, the support and monitoring technology has changed dramatically over the past 30 years. Most recently newer dual lumen VV cannulas for respiratory support have been introduced and centrifugal pumps are increasingly being used to support pediatric patients. There have been advances in anticoagulation strategies as well as the development of more biocompatible circuits and oxygenators. Near infrared spectroscopy monitoring is increasingly being used during ECMO support. With the exception of a VV cannulation strategy none of these technologies has yet been shown to improve neurologic outcome[34].

REHABILITATION

An intriguing development in the care of the ECMO patient is the concept of active rehabilitation during ECMO support. Critical illness of any sort leads to deconditioning due to immobility and this is especially problematic with highly invasive support technologies. If patients can be exercised and kept mobile during mechanical support they may be more able to progress quickly once liberated from invasive support. There have been several small series of patients who received active physical or occupational therapy, advancing to ambulation, while undergoing ECMO support[58-60]. Thus far this strategy has been limited to older patients supported for respiratory indications, almost exclusively with single dual lumen catheters.

CONCLUSION

Neurologic outcomes after ECMO support vary by patient age, type of support, indications for support, and underlying diagnosis. There are not widely accepted standards for initiation of ECMO outside of the neonatal respiratory population so severity of illness and underlying conditions among patients supported by ECMO may vary widely between institutions. Acute severe neurologic complications are more prevalent in neonates and children than adults. The long-term impact of ECMO sup-

port on development, school performance, and quality of life is poorly defined and needs further study. Where it has been evaluated, ECMO appears to be cost effective.

REFERENCES

1 ECLS Registry Report: International Summary January 2013. Available from: URL: http: //www.elsonet.org/index.php/registry/statistics/limited.html

2 **Keenan HT**, Hooper SR, Wetherington CE, Nocera M, Runyan DK. Neurodevelopmental consequences of early traumatic brain injury in 3-year-old children. *Pediatrics* 2007; **119**: e616-623 [DOI: 10.1542/peds.2006-2313]

3 **Tabbutt S**, Nord AS, Jarvik GP, Bernbaum J, Wernovsky G, Gerdes M, Zackai E, Clancy RR, Nicolson SC, Spray TL, Gaynor JW. Neurodevelopmental outcomes after staged palliation for hypoplastic left heart syndrome. *Pediatrics* 2008; **121**: 476-483 [PMID: 18310195 DOI: 10.1542/peds.2007-1282]

4 **Hofkosh D**, Thompson AE, Nozza RJ, Kemp SS, Bowen A, Feldman HM. Ten years of extracorporeal membrane oxygenation: neurodevelopmental outcome. *Pediatrics* 1991; **87**: 549-555 [PMID: 1707157]

5 **Ibrahim AE**, Duncan BW, Blume ED, Jonas RA. Long-term follow-up of pediatric cardiac patients requiring mechanical circulatory support. *Ann Thorac Surg* 2000; **69**: 186-192 [PMID: 10654511 DOI: 10.1016/S0003-4975(99)01194-7]

6 **Hamrick SE**, Gremmels DB, Keet CA, Leonard CH, Connell JK, Hawgood S, Piecuch RE. Neurodevelopmental outcome of infants supported with extracorporeal membrane oxygenation after cardiac surgery. *Pediatrics* 2003; **111**: e671-e675 [PMID: 12777584 DOI: 10.1542/peds.111.6.e671]

7 **Taylor AK**, Cousins R, Butt WW. The long-term outcome of children managed with extracorporeal life support: an institutional experience. *Crit Care Resusc* 2007; **9**: 172-177 [PMID: 17536987]

8 **Costello JM**, O'Brien M, Wypij D, Shubert J, Salvin JW, Newburger JW, Laussen PC, Arnold JH, Fynn-Thompson F, Thiagarajan RR. Quality of life of pediatric cardiac patients who previously required extracorporeal membrane oxygenation. *Pediatr Crit Care Med* 2012; **13**: 428-434 [PMID: 22067987 DOI: 10.1097/PCC.0b013e318238ba21]

9 **Danzer E**, Gerdes M, D'Agostino JA, Partridge EA, Hoffman-Craven CH, Bernbaum J, Rintoul NE, Flake AW, Adzick NS, Hedrick HL. Preschool neurological assessment in congenital diaphragmatic hernia survivors: outcome and perinatal factors associated with neurodevelopmental impairment. *Early Hum Dev* 2013; **89**: 393-400 [PMID: 23333410 DOI: 10.1016/j.earlhumdev.2012.12.009.]

10 **Lidegran MK**, Mosskin M, Ringertz HG, Frenckner BP, Lindén VB. Cranial CT for diagnosis of intracranial complications in adult and pediatric patients during ECMO: Clinical benefits in diagnosis and treatment. *Acad Radiol* 2007; **14**: 62-71 [DOI: 10.1016/j.acra.2006.10.004]

11 **Barrett CS**, Bratton SL, Salvin JW, Laussen PC, Rycus PT, Thiagarajan RR. Neurological injury after extracorporeal membrane oxygenation use to aid pediatric cardiopulmonary resuscitation. *Pediatr Crit Care Med* 2009; **10**: 445-451 [PMID: 19451851 DOI: 10.1097/PCC.0b013e318198bd85]

12 **Rollins MD**, Hubbard A, Zabrocki L, Barnhart DC, Bratton SL. Extracorporeal membrane oxygenation cannulation trends for pediatric respiratory failure and central nervous system injury. *J Pediatr Surg* 2012; **47**: 68-75 [PMID: 22244395 DOI: 10.1016/j.jpedsurg.2011.10.017]

13 **Hardart GE**, Fackler JC. Predictors of intracranial hemorrhage during neonatal extracorporeal membrane oxygenation. *J Pediatr* 1999; **134**: 156-159 [PMID: 9931522 DOI: 10.1016/S0022-3476(99)70408-7]

14 **Hervey-Jumper SL**, Annich GM, Yancon AR, Garton HJ, Muraszko KM, Maher CO. Neurological complications of extracorporeal membrane oxygenation in children. *J Neurosurg Pediatr* 2011; **7**: 338-344 [PMID: 21456903 DOI: 10.3171/2011.1.PEDS10443]

15 **Liesemer K**, Bratton SL, Zebrack CM, Brockmeyer D, Statler KD. Early post-traumatic seizures in moderate to severe pediatric traumatic brain injury: rates, risk factors, and clinical features. *J Neurotrauma* 2011; **28**: 755-762 [PMID: 21381863 DOI: 10.1089/neu.2010.1518]

16 **Bartlett RH**, Gazzaniga AB, Jefferies MR, Huxtable RF, Haiduc NJ, Fong SW. Extracorporeal membrane oxygenation (ECMO) cardiopulmonary support in infancy. *Trans Am Soc Artif Intern Organs* 1976; **22**: 80-93 [PMID: 951895]

17 **Bartlett RH**, Gazzaniga AB, Toomasian J, Coran AG, Roloff D, Rucker R. Extracorporeal membrane oxygenation (ECMO) in neonatal respiratory failure. 100 cases. *Ann Surg* 1986; **204**: 236-245 [PMID: 3530151 DOI: 10.1097/00000658-198609000-00003]

18 **Zapol WM**, Snider MT, Hill JD, Fallat RJ, Bartlett RH, Edmunds LH, Morris AH, Peirce EC, Thomas AN, Proctor HJ, Drinker PA, Pratt PC, Bagniewski A, Miller RG. Extracorporeal membrane oxygenation in severe acute respiratory failure. A randomized prospective study. *JAMA* 1979; **242**: 2193-2196 [PMID: 490805 DOI: 10.1001/jama.1979.03300200023016]

19 **Smith K**, McMullan M, Bratton SL, Goldbloom K, Rycus P, Kinsella J, Brogan TV. Patient age alters the influence of gestational age on coutcome in neonates with respiratory failure who receive extracorporeal life support. *Journal of perinatology* 2013; In press

20 **del Nido PJ**, Dalton HJ, Thompson AE, Siewers RD. Extracorporeal membrane oxygenator rescue in children during cardiac arrest after cardiac surgery. *Circulation* 1992; **86**: Ⅱ 300-Ⅱ 304 [PMID: 1424017]

21 **Polito A**, Barrett CS, Wypij D, Rycus PT, Netto R, Cogo PE, Thiagarajan RR. Neurologic complications in neonates supported with extracorporeal membrane oxygenation. An analysis of ELSO registry data. *Intensive Care Med* 2013; **39**: 1594-1601 [PMID: 23749154 DOI: 10.1007/s00134-013-2985-x]

22 **Schumacher RE**, Palmer TW, Roloff DW, LaClaire PA, Bartlett RH. Follow-up of infants treated with extracorporeal membrane oxygenation for newborn respiratory failure. *Pediatrics* 1991; **87**: 451-457 [PMID: 1707156]

23 **Waitzer E**, Riley SP, Perreault T, Shevell MI. Neurologic outcome at school entry for newborns treated with extracorporeal membrane oxygenation for noncardiac indications. *J Child Neurol* 2009; **24**: 801-806 [PMID: 19196874 DOI: 10.1177/0883073808330765]

24 **Petrou S**, Bischof M, Bennett C, Elbourne D, Field D, McNally H. Cost-effectiveness of neonatal extracorporeal membrane oxygenation based on 7-year results from the United Kingdom Collaborative ECMO Trial. *Pediatrics* 2006; **117**: 1640-1649 [PMID: 16651318 DOI: 10.1542/peds.2005-1150]

25 **Fligor BJ**, Neault MW, Mullen CH, Feldman HA, Jones DT. Factors associated with sensorineural hearing loss among survivors of extracorporeal membrane oxygenation therapy. *Pediatrics* 2005; **115**: 1519-1528 [PMID: 15930212 DOI: 10.1542/peds.2004-0247]

26 **Murray M**, Nield T, Larson-Tuttle C, Seri I, Friedlich P. Sensorineural hearing loss at 9-13 years of age in children with a history of neonatal extracorporeal membrane oxygenation. *Arch Dis Child Fetal Neonatal Ed* 2011; **96**: F128-F132 [PMID: 20971719 DOI: 10.1136/adc.2010.186395]

27 **Parish AP**, Bunyapen C, Cohen MJ, Garrison T, Bhatia J. Seizures as a predictor of long-term neurodevelopmental outcome in survivors of neonatal extracorporeal membrane oxygenation (ECMO). *J Child Neurol* 2004; **19**: 930-934 [PMID: 15704865 DOI: 10.1177/088307380401901204 01]

28 **Campbell LR**, Bunyapen C, Gangarosa ME, Cohen M, Kanto WP. Significance of seizures associated with extracor-

poreal membrane oxygenation. *J Pediatr* 1991; **119**: 789-792 [PMID: 1941388 DOI: 10.1016/S0022-3476(05)80304-X]
29 **Long SH**, Galea MP, Eldridge BJ, Harris SR. Performance of 2-year-old children after early surgery for congenital heart disease on the Bayley Scales of Infant and Toddler Development, Third Edition. *Early Hum Dev* 2012; **88**: 603-607 [PMID: 22336496 DOI: 10.1016/j.earlhumdev.2012.01.007]
30 **Snookes SH**, Gunn JK, Eldridge BJ, Donath SM, Hunt RW, Galea MP, Shekerdemian L. A systematic review of motor and cognitive outcomes after early surgery for congenital heart disease. *Pediatrics* 2010; **125**: e818-e827 [PMID: 20231182 DOI: 10.1542/peds.2009-1959]
31 **Neufeld RE**, Clark BG, Robertson CM, Moddemann DM, Dinu IA, Joffe AR, Sauve RS, Creighton DE, Zwaigenbaum L, Ross DB, Rebeyka IM. Five-year neurocognitive and health outcomes after the neonatal arterial switch operation. *J Thorac Cardiovasc Surg* 2008; **136**: 1413-1421, 1421.e1-1421.e2 [PMID: 19114183 DOI: 10.1016/j.jtcvs.2008.05.011]
32 **Miller SP**, McQuillen PS, Hamrick S, Xu D, Glidden DV, Charlton N, Karl T, Azakie A, Ferriero DM, Barkovich AJ, Vigneron DB. Abnormal brain development in newborns with congenital heart disease. *N Engl J Med* 2007; **357**: 1928-1938 [PMID: 17989385 DOI: 10.1056/NEJMoa067393]
33 **Cengiz P**, Seidel K, Rycus PT, Brogan TV, Roberts JS. Central nervous system complications during pediatric extracorporeal life support: incidence and risk factors. *Crit Care Med* 2005; **33**: 2817-2824 [PMID: 16352965 DOI: 10.1097/01.CCM.0000189940.70617.C3]
34 **Zabrocki LA**, Brogan TV, Statler KD, Poss WB, Rollins MD, Bratton SL. Extracorporeal membrane oxygenation for pediatric respiratory failure: Survival and predictors of mortality. *Crit Care Med* 2011; **39**: 364-370 [PMID: 20959787 DOI: 10.1097/CCM.0b013e3181fb7b35]
35 Factors associated with outcomes of patients on extracorporeal membrane oxygenation support: a 5-year cohort study. *Crit Care* 2013; **17**: R73 [PMID: 23594433 DOI: 10.1186/cc12681]
36 **Peek GJ**, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, Hibbert CL, Truesdale A, Clemens F, Cooper N, Firmin RK, Elbourne D. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. *Lancet* 2009; **374**: 1351-1363 [PMID: 19762075 DOI: 10.1016/S0140-6736(09)61069-2]
37 **Thiagarajan RR**, Laussen PC, Rycus PT, Bartlett RH, Bratton SL. Extracorporeal membrane oxygenation to aid cardiopulmonary resuscitation in infants and children. *Circulation* 2007; **116**: 1693-1700 [PMID: 17893278 DOI: 10.1161/CIRCULATIONAHA.106.680678]
38 **Kane DA**, Thiagarajan RR, Wypij D, Scheurer MA, Fynn-Thompson F, Emani S, del Nido PJ, Betit P, Laussen PC. Rapid-response extracorporeal membrane oxygenation to support cardiopulmonary resuscitation in children with cardiac disease. *Circulation* 2010; **122**: S241-S248 [PMID: 20837920 DOI: 10.1161/CIRCULATIONAHA.109.928390]
39 **Jaggers JJ**, Forbess JM, Shah AS, Meliones JN, Kirshbom PM, Miller CE, Ungerleider RM. Extracorporeal membrane oxygenation for infant postcardiotomy support: significance of shunt management. *Ann Thorac Surg* 2000; **69**: 1476-1483 [PMID: 10881826 DOI: 10.1016/S0003-4975(00)01330-8]
40 **Chow G**, Koirala B, Armstrong D, McCrindle B, Bohn D, Edgell D, Coles J, de Veber G. Predictors of mortality and neurological morbidity in children undergoing extracorporeal life support for cardiac disease. *Eur J Cardiothorac Surg* 2004; **26**: 38-43 [PMID: 15200977 DOI: 10.1016/j.ejcts.2004.04.010]
41 **Morris MC**, Ittenbach RF, Godinez RI, Portnoy JD, Tabbutt S, Hanna BD, Hoffman TM, Gaynor JW, Connelly JT, Helfaer MA, Spray TL, Wernovsky G. Risk factors for mortality in 137 pediatric cardiac intensive care unit patients managed with extracorporeal membrane oxygenation. *Crit Care Med* 2004; **32**: 1061-1069 [PMID: 15071402 DOI: 10.1097/01.CCM.0000119425.04364.CF]
42 **Lequier L**, Joffe AR, Robertson CM, Dinu IA, Wongswadiwat Y, Anton NR, Ross DB, Rebeyka IM. Two-year survival, mental, and motor outcomes after cardiac extracorporeal life support at less than five years of age. *J Thorac Cardiovasc Surg* 2008; **136**: 976-983.e3 [PMID: 18954639 DOI: 10.1016/j.jtcvs.2008.02.009]
43 **Morris MC**, Wernovsky G, Nadkarni VM. Survival outcomes after extracorporeal cardiopulmonary resuscitation instituted during active chest compressions following refractory in-hospital pediatric cardiac arrest. *Pediatr Crit Care Med* 2004; **5**: 440-446 [PMID: 15329159 DOI: 10.1097/01.PCC.0000137356.58150.2E]
44 **Alsoufi B**, Al-Radi OO, Nazer RI, Gruenwald C, Foreman C, Williams WG, Coles JG, Caldarone CA, Bohn DG, Van Arsdell GS. Survival outcomes after rescue extracorporeal cardiopulmonary resuscitation in pediatric patients with refractory cardiac arrest. *J Thorac Cardiovasc Surg* 2007; **134**: 952-959.e2 [PMID: 17903513 DOI: 10.1016/j.jtcvs.2007.05.054]
45 **Huang SC**, Wu ET, Chen YS, Chang CI, Chiu IS, Wang SS, Lin FY, Ko WJ. Extracorporeal membrane oxygenation rescue for cardiopulmonary resuscitation in pediatric patients. *Crit Care Med* 2008; **36**: 1607-1613 [PMID: 18434885 DOI: 10.1097/CCM.0b013e318170b82b]
46 **Prodhan P**, Fiser RT, Dyamenahalli U, Gossett J, Imamura M, Jaquiss RD, Bhutta AT. Outcomes after extracorporeal cardiopulmonary resuscitation (ECPR) following refractory pediatric cardiac arrest in the intensive care unit. *Resuscitation* 2009; **80**: 1124-1129 [PMID: 19695762 DOI: 10.1016/j.resuscitation.2009.07.004]
47 **Gottdiener JS**, Brown J, Zoltick J, Fletcher RD. Left ventricular hypertrophy in men with normal blood pressure: relation to exaggerated blood pressure response to exercise. *Ann Intern Med* 1990; **112**: 161-166 [PMID: 2136981 DOI: 10.1007/s00134-011-2168-6]
48 **Huang SC**, Wu ET, Wang CC, Chen YS, Chang CI, Chiu IS, Ko WJ, Wang SS. Eleven years of experience with extracorporeal cardiopulmonary resuscitation for paediatric patients with in-hospital cardiac arrest. *Resuscitation* 2012; **83**: 710-714 [PMID: 22306256 DOI: 10.1016/j.resuscitation.2012.01.031]
49 **Joffe AR**, Lequier L, Robertson CM. Pediatric outcomes after extracorporeal membrane oxygenation for cardiac disease and for cardiac arrest: a review. *ASAIO J* 2012; **58**: 297-310 [PMID: 22643323 DOI: 10.1097/MAT.0b013e31825a21ff]
50 **Fiser DH**, Long N, Roberson PK, Hefley G, Zolten K, Brodie-Fowler M. Relationship of pediatric overall performance category and pediatric cerebral performance category scores at pediatric intensive care unit discharge with outcome measures collected at hospital discharge and 1- and 6-mo follow-up assessments. *Crit Care Med* 2000; **28**: 2616-2620 [PMID: 10921604 DOI: 10.1097/00003246-200007000-00072]
51 **Raymond TT**, Cunnyngham CB, Thompson MT, Thomas JA, Dalton HJ, Nadkarni VM. Outcomes among neonates, infants, and children after extracorporeal cardiopulmonary resuscitation for refractory inhospital pediatric cardiac arrest: a report from the National Registry of Cardiopulmonary Resuscitation. *Pediatr Crit Care Med* 2010; **11**: 362-371 [PMID: 19924027 DOI: 10.1097/PCC.0b013e3181c0141b]
52 **Nadkarni VM**, Larkin GL, Peberdy MA, Carey SM, Kaye W, Mancini ME, Nichol G, Lane-Truitt T, Potts J, Ornato JP, Berg RA. First documented rhythm and clinical outcome from in-hospital cardiac arrest among children and adults. *JAMA* 2006; **295**: 50-57 [PMID: 16391216 DOI: 10.1001/jama.295.1.50]
53 **Turek JW**, Andersen ND, Lawson DS, Bonadonna D, Turley RS, Peters MA, Jaggers J, Lodge AJ. Outcomes before and after implementation of a pediatric rapid-response extracorporeal membrane oxygenation program. *Ann Thorac Surg*

2013; **95**: 2140-2146; discussion 2140-2147 [PMID: 23506632 DOI: 10.1016/j.athoracsur.2013.01.050]
54 **Als LC**, Nadel S, Cooper M, Pierce CM, Sahakian BJ, Garralda ME. Neuropsychologic function three to 6 mo following admission to the PICU with meningoencephalitis, sepsis, and other disorders: a prospective study of school-aged children. *Crit Care Med* 2013; **41**: 1094-1103 [PMID: 23385103 DOI: 10.1097/CCM.0b013e318275d032]
55 **Ebrahim S**, Singh S, Hutchison JS, Kulkarni AV, Sananes R, Bowman KW, Parshuram CS. Adaptive behavior, functional outcomes, and quality of life outcomes of children requiring urgent ICU admission. *Pediatr Crit Care Med* 2013; **14**: 10-18 [PMID: 23132399 DOI: 10.1097/PCC.0b013e31825b64b3]
56 **Conlon NP**, Breatnach C, O'Hare BP, Mannion DW, Lyons BJ. Health-related quality of life after prolonged pediatric intensive care unit stay. *Pediatr Crit Care Med* 2009; **10**: 41-44 [PMID: 19057434 DOI: 10.1097/PCC.0b013e31819371f6]
57 **Mahle WT**, Forbess JM, Kirshbom PM, Cuadrado AR, Simsic JM, Kanter KR. Cost-utility analysis of salvage cardiac extracorporeal membrane oxygenation in children. *J Thorac Cardiovasc Surg* 2005; **129**: 1084-1090 [PMID: 15867784 DOI: 10.1016/j.jtcvs.2004.08.012]
58 **Garcia JP**, Kon ZN, Evans C, Wu Z, Iacono AT, McCormick B, Griffith BP. Ambulatory veno-venous extracorporeal membrane oxygenation: innovation and pitfalls. *J Thorac Cardiovasc Surg* 2011; **142**: 755-761 [PMID: 21924145 DOI: 10.1016/j.jtcvs.2011.07.029]
59 **Rehder KJ**, Turner DA, Hartwig MG, Williford WL, Bonadonna D, Walczak RJ, Davis RD, Zaas D, Cheifetz IM. Active rehabilitation during extracorporeal membrane oxygenation as a bridge to lung transplantation. *Respir Care* 2013; **58**: 1291-1298 [PMID: 23232742 DOI: 10.4187/respcare.02155]
60 **Rahimi RA**, Skrzat J, Reddy DR, Zanni JM, Fan E, Stephens RS, Needham DM. Physical rehabilitation of patients in the intensive care unit requiring extracorporeal membrane oxygenation: a small case series. *Phys Ther* 2013; **93**: 248-255 [PMID: 23104895 DOI: 10.2522/ptj.20120336]

P- Reviewers: Cavanna AE, Orlacchio A, Rodrigues MG
S- Editor: Gou SX **L- Editor:** A **E- Editor:** Liu XM

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5492/wjccm.v2.i4.48

World J Crit Care Med 2013 November 4; 2(4): 48-55
ISSN 2220-3141 (online)

ORIGINAL ARTICLE

Molecular targeting therapy using bevacizumab for peritoneal metastasis from gastric cancer

Keishiro Aoyagi, Kikuo Kouhuji, Motoshi Miyagi, Junya Kizaki, Taro Isobe, Kousuke Hashimoto, Kazuo Shirouzu

Keishiro Aoyagi, Kikuo Kouhuji, Motoshi Miyagi, Junya Kizaki, Taro Isobe, Kousuke Hashimoto, Kazuo Shirouzu, Department of Surgery, Kurume University School of Medicine, Kurume, Fukuoka 830-0011, Japan
Author contributions: Aoyagi K was responsible for writing and revising the full text original article in response to an invitation from an editorial office member of the *WJCCM*; Kouhuji K instructed the research and clinical work; Miyagi M established the high potential peritoneal dissemination cell line MKN-45P; Kizaki J cultured the two cell lines and measured the concentration of various cytokines; Isobe T performed the *in vivo* study on the peritoneal dissemination model using nude mice; Hasimoto K performed the pathological study on the intra-abdominal tumor of nude mice; and Professor Shirouzu K, Chair of the Department of Surgery, provided useful critique and advice for our research.
Correspondence to: Keishiro Aoyagi, Assistant Professor, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan. keishiro@med.kurume-u.ac.jp
Telephone: +81-942-353311 Fax: +81-942-340709
Received: May 2, 2013 Revised: June 5, 2013
Accepted: July 4, 2013
Published online: November 4, 2013

Abstract

AIM: To clarify the significance of vascular endothelial growth factor (VEGF) in peritoneal metastasis from gastric cancer, using the gastric cancer cell line MKN-45 compared with the high potential peritoneal dissemination gastric cancer cell line MKN-45P.

METHODS: The supernatant of culture medium of MKN-45 cells or MKN-45P cells was collected and the concentrations were measured of various cytokines, matrix metalloproteinases, growth factor and angiogenic factors, including VEGF. We performed an initial pilot study to explore whether bevacizumab, a humanized monoclonal antibody against VEGF, had any suppressive effect on the peritoneal dissemination from gastric cancer in an experimental nude mouse model of peritoneal metastasis.

RESULTS: The concentrations of interleukin-6 (IL-6), IL-8, VEGF and matrix metalloproteinase-2 protein in the culture supernatant were each significantly higher than each of those for MKN-45. In the *in vivo* study, the volume of ascites and the mitotic index were significantly lower in the therapy group than in the non-therapy group. The survival curve of the therapy group was significantly higher than that of the non-therapy group. These results suggested that VEGF was correlated with peritoneal metastasis from gastric cancer.

CONCLUSION: Findings suggested that bevacizumab for inhibiting VEGF could suppress peritoneal dissemination from gastric cancer.

Key words: Gastric cancer; Peritoneal metastasis; Vascular endothelial growth factor; MKN-45P; Bevacizumab

Aoyagi K, Kouhuji K, Miyagi M, Kizaki J, Isobe T, Hashimoto K, Shirouzu K. Molecular targeting therapy using bevacizumab for peritoneal metastasis from gastric cancer. *World J Crit Care Med* 2013; 2(4): 48-55 Available from: URL: http://www.wjgnet.com/2220-3141/full/v2/i4/48.htm DOI: http://dx.doi.org/10.5492/wjccm.v2.i4.48

INTRODUCTION

Peritoneal metastasis is the most common form of recurrence from gastric cancer and is associated with a poor prognosis. Therefore, the management of any dissemination in the peritoneal cavity is important in the treatment of gastric cancer. However, there is as yet no effective treatment against peritoneal metastasis from gastric cancer. The development of peritoneal metastasis

is a multistep process, beginning with the detachment of cancer cells from the primary tumor, their attachment to peritoneal mesothelial cells, retraction of the mesothelial cells, and exposure of the basement membrane. After attachment to the basement membrane, the cancer cells degrade in the extracellular matrix and then proliferate[1-3]. Finally, the cancer cells induce angiogenesis and lymphangiogenesis. Many cytokines, growth factors, matrix metalloproteinases and angiogenic factors play important roles in these steps. Tumor growth requires new vessel formation and this is driven predominantly by vascular endothelial growth factor (VEGF), the most potent angiogenic molecule known and the principle target for antiangiogenic therapy. VEGF levels in malignant ascites are remarkably elevated[4]. VEGF has been reported to enhance vascular permeability and angiogenesis in the abdominal wall and contributes to the establishment of peritoneal dissemination with malignant ascites[4,5]. In ovarian cancer, three pathological events are thought to cause malignant ascites: obstruction of the lymphatic vessels by tumor cells inhibiting lymphatic drainage from the peritoneal cavity; hyperpermeability of microvessels lining the peritoneal cavity; and angiogenesis[6]. In gastric cancer, there was a tendency for the tumor/normal ratio of VEGF mRNA to be correlated with distant metastasis[7] and positive expression of tissue VEGF, circulating VEGF, VEGF-C and VEGF-D were each associated with poor prognosis in resected gastric cancer[8]. We have previously reported that tissue VEGF was a useful indicator of peritoneal recurrence of gastric cancer[9]. The aim of the present study was to clarify the significance of VEGF in peritoneal metastasis from gastric cancer. We compared cytokines, matrix metalloproteinases (MMPs) and VEGF in the gastric cancer cell line MKN-45 and in the high potential peritoneal dissemination gastric cancer cell line MKN-45P, using an enzyme-linked immunosorbent assay (ELISA) method. Furthermore, we investigated whether administration of VEGF antibody could prevent peritoneal metastasis from gastric cancer. Bevacizumab is a humanized monoclonal antibody against VEGF and was the first commercially available angiogenesis inhibitor. We investigated whether bevacizumab had a suppressive effect on peritoneal dissemination from gastric cancer, experimentally, using a mouse peritoneal metastasis model.

MATERIALS AND METHODS

Cell lines

We used the high potential peritoneal dissemination cell line MKN-45P, established from the human gastric cancer cell line MKN-45 (derived from a poorly differentiated adenocarcinoma in a 62 year old woman; Health Science Research Resources Bank, Tokyo, Japan) in our institute, as described previously[10]. Briefly, nude mice (BALB/c nu/nu) were subcutaneously inoculated with MKN-45 cells and the subcutaneous nodules were removed and injected into other nude mice intraperitoneally. The cancer cells from the peritoneal nodules were injected into the abdominal cavity of other mice. The process was continued through to a seventh generation. The resulting high potential peritoneal dissemination cell line was named MKN-45P. MKN-45 and MKN-45P cells were each maintained in RPMI-1640 medium (Nihon Seiyaku Co., Komaki, Aichi, Japan) supplemented with 10% heat inactivated fetal bovine serum (FBS) (Gibco Uxbridge, Middlesex, United Kingdom), 2 mmol/L-glutamine and penicillin-streptomycin (50 IU/mL and 50 μg/mL, respectively) at 37.0 ℃ in humidified air with 5% CO_2.

Measurement of cytokines in conditioned medium

For measurement of cytokines in conditioned medium, the MKN-45 cells (1×10^6 cells/10 mL) or MKN-45P cells (1×10^6 cells/10 mL) were placed in 100 mm tissue culture dishes (IWAKI Co., Funabashi, Chiba, Japan) and cultured for 72 h in medium containing 10% FBS at 37.0 ℃ in humidified air with 5% CO_2. The number of cells in each cell line was evaluated visually at 12, 24, 48 and 72 h (values: mean of three fields). The supernatant was then collected and the concentrations of interleukin-1β (IL-1β), IL-6, IL-8, IL-10, hepatocyte growth factor (HGF), transforming growth factor-β1 (TGF-β1), VEGF, MMP-2, MMP-9 and tissue inhibitor of metalloproteinases-1 (TIMP-1) proteins were each measured using the ELISA method (IL-1β, IL-8 and IL-10: Bio Source Europe S. A., Nivelles, Belgium; IL-6: Fujirebio Inc., Tokyo, Japan; HGF: Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan; TGF-β1 and VEGF: RD System Inc., Minneapolis, MN, United States; MMP-2, MMP-9 and TIMP-1: Daiichi Fine Chemical Co. Ltd., Takaoka, Toyama, Japan). Each cytokine was measured in 5 samples and the means of these were compared between the MKN-45 cells and the MKN-45P cells.

Animals

4 wk old athymic male BALB/c nu/nu nude mice, each weighing 18 g, were obtained from CLEA (Tokyo, Japan). The mice were housed in cages under specific pathogen-free conditions and provided with sterilized food and water ad libitum.

Drugs

The humanized murine monoclonal antibody against human VEGF (bevacizumab, Avastin) was purchased from Genetech (San Francisco, CA, United States).

Experimental design

The experimental group consisted of 5 wk old male mice (n = 10). We determined a working concentration of bevacizumab according to Wildiers *et al*[11]. On day 0, we injected 1×10^7 MKN-45P cells into the abdominal cavity of each mouse, followed by a single intraperitoneal (*ip*) injection of 200 μg bevacizumab in 1 mL saline on day 0 and day 4. On day 21, five mice were sacrificed under ether anesthesia; these were weighed and then we

Table 1 Comparison of cytokines between MKN-45 and MKN-45P

	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	VEGF (pg/mL)	MMP-2 (ng/mL)	TIMP-1 (ng/mL)
MKN-45	0.9 ± 0.7	1.2 ± 0.7	381.9 ± 147.1	1335.0 ± 624.3	0.3 ± 0.1	2.7 ± 1.8
MKN-45P	0.4 ± 0.2	2.9 ± 0.6	891.4 ± 210.2	3806.0 ± 229.8	0.7 ± 0.5	6.0 ± 4.0
P value	0.109	0.045	0.011	0.013	0.021	0.126

IL: Interleukin; VEGF: Vascular endothelial growth factor; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitor of metalloproteinase.

calculated the mean number of tumor nodules in a 1 cm^2 area in three fields on the mesentery and calculated the volume of ascites. We also extracted retroperitoneal tissues for histological examination. Another five mice were monitored until they died and the survival rate was calculated using the Kaplan-Meier method. A matching number of control mice were given 1 mL of drug-free saline.

Histology

After extraction, the retroperitoneal tissues were fixed for 12 h in 10% neutral buffered formaldehyde, then cut every 5 mm horizontally and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin (HE) and examined using light microscopy. We counted the frequency of hydronephrosis on the retroperitoneal tissues. The mitotic index was defined as the mean number of mitotic figures in a 400 times magnified field from ten arbitrary microscopic fields.

Immunohistochemistry

VEGF was analyzed using immunohistochemical staining and the avidin-biotin-peroxidase complex technique (Vectastain ABC Kit; Vector, Burlingame, CA, United States). Briefly, 3 μm thick sections of the formalin-fixed paraffin-embedded tissue specimens were deparaffinized and dehydrated. The sections were washed with phosphate-buffered saline (PBS), treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase, and then incubated with primary antibody in a humidified chamber at 4 ℃ overnight. As the primary antibody, rabbit polyclonal antibody A-20 was used (Santa Cruz Biotechnology, Santa Cruz, CA, United States) for VEGF, diluted at 1:200. Sections were washed three times with PBS, then incubated with biotinylated horse anti-rabbit immunoglobulin G antibody for 30 min, washed again three times with PBS, and then incubated with avidin-biotinylated peroxidase complex for 30 min. After three additional washings with PBS, staining was developed by incubating the sections in 3-amino-9-ethylcarbazole (Vector) for 5 min. The sections were then counterstained with hematoxylin and mounted. The cell types showing positive staining for VEGF were defined morphologically by H and E staining, using serial sections. VEGF expression was classified as one of three categories using a method modified from the literature that was previously used on gastric tissue[9], depending on the percentage of tumor cells stained: category 1 being less than 30% of cells stained; category 2 being from 30% to 49% stained; and category 3 being 50% or more cells stained.

Ethics

This study was approved by Kurume University Institutional Animal Care and Use Committee of Ethics.

Statistical analysis

Student's *t*-test and the χ^2 test were used to analyze the data for any significant difference and any difference was considered statistically significant when the *P* value was less than 0.05. The cumulative survival rate was calculated using the Kaplan-Meier method. The significance of any difference between the survival curves was determined using the log-rank test and any difference was considered significant at the 5% level.

RESULTS

Measurement of cytokines in condition medium

The number of MKN-45 or MKN-45P cells was counted at 24, 48 and 72 h. There was no difference in the number of cancer cells between the two cell lines.

The concentrations of cytokines in conditioned media from MKN-45 and from MKN-45P are shown in Table 1. The concentrations of IL-6, IL-8, VEGF and MMP-2 protein in the culture supernatants from MKN-45P were each significantly higher than each of those from MKN-45 ($P = 0.045$, $P = 0.011$, $P = 0.013$ and $P = 0.021$, respectively) (Table 1).

Peritoneal dissemination model

Peritoneal dissemination with bloody ascites was recognized in all five mice using the MKN-45P cell line (Figure 1A). Numerous nodules were seen on the mesentery (Figure 1B). We confirmed histologically that the nodule in the peritoneum was composed of cancer cells. All five mice in the non-therapy group were cachexic; however, there was no significant difference in body weight ($P = 0.591$) and no difference in the number of peritoneal nodules ($P = 0.783$) between the therapy and non-therapy group. The volume of ascites in the therapy group was significantly less than that in the non-therapy group ($P = 0.042$). No side-effects of bevacizumab were evident, such as bleeding, bowel perforation or thrombosis (Table 2).

Histopathological findings

In the therapy group, two right kidneys (40%) and one

Table 2 Number of tumor nodules, volume of ascites, body weight, frequency of hydronephrosis and mitotic index in the mice treated with bevacizumab and the untreated mice

Treatment	*n*	Tumor nodules	Ascites (mL)	Body weight (g)	Hydronephrosis		Mitosis index
					Right kidney	Left kidney	
Non-therapy	5	16.64 ± 5.06[1]	0.60 ± 0.51[2]	12.21 ± 0.51[3]	4 (80)	2 (40)	21.0 ± 5.7[b]
Therapy	5	17.66 ± 3.45	0.04 ± 0.03	12.41 ± 0.61	2 (40)	1 (20)	9.6 ± 2.1

[1]$P = 0.783$; [2]$P = 0.042$; [3]$P = 0.591$; [b]$P < 0.01$ *vs* therapy group.

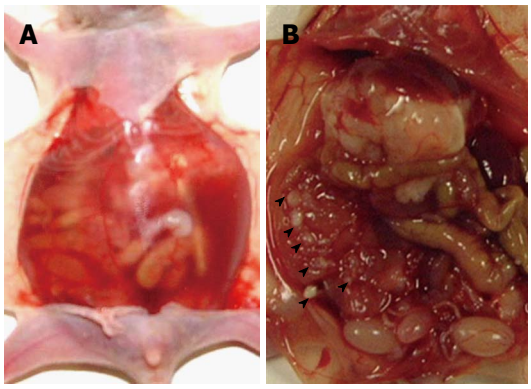

Figure 1 Peritoneal dissemination model. A: Bloody ascites was recognized in the peritoneal cavity of the peritoneal dissemination model, using the MKN-45P cell line; B: Numerous tumor nodules (arrowheads) were recognized on the mesentery.

left kidney (20%) showed hydronephrosis. In the non-therapy group, four right kidneys (80%) and two left kidneys (40%) showed hydronephrosis. The frequency of hydronephrosis in the therapy group was lower than that in the non-therapy group (Table 2). In the therapy group, the grade of hydronephrosis was mild and only a small amount of tissue was recognized in the retroperitoneum. In contrast, in the non-therapy group, the grade of hydronephrosis was severe and a large amount of tumor tissue was recognized in the retroperitoneum (Figure 2). High magnification examination of the tumor tissue revealed a lower number of mitoses in the therapy group than in the control group (Figure 3).

Immunohistochemical findings

Immunoreactivity for VEGF was mainly identified as supranuclear staining or diffuse staining in the cytoplasm of the cancer cells (Figure 4). Based on the percentage of positive tumor staining, all of the five mice in the therapy group were in category 2, whereas all of the five mice in the non-therapy group were in category 3 (Figure 4).

Mitotic index

The mitotic index was 9.6 ± 2.1 in the therapy group and this was significantly lower than that of 21.0 ± 5.7 in the non-therapy group ($P < 0.01$) (Table 2).

Survival curves

We investigated the findings for any correlation between survival and bevacizumab treatment. The median survival of the treated mice was 30.8 d and that of the untreated mice was 26.6 d The survival of the therapy group was significantly longer than that of the non-therapy group ($P = 0.005$) (Figure 5).

DISCUSSION

Research in the field of tumor angiogenesis has provided a foundation for radical development in the management and treatment of human cancers. VEGF is the most sensitive angiogenic factor and is expressed in cancer cells. Several clinical trials have confirmed that targeting the vascular VEGF/VEGF receptor pathway can show some clinical benefit. VEGF was initially described as a vascular permeability factor by Senger *et al*[12] in 1983 and was later cloned and found to be homologous to VEGF by Ferrara *et al*[13]. VEGF has been reported to enhance the permeability of tumor vessels[5], to induce serine protease or metalloproteases[14,15], to inhibit apoptosis in endothelial cells[16,17], and to inhibit the maturation of dendritic cells[18]. Since then, several randomized trials have shown a clinical benefit by various VEGF-targeted agents in patients with metastatic colorectal cancer, advanced non small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma and metastatic breast cancer[19]. VEGF-targeted therapy has thus become an important treatment option for several human malignancies.

Peritoneal metastasis in gastric cancer takes place through a multistep process involving the detachment of cancer cells from the primary tumor, their attachment to the distant peritoneum, invasion into the subperitoneal space, proliferation and angiogenesis[1-3]. Angiogenesis is a key step in the various stages of human cancer development and dissemination. Previous reports have indicated that the presence of angiogenic factors is an essential event in the development of peritoneal metastasis[20-22].

In gastric cancer, there is a tendency for the tumor/normal ratio of VEGF mRNA to be correlated with distant metastasis[7]. Positive expression of tissue VEGF, circulating VEGF, VEGF-C and VEGF-D were each associated with poor prognosis in resected gastric cancer[8]. We have previously reported that tissue VEGF was a useful indicator of peritoneal recurrence from gastric cancer[9]. In our immunohistochemical study on clinical specimens, the VEGF score of patients with peritoneal recurrence was significantly higher than that of patients without peritoneal recurrence and the VEGF score was

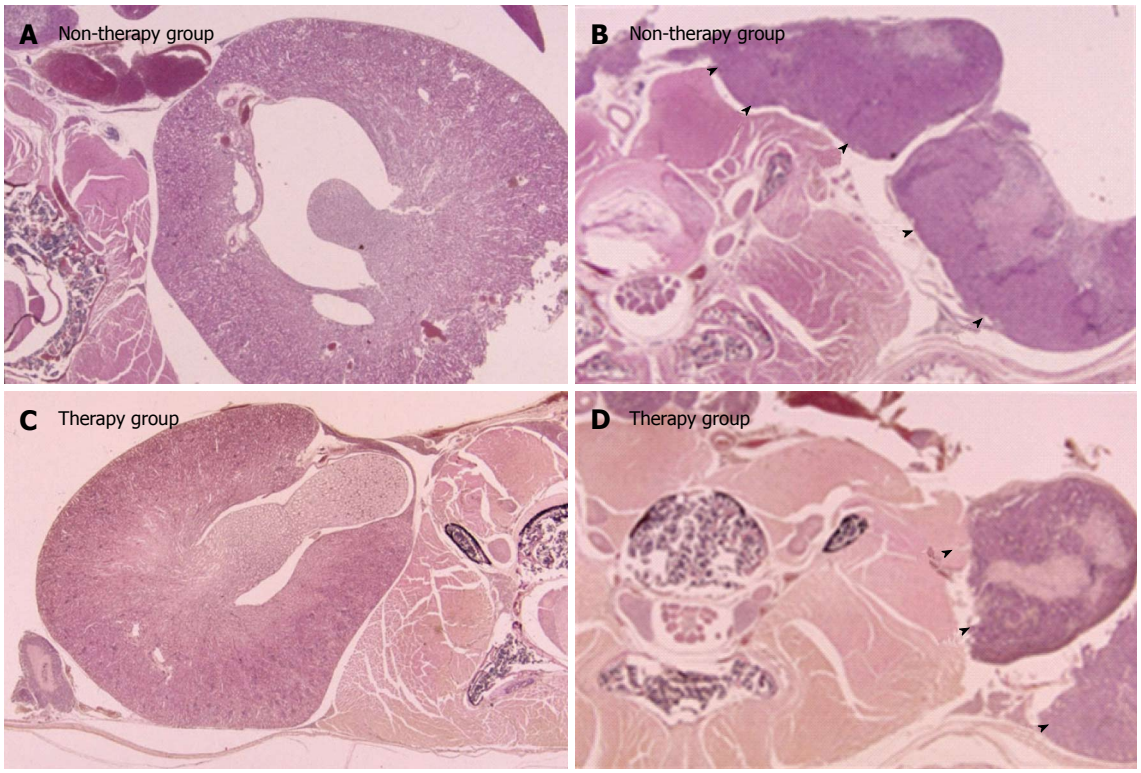

Figure 2 Macroscopic and histological findings of the retroperitoneal tissue. A: Hydronephrosis in the left kidney in the non-therapy group (HE, × 40); B: A large amount of tumor tissues (arrowheads) was recognized on the retroperitoneum in the non-therapy group (HE, × 40); C: Mild hydronephrosis in the right kidney in the therapy group (HE, × 40); D: A small amount of tumor tissues (arrowheads) was recognized on the retroperitoneum in the therapy group (HE, × 40). HE staining, with low magnification, on the cut surface of retroperitoneal tissues in the non-therapy group and the therapy group.

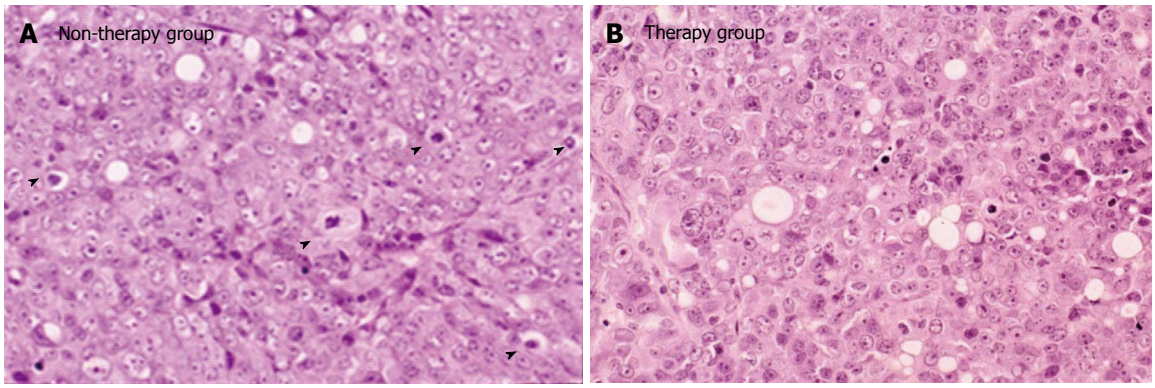

Figure 3 High magnification of tumor tissue. High magnification revealed tumor tissue in both the non-therapy group (A) and in the therapy (B) group. The number of mitoses (arrowheads) in the non-therapy group was larger than that in the therapy group (HE, × 400).

a significant parameter of peritoneal recurrence, suggesting that VEGF was correlated with peritoneal metastasis from gastric cancer and that VEGF was a useful indicator of peritoneal recurrence[9].

The present study reveals that the concentrations of IL-6, IL-8, VEGF and MMP-2 protein in the culture supernatant of MKN-45P are each significantly higher than each of those of MKN-45. IL-6 has been reported as a prognostic factor in gastric carcinoma and is significantly correlated with the incidence of lymph node metastasis and liver metastasis[23]. IL-8 has been reported as a prognostic factor in gastric carcinoma and is significantly correlated with the depth of invasion and vessel infiltration[24]. IL-6 and IL-8 are each related to the accomplishment of peritoneal dissemination by inducement of angiogenesis[25,26].

Degradation of the extracellular matrix is considered to be a prerequisite for peritoneal metastasis and MMPs are thought to play an important role in this process[27,28]. There are many reports that highly invasive cancer cells with a high potential for metastasis stimulate the production of MMPs[27] and that MMP-2 is significantly correlated with depth of invasion, lymph node metastasis and distant metastasis from gastric cancer[29].

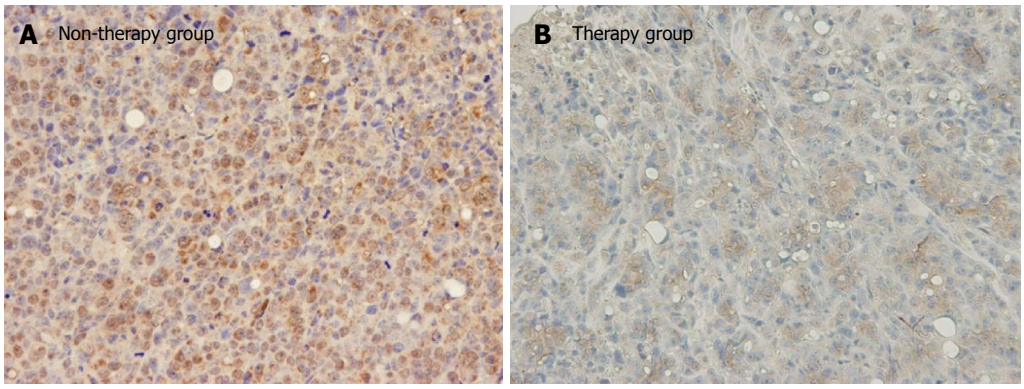

Figure 4 Immunohistochemical staining of tumor tissue. Immunoreactivity for vascular endothelial growth factor (VEGF) was mainly identified as supranuclear staining or diffused staining in the cytoplasm of cancer cells. A: All 5 mice in the non-therapy group according to the percentage of positive tumor staining were in category 3, with 50% or more cells stained (VEGF, × 200); B: All 5 mice in the therapy group were in category 2, with 30 to 49% stained (VEGF, × 200).

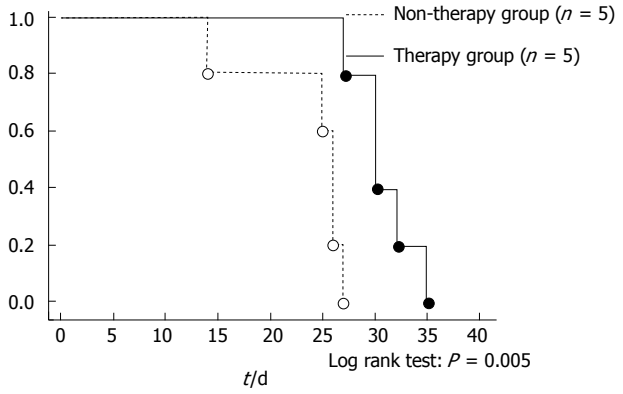

Figure 5 Survival curves. Survival curves of the bevacizumab-treated (n = 5), and untreated mice (n = 5). The mean survival duration of the treated mice was 30.8 d and of the untreated mice was 23.6 d (P = 0.005).

These studies have provided clear evidence that VEGF is an essential element in the development of peritoneal metastasis. Accordingly, we investigated whether VEGF antibody might prevent peritoneal metastasis from gastric cancer.

Bevacizumab is a monoclonal antibody against VEGF that inhibits tumor growth by blocking angiogenesis. Cancer cells transferred with VEGF have been found to have an increased potential for the development of tumorigenesis in a xenograft model[21]. According to several reports, antiangiogenic agents can decrease tumor vessel permeability and prevent tumor growth[11,30,31]. Jain *et al*[31] have reported that antiangiogenic therapy normalized tumor vessels and reduced interstitial fluid pressure, which finally decreased malignant ascites. In the present study, all the mice in the non-therapy group were cachexic. However, there was no significant difference in body weight between the therapy group and the non-therapy group because the volume of ascites in the therapy group was significantly less than that in the non-therapy group. These findings suggested that bevacizumab suppressed cell proliferative activity by inhibiting angiogenesis of VEGF, thus contributing to the smaller amount of tumor tissue and the low incidence of hydronephrosis in the therapy group. Although the number of peritoneal nodules did not differ significantly between the two groups, the nodules on the mesentery in the treated group appeared to have been smaller but these were too small to be measured or weighed. The tumors on the retroperitoneum in the non-therapy group were larger than those in the therapy group and large tumors need new blood vessels for their growth. On immunohistochemical staining, the percentage of tumor cells stained for VEGF in the therapy group was lower than that in the non-therapy group. The mitotic index in the therapy group was also significantly lower than that in the non-therapy group. These results suggested that bevacizumab might suppress the vascular permeability effect and the cell proliferative activity by inhibiting angiogenesis of VEGF and thereby prolonging survival in the mice in the therapy group.

The findings from the present study indicate that the addition of bevacizumab to standard treatment might prolong the survival of gastric cancer patients, especially those with peritoneal metastasis. In conclusion, combination of bevacizumab with anticancer drugs may suppress peritoneal dissemination from gastric cancer.

The results from the present study show that VEGF was correlated with peritoneal metastasis from gastric cancer. Accordingly, using bevacizumab to inhibit VEGF may suppress peritoneal dissemination from gastric cancer. Therefore, combination of bevacizumab with anticancer drugs might suppress peritoneal dissemination from gastric cancer.

COMMENTS

Background

The therapy for peritoneal metastasis is the most important treatment to improve the prognosis of advanced gastric cancer. However, there is yet no effective treatment against peritoneal metastasis from gastric cancer. The relationship between vascular endothelial growth factor (VEGF) and peritoneal metastasis has been reported. Therefore, the authors investigated whether bevacizumab, a humanized monoclonal antibody against VEGF, had a suppressive effect on peritoneal dissemination from gastric cancer, experimentally, using a mouse peritoneal metastasis model.

Research frontiers

The research hot spot is suppression of peritoneal metastasis and prolonging the survival of peritoneal metastasis by bevacizumab.

Innovations and breakthroughs

The authors proved that bevacizumab reduced the volume of ascites and decreased the proliferative activity of cancer cells on the peritoneum macroscopically and microscopically. So, this research proved the suppressive effect of bevacizumab for peritoneal metastasis from gastric cancer more clearly than other similar articles. Moreover, hydronephrosis is one of the most popular events of peritoneal metastasis from gastric cancer clinically. In this research, the authors focused on the hydronephrosis and proved the suppressive effect of bevacizumab for hydronephrosis to reduce the tumor volume on the retroperitoneum.

Applications

The results show that using bevacizumab to inhibit the VEGF may suppress peritoneal dissemination from gastric cancer. Therefore, bevacizumab could be used in preventing peritoneal recurrence and the combination of bevacizumab with anticancer drugs may suppress peritoneal dissemination from gastric cancer.

Peer review

The authors think that the design of this study is good and they analyze the effect of molecular targeting therapy for VEGF against peritoneal metastasis from gastric cancer. The results are interesting and suggest that bevacizumab could be used in preventing peritoneal recurrence. The combination of bevacizumab with anticancer drugs may suppress peritoneal dissemination from gastric cancer.

REFERENCES

1 **Yonemura Y**, Endo Y, Yamaguchi T, Fujimura T, Obata T, Kawamura T, Nojima N, Miyazaki I, Sasaki T. Mechanisms of the formation of the peritoneal dissemination in gastric cancer. *Int J Oncol* 1996; **8**: 795-802 [PMID: 21544429]

2 **Yonemura Y**. Peritoneal dissemination. Tokyo: Health Publishers, 1996

3 **Liotta LA**. Tumor invasion and metastases--role of the extracellular matrix: Rhoads Memorial Award lecture. *Cancer Res* 1986; **46**: 1-7 [PMID: 2998604]

4 **Zebrowski BK**, Liu W, Ramirez K, Akagi Y, Mills GB, Ellis LM. Markedly elevated levels of vascular endothelial growth factor in malignant ascites. *Ann Surg Oncol* 1999; **6**: 373-378 [PMID: 10379858 DOI: 10.1007/s10434-999-0373-0]

5 **Dvorak HF**, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995; **146**: 1029-1039 [PMID: 7538264]

6 **Belotti D**, Paganoni P, Manenti L, Garofalo A, Marchini S, Taraboletti G, Giavazzi R. Matrix metalloproteinases (MMP9 and MMP2) induce the release of vascular endothelial growth factor (VEGF) by ovarian carcinoma cells: implications for ascites formation. *Cancer Res* 2003; **63**: 5224-5229 [PMID: 14500349]

7 **Ryu KH**, Shim KN, Jung SA, Yoo K, Joo YH, Lee JH. Significance of preoperative tissue levels of vascular-endothelial cadherin, liver-intestine cadherin and vascular endothelial growth factor in gastric cancer. *Korean J Gastroenterol* 2012; **60**: 229-241 [PMID: 23089909 DOI: 10.4166/kjg.2012.60.4.229]

8 **Liu L**, Ma XL, Xiao ZL, Li M, Cheng SH, Wei YQ. Prognostic value of vascular endothelial growth factor expression in resected gastric cancer. *Asian Pac J Cancer Prev* 2012; **13**: 3089-3097 [PMID: 22994715 DOI: 10.7314/APJCP.2012.13.7.3089]

9 **Aoyagi K**, Kouhuji K, Yano S, Miyagi M, Imaizumi T, Takeda J, Shirouzu K. VEGF significance in peritoneal recurrence from gastric cancer. *Gastric Cancer* 2005; **8**: 155-163 [PMID: 16086118 DOI: 10.1007/s10120-005-0329-4]

10 **Miyagi M**, Aoyagi K, Kato S, Shirouzu K. The TIMP-1 gene transferred through adenovirus mediation shows a suppressive effect on peritoneal metastases from gastric cancer. *Int J Clin Oncol* 2007; **12**: 17-24 [PMID: 17380436 DOI: 10.1007/s10147-006-0616-z]

11 **Wildiers H**, Guetens G, De Boeck G, Verbeken E, Landuyt B, Landuyt W, de Bruijn EA, van Oosterom AT. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. *Br J Cancer* 2003; **88**: 1979-1986 [PMID: 12799646 DOI: 10.1038/sj.bjc.6601005]

12 **Senger DR**, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science* 1983; **219**: 983-985 [PMID: 6823562 DOI: 10.1126/science.6823562]

13 **Ferrara N**, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 1989; **161**: 851-858 [PMID: 2735925 DOI: 10.1016/0006-291X(89)92678-8]

14 **Pepper MS**, Ferrara N, Orci L, Montesano R. Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. *Biochem Biophys Res Commun* 1991; **181**: 902-906 [PMID: 1755866 DOI: 10.1016/0006-291X(91)91276-I]

15 **Unemori EN**, Ferrara N, Bauer EA, Amento EP. Vascular endothelial growth factor induces interstitial collagenase expression in human endothelial cells. *J Cell Physiol* 1992; **153**: 557-562 [PMID: 1447317]

16 **Shaheen RM**, Davis DW, Liu W, Zebrowski BK, Wilson MR, Bucana CD, McConkey DJ, McMahon G, Ellis LM. Antiangiogenic therapy targeting the tyrosine kinase receptor for vascular endothelial growth factor receptor inhibits the growth of colon cancer liver metastasis and induces tumor and endothelial cell apoptosis. *Cancer Res* 1999; **59**: 5412-5416 [PMID: 10554007]

17 **Gerber HP**, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, Ferrara N. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 1998; **273**: 30336-30343 [PMID: 9804796 DOI: 10.1074/jbc.273.46.30336]

18 **Lissoni P**, Malugani F, Bonfanti A, Bucovec R, Secondino S, Brivio F, Ferrari-Bravo A, Ferrante R, Vigoré L, Rovelli F, Mandalà M, Viviani S, Fumagalli L, Gardani GS. Abnormally enhanced blood concentrations of vascular endothelial growth factor (VEGF) in metastatic cancer patients and their relation to circulating dendritic cells, IL-12 and endothelin-1. *J Biol Regul Homeost Agents* 2001; **15**: 140-144 [PMID: 11501971]

19 **Ellis LM**, Hicklin DJ. Pathways mediating resistance to vascular endothelial growth factor-targeted therapy. *Clin Cancer Res* 2008; **14**: 6371-6375 [PMID: 18927275 DOI: 10.1158/1078-0432.CCR-07-5287]

20 **Yoneda J**, Kuniyasu H, Crispens MA, Price JE, Bucana CD, Fidler IJ. Expression of angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. *J Natl Cancer Inst* 1998; **90**: 447-454 [PMID: 9521169 DOI: 10.1093/jnci/90.6.447]

21 **Kondo Y**, Arii S, Mori A, Furutani M, Chiba T, Imamura M. Enhancement of angiogenesis, tumor growth, and metastasis by transfection of vascular endothelial growth factor into LoVo human colon cancer cell line. *Clin Cancer Res* 2000; **6**: 622-630 [PMID: 10690548]

22 **Mori A**, Arii S, Furutani M, Mizumoto M, Uchida S, Furuyama H, Kondo Y, Gorrin-Rivas MJ, Furumoto K, Kaneda Y, Imamura M. Soluble Flt-1 gene therapy for peritoneal metastases using HVJ-cationic liposomes. *Gene Ther* 2000; **7**: 1027-1033 [PMID: 10871751 DOI: 10.1038/sj.gt.3301202]

23 **Ashizawa T**, Okada R, Suzuki Y, Takagi M, Yamazaki T, Sumi T, Aoki T, Ohnuma S, Aoki T. Clinical significance of interleukin-6 (IL-6) in the spread of gastric cancer: role of IL-6 as a prognostic factor. *Gastric Cancer* 2005; **8**: 124-131 [PMID: 15864720 DOI: 10.1007/s10120-005-0315-x]

24 **Kido S**, Kitadai Y, Hattori N, Haruma K, Kido T, Ohta M,

Tanaka S, Yoshihara M, Sumii K, Ohmoto Y, Chayama K. Interleukin 8 and vascular endothelial growth factor -- prognostic factors in human gastric carcinomas? *Eur J Cancer* 2001; **37**: 1482-1487 [PMID: 11506954 DOI: 10.1016/S0959-8049(01)00147-2]

25 **Huang SP**, Wu MS, Wang HP, Yang CS, Kuo ML, Lin JT. Correlation between serum levels of interleukin-6 and vascular endothelial growth factor in gastric carcinoma. *J Gastroenterol Hepatol* 2002; **17**: 1165-1169 [PMID: 12453275 DOI: 10.1046/j.1440-1746.2002.02873.x]

26 **Kitadai Y**, Haruma K, Sumii K, Yamamoto S, Ue T, Yokozaki H, Yasui W, Ohmoto Y, Kajiyama G, Fidler IJ, Tahara E. Expression of interleukin-8 correlates with vascularity in human gastric carcinomas. *Am J Pathol* 1998; **152**: 93-100 [PMID: 9422527]

27 **Nagase H**, Woossner JF Jr. Matrix metallopreteinases. *J Biol Chem* 1999; **274**: 21492-21494 [DOI: 10.1074/jbc.274.31.21491]

28 **Mizutani K**, Kofuji K, Shirouzu K. The significance of MMP-1 and MMP-2 in peritoneal disseminated metastasis of gastric cancer. *Surg Today* 2000; **30**: 614-621 [PMID: 10930227 DOI: 10.1007/s005950070101]

29 **Mönig SP**, Baldus SE, Hennecken JK, Spiecker DB, Grass G, Schneider PM, Thiele J, Dienes HP, Hölscher AH. Expression of MMP-2 is associated with progression and lymph node metastasis of gastric carcinoma. *Histopathology* 2001; **39**: 597-602 [PMID: 11903578 DOI: 10.1046/j.1365-2559.2001.01306.x]

30 **Ferrara N**, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov* 2004; **3**: 391-400 [PMID: 15136787 DOI: 10.1038/nrd1381]

31 **Jain RK**, Tong RT, Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model. *Cancer Res* 2007; **67**: 2729-2735 [PMID: 17363594 DOI: 10.1158/0008-5472.CAN-06-4102]

P- Reviewers: Cimpean AM, Lu XM, Li Q, Tamiya M
S- Editor: Gou SX **L- Editor**: Roemmele A **E- Editor:** Liu XM

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5492/wjccm.v2.i4.56

World J Crit Care Med 2013 November 4; 2(4): 56-64
ISSN 2220-3141 (online)

BRIEF ARTICLE

Pyruvate-fortified resuscitation stabilizes cardiac electrical activity and energy metabolism during hypovolemia

Hunaid A Gurji, Daniel W White, Besim Hoxha, Jie Sun, Albert H Olivencia-Yurvati, Robert T Mallet

Hunaid A Gurji, Daniel W White, Jie Sun, Albert H Olivencia-Yurvati, Robert T Mallet, Departments of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, TX 76107, United States
Besim Hoxha, Olivencia-Yurvati, Robert T Mallet, Departments of Surgery, University of North Texas Health Science Center, Fort Worth, TX 76107, United States
Albert H Olivencia-Yurvati, Robert T Mallet, Cardiovascular Research Institute, University of North Texas Health Science Center, Fort Worth, TX 76107, United States
Robert T Mallet, Institute for Aging and Alzheimer's Research, University of North Texas Health Science Center, Fort Worth, TX 76107, United States
Author contributions: Gurji HA, White DW, Hoxha B, Olivencia-Yurvati AH, Mallet RT designed the research; Gurji HA, White DW, Hoxha B conducted the surgical preparation and experimental protocols; Gurji HA, Sun J performed analytical measurements; Gurji HA, Sun J analyzed the data and prepared the figures; Gurji HA, Olivencia-Yurvati AH, Mallet RT wrote the manuscript.
Supported by Grant #W911NF0910086 from the United States Department of Defense; Predoctoral fellowships from the Graduate School of Biomedical Sciences, University of North Texas Health Science Center to Gurji HA and White DW
Correspondence to: Robert T Mallet, PhD, Department of Integrative Physiology, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107-2699, United States. robert.mallet@unthsc.edu
Telephone: +1-817-7352260 Fax: +1-817-7355084
Received: May 7, 2013 Revised: July 20, 2013
Accepted: August 12, 2013
Published online: November 4, 2013

Abstract

AIM: To test the hypothesis that fluid resuscitation with Ringer's solution enriched with pyruvate (PR), a physiological antioxidant and energy substrate, affords protection of myocardial metabolism and electrophysiological performance superior to lactated Ringer's (LR) during hypovolemia and hindlimb ischemia-reperfusion.

METHODS: Male domestic goats (25-30 kg) were exsanguinated to a mean arterial pressure of 48 ± 1 mmHg. Right hindlimb ischemia was imposed for 90 min by applying a tourniquet and femoral crossclamp. LR or PR, infused *iv*, delivered 0.05 mmol/kg per minute *L*-lactate or pyruvate, respectively, from 30 min hindlimb ischemia until 30 min post-ischemia. Time controls (TC) underwent neither hemorrhage, hindlimb ischemia nor resuscitation. Goats were sacrificed and left ventricular myocardium biopsied at 90 min fluid resuscitation (n = 6 per group) or 3.5 h later (n = 9 LR, 10 PR, 8 TC).

RESULTS: Myocardial 8-isoprostane content, phosphocreatine phosphorylation potential, creatine kinase activity, and heart rate-adjusted QT interval (QTc) variability were evaluated at 90 min resuscitation and 3.5 h post-resuscitation. PR sharply lowered pro-arrhythmic QTc variability *vs* LR ($P < 0.05$); this effect persisted 3.5 h post-resuscitation. PR lowered myocardial 8-isoprostane content, a product of oxidative stress, by 39 and 37% during and 3.5 h after resuscitation, respectively, *vs* LR. Creatine kinase activity fell 42% post-LR *vs* TC ($P < 0.05$), but was stable post-PR ($P < 0.02$ *vs* post-LR). PR doubled phosphocreatine phosphorylation potential, a measure of ATP free energy state, *vs* TC and LR ($P < 0.05$); this energetic enhancement persisted 3.5 h post-resuscitation.

CONCLUSION: By augmenting myocardial energy state and protecting creatine kinase activity, pyruvate-enriched resuscitation stabilized cardiac electrical function during central hypovolemia and hindlimb ischemia-reperfusion.

Key words: Creatine kinase; Electrocardiogram; Hypovolemia; 8-Isoprostane; Phosphocreatine; Reactive oxygen species; Ringer's lactate

Core tip: In goats subjected to exsanguination-induced hypovolemia and tourniquet-imposed hindlimb ischemia-reperfusion, intravenous resuscitation with Ringer's lactate produced marked electrocardiographic instability, lipid peroxidation and inactivation of the critical creatine kinase system, which supplies energy for membrane ion transport. In comparison with lactated Ringer's, resuscitation enriched with the natural antioxidant and energy substrate pyruvate stabilized cardiac rhythm, prevented lipid peroxidation, preserved creatine kinase activity and augmented myocardial energy reserves. Importantly, these favorable effects persisted for at least 3.5 h after terminating pyruvate-enriched resuscitation. Thus, pyruvate-enriched resuscitation prevented creatine kinase inactivation by oxidative stress, thereby preventing cardiac rhythm disturbances after central hypovolemia.

Gurji HA, White DW, Hoxha B, Sun J, Olivencia-Yurvati AH, Mallet RT. Pyruvate-fortified resuscitation stabilizes cardiac electrical activity and energy metabolism during hypovolemia. *World J Crit Care Med* 2013; 2(4): 56-64 Available from: URL: http://www.wjgnet.com/2220-3141/full/v2/i4/56.htm DOI: http://dx.doi.org/10.5492/wjccm.v2.i4.56

INTRODUCTION

Central hypovolemia and systemic hypotension can compromise myocardial perfusion, thereby depleting cellular energy reserves and generating cytotoxic reactive oxygen species (ROS). Interventions to stabilize blood pressure after hemorrhage include fluid resuscitation to expand intravascular volume and application of tourniquets to slow bleeding from wounded extremities[1]. Tourniquets impose ischemia on the distal tissue; reintroduction of oxygenated blood upon tourniquet removal triggers massive production of ROS which provoke systemic inflammation[2,3].

In myocardium, ROS can destabilize electrophysiological function by decreasing mitochondrial membrane potential[4], disrupting ATP production[5,6] and increasing K^+ flux through sarcolemmal ATP-sensitive potassium (K_{ATP}) channels[4,5]. Creatine kinase (CK), which catalyzes high energy phosphate shuttling from the mitochondria to the cytosol, can be reversibly inactivated by ROS[6-8], potentially producing a pro-arrhythmic state[7]. Dzeja *et al*[9] proposed that CK inactivation by ROS causes ADP accumulation that opens K_{ATP} channels, thereby shortening cardiac action potentials and provoking arrhythmias. CK was shown to be physically associated with the SUR2A subunit of the sarcolemmal K_{ATP} channel[10], raising the possibility that changes in CK activity may directly modulate K_{ATP} channel current. Thus, CK inactivation by ROS may contribute to cardiac electrical instability.

Ringer's lactate ranks among the mainstay fluids for resuscitation of trauma victims[11]. A non-antioxidant, lactate does not protect myocardium from oxidative stress[12-14]. In contrast, pyruvate is a potent physiological antioxidant[7,13,15,16]. Accordingly, fluid resuscitation with pyruvate-enriched Ringer's solution could dampen ROS formation in the myocardium, protecting CK and ameliorating cardiac electrical instability during hypovolemia and tourniquet application and release.

This study tested the hypothesis that substituting pyruvate resuscitation for lactate can suppress myocardial ROS formation, preserve CK activity and ATP metabolism and, thus, maintain cardiac electrical stability in goats subjected to hemorrhage and hindlimb ischemia-reperfusion. We also examined whether these beneficial effects would persist at least 3.5 h after completing pyruvate-enriched resuscitation. This study revealed that pyruvate-enhanced Ringer's resuscitation effectively and persistently suppressed ROS formation in the myocardium, prevented loss of CK activity, augmented energy reserves, and stabilized cardiac electrical rhythm more effectively than lactated Ringer's.

MATERIALS AND METHODS

Animal experimentation was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center and conducted in accordance with the Guide to the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996) and the Position of the American Heart Association on Research Animal Use. Forty-five male Boer goats, 25-30 kg, were randomly assigned to time control (TC), lactate Ringer's (LR) resuscitation, or pyruvate Ringer's (PR) resuscitation.

Surgical preparation

Goats were anesthetized by *iv* injection of ketamine (5 mg/kg) and midazolam (0.2 mg/kg), intubated, and mechanically ventilated with 1%-2% isoflurane supplemented with 100% O_2. The right carotid artery, jugular vein, and femoral vein were isolated and cannulated with saline-filled polyurethane catheters. A pressure transducer (Argon Medical Devices; Athens, TX) was connected to the arterial catheter to monitor blood pressure and heart rate. Blood O_2 saturation was continuously monitored by pulse oximetry. Anticoagulant heparin (650 U/kg) was injected *iv* to prevent thrombosis within the catheters. Needle electrodes were applied to the forelimbs and left hindlimb. Standard lead 2 electrocardiogram was captured with a National Instruments analog-to-digital acquisition board sampling at 500 Hz and stored on a Dell laptop running Windaq (Dataq, Akron, OH) acquisition software.

Hemorrhage, hindlimb ischemia, and fluid resuscitation

Mean arterial pressure was lowered to 48 ± 1 mmHg by controlled blood withdrawal (20 mL/min) from the jugular vein (total withdrawal 220 ± 13 mL, *i.e.*, 8.5 ± 0.6 mL/kg). The target mean arterial pressure was chosen to impose moderately severe hypotension while avoiding irreversible hemodynamic collapse. Next, the right femoral

artery was occluded with an atraumatic vasoclamp and a veterinary tourniquet was applied to the right hindlimb proximal to the femoral cutdown to impose hindlimb ischemia. The tourniquet was placed and tightened until the locking mechanism could no longer be advanced. 30 min later, resuscitation was administered for 90 min by jugular venous infusion (10 mL/min) of freshly prepared Ringer's solution containing the sodium salts of pyruvate or the natural lactate stereoisomer, *L*-lactate (Sigma, St. Louis MO)[17] at concentrations (110-150 mmol/L) adjusted to deliver 0.05 mmol pyruvate or lactate/kg per minute. At 60 min of resuscitation, the vasoclamp and tourniquet were released to reperfuse the hindlimb. Time control (TC) goats were surgically prepared and instrumented, but were not subjected to hemorrhage, resuscitation, or hindlimb ischemia. Left ventricular myocardium was biopsied at 90 min fluid resuscitation (n = 6 per group), or 3.5 h after resuscitation, *i.e.,* 4 h after hindlimb reperfusion (n = 9 LR, 10 PR). In TC experiments, myocardium was harvested at times corresponding to 90 min resuscitation (n = 6) and 3.5 h post-resuscitation (n = 8).

QTc interval variability

Variability of the QT interval provides a measure of pro-arrhythmic electrical instability[18,19]. The QT interval was the time from the initial deflection from the isoelectric PR segment to the point at which voltage returned to the isoelectric TP segment. No U waves were detected in the electrocardiograms. The Bazett equation[20] was used to adjust QT intervals for variations in heart rate, yielding corrected QT (QTc) intervals. A minimum of 20 consecutive cardiac cycles per timepoint were analyzed; the standard deviation of the QTc values represented QTc interval variability[21].

Myocardial metabolites and enzymes

Left ventricular myocardium was snap-frozen *in situ* with liquid N_2-precooled Wollenberger tongs, quickly excised, immersed in liquid N_2 and stored at -80 ℃. These biopsies were pulverized under liquid N_2, and then phosphocreatine (PCr), creatine (Cr), inorganic phosphate (P_i), ATP, pyruvate, lactate and citrate were extracted in 0.3 mol/L $HClO_4$[22,23] and measured by spectrophotometry[24-30]. Intracellular free ADP concentration ([ADP]) was estimated from the CK equilibrium[31], where [ADP] = {[ATP] [Cr]}/{[PCr] K_{CK}/[H^+]}. The equilibrium constant for CK was taken to equal 5.18×10^{-10} mol/L at a cytosolic free Mg^{2+} concentration of 0.6 mmol/L which was estimated from indicator metabolite analyses in guinea-pig myocardium[32]. Intracellular [H^+] was assumed to equal 63×10^{-10} mol/L, based on measurements in *in situ* canine left ventricular myocardium[33]. Phosphocreatine phosphorylation potential, [PCr]/{[Cr] [P_i]}, provided a measure of the free energy state of ATP in the myocardium[22,23]. The intracellular space estimates used to calculate the phosphorylation potential were calculated as 1 – [(dry mass/wet mass) + extracellular volume], where extracellular volume was taken as 0.2 mL/g wet mass based on measurements in *in situ* canine myocardium[22].

Proteins in frozen myocardium were extracted in phosphate buffer[34]. Protein concentrations in the extracts were measured colorimetrically[35] with a Coomassie Plus Bradford kit (Pierce, Rockford, IL). CK and lactate dehydrogenase (LDH) activities were spectrophotometrically assayed[36,37] and expressed as IU/mg protein.

Isolation and analysis of creatine kinase MB isoform

Left ventricular protein extracts, 6 μg per sample, were electrophoretically separated in a gel composed of 30 mL 1.5% agarose in 30 mM barbital buffer (pH 8.6) at 70 V for 4 h at 4 ℃. Next, gels were incubated at 37 ℃ in a reaction buffer containing 100 mmol/L Tris-HCl (pH 7.4), 5 mmol/L $MgCl_2$, 5 mmol/L glucose, 1 mmol/L ADP, 15 mmol/L PCr, 3 mmol/L AMP, 10 mmol/L *N*-acetylcysteine, 0.3 mmol/L $NADP^+$, 10 μg/mL glucose-6-phosphate dehydrogenase, and 10 μg/mL hexokinase for 20 min. Lastly, gels were imaged in ultraviolet light in an Alpha Innotech Fluorchem Imaging system. Band densitometry was performed with AlphaEase Fluorchem. Activities of the CK_{MB} isoform in the LR and PR long protocols were identified from the position of standard CK_{MB} and normalized to the long protocol mean TC value.

Myocardial 8-isoprostane

A product of phospholipid oxidation by ROS, 8-isoprostane provided a stable marker of oxidative stress[38]. 8-isoprostane was extracted from frozen myocardium and assayed at 412 nm in a 96 well plate reader (BioTek KCJunior, Winooski, VT) using an ELISA kit (Cayman Chemical, Ann Arbor, MI). 8-isoprostane contents in the LR, PR and long protocol TC groups were normalized to the short protocol TC values.

Statistical analysis

Presented values are mean ± SD. QTc variability was analyzed using a two-factor (treatment, time) ANOVA with repeated measures. All other variables were analyzed by single-factor (treatment) ANOVA. When ANOVA detected statistically significant effects, Student-Newman Keuls *post hoc* tests were applied to identify the specific between-group differences. Statistical analyses were performed using SigmaStat v10. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Effects of pyruvate- vs lactate-enriched fluid resuscitation on systemic arterial pressure

Controlled exsanguination of the goats lowered mean arterial pressure from 100 to 48-50 mmHg (Figure 1). Intravenous LR resuscitation, initiated 30 min after exsanguination and imposition of hindlimb ischemia, restored mean arterial pressure to that of time control goats. PR resuscitation was more effective than LR; at 90 min re-

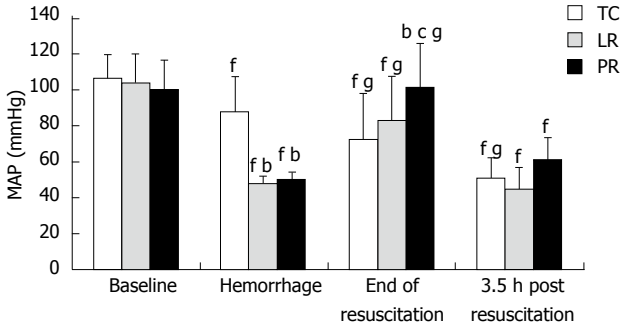

Figure 1 Systemic arterial pressure during hemorrhage, resuscitation and recovery. Values are mean ± SD from 8 time control (TC), 9 lactate Ringer's (LR), and 10 pyruvate Ringer's (PR) experiments. $^{b}P < 0.01$ *vs* TC; $^{c}P < 0.05$ *vs* LR; $^{f}P < 0.01$ *vs* baseline of same group; $^{g}P < 0.05$ *vs* pre-resuscitation hemorrhage of same group.

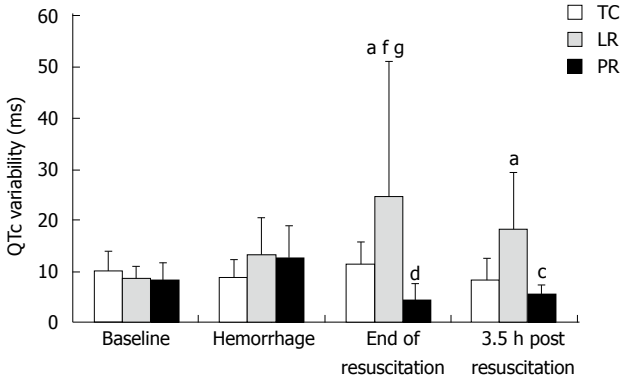

Figure 2 QTc variability during fluid resuscitation and recovery. Values are mean ± SD from 8 time control (TC), 9 lactate Ringer's (LR), and 10 pyruvate Ringer's (PR) experiments. $^{a}P < 0.05$ *vs* TC; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* LR; $^{f}P < 0.01$ *vs* baseline of same group; $^{g}P < 0.05$ *vs* pre-resuscitation hemorrhage of same group.

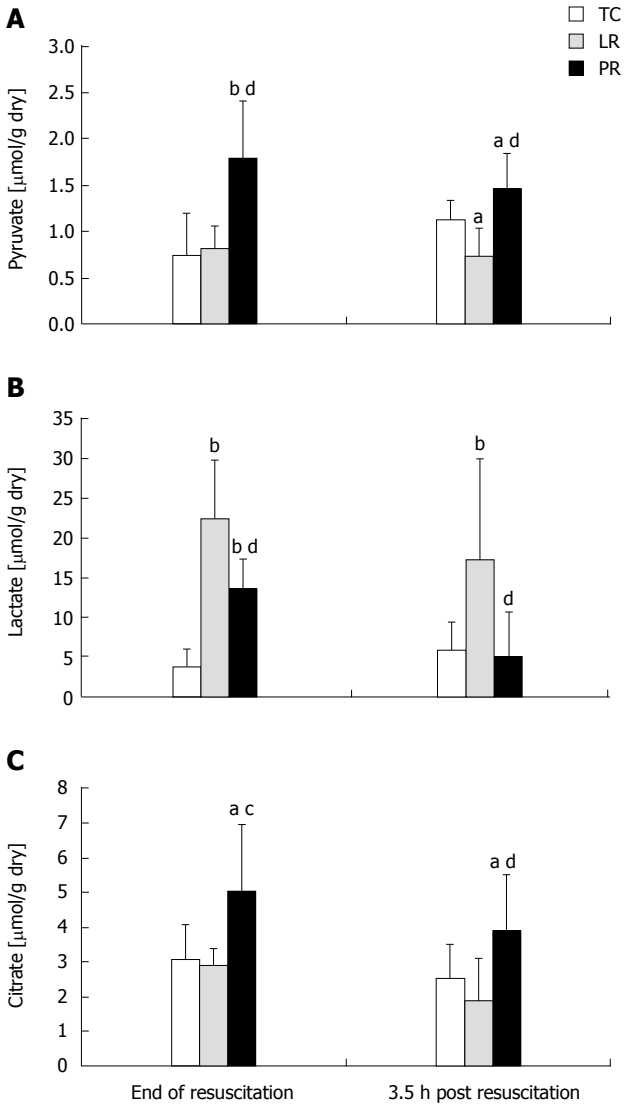

Figure 3 Myocardial pyruvate (A), lactate (B) and citrate (C) contents. Values (mean ± SD) are from left ventricular myocardium biopsied at 90 resuscitation ($n = 6$ per group) or at 3.5 h post resuscitation [8 time control (TC), 9 lactate Ringer's (LR), and 10 pyruvate Ringer's (PR)]. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* TC; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* LR.

suscitation, mean arterial pressure in the PR-resuscitated goats was appreciably higher than that of LR ($P < 0.05$) and TC ($P < 0.05$) goats. Mean arterial pressure fell after resuscitation. At 3.5 h post-resuscitation, the difference in mean arterial pressure in PR *vs* LR goats persisted but was no longer statistically significant ($P = 0.084$).

Cardiac electrical instability

Increased variability of the heart rate adjusted ventricular depolarization-repolarization period, *i.e.* QTc interval, indicates cardiac electrical instability[39]. QTc variability sharply increased during LR resuscitation (Figure 2) to twice the TC value ($P < 0.05$). In contrast, PR lowered QTc variability to 18% of the respective LR value ($P < 0.01$). QTc variability was still elevated 3.5 h after LR resuscitation *vs* TC ($P < 0.05$) but remained suppressed post-PR *vs* post-LR ($P < 0.05$). Hence, PR stabilizes cardiac electrical function both during and for 3.5 h after its administration.

Myocardial pyruvate, lactate and citrate

In the cytosol, LDH maintains an equilibrium between pyruvate and lactate[13]; in the mitochondria, pyruvate carboxylation generates oxaloacetate and, thus, Krebs cycle intermediates including citrate[15,16]. To assess pyruvate metabolism, pyruvate, lactate, and citrate were measured in left ventricular myocardium at 90 min resuscitation and 3.5 h post-resuscitation, *i.e.*, 30 min and 4 h hindlimb reperfusion. As expected, pyruvate content (Figure 3A) was higher during PR *vs* both LR resuscitation ($P < 0.01$) and the corresponding TC value ($P < 0.01$). Notably, pyruvate content remained elevated for 3.5 h after PR resuscitation. Lactate contents (Figure 3B) were increased by LR and, to a lesser extent, PR ($P < 0.01$ *vs* TC); the latter result indicated conversion of pyruvate to lactate in PR-resuscitated myocardium. Lactate content remained elevated 3.5 h after LR resuscitation ($P < 0.01$ *vs* TC) but subsided post-PR ($P < 0.01$ *vs* LR). Myocardial citrate content (Figure 3C) paralleled pyruvate content. Thus, PR resuscitation increased citrate content by 62% and 77% *vs* TC and LR, respectively ($P < 0.05$).

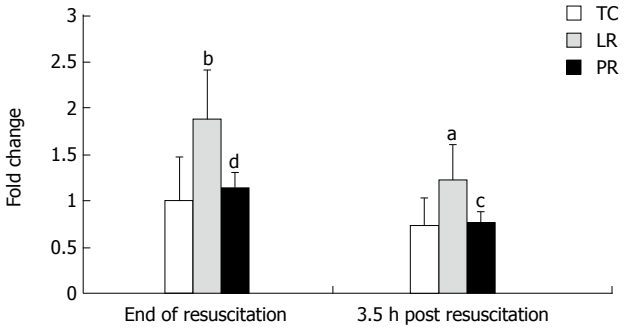

Figure 4 Myocardial 8-isoprostane content. Values (mean ± SD) are normalized to the 'end of resuscitation' time control (TC) values [8 time control (TC), 9 lactate Ringer's (LR), and 10 pyruvate Ringer's (PR)]. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* TC; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* lactate Ringer's (LR).

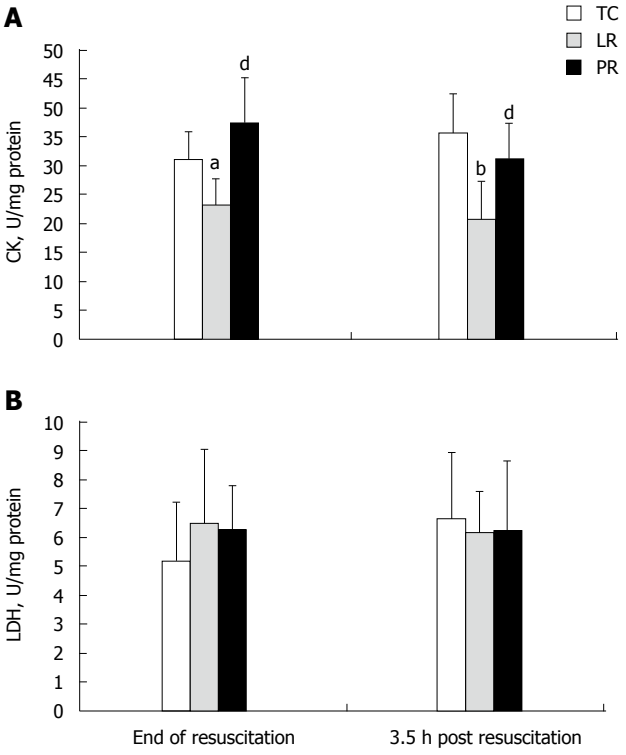

Figure 5 Myocardial creatine kinase (A) and lactate dehydrogenase (B) activities. Enzyme activities were measured in 8 time control (TC), 9 lactate Ringer's (LR) and 10 pyruvate Ringer's (PR) experiments. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* TC; $^{d}P < 0.01$ *vs* LR. CK: Creatine kinase; LDH: Lactate dehydrogenase.

Citrate content fell by 22% during the 3.5 h after PR resuscitation, but remained well above the respective post-LR ($P < 0.01$) and TC ($P < 0.05$) contents.

8-Isoprostane

Oxidative stress is potentially arrhythmogenic[4,40]. The lipid peroxide 8-isoprostane is a marker of oxidative stress and antioxidant deficiency[7,38]. The combination of hemorrhage, LR resuscitation and hindlimb ischemia-reperfusion imposed oxidative stress on the myocardium: 8-isoprostane content, measured 30 min after hindlimb reperfusion, increased 88% ($P < 0.01$) *vs* TC (Figure 4). In contrast, PR resuscitation suppressed oxidative stress: 8-isoprostane content in the PR group was 39% below the LR value ($P < 0.01$), and did not differ from TC (Figure 4). This pattern persisted until 4 h of hindlimb reperfusion: 8-isoprostane content in the post-LR myocardium was 65% above the TC value ($P < 0.05$), but in the post-PR myocardium, 8-isoprostane content was 37% below post-LR content ($P < 0.05$) and similar to that of TC. Thus, the combination of hemorrhage, LR resuscitation, and hindlimb ischemia-reperfusion imposed oxidative stress in left ventricular myocardium which was prevented by PR resuscitation.

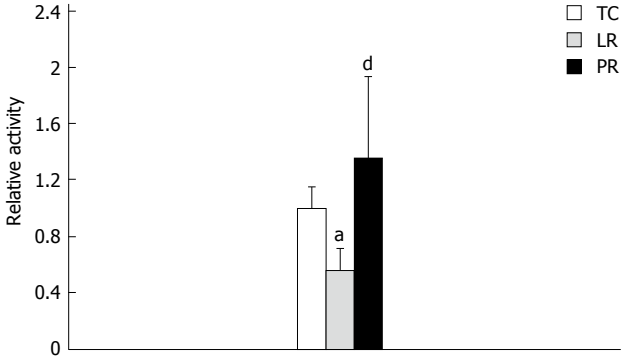

Figure 6 Myocardial creatine kinase MB isoenzyme activities. Creatine kinase-MB activities, measured in 8 time control (TC), 9 lactate Ringer's (LR) and 10 pyruvate Ringer's (PR) experiments, are expressed relative to the TC value. $^{a}P < 0.05$ *vs* TC; $^{d}P < 0.01$ *vs* LR.

Creatine kinase activity

CK plays a pivotal role in myocardial energy metabolism by shuttling high energy phosphates from the mitochondria to the myofilaments and membrane ion pumps[41]. Myocardial CK activity fell 25% ($P < 0.05$) during LR resuscitation *vs* TC (Figure 5A), but PR resuscitation preserved CK activity ($P < 0.01$ *vs* LR). These treatment effects persisted for 3.5 h after resuscitation: CK activity post-LR was 42% below the respective TC value ($P < 0.001$), but remained robust 3.5 h after PR resuscitation ($P < 0.01$ *vs* LR). Examination of CK_{MB} isoenzyme activity (Figure 6) revealed that LR resuscitation was unable to protect the cardiac specific isoenzyme from inactivation, but PR resuscitation preserved CK_{MB} activity ($P < 0.001$ *vs* LR). Activities of LDH, which is released from damaged cardiomyocytes[42], did not differ among the treatments, and remained stable throughout the protocol (Figure 5B). Thus, loss of CK activity after LR resuscitation probably was not due to CK leakage from damaged cardiomyocytes.

Myocardial energy metabolites

The combination of hemorrhage, hindlimb ischemia-reperfusion, and LR resuscitation slightly depleted myocardial ATP (Figure 7A) at 3.5 h post-resuscitation ($P < 0.05$ *vs* TC). PR resuscitation not only stabilized but even increased ATP content ($P < 0.01$ *vs* LR and $P < 0.05$ *vs* TC). At 3.5 h post-PR, ATP content remained 20% above the post-LR value ($P < 0.05$; Figure 7A). Resus-

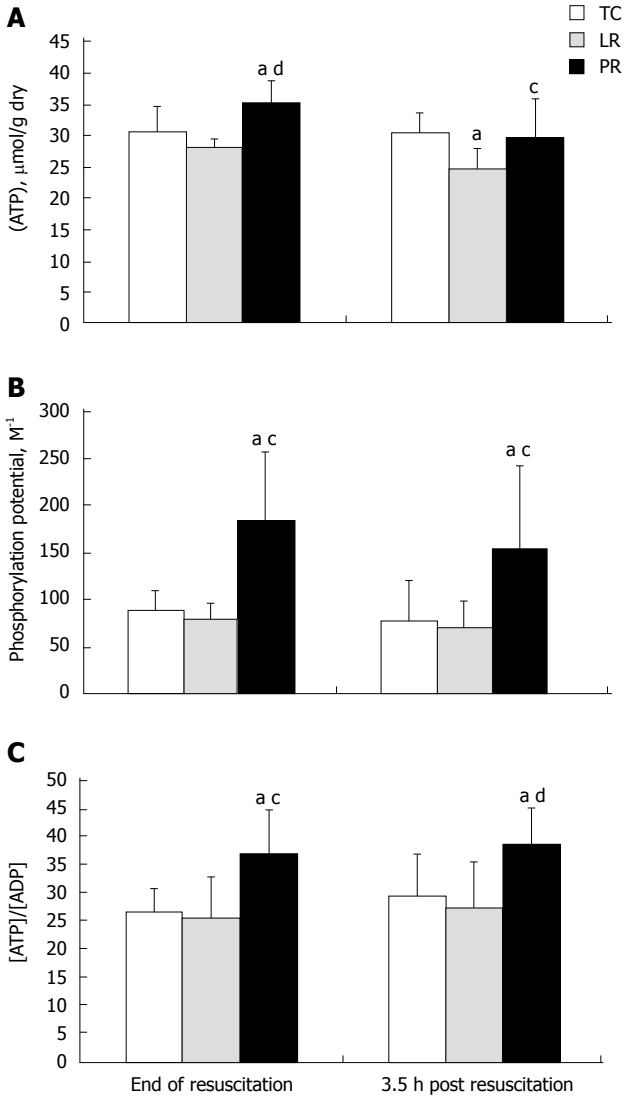

Figure 7 Myocardial ATP content (A), phosphorylation potential (B) and ATP/ADP ratio (C). Metabolites were measured in the same biopsies [8 time control (TC), 9 lactate Ringer's (LR), and 10 pyruvate Ringer's (PR)]. $^{a}P < 0.05$ *vs* TC; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* LR.

citation with PR sharply increased myocardial phosphocreatine phosphorylation potential, *i.e.* [PCr]/{[Cr] [P_i]}, *vs* LR-resuscitated and TC myocardium ($P < 0.05$), an effect that persisted 3.5 h after resuscitation (Figure 7B). Another measure of myocardial energetics, the ATP/free ADP concentration ratio (Figure 7C) defines the poise of the cytosolic free adenylate system[31]. PR resuscitation increased ATP/ADP ratio by 30%-40% ($P < 0.05$) *vs* LR-resuscitated and TC myocardium. Enhancement of ATP/ADP, like [PCr]/{[Cr] [P_i]}, persisted 3.5 h after PR resuscitation. Thus, PR resuscitation stabilized myocardial energetics more effectively than LR; importantly, these favorable effects persisted for 3.5 h after pyruvate administration.

DISCUSSION

This study tested the hypothesis that resuscitation with a pyruvate-fortified Ringer's solution during hypovolemia and hindlimb ischemia-reperfusion would stabilize cardiac electrical function and preserve creatine kinase activity in the face of oxidative stress, and maintain myocardial energy reserves in a manner superior to Ringer's lactate. In addition, we proposed that these beneficial effects would be evident not only during pyruvate administration, but also would persist another 3.5 h. Compared to LR, PR resuscitation stabilized cardiac electrical activity, suppressed lipid peroxidation, prevented CK inactivation, preserved ATP content and augmented myocardial phosphorylation potential and [ATP]/[ADP] ratio. Importantly, these myriad favorable effects of PR persisted at least 3.5 h after its administration. PR also restored arterial pressure more effectively than LR, although this effect was no longer statistically significant 3.5 h post-resuscitation.

Pyruvate provides antioxidative protection against ROS

The combination of hemorrhage, systemic hypotension, hindlimb ischemia-reperfusion and LR resuscitation imposed oxidative stress on the myocardium. By chemically modifying cellular components, ROS can impair cellular metabolism and compromise function[6,16,43]. A natural antioxidant, pyruvate dampened myocardial ROS formation both during and for 3.5 h after its administration. LR resuscitation was comparatively ineffective at suppressing ROS. Previous studies of pyruvate-enriched resuscitative fluids for treatment of hemorrhagic shock also have documented pyruvate's antioxidant capabilities[12,13,44]. Pyruvate detoxifies ROS in direct, non-enzymatic reactions[15,45]. In addition, pyruvate carboxylation generates Krebs cycle intermediates, producing citrate which, by inhibiting phosphofructokinase, diverts glucose-6-phosphate into the hexose-monophosphate pathway, the source of NADPH to maintain the endogenous antioxidant glutathione[15].

Pyruvate protects creatine kinase

Studies of myocardial ischemia-reperfusion have implicated ROS in the inhibition of metabolic enzymes and have shown that pyruvate preserves these enzymes in parallel with enhanced glutathione redox state[8,46]. Sharma *et al*[8] demonstrated that ROS inactivated key glycolytic and Krebs cycle enzymes in canine myocardium during cardiac arrest, but pyruvate infusion restored these enzyme activities. Cardioplegia-arrested swine myocardium reperfused for 3 min with whole blood had lower glutathione redox state and activities of CK and other enzymes than myocardium reperfused with pyruvate-enriched blood[7]. In the present study, PR protected myocardial CK activity more effectively than LR, and this effect persisted 3.5 h after PR administration. Creatine kinase is physically associated with SUR2A subunits of sarcolemmal K_{ATP} channels[10]. CK inactivation could open these channels and promote potassium efflux, thereby shortening the action potential and favoring proarrhythmic afterdepolarizations. Thus, preservation

of CK by PR may have helped to stabilize action potential duration and cardiac electrical function. Loss of the cytosolic enzyme LDH indicates sarcolemmal rupture. Because LDH activity was stable, the loss of myocardial CK activity in LR-resuscitated goats likely was not due to sarcolemmal rupture but rather inactivation by ROS. In that case, PR preservation of CK activity could be ascribed specifically to pyruvate's antioxidant properties.

Pyruvate stabilizes cardiac electrical function: role of ATP: ADP ratio

Victims of hemorrhage are at risk of developing cardiac electrical instability[47] which can be monitored by assessing QTc variability[18,48-50]. ROS have been implicated in the pathogenesis of arrhythmias, and it has been suggested that the antioxidative properties of certain medications could suppress ROS-mediated arrhythmogenesis[40]. Several studies have identified K^+ channels that are sensitive to oxyradical attack[4,51]. Zhang *et al*[52] elegantly demonstrated that ROS could dampen the delayed rectifier and HERG K^+ channel activities, thereby prolonging the action potential. Inactivation of the transient outward K^+ channel by hydrogen peroxide[53,54] could prolong the QT interval and action potential duration. However, *S*-nitrosylation of cardiac ion channels can either attenuate or prolong the action potential[52]. Thus, the temporal variability of repolarization produced by oxyradical attack on various cardiac ion channels can place the myocardium in a proarrhythmic state. Pyruvate supplementation can prevent ST segment elevation in myocardial ischemia-reperfusion[55,56], but pyruvate's influence on cardiac repolarization has not been reported. In this study, pyruvate-enriched resuscitation prevented the increased instability of repolarization produced by lactated Ringer's resuscitation following hypovolemia and hindlimb ischemia-reperfusion. Importantly, this anti-arrhythmic protection persisted at least 3.5 h after pyruvate administration.

Resuscitation with PR augmented ATP content and PCr phosphorylation potential *vs*. LR, both during and 3.5 h after resuscitation. These favorable energetic effects are likely due, in large part, to pyruvate's antioxidative properties as a ROS scavenger and stabilizer of glutathione redox state[16], thereby protecting metabolic enzymes, combined with its energy-yielding capabilities as a readily oxidized fuel. Indeed, pyruvate- but not lactate-enriched perfusion bolstered PCr phosphorylation potential of post-ischemic guinea-pig myocardium[57]. Studies of the energy-generating properties of pyruvate have focused on responses during pyruvate supplementation[6,16]. This study is the first to demonstrate that pyruvate's enhancement of myocardial energy state persists at least 3.5 h after pyruvate administration.

The dynamic balance between ATP and ADP modulates the gating properties of the sarcolemmal K_{ATP} channel, a sensor of cellular energy state[58]. Zhou *et al*[5] demonstrated that a decrease in ATP/ADP ratio opened K_{ATP} channels and shortened action potentials; restoring ATP/ADP inactivated the channels. Crawford *et al*[10] demonstrated that ATP dampened K_{ATP} channel activity, but its conversion to ADP activated the channel. Accordingly, increased myocardial ATP/ADP ratio may have contributed importantly to PR-induced stabilization of cardiac electrical activity. This study provides the first *in vivo* evidence that pyruvate administration can minimize the cardiac electrophysiological consequences of hemorrhagic shock and tourniquet-imposed ischemia-reperfusion of extremities.

Limitations

This project aimed to decipher the mechanism linking pyruvate-enriched resuscitation to cardiac electrical stabilization. *In vitro* experiments are required to define the contributions of the K_{ATP} channel and possibly other membrane ion channels and pumps to the heart's electrophysiological responses to PR *vs* LR. CK is present in various subcellular locations within cardiomyocytes, including the mitochondrial inter-membrane space[41], adjacent to the myofilaments[41] and in association with membrane ion pumps and K_{ATP} channels[9,10]. Although total CK activity was assessed in this study, the enzyme could not be measured at its different subcellular locations, so the specific impact of LR *vs* PR resuscitation on K_{ATP}-associated CK is undefined. The cardiac effects of PR *vs* LR resuscitation beyond the first 4 h recovery, pyruvate-enrichment of other resuscitative media, including colloidal fluids, and the efficacy of esterified pyruvate derivatives, *e.g.*, ethyl pyruvate[59,60] have not been examined in this model of hemorrhagic shock.

This study revealed several beneficial effects of pyruvate-enriched crystalloid resuscitation during central hypovolemia and hindlimb ischemia-reperfusion. Pyruvate-enriched Ringer's effectively scavenged ROS, protecting CK and augmenting myocardial energy state during and 3.5 h after PR resuscitation. These actions may have contributed to the observed pyruvate stabilization of cardiac electrical function. Fluid resuscitation is an important therapeutic strategy for central hypovolemia. These findings support development of resuscitative fluids enriched with pyruvate or its derivatives, *e.g.*, ethyl pyruvate, to improve treatment and outcomes in combat casualties and civilian trauma victims.

ACKNOWLEDGMENTS

The skillful technical assistance of Arthur G Williams, BS, Linda Howard, Shirley Nelson, RVAT, Diana R Schulz, MS, Sheldon Gaines, BS and Luis Dlouhy, BS, is gratefully acknowledged. This study was conducted in partial fulfillment of the requirements for the PhD degree for HAG.

COMMENTS

Background

Central hypovolemia and systemic hypotension can compromise myocardial

perfusion, thereby depleting cellular energy reserves and generating cytotoxic reactive oxygen species (ROS). Creatine kinase (CK), which catalyzes high energy phosphate shuttling from the mitochondria to the cytosol, can be reversibly inactivated by ROS[6-8], potentially producing a pro-arrhythmic state[7].

Research frontiers

Ringer's lactate ranks among the mainstay fluids for resuscitation of trauma victims. A non-antioxidant, lactate does not protect myocardium from oxidative stress. In contrast, pyruvate is a potent physiological antioxidant. Accordingly, fluid resuscitation with pyruvate-enriched Ringer's solution could dampen ROS formation in the myocardium, protecting CK and ameliorating cardiac electrical instability during hypovolemia and tourniquet application and release.

Innovations and breakthroughs

This study tested the hypothesis that substituting pyruvate resuscitation for lactate can suppress myocardial ROS formation, preserve CK activity and ATP metabolism and, thus, maintain cardiac electrical stability in goats subjected to hemorrhage and hindlimb ischemia-reperfusion.

Applications

This study revealed several beneficial effects of pyruvate-enriched crystalloid resuscitation during central hypovolemia and hindlimb ischemia-reperfusion. Pyruvate-enriched Ringer's effectively scavenged ROS, protecting CK and augmenting myocardial energy state during and 3.5 h after pyruvate Ringer's resuscitation.

Peer review

This was a well written manuscript and suitable for publication. The hypothesis is one of constant debate in the clinical literature and I think their experiment was well performed in a good/appropriate animal model. Their methods and results appear well supported.

REFERENCES

1 **Kragh JF**, Walters TJ, Baer DG, Fox CJ, Wade CE, Salinas J, Holcomb JB. Survival with emergency tourniquet use to stop bleeding in major limb trauma. *Ann Surg* 2009; **249**: 1-7 [PMID: 19106667 DOI: 10.1097/SLA.0b013e31818842ba]

2 **Concannon MJ**, Kester CG, Welsh CF, Puckett CL. Patterns of free-radical production after tourniquet ischemia: implications for the hand surgeon. *Plast Reconstr Surg* 1992; **89**: 846-852 [PMID: 1561256]

3 **Saeed SA**, Waqar MA, Zubairi AJ, Bhurgri H, Khan A, Gowani SA, Waqar SN, Choudhary MI, Jalil S, Zaidi AH, Ara I. Myocardial ischaemia and reperfusion injury: reactive oxygen species and the role of neutrophil. *J Coll Physicians Surg Pak* 2005; **15**: 507-514 [PMID: 16202368]

4 **Brown DA**, Aon MA, Frasier CR, Sloan RC, Maloney AH, Anderson EJ, O'Rourke B. Cardiac arrhythmias induced by glutathione oxidation can be inhibited by preventing mitochondrial depolarization. *J Mol Cell Cardiol* 2010; **48**: 673-679 [PMID: 19962380]

5 **Zhou L**, Cortassa S, Wei AC, Aon MA, Winslow RL, O' Rourke B. Modeling cardiac action potential shortening driven by oxidative stress-induced mitochondrial oscillations in guinea pig cardiomyocytes. *Biophys J* 2009; **97**: 1843-1852 [PMID: 19804714 DOI: 10.1016/j.bpj.2009.07.029]

6 **Knott EM**, Ryou MG, Sun J, Heymann A, Sharma AB, Lei Y, Baig M, Mallet RT, Olivencia-Yurvati AH. Pyruvate-fortified cardioplegia suppresses oxidative stress and enhances phosphorylation potential of arrested myocardium. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1123-H1130 [PMID: 15908464 DOI: 10.1152/ajpheart.00322.2005]

7 **Knott EM**, Sun J, Lei Y, Ryou MG, Olivencia-Yurvati AH, Mallet RT. Pyruvate mitigates oxidative stress during reperfusion of cardioplegia-arrested myocardium. *Ann Thorac Surg* 2006; **81**: 928-934 [PMID: 16488697 DOI: 10.1016/j.athoracsur.2005.08.046]

8 **Sharma AB**, Sun J, Howard LL, Williams AG, Mallet RT. Oxidative stress reversibly inactivates myocardial enzymes during cardiac arrest. *Am J Physiol Heart Circ Physiol* 2007; **292**: H198-H206 [PMID: 16920803 DOI: 10.1152/ajpheart.00698.2006]

9 **Dzeja PP**, Terzic A. Phosphotransfer reactions in the regulation of ATP-sensitive K+ channels. *FASEB J* 1998; **12**: 523-529 [PMID: 9576479]

10 **Crawford RM**, Ranki HJ, Botting CH, Budas GR, Jovanovic A. Creatine kinase is physically associated with the cardiac ATP-sensitive K+ channel in vivo. *FASEB J* 2002; **16**: 102-104 [PMID: 11729098]

11 **Peerless JR**. Fluid management of the trauma patient. *Curr Opin Anaesthesiol* 2001; **14**: 221-225 [PMID: 17016405 DOI: 10.1097/00001503-200104000-00015]

12 **Flaherty DC**, Hoxha B, Sun J, Gurji H, Simecka JW, Mallet RT, Olivencia-Yurvati AH. Pyruvate-fortified fluid resuscitation improves hemodynamic stability while suppressing systemic inflammation and myocardial oxidative stress after hemorrhagic shock. *Mil Med* 2010; **175**: 166-172 [PMID: 20358705]

13 **Bassenge E**, Sommer O, Schwemmer M, Bünger R. Antioxidant pyruvate inhibits cardiac formation of reactive oxygen species through changes in redox state. *Am J Physiol Heart Circ Physiol* 2000; **279**: H2431-H2438 [PMID: 11045981]

14 **Mauriz JL**, González P, Jorquera F, Olcoz JL, González-Gallego J. Caspase inhibition does not protect against liver damage in hemorrhagic shock. *Shock* 2003; **19**: 33-37 [PMID: 12558141 DOI: 10.1097/01.shk.0000048074.51778.24]

15 **Mallet RT**. Pyruvate: metabolic protector of cardiac performance. *Proc Soc Exp Biol Med* 2000; **223**: 136-148 [PMID: 10654616 DOI: 10.1046/j.1525-1373.2000.22319.x]

16 **Mallet RT**, Sun J. Antioxidant properties of myocardial fuels. *Mol Cell Biochem* 2003; **253**: 103-111 [PMID: 14619960]

17 **Kuze S**, Naruse T, Yamazaki M, Hirota K, Ito Y, Miyahara T. Effects of sodium L-lactate and sodium racemic lactate on intraoperative acid-base status. *Anesth Analg* 1992; **75**: 702-707 [PMID: 1416121 DOI: 10.1213/00000539-199211000-00008]

18 **Zabel M**, Portnoy S, Franz MR. Electrocardiographic indexes of dispersion of ventricular repolarization: an isolated heart validation study. *J Am Coll Cardiol* 1995; **25**: 746-752 [PMID: 7860924 DOI: 10.1016/0735-1097(94)00446-W]

19 **Atiga WL**, Calkins H, Lawrence JH, Tomaselli GF, Smith JM, Berger RD. Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. *J Cardiovasc Electrophysiol* 1998; **9**: 899-908 [PMID: 9786070 DOI: 10.1111/j.1540-8167.1998.tb00130.x]

20 **Bazett HC**. An analysis of the time-relations of electrocardiograms. *Heart* 1920; **7**: 353-370 [DOI: 10.1053/euhj.1998.1276]

21 **Krauss TT**, Mäuser W, Reppel M, Schunkert H, Bonnemeier H. Gender effects on novel time domain parameters of ventricular repolarization inhomogeneity. *Pacing Clin Electrophysiol* 2009; **32** Suppl 1: S167-S172 [PMID: 19250086]

22 **Itoya M**, Mallet RT, Gao ZP, Williams AG, Downey HF. Stability of high-energy phosphates in right ventricle: myocardial energetics during right coronary hypotension. *Am J Physiol* 1996; **271**: H320-H328 [PMID: 8760191]

23 **Yi KD**, Downey HF, Bian X, Fu M, Mallet RT. Dobutamine enhances both contractile function and energy reserves in hypoperfused canine right ventricle. *Am J Physiol Heart Circ Physiol* 2000; **279**: H2975-H2985 [PMID: 11087255]

24 **Heinz F**, Weiβer H. Creatine phosphate. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅷ: 507-514

25 **Wahlefeld AW**, Siedel J. Creatine and creatinine. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅷ: 500-506

26 **Gawehn K**. Inorganic phosphate. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅶ: 552-558

27 **Trautschold I**, Lamprecht W, Schweitzer G. ATP: uv-method with hexokinase and glucose-6-phosphate dehydrogenase. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅶ: 346-357

28 **Lamprecht W**, Heinz F. Pyruvate. In: Bergmeyer HU. Methods

of Enzymatic Analysis. New York: Academic Press, 1983; Ⅵ: 570-577
29 **Noll F**. L-(+)-lactate. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅵ: 582-588
30 **Möllering H**. Citrate. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅶ: 2-12
31 **Veech RL**, Lawson JW, Cornell NW, Krebs HA. Cytosolic phosphorylation potential. *J Biol Chem* 1979; **254**: 6538-6547 [PMID: 36399]
32 **Mallet RT**, Kang YH, Mukohara N, Bünger R. Use of cytosolic metabolite patterns to estimate free magnesium in normoxic myocardium. *Biochim Biophys Acta* 1992; **1139**: 239-247 [PMID: 1627662 DOI: 10.1016/0925-4439(92)90140-l]
33 **Tune JD**, Mallet RT, Downey HF. Insulin improves cardiac contractile function and oxygen utilization efficiency during moderate ischemia without compromising myocardial energetics. *J Mol Cell Cardiol* 1998; **30**: 2025-2035 [PMID: 9799656 DOI: 10.1006/jmcc.1998.0763]
34 **Braasch W**, Gudbjarnason S, Puri PS, Ravens KG, Bing RJ. Early changes in energy metabolism in the myocardium following acute coronary artery occlusion in anesthetized dogs. *Circ Res* 1968; **23**: 429-438 [PMID: 5676453]
35 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051]
36 **Gerhardt W**. Creatine kinase. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅲ: 508-518
37 **Wahlefeld AW**. Lactate dehydrogenase: UV-method with L-lactate and NAD. In: Bergmeyer HU. Methods of Enzymatic Analysis. New York: Academic Press, 1983; Ⅲ: 126-133
38 **Morrow JD**. The isoprostanes-unique products of arachidonate peroxidation: their role as mediators of oxidant stress. *Curr Pharm Des* 2006; **12**: 895-902 [PMID: 16533158 DOI: 10.2174/138161206776055985]
39 **Hohnloser SH**, van de Loo A, Arendts W, Zabel M, Just H. QT-dispersion in the surface ECG as a parameter of increased electrical vulnerability in acute myocardial ischemia. *Z Kardiol* 1993; **82**: 678-682 [PMID: 7904787]
40 **Tomaselli GF**, Barth AS. Sudden cardio arrest: oxidative stress irritates the heart. *Nat Med* 2010; **16**: 648-649 [PMID: 20526319]
41 **Bessman SP**, Yang WC, Geiger PJ, Erickson-Viitanen S. Intimate coupling of creatine phosphokinase and myofibrillar adenosinetriphosphatase. *Biochem Biophys Res Commun* 1980; **96**: 1414-1420 [PMID: 6449202 DOI: 10.1126/science.6450446]
42 **Vanden Hoek TL**, Shao Z, Li C, Zak R, Schumacker PT, Becker LB. Reperfusion injury on cardiac myocytes after simulated ischemia. *Am J Physiol* 1996; **270**: H1334-H1341 [PMID: 8967373]
43 **Flaherty DC**, Hoxha B, Sun J, Gurji H, Simecka JW, Olivencia-Yurvati AH, Mallet RT. Pyruvate-enriched resuscitation protects hindlimb muscle from ischemia-reperfusion injury. *Mil Med* 2010; **175**: 1020-1026 [PMID: 21265313]
44 **Mongan PD**, Capacchione J, West S, Karaian J, Dubois D, Keneally R, Sharma P. Pyruvate improves redox status and decreases indicators of hepatic apoptosis during hemorrhagic shock in swine. *Am J Physiol Heart Circ Physiol* 2002; **283**: H1634-H1644 [PMID: 12234818 DOI: 10.1152/ajpheart.01073.2001]
45 **Vásquez-Vivar J**, Denicola A, Radi R, Augusto O. Peroxynitrite-mediated decarboxylation of pyruvate to both carbon dioxide and carbon dioxide radical anion. *Chem Res Toxicol* 1997; **10**: 786-794 [PMID: 9250413 DOI: 10.1021/tx970031g]
46 **Powell SR**, Wang P, Katzeff H, Shringarpure R, Teoh C, Khaliulin I, Das DK, Davies KJ, Schwalb H. Oxidized and ubiquitinated proteins may predict recovery of postischemic cardiac function: essential role of the proteasome. *Antioxid Redox Signal* 2005; **7**: 538-546 [PMID: 15889999 DOI: 10.1089/ars.2005.7.538]
47 **Frontera JA**, Parra A, Shimbo D, Fernandez A, Schmidt JM, Peter P, Claassen J, Wartenberg KE, Rincon F, Badjatia N, Naidech A, Connolly ES, Mayer SA. Cardiac arrhythmias after subarachnoid hemorrhage: risk factors and impact on outcome. *Cerebrovasc Dis* 2008; **26**: 71-78 [PMID: 18525201 DOI: 10.1159/000135711]
48 **Hanci V**, Gül S, Dogan SM, Turan IO, Kalayci M, Açikgöz B. Evaluation of P wave and corrected QT dispersion in subarachnoid haemorrhage. *Anaesth Intensive Care* 2010; **38**: 128-132 [PMID: 20191788]
49 **Ozdemir D**, Ozdemir N, Unal N, Tektas S. QTc dispersion in children with severe head trauma. *Pediatr Emerg Care* 2005; **21**: 658-661 [PMID: 16215468 DOI: 10.1097/01.pec.0000181427.25342.e0]
50 **Macmillan CS**, Andrews PJ, Struthers AD. QTc dispersion as a marker for medical complications after severe subarachnoid haemorrhage. *Eur J Anaesthesiol* 2003; **20**: 537-542 [PMID: 12884986]
51 **Gonzalez DR**, Treuer A, Sun QA, Stamler JS, Hare JM. S-Nitrosylation of cardiac ion channels. *J Cardiovasc Pharmacol* 2009; **54**: 188-195 [PMID: 19687749]
52 **Zhang Y**, Sun X, Zhang Y, Wang J, Lu Y, Yang B, Wang Z. Potential therapeutic value of antioxidants for abnormal prolongation of QT interval and the associated arrhythmias in a rabbit model of diabetes. *Cell Physiol Biochem* 2011; **28**: 97-102 [PMID: 21865852 DOI: 10.1159/000331718]
53 **Lu Z**, Abe J, Taunton J, Lu Y, Shishido T, McClain C, Yan C, Xu SP, Spangenberg TM, Xu H. Reactive oxygen species-induced activation of p90 ribosomal S6 kinase prolongs cardiac repolarization through inhibiting outward K+ channel activity. *Circ Res* 2008; **103**: 269-278 [PMID: 18599872 DOI: 10.1161/CIRCRESAHA.107.166678]
54 **Rozanski GJ**, Xu Z. Glutathione and K(+) channel remodeling in postinfarction rat heart. *Am J Physiol Heart Circ Physiol* 2002; **282**: H2346-H2355 [PMID: 12003845 DOI: 10.1152/ajpheart.00894.2001]
55 **Liedtke AJ**, Nellis SH, Neely JR, Hughes HC. Effects of treatment with pyruvate and tromethamine in experimental myocardial ischemia. *Circ Res* 1976; **39**: 378-387 [PMID: 954168]
56 **Sharma AB**, Knott EM, Bi J, Martinez RR, Sun J, Mallet RT. Pyruvate improves cardiac electromechanical and metabolic recovery from cardiopulmonary arrest and resuscitation. *Resuscitation* 2005; **66**: 71-81 [PMID: 15993732 DOI: 10.1016/j.resuscitation.2004.12.016]
57 **Bünger R**, Mallet RT, Hartman DA. Pyruvate-enhanced phosphorylation potential and inotropism in normoxic and postischemic isolated working heart. Near-complete prevention of reperfusion contractile failure. *Eur J Biochem* 1989; **180**: 221-233 [PMID: 2707262 DOI: 10.1111/j.1432-1033.1989.tb14637.x]
58 **Noma A**. ATP-regulated K+ channels in cardiac muscle. *Nature* 1983; **305**: 147-148 [PMID: 6310409 DOI: 10.1038/305147a0]
59 **Fink MP**. Ethyl pyruvate: a novel anti-inflammatory agent. *Crit Care Med* 2003; **31**: S51-S56 [PMID: 12544977 DOI: 10.1097/01.CCM.0000042476.32014.43]
60 **Cai B**, Chen F, Lin X, Miller E, Szabo C, Deitch EA, Ulloa L. Anti-inflammatory adjuvant in resuscitation fluids improves survival in hemorrhage. *Crit Care Med* 2009; **37**: 860-868 [PMID: 19237889]

P- Reviewer Firstenberg MS **S- Editor** Zhai HH
L- Editor A **E- Editor** Liu XM

World Journal of Critical Care Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
www.wjgnet.com

World J Crit Care Med 2013 November 4; 2(4): I-V
ISSN 2220-3141 (online)

GENERAL INFORMATION

World Journal of Critical Care Medicine (*World J Crit Care Med*, *WJCCM*, online ISSN 2220-3141, DOI: 10.5492) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJCCM covers topics concerning severe infection, shock and multiple organ dysfunction syndrome, infection and anti-infection treatment, acute respiratory distress syndrome and mechanical ventilation, acute kidney failure, continuous renal replacement therapy, rational nutrition and immunomodulation in critically ill patients, sedation and analgesia, cardiopulmonary cerebral resuscitation, fluid resuscitation and tissue perfusion, coagulant dysfunction, hemodynamic monitoring and circulatory support, ICU management and treatment control, and application of bronchofiberscopy in critically ill patients. The current columns of *WJCCM* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography.

We encourage authors to submit their manuscripts to *WJCCM*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJCCM is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial borad members or peer reivewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJCCM* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in critical care medicine; (12) Brief Articles: To briefly report the novel and innovative findings in critical care medicine; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, e.g., the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJCCM*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of critical care medicine; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Critical Care Medicine

ISSN

ISSN 2220-3141 (online)

Launch date

February 4, 2012

Frequency
Quarterly

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Critical Care Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Publisher
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Telephone: +852-58042046
Fax: +852-31158812
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Production center
Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

Representative office
USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/2220-3141/g_info_20100722180909.htm.

Indexed and Abstracted in
Digital Object Identifier.

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJCCM* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory ani-

mals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-3141/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjccm@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, *e.g.*, Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned

clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P <$ 0.05, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, *e.g.*, PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-3141/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-3141/g_info_20100725073726.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/2220-3141/g_info_20100725073445.htm.

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJCCM is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 600 USD per article. All invited articles are published free of charge.

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com